FINAL TECHNICAL REPORT COVER PAGE

LONGITUDINAL EFFECTS OF PROLONGED OPIOID USE ON CORTICAL BONE REMODELING IN A RABBIT MODEL

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Scientific Approach: Description of Study Sample and Progress Made

Objective 1: To characterize, for the first time, cortical bone microstructural changes in a rabbit opioid model.

Activity 1 – *Ethics application, animal handling training, animal ordering, acclimation*
Dates: 1 January – 30 April 2019
Progress: 100% Complete

A detailed animal protocol (Protocol #: 18-11-12 ARC) was written by Dr. Andronowski and approved by The University of Akron Institutional Animal Care and Use Committee (IACUC) on 12/10/2018 (**Appendix I**). Due to the substantial time and effort required for devising animal protocols, and in conjunction with the IACUC's monthly meeting schedule, Dr. Andronowski began the protocol writing process in October 2018 to ensure the necessary approvals were in place for the beginning of the funding period (01/01/2019). In-person training with The University of Akron Research Vivarium (UARV) attending veterinarian, Dr. Stanley Dannemiller, in proper ethical care, handling, and use of laboratory animals was completed by the PI, Post-doctoral Fellow (Dr. Mary Cole), Graduate Research Assistant (Reed Davis), Undergraduate Research Assistant (Adam Schuller), and Tiered Mentoring Students/unpaid undergraduate laboratory volunteers (Gina Tubo and Abigail LaMarca) on 03/11/2019 (**Appendix II**). In-person Hazardous Substances training was provided by UA Health and Safety representative, Alex Stakleff, to Andronowski, Schuller, Davis, Tubo, and LaMarca on 02/01/2019. UARV facility training was also provided by vivarium manager, Beth Kenaga, to the above research team members on 02/01/2019, and to Cole and LaMarca on 03/11/2019.

Skeletally mature, 6-month-old (2.3-3.0 kg), male New Zealand White rabbits were ordered from Covance Research Products Inc. on 3/19/2019 (**Appendix III**) and delivered to UARV on 04/16/2019, as per our projected timeline (**Appendix IV)**. Male animals were selected to avoid the potential influence of female hormone cycles on bone physiology. The rabbits were individually housed in rabbit batteries to allow for some interaction, while keeping the animals lodged separately (**Appendix V**). The rabbits were quarantined and habituated to the testing conditions for a two-week period following their arrival at the UARV (04/16/2019 – 04/31/2019). The rabbits were randomly divided (using a random number generator) into three groups of 7 animals each: morphine, fentanyl, and controls. The control group was further randomly divided into saline vehicle (n=3) and transdermal patch groups (n=4). These group sizes match the mean numbers employed in previous characterizations of this model for cancellous bone or cortical geometry/density[1–5]. After the acclimation period, the experimental treatments for the opioid groups (morphine and fentanyl) began and continued for eight weeks.

Activity 2 – *Animal Dosing*
Dates: 1 May – 26 June 2019
Progress: 100% Complete

The proposed opioid dosing levels are consistent with clinical recommendations for analgesia in rabbits and were finalized with the UARV veterinarian. The morphine sulfate group received a dose of 3 mg/kg/day via subcutaneous injection. The control group was administered saline at a dose of 3 mg/kg/day. A proposed

change affecting the delivery of fentanyl from subcutaneous injection to transdermal patch administration was proposed by Dr. Andronowski and approved by the IACUC on 04/07/2019 (**Appendix VI**). According to literature published by Foley et al.[6] and Jain et al.[7] there is demonstrable evidence that transdermal patch delivery of fentanyl resulted in a detectable change in bone metrics. We proposed this modification to eliminate the lack of consensus in recommended injectable fentanyl dosing from various professional consultants, and to reduce risk of accidental exposure during administration of the narcotics. Further, this change will address the concerns of the IACUC regarding injections being handled by trainees (Undergraduate and Graduate Students) while the Principal Investigator is attending conferences, or if she should be sick, or otherwise unable to attend dosing periodically throughout the duration of the study.

The transdermal patch administration reduced the need to dose the animals each day and instead acted as a slow-release delivery agent. A 25-ug/h slow-release transdermal fentanyl patch (Henry Schein Inc.) was placed on the dorsum of the rabbits in the interscapular region in the fentanyl experimental group every third day, according to manufacturer's instructions. This size patch and specific dosage was selected based on data obtained by Foley et al.[6] and Jain et al.[7] The patches were adhered via adhesives associated with the patch manufacturing. Patches were further secured by applying a medical-grade Tegaderm transparent film dressing (used in clinical settings to adhere IVs and heal burns, etc.). A placebo patch (Tegaderm film dressing) was placed on each control patch animal in a manner comparable to the experimental animals. For a detailed justification of this change from the agency approved experimental plan, please refer to the approved VVC Modification (**Appendix VI**).

A second proposed change affecting the fentanyl experimental group was initiated that differed from the agency approved plan. A VVC Modification was put forward to cover the drug eluting transdermal patches (fentanyl) and control patches (Tegaderm) in order to prevent the animals from chewing, removing, and/or ingesting these from the interscapular region. The removal of patches by the animals was a common issue faced during the experimental component of the study and we were concerned for the well-being of these animals. Following consultation with the UARV veterinarian, Dr. Stan Dannemiller, a prescription was provided for the use of rabbit jackets (and other wraps) via email on 05/03/2019. The proposed modification of rabbit jackets on all treatment and control transdermal patch animals was approved on 05/04/2019 (**Appendix VII**).

All animals further underwent subcutaneous injection with a bone-labelling fluorochrome, calcein, to facilitate *ex vivo* dynamic histomorphometry following euthanasia. Calcein was administered at a level 10/mg/kg[8] after two weeks (days 13 and 14), four weeks (days 27 and 28), six weeks (days 41 and 42), and eight weeks[9,10] of the opioid regimens. It is important to note that the injection of calcein at this dose level is not associated with any undesirable side effects[11]. The fourth calcein dosing was administered two days and one day prior to intraperitoneal injections containing a euthanasia solution, pentobarbital sodium (Fatal-Plus). The euthanasia solution was delivered in the UARV necropsy suite at a level of 125 mg/kg per animal. In-person training with the UARV attending veterinarian, Dr. Stanley Dannemiller, in intraperitoneal injections and bilateral pneumothorax for euthanasia was completed by the PI, Post-doctoral Fellow (Dr. Mary Cole), Graduate Research Assistant (Reed Davis), Undergraduate Research Assistant (Adam Schuller), and Tiered Mentoring Student (Abigail LaMarca) prior to euthanasia on 06/27/2019 (**Appendix VIII**).

Overall, *Activities 1 and 2* proceeded 100% on track in accordance with our proposed project timeline (**Appendix IV**).

Objective 2: To optimize 3D micro-CT imaging of remodeling events in an opioid rabbit model.

Activity 3: Micro-CT imaging and histomorphometry
Projected Completion Dates: 08/19 – 01/20
Micro-CT imaging Progress: 100% as per proposed timeline
Histomorphometry Progress: 100% completed; began January 2020, delayed by COVID-19 pandemic
Progress: 100% as per proposed revised timeline

Micro-CT Imaging (ex vivo): The left femur of each rabbit was visualized with non-destructive 3D micro-CT imaging followed by traditional thin-section histology. Immediately following euthanasia, the left femur and tibia were dissected from each animal, wrapped in sterile saline-soaked gauze, and stored in a sample tube in a -80° C freezer. The remainder of the animal was sealed in plastic under vacuum and frozen for future bone and tissue research. To prepare for micro-CT imaging, all members of the research team have completed radiation generating equipment training (RGE Training), as well as two to three sessions of individualized, hands-on training for independent operation of the micro-CT instrument. Micro-CT imaging was performed using a SkyScan 1172 micro-CT (Bruker Micro-CT) desktop system located in the Surface and Optical Analysis Facility within the Polymer Innovation Center at The University of Akron. Since this system is shared by users from various departments at The University of Akron, a conservative estimate of 6-9 months for imaging was estimated (**Appendix IV**).

Dr. Andronowski developed and tested a micro-CT scanning protocol using bone tissue samples from her concurrent projects. Each femur and tibia were mounted to a brass sample holder via dental wax and secured with Parafilm to ensure the sample does not move during scanning. Anterior and Medial sides were marked with clay inclusions in a parafilm wrapping that would be visible on the scanned image to orient the sample during image analysis. The midshaft diaphysis was imaged at 5.49 μm voxel size (in three stacked sections) to allow for detection of the vascular canal network and the presence of BMU cutting cones, signifying remodeling activity. The previously optimized micro-CT imaging protocol was applied and includes the use of a Hamamatsu 100/250 optical camera to take 1992 projection images spanning 180 degrees of rotation at 74 kilovolts (kV) and 134 source current (uA). The population density of vascular pore systems was volumetrically assessed within the VOI (pores/mm^3). Additional micro-CT parameters included % porosity, canal number, canal connectivity density, canal diameter, and canal separation[12].

Conventional histomorphometry was performed to further characterize cortical bone differences among groups. The measured section of the left distal femur, which was scanned using micro-CT, was removed in a 10 mm section, dehydrated and embedded in methyl methacrylate (MMA) according to a protocol developed in the Andronowski Lab (**Appendix IX**). Frost[13] recommends that 50 mm^2 of bone be evaluated, which requires 2 to 5 serial thin-sections. In our experience, and that of colleagues, evaluating two thin sections is adequate to account for the variation in microstructures between sections. A diamond wire saw was employed to cut two thin sections (~60 – 90 μm thick) from each embedded block, which were mounted on glass slides. Thin sections were visualized on an Olympus BX51 microscope by capturing overlapping microscopic images at 200x with brightfield with Differential Interference Contrast (DIC) and the

associated cellSens Entry software 1.16 (Olympus). The overlapping microscopic images were stitched together into a composite image of the cross-section using Microsoft Image Composite Editor and Photoshop 2020. Cross-sectional microscopic images were similarly captured and photomerged at 200x using circularly polarized light, and at 100x using fluorescence with a FITC filter cube. After photomerging, the DIC, circularly polarized, and fluorescence images were aligned with one another in Photoshop 2020. These cross-sections were then concurrently anatomically aligned to match the orientation of the corresponding micro-CT data using a 2D slice of the cortical shell extracted from the micro-CT image processing workflow. Cross-sectional images were then cleared external to periosteal and endosteal borders. The circularly polarized image was used to identify and isolate the remodeling area, which was the circumferential intracortical region of secondarily remodeled (Haversian) bone, using the software ORS Dragonfly v.4.1 (Object Research Systems, Montréal, Canada). A custom ImageJ toolkit, OsteoFlo, was developed by Drs. Cole and Andronowski to semi-automatically extract classifications (e.g., vascular pore type, single/double/triple labeled osteons), morphometric parameters (e.g., areal fraction, population density, size, and shape parameters), and remodeling parameters (e.g., number of active remodeling centers, osteonal mineral apposition rate, osteon wall thickness, activation frequency) from the DIC and fluorescence images. These parameters were assessed for whole cross-sections and for anatomical quadrants (Anterior, Medial, Posterior, Lateral) within cross-sections.

Objective 3: Identify if prolonged opioid use is discernable in cortical bone microstructural features used in histological age-at-death estimation.

Activity 4: Data Analysis
Projected Completion Dates: 02/20 – 06/20
Revised Completion Dates: 07/21 – 09/21
Progress: 100% as per proposed revised timeline

Statistical analyses were accomplished using R statistical software (The R Foundation, v. 4.1.1). Micro-CT data, which described the 3D morphometry of vascular pore networks, were assessed using Linear Mixed Models (LMM), to control for the repeated measure of using the femur and tibia from the same individuals as a random effect. Each morphometric variable was tested for a significant response to the fixed effects of drug group, bone type (femur or tibia), and anatomical region. Histological data, which describe the 2D morphometry and aspects of remodeling rate and frequency in the femur specifically, were assessed using ANOVAs. Each morphometric variable was tested for a significant response to the fixed effects of drug group and anatomical region. Appropriate post-hoc analyses tested for significant differences between pairs of drug groups and pairs of anatomical regions, and tests for model importance and goodness-of fit (R^2, effect size, power analyses) were also performed. A significance level of $p \leq 0.05$ was used for testing.

Data analysis proceeded on schedule beginning July 2021, according to the revised project timeline.

Activity 5: Manuscript preparation and knowledge dissemination
Projected Completion Dates: 07/20 – 12/20
Revised Completion Dates: 07/21 – 09/21
Progress: 100% as per proposed revised timeline

Planned Scholarly Product

Planned scholarly products include manuscript submissions to competitive peer-reviewed scientific journals. The results of the proposed application and limitations of this research will be published in journals targeting forensic specialists such as forensic anthropologists and archaeologists, missing persons detectives, and crime scene personnel. To reach forensic practitioners and the general forensic science community, scientific journals will include *Journal of Forensic Sciences*, *Forensic Anthropology*, and *Forensic Science International.*

The proposed work is also of interest to the bone biology and biomedical imaging communities. As such, Dr. Andronowski intends to target these groups through discipline specific journals such as the *Journal of Anatomy, Bone, Micron,* and the *Anatomical Record*.

Dissemination Strategy

The ultimate goal of the Andronowski Group is to further understandings of how bone remodeling is related to age-related change using high-resolution imaging modalities (e.g., micro-CT and SR micro-CT), while simultaneously generating and disseminating new scientific knowledge. To broadly disseminate the findings and reach a wide variety of specialists within forensic science and the criminal justice system, law enforcement and legal personnel, medical examiners, forensic anthropologists and forensic archaeologists, initial results will be presented at the annual American Academy of Forensic Sciences conference. For a more targeted audience, results will be presented at the annual Canadian Bone and Joint Conference hosted by the University of Western Ontario's Bone and Joint Institute. These conferences bring together experts from across disciplines committed to interdisciplinary and high-impact research related to bone-affecting conditions and their treatment.

In addition, Dr. Andronowski routinely travels to the Canadian Light Source (CLS) synchrotron facility located on The University of Saskatchewan campus to run synchrotron imaging experiments and collaborate on other research projects. She will further disseminate the information learned through this venue and reach a number of bone imaging specialists and musculoskeletal researchers in the Department of Anatomy, Physiology, and Pharmacology. Lab members Dr. Andronowski, Dr. Cole, Reed Davis, and Gina Tubo traveled to the CLS in August 2019 and December 2019 for experimental beam time on a concurrent project. Further planned synchrotron experiments were on hold from April 2020 – June 2021. The Andronowski Lab was able to travel to CLS once again in September 2021 for imaging time related to various other research projects.

A manuscript documenting the findings from *Activities 1 and 2* titled 'Rabbits (*Oryctolagus cuniculus*) as a Model System for Longitudinal Experimental Opioid Treatments: Implications for Orthopedic and Biomedical Research' was published in a special issue in the journal *Osteology* (**Appendix X**). All members of the research team contributed. Additional manuscripts describing micro-CT and histology results, and the associated software packages developed for this software, are currently in preparation. Further manuscript preparations and data dissemination proceeded on schedule as per the revised project timeline.

Activity 6: Project management (Andronowski)
Projected Completion Dates: 01/19 – 09/21
Progress: 100% complete as per proposed timeline

The current study was managed through the Department of Biology at The University of Akron. The Principal Investigator (Dr. Andronowski) provided overall project direction and coordination, contributed to methods and data review. Andronowski prepared quarterly and semi-annual reports to the Office of Justice Programs (OJP), conference abstracts, and data for journal submissions and other forms of dissemination. She was further responsible for overall project management and coordination. Dr. Andronowski trained and supervised a Post-doctoral Fellow (Dr. Mary Cole), Graduate Research Assistant (Reed Davis), Undergraduate Research Assistant (Adam Schuller), and Tiered Mentoring Undergraduate Students (Gina Tubo, Abigail LaMarca, and Josh Taylor) at The University of Akron, certified that milestones were met, and ensured the timely submission of quarterly and semi-annual reports to OJP.

Project management proceeded on schedule with 100% completion at of the end of the no-cost extension (08/31/2021).

II. ACCOMPLISHMENTS

2.1. What are the major goals and objectives of the project?

The **purpose** of the project, as stated in the approved application, is to develop a longitudinal model for studying the effects of prolonged drug exposure, specifically opioids, on cortical bone remodeling in an animal — the rabbit — which remodels its cortical bone in a manner comparable to humans. The **ultimate goal** is to describe how analgesic drugs, particularly morphine and fentanyl, affect microscopic structures of cortical bone used in histological age estimation methods in forensic anthropology. The current work is highly important and culturally relevant as the misuse and addiction to opioids (and synthetic opioids) is a serious public health crisis nationwide. **Given the limited data available related to the longitudinal impact of opioid abuse on bone remodeling, this study is working to improve the applicability of histological age-estimation methods and improve scientific standards within the field of forensic anthropology.** The implications are critical given that many of the skeletal remains examined by forensic anthropologists come from marginalized backgrounds with substance abuse issues and overall poor health. Thus, histological methods developed on healthy cases may prove ineffective in the analysis of such individuals.

To address this overarching goal, Dr. Andronowski developed and implemented a high resolution three-dimensional (3D) imaging model capable of tracking cortical bone remodeling events to serve as a platform for assessing the effects of morphine and fentanyl on Basic Multicellular Unit (BMU) activity. As BMUs are clusters of cells that essentially "tunnel" through bone tissues to enact repair and remodeling, they are excellent biomarkers of bone microstructural changes. Their activity, however, has been rarely monitored histologically and high-resolution imaging techniques offer a new avenue for understanding their spatio-temporal behavior. The use of high-resolution micro-CT imaging enables a scale of analysis that will allow **two central research questions** to be addressed: 1) What are the effects of prolonged morphine and

fentanyl use on cortical bone remodeling? Will cortical bone microstructure vary randomly with respect to opioid use, or will it be correlated? 2) Are the effects of prolonged opioid use discernable in cortical bone microstructural features used in histological age-at-death estimation?

The current project has three primary objectives to: 1) characterize, for the first time, cortical bone microstructural changes in a rabbit opioid model, 2) optimize 3D micro-Computed Tomography (micro-CT or μCT) based imaging of remodeling events in a rabbit model, and 3) identify if prolonged opioid use is discernable in cortical bone microstructural features used in histological age-at-death estimation. Their status within the proposed timeline and the scientific approach employed is detailed. **Appendix IV** further provides a visual representation of the revised project timeline, outlines completed activities, and milestones for the animal procedures and overall study.

The initial administrative and experimental phases of this project were reported in the semi-annual report submitted in July 2019. This involved the completion of *Activity 1* (Ethics application for IACUC, animal handling training, animal ordering and acclimation) and *Activity 2* (Animal dosing) as described in the original project timeline. The first objective, encompassing *Activities 1 and 2* per the project timeline, was 100% completed during the first reporting period ending 06/31/19. In the subsequent period (07/01/19 – 12/31/19), all micro-CT imaging related to *Activity 3* was completed as projected in the original project timeline. For reporting period (01/01/20 – 06/30/20), micro-CT image processing and statistical analyses related to *Activity 4* were completed according to the revised project timeline as described below. During the reporting period (07/01/20 – 12/31/20), tasks related to the histomorphometry experiments of *Activity 3* were completed including methyl methacrylate (MMA) embedding, microscopic slide preparation, digital annotation of the sections, and histological analysis protocol development. For the reporting period (01/01/21 – 06/30/21), preparations for histological analyses and data analysis related to the histology experiments of *Activity 3* were completed. Under the no-cost extension, tasks related to *Activity 4* and *5* concerning histomorphometric data analysis, manuscript drafts, and knowledge dissemination were finalized.

2.2. What was accomplished under these goals?

2.2.1. Major Activities

Objective 1: To characterize, for the first time, cortical bone microstructural changes in a rabbit opioid model.

Activity 1 – *Ethics application, animal handling training, animal ordering, acclimation*
Dates: 1 January – 30 April 2019
Progress: 100% Complete

A detailed animal protocol (Protocol #: 18-11-12 ARC) was written by Dr. Andronowski and approved by The University of Akron Institutional Animal Care and Use Committee (IACUC) committee on 12/10/2018 (**Appendix I**). In-person training with The University of Akron Research Vivarium (UARV) attending veterinarian, Dr. Stanley Dannemiller, in proper ethical care, handling, and use of laboratory animals was completed by the PI, Post-doctoral Fellow (Dr. Mary Cole), Graduate Research Assistant (Reed Davis),

and Undergraduate Research Assistant (Adam Schuller) on 03/11/2019 (**Appendix II**). In-person Hazardous Substances training was provided by UA Health and Safety representative, Alex Stakleff, to Andronowski, Schuller, and Davis on 02/01/2019. UARV facility training was also provided by vivarium manager, Beth Kenaga, to the above research team members on 02/01/2019, and to Cole and LaMarca on 03/11/2019.

Project supplies and consumables were ordered in preparation for the arrival of the live rabbits and their experimental treatments. These items included (but are not limited to): enrichment toys, syringes/needles for administration of pharmaceutical agents, and water bottles and food troughs. We also purchased the pharmaceutical agents (morphine sulphate and fentanyl) through the UARV.

Activity 2 – *Animal Dosing*
Dates: 1 May – 26 June 2019
Progress: 100% Complete

The Animal Dosing phase (Activity 2) began 05/01/2019 following the two-week acclimation period. The rabbits were consistently dosed in order from the least potent experimental agent (saline) to the most potent (fentanyl), thus following the regimen of control, morphine, and fentanyl groups. Each individual subject was randomly assigned to a group of seven rabbits following acclimation in order to eliminate observable difference within or between groups for the experimental portion of the study. This ensured that any significant distinctions in bone micromorphology following the dosing period can be attributed to experimental manipulation. Within the control group, animals were randomly assigned to either the saline injection subgroup (n=3) or the transdermal patch control counterpart (n=4). In the control group, the saline injection subgroup rabbits were administered a subcutaneous bolus of saline, equal in volume to what the animal would receive if it were being dosed with morphine, through injection at the interscapular region. The patch control subgroup was treated by applying a quarter-sized amount of topical 20% isopropyl myristate in sterile saline (a skin softening agent) followed by an adhesive Tegaderm patch on the interscapular region. Morphine group rabbits were dosed with a subcutaneous bolus of morphine sulphate at 3 mg/kg/day injected at the interscapular region. Fentanyl group rabbits were dosed through application of 25 mcg/h transdermal fentanyl to bare skin at the interscapular region covered by a larger adhesive Tegaderm patch. All rabbits were shaved with Oster electric clippers using a 10-blade to remove the initial bulk of hair and a 40-blade to clip the finely-textured undercoat as needed. Injection of saline or morphine sulphate occurred every day between 8:00 and 11:00 A.M. while novel patch application followed a 72-hour dosing regimen, with application every third day. If the animal was observed to have removed its experimental treatment, a new transdermal fentanyl patch and/or adhesive Tegaderm covering was applied.

Objective 2: To optimize 3D micro-CT imaging of remodeling events in an opioid rabbit model.

Activity 3: Micro-CT imaging and histomorphometry
Projected Completion Dates: 08/19 – 04/20
Micro-CT imaging Progress: 100% as per proposed timeline
Histomorphometry Progress: 100% completed as per revised project timeline; began January 2020 (delayed by COVID-19 pandemic)

Micro-CT Imaging (ex vivo): Immediately following euthanasia, the left femur and tibia were dissected from each animal, wrapped in sterile saline-soaked gauze, and stored in a sample tube in a -20° C freezer. The remainder of the animal was sealed in plastic under vacuum and frozen in a -80° C freezer for future bone and tissue research. To prepare for micro-CT imaging, all members of the research team completed radiation generating equipment training (RGE Training), as well as two to three sessions of individualized, hands-on training for independent operation of the micro-CT instrument. Micro-CT imaging was performed using a SkyScan 1172 micro-CT (Bruker Micro-CT) desktop system located in the Surface and Optical Analysis Facility within the National Polymer Innovation Center (NPIC) at The University of Akron. Since this system is shared by users from various departments at The University of Akron, a conservative estimate of 6-9 months for imaging was estimated and carried out. Imaging was completed by Dr. Andronowski, Dr. Cole, and Reed Davis. For all 21 rabbits, micro-CT scans were captured at the midshaft femur, midshaft tibia, and proximal tibio-fibula. Tibial midshaft imaging was completed to assess interskeletal variability among porosity parameters in respect to the femoral midshaft. The proximal tibio-fibula data will provide further data on changes to trabecular bone morphology due to opioid exposure.

Dr. Andronowski developed and tested a micro-CT scanning protocol using bone tissue samples from her concurrent projects. Each femur and tibia was mounted to a brass sample holder via dental wax and secured with Parafilm to ensure the sample did not move during scanning. A previously optimized micro-CT imaging protocol was applied and included the use of a Hamamatsu 100/250 optical camera to take 1992 projection images spanning 180 degrees of rotation at 74 kilovolts (kV).

To set scan parameters, all extracted femora and tibiae were measured for length, with rabbit #4 possessing the largest femur and tibia. Specimens from this animal were chosen for preliminary imaging to ensure that all remaining femora and tibiae fit within the gantry and Field of View (FOV) of the μCT system. To optimize resolution, the midshaft femur of rabbit #4 was visualized at voxel sizes of 4.57 μm, 7.45 μm, and 9.93 μm. The maximum resolution of 4.57 μm was chosen because it closely accommodated the femoral midshaft within the FOV. The minimum resolution of 9.93 μm reflects previous recommendations of at least 10 μm resolution for visualizing human cortical pore networks to maintain structural parameters[14]. A lower resolution (e.g., 9.93 μm) can visualize a greater length of bone, but structures such as cortical pores will not be as well resolved. The resulting X-ray projections were reconstructed into tomographic cross-sectional slices using Nrecon software (Bruker). An image processing workflow developed by Dr. Cole extracted the cortical pore network for the three-dimensional (3D) visualization and morphometric quantification. We found that the larger voxel sizes (7.45 μm and 9.93 μm) generated substantial image noise that could not be easily distinguished from smaller pores through image processing. Three-dimensional visualization of pore networks showed a substantial reduction in the pore systems reconstructed at 7.45 μm and 9.93 μm, compared to 4.57 μm. Specifically, pore networks visible at 4.57

µm covered the entire cross-sectional thickness as expected, while pore networks visible at 7.45 µm and 9.93 µm were largely restricted to the expanded pore systems adjacent to the endosteum **(Figure 1).** Accordingly, a higher resolution increased the number of pore systems with at least two segments, from 403 systems at 9.93 µm, to 1,178 systems at 7.45 µm, to 2,210 systems at 4.57 µm.

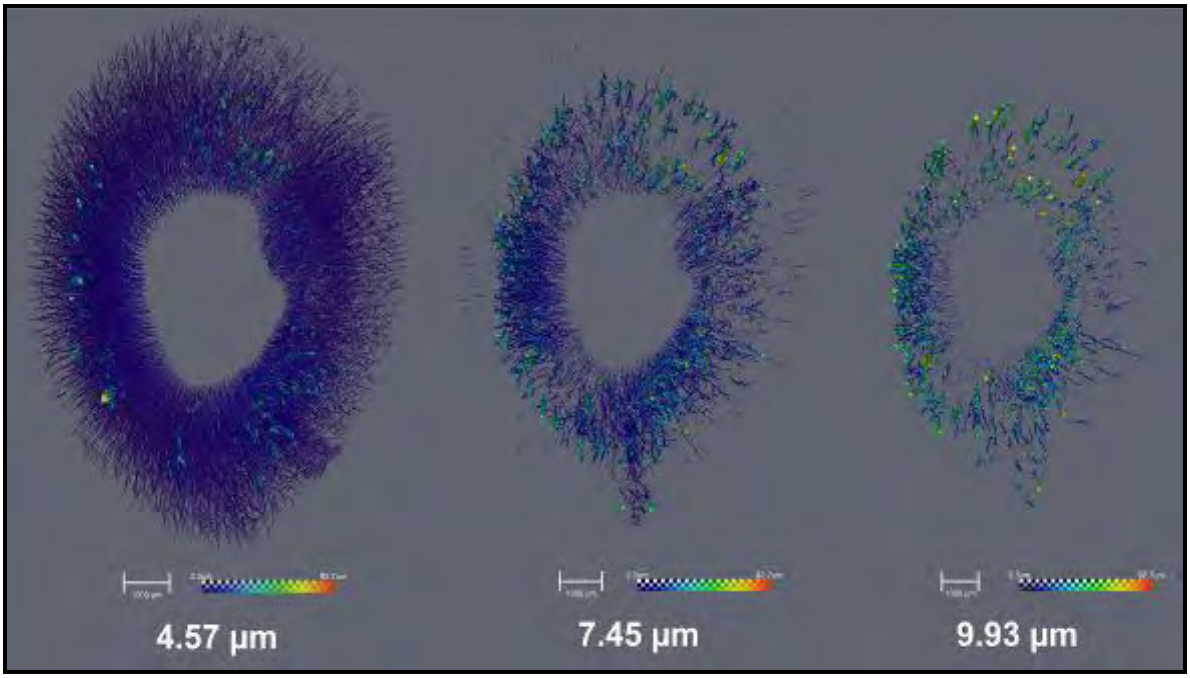


Figure 1: Cortical pore networks from the femur decrease in visibility and cross-sectional coverage at lower micro-CT imaging resolutions. Color scale represents local pore thickness. Scale bar is 1000 µm.

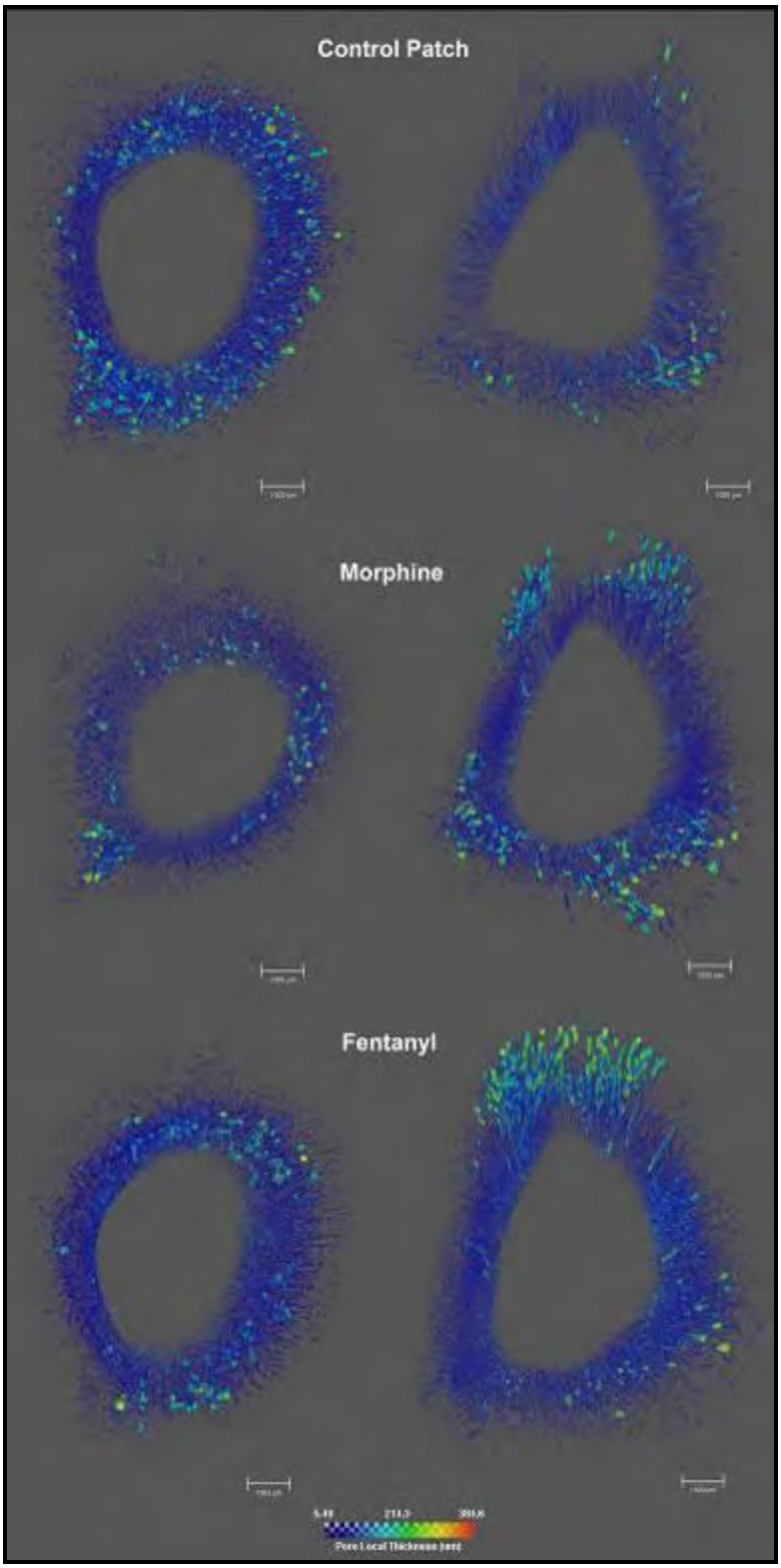


Figure 2: Cortical pore networks from femora (left) and tibiae (right) from each group, with micro-CT imaging resolution set at 5.49 µm. Color scale represents local pore thickness. Scale bar is 1000 µm.

During reporting period 07/01/19 – 12/31/19, all femoral and tibial midshafts (n = 42) were imaged at 5.49 µm resolution **(Figure 2)** as this was the highest resolution where all samples consistently fit within the FOV. This scan resolution visualizes a bone length of 7.318 mm. At lower resolutions (e.g., 4.57 µm) larger femoral or tibial midshafts could not be reliably positioned wholly within the FOV. Femora and tibiae were prepared and scanned according to the protocol described in the attached **Appendix XI** entitled "Rabbit Opioid SkyScan µCT SOP". Due to length, the tibio-fibula could not stand fully upright on the SkyScan stage within the gantry. Thus, the distal end of each tibio-fibula was removed using an Isomet Saw to reduce bone length to 87 mm, which could fit within the SkyScan gantry. Femora were scanned at the midshaft, while tibio-fibulae were scanned at the midshaft of the isolated tibia prior to its merging with the fibula. Additionally, all proximal tibio-fibulae (n = 21) were scanned at a resolution of 10.98 µm, which was the maximum resolution that could consistently fit this region fully within the FOV. This scan resolution visualizes a bone length of 14.636 mm. Scans of the proximal tibio-fibula were used for analysis of trabecular architecture by Graduate Assistant, Reed Davis, in the upcoming study periods (**Figure 3**). In all scans, anterior and medial clay inclusions were inserted within the bone's Parafilm wrapping to mark anatomical orientation for the subsequent tomographic reconstructions.

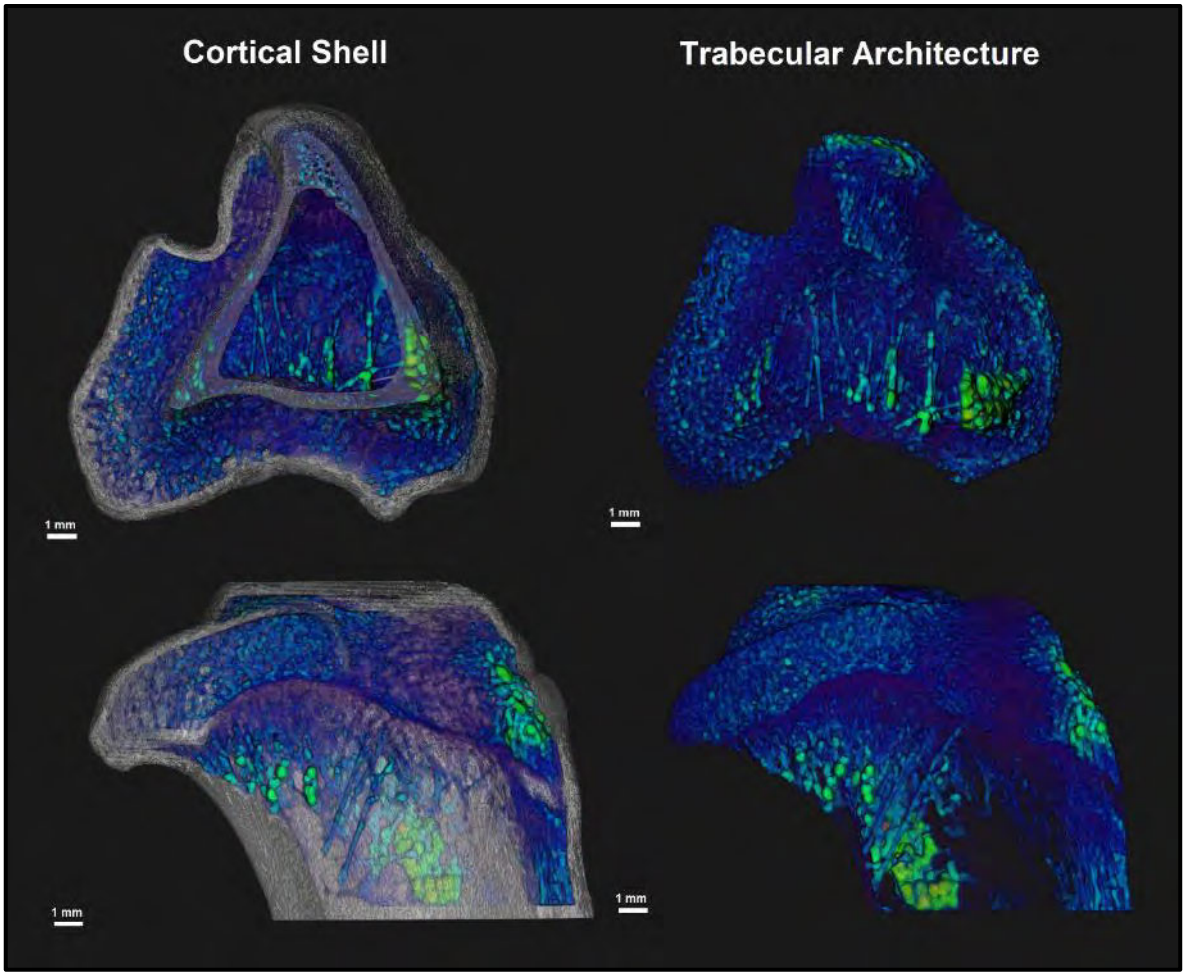


Figure 3: Trabecular architecture visible from inferior (top) and medial (bottom) views of the proximal left tibia from a control patch rabbit. Color scale corresponds to volume thickness. Scale bar is 1000 µm.

An image processing workflow was developed by Dr. Cole to extract the cortical pore network from femoral and tibial midshaft scans. This workflow is automatically processed through custom macros written for

ImageJ (NIH) and CTAnalyser (Bruker) as described in the attached **Appendix XII** "Rabbit Opioid μCT Image Processing SOP". In brief, this workflow involves tomographic reconstruction, anatomical orientation, longitudinal alignment, extraction of total area and cortical area masks, and low-contrast filter extraction of the pore network. Pore morphometric variables, broadly associated with pore density, volume, connectivity, and orientation, are assessed within the boundaries of the cortical area mask using CTAnalyser. These morphometric variables are reported both for the aggregate pore network and for individual pore systems. CTAnalyser also reports variables associated with the cross-sectional geometry of the cortical area, using the total area mask as the region of interest.

Dr. Cole developed an additional macro for ImageJ that divides each cortical area mask into anterior, posterior, medial, and lateral regional quadrants using the section centroid. When a given regional mask is loaded as the region of interest for a pore network in CTAnalyser, the reported morphometry is limited to that region. These data will facilitate analysis of regional variation in pore distribution and morphometry, which may be associated with the drug treatment group. This regional image processing workflow is described in the attached **Appendix XIII** "Femur and Tibia Quadrant Regional Processing SOP". As regional quadrants artificially truncate cortical bone and pore network branching at their boundaries, cross-sectional geometry was not carried out for regional comparisons. All specialized image processing macros developed for this project are contained in **Appendices XIV – XV** "CTAnalyser Macros" and "ImageJ Macros".

During reporting period 01/01/20 – 06/30/20, skeletonization analysis of the binarized pore network was carried out to examine the branching patterns of pore systems. The Auto-Skeleton module of Amira 6.4.0 was employed, as described in the attached **Appendix XVI** "Skeletonization SOP". Skeletonization data collection was performed by undergraduate student Kassidy Wilson for her undergraduate honors thesis, with assistance from Drs. Andronowski and Cole.

During reporting period 07/01/20 – 12/31/20, we made significant process on the histomorphometry component of *Activity 3*. This phase, described below, began on target in January 2020 as proposed in the original project timeline. Due to the COVID-19 pandemic, however, this component of the project was slowed and a no-cost extension was filed. An explanation and revised plan for the histomorphometry experiments is presented below.

Description of Extenuating Circumstances Leading to Delays in Histomorphometry Experiments

On 03/11/2020, the World Health Organization declared the novel coronavirus outbreak (COVID-19) a global pandemic. As a result, The University of Akron cancelled in-person classes that day and in-person lab work was ordered to cease immediately. As per our project timeline, our research team was preparing rabbit femoral bone thin-sections for dynamic histomorphometry (*Activity 3.4*). This process involved daily lab work that included bone dehydration, methyl methacrylate (MMA) infiltration and curing, sectioning via the in-house Well Diamond Wire Saw, and slide mounting. The stalling of in-lab operations and the shutdown of The University of Akron halted the progress of *Activity 3.4* indefinitely.

The governor of Ohio declared a "Stay-at-Home" order on 03/23/2020 which was extended through 06/04/2020. On 05/21/2020, The University of Akron administration announced a 'Researcher Return to

Work' initiative and Principal Investigators were invited to create individual Return to Work plans for their groups for review by administrators and unit heads. The document Dr. Andronowski created (**Appendix XVII:** "Researcher Return to Work") outlined the guidelines and expectations of Andronowski Lab members for a safe return to lab activities during the ongoing COVID-19 pandemic. On 06/08/2020, the plan was approved with restrictions related to limits on laboratory personnel numbers and office use and occupancy, the implementation of shift work, and personal protective equipment requirements.

Due to current social/physical distancing guidelines requiring separation distances of 6 feet or more, no more than 2-3 individuals are permitted in Dr. Andronowski's main lab space at any given time. Lab procedures requiring more than one person in close proximity are to be minimized as much as possible. Due to disruptions in supply chains, the ordering and delivery of consumables, stains, PPE, and lab equipment has also been significantly slowed leading to experiment delays. As a result, our bone histomorphometry specimen preparation and the subsequent analyses has been slowed.

A detailed timeline narrative and implementation timeline (**Appendix XVIII: "**Projected MMA Timeline") have been prepared that outline the revised trajectory for *Activities 3.4* and *4.2* in line with our lab safety guidelines. As of 08/31/2021, we are on schedule with the revised project timeline, submitted our first paper from the project for publication, and are finishing additional manuscript drafts for submission.

Conventional histomorphometry was performed to further characterize cortical bone differences among groups. Each member of the research team has experience with the traditional histological preparation of bone tissue, having applied it to other research projects. Our embedding and sectioning protocol was developed and tested by Drs. Andronowski and Cole on pig ribs and on Mini Rex rabbit femora and tibiae mid-shafts. The specimens were dehydrated and embedded in MMA according to a protocol developed by the Andronowski Lab (**Appendix IX:** "MMA Embedding SOP"). A precision diamond wire saw (Well Wire Saws, Norcross, GA) was employed to cut two transverse cross-sections, followed by sequential longitudinal sections, that were ~ 60 – 90 µm thick. Frost[13] recommends that 50 mm^2 of bone be evaluated, which requires 2 to 5 serial sections. In our experience, evaluating two thin- sections is adequate to account for the variation in microstructures between sections. The thin-sections were mounted on glass slides, cover slipped, and dried. The finished slides were imaged using an Olympus BX51 fluorescence microscope fitted with Differential Interference Contrast (DIC). Digital brightfield microscopic images were captured at 200x magnification and DIC using the associated cellSens Entry software 1.16 (Olympus) and stitched together into a composite image of the section using Microsoft Image Composite Editor and Photoshop 2020. Circularly polarized light images were further captured at 200x for each slide to aid in distinguishing osteon boundaries. Finally, images of each slide were captured at 100x under fluorescence using a FITC filter cube for visualization of the calcein labels. The microscopic imaging and digital stitching produced DIC, circularly polarized, and fluorescent image of each complete cross-section.

During 08/20 of reporting period (06/30/20 – 12/31/20), thin-section mounting and microscopic imaging testing began and was completed by 09/20. Microscopic imaging of the prepared cross-sections began in 09/20 with the assistance of Undergraduate Research Assistant, Joshua Taylor. Protocol development was initiated following traditional histomorphometric parameters outlined in Jaworski and Lok[15]. The microscope and the cellSens Entry software scales were calibrated prior to the evaluation of thin-sections. Standard nomenclature for histological variables was followed[16] during protocol development.

Histomorphometric parameters relating to histological age-estimation are described in **Appendix XIX: Histomorphometric Variables.**

On 01/21, photomerging of the DIC brightfield, circularly polarized, and fluorescent images was initiated. This aspect was primarily conducted by Graduate Research Assistant, Joshua Taylor. In 02/21, Taylor trained and supervised two undergraduate students to assist. Upon completion, the analytical focus shifted to overlaying the images in Adobe Photoshop v.23.0.0 (Adobe, San Jose, California). This allowed for the DIC, circularly polarized, and fluorescence images from the same slide to be oriented identically and with matching coordinates for all microstructural features. Subsequently, these images were anatomically oriented in Adobe Photoshop in alignment with a binarized outline of the cortical bone cross-section from the previously processed micro-CT images of each sample. All images were oriented in a clockwise manner from the top of the image: Anterior, Medial, Posterior, Lateral (**Figure 4**). Finally, the areas of each image were cleared outside the periosteal border and within the endosteal border, using the Wand Tool and Freehand Selection Tool in ImageJ (**Figure 5**).

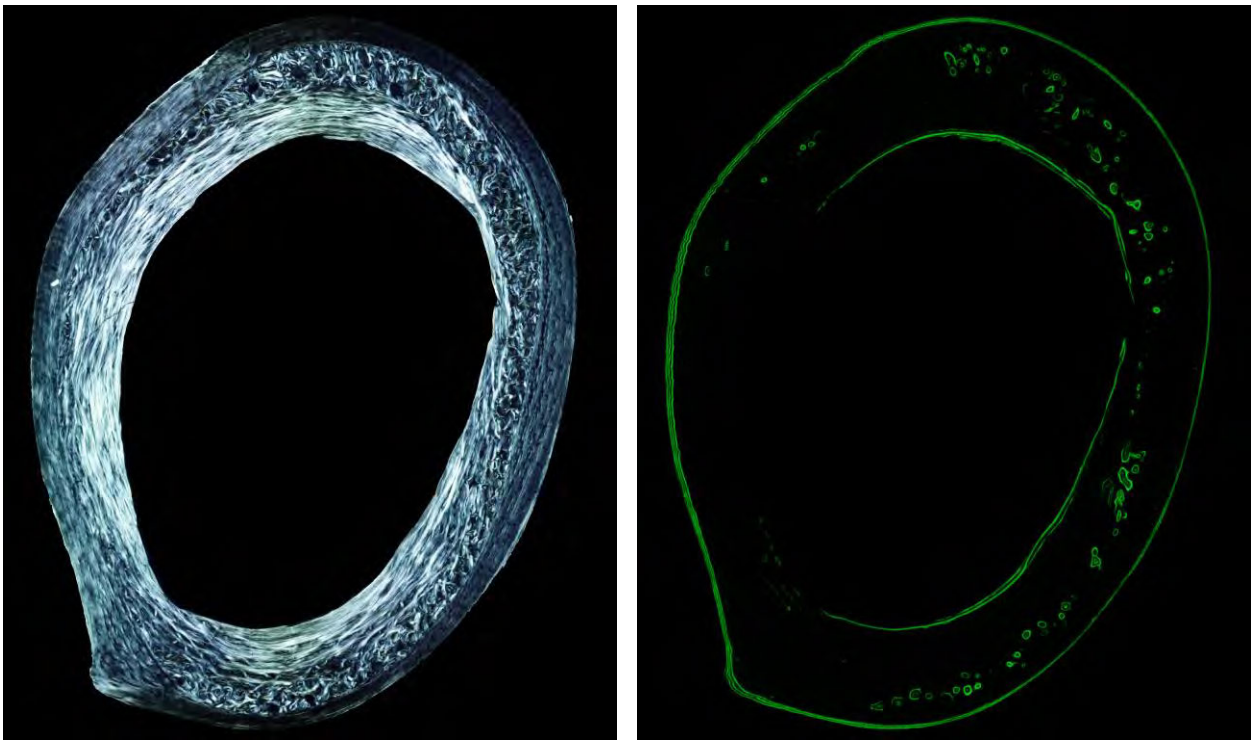


Figure 4: Circularly polarized image (left) and fluorescence image (right) aligned with DIC image in Figure 5, Sample 1LF_85µm.

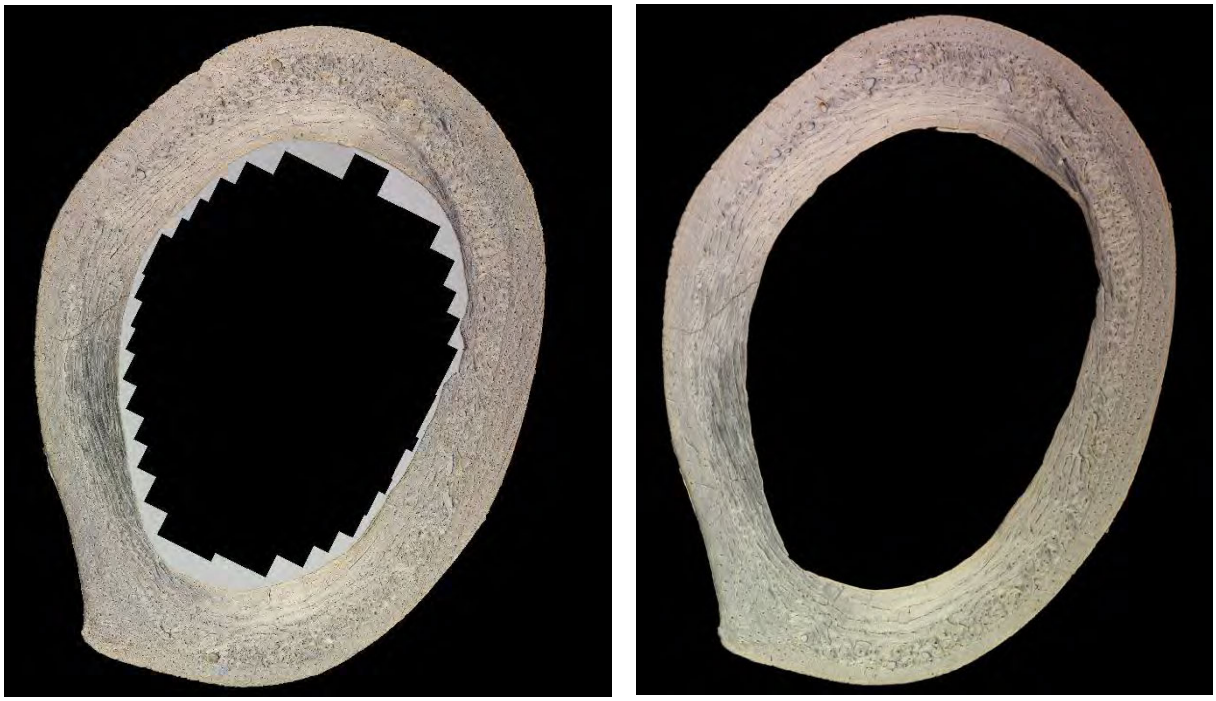


Figure 5: DIC image before (left) and after (right) clearing the endosteal border, Sample 1LF_85µm.

Dr. Cole recruited the assistance of an additional undergraduate student to aid in the isolation of the remodeling area of all polarized images using the software Dragonfly. The remodeling area was exported both as a binarized image TIFF file, and as a region of interest (ROI) file for import into ImageJ (**Figure 6**). Final preparation for histomorphometric analysis was completed by 06/21.

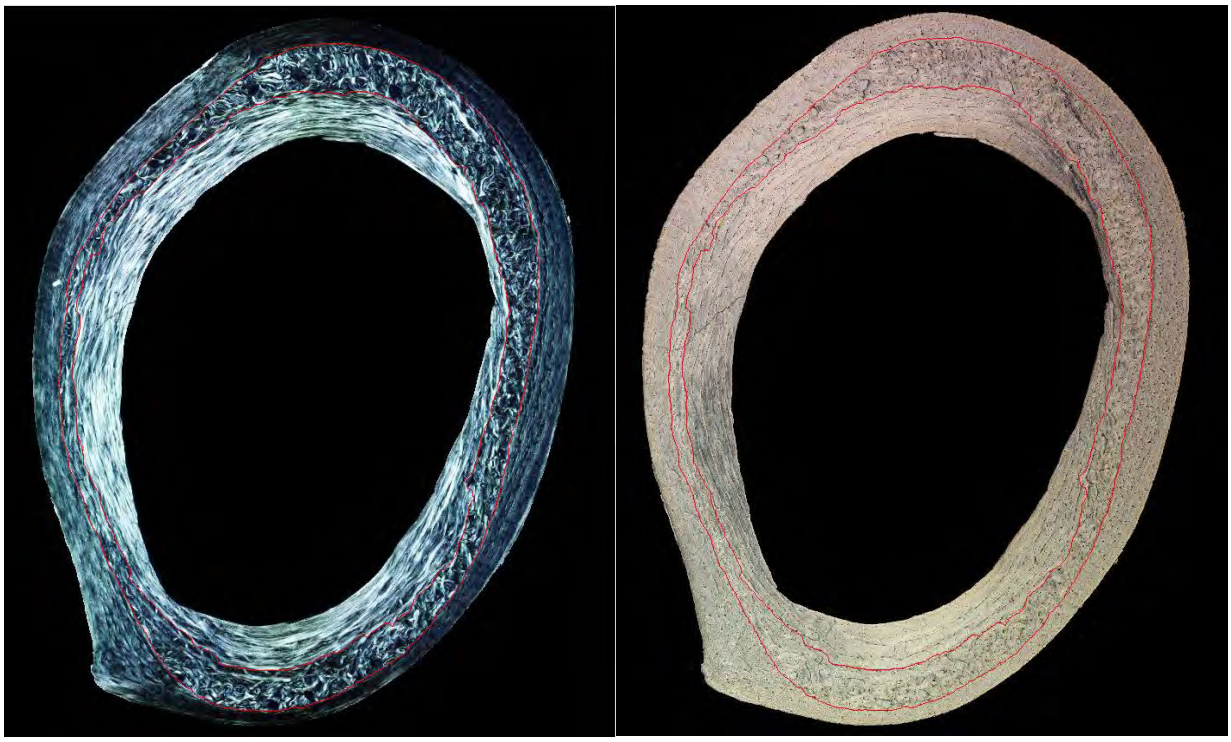


Figure 6: Circularly polarized image (left) and DIC image (right) with remodeling area selected (red), Sample 1LF_85µm.

During the 01/01/21 – 06/30/21 reporting period, Dr. Cole developed custom ImageJ macros for the automated extraction, manual correction, and automated regional subdivision and morphometric analysis of cortical pores from DIC images. Macros for extraction, manual correction, and analysis of osteons were developed during the 06/30/2021 – 08/31/2021 reporting period. The combined software toolkit for ImageJ was named "OsteoFlo" (**Figure 7**).

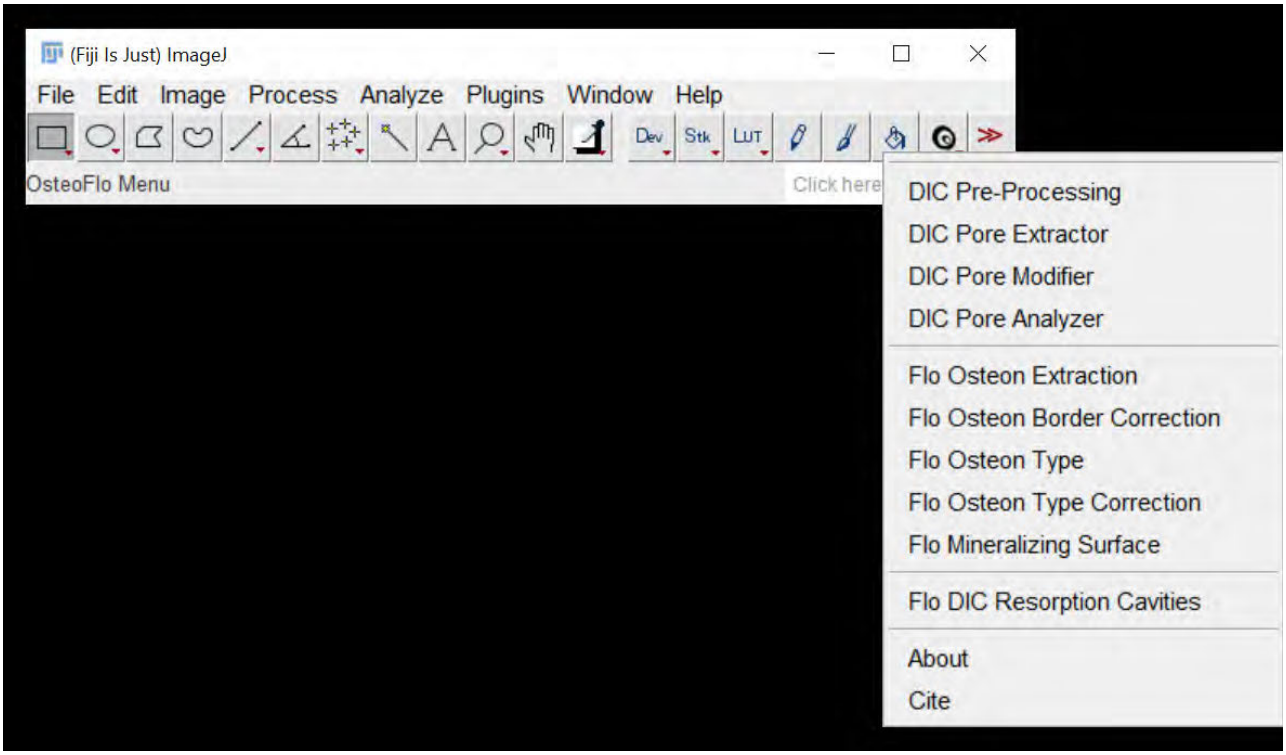


Figure 7: The OsteoFlo toolkit interface loaded in ImageJ.

ImageJ macros for pore analysis included the following:

DIC Pre-Processing (Fully Automated): The DIC image is evenly illuminated through application of a high pass filter, contrast enhancement, and background subtraction. This macro removes variation in sample illumination over the cross-section caused by photomerging artifacts (**Figure 8**).

Figure 8: DIC image before (left) and after (right) pre-processing for Sample 1LF_85µm.

Pore Extractor (Fully Automated): The user loads the pre-processed DIC image and the remodeling area region of interest (ROI), which was previously extracted from circularly polarized images. Within this remodeling area ROI, probable pore ROIs are extracted from the DIC image using an Intermodes auto-threshold, particle size and circularity thresholding, and binary closing. The user can inspect the proposed selections and modify the particle size and circularity thresholds before extraction. Pores are extracted within the remodeling area ROI, which is known to experience secondary bone remodeling during life, as indicated by the Haversian (secondary) bone visible on the circularly polarized image. This ROI restriction excludes the periosteal and endosteal areas of primary bone containing the cortical pores of primary osteons, which are formed during initial growth.

Pore Modifier (Manual): Automatically selected pore ROIs exported by Pore Extractor are manually inspected and edited (**Figure 9**) using custom keyboard shortcuts that facilitate manual pore selection and image navigation. These shortcuts include:

- One-click access to ImageJ tools, including the Wand Tool, Wand Tool Options (for pixel brightness tolerance adjustment), Freehand Selection Tool, Zoom Tool, Scrolling Tool, and Selection Brush Tool
- Toggling ROI labels and ROI selections on and off as image overlays
- Resetting the Wand Tool tolerance to zero
- Splitting joined ROIs after separation with the Selection Brush Tool
- Filling enlarged ROIs after border expansion with the Selection Brush Tool
- Decreasing or increasing Selection Brush Tool size by factors of 5 pixels
- Reverting an ROI to its original shape after manual modification
- Auto-saving the current ROI set to the user's file system

Due to its Auto-Save function, Pore Modifier may be run repeatedly on the same ROI set, spreading the manual correction over multiple user sessions for particularly large images.

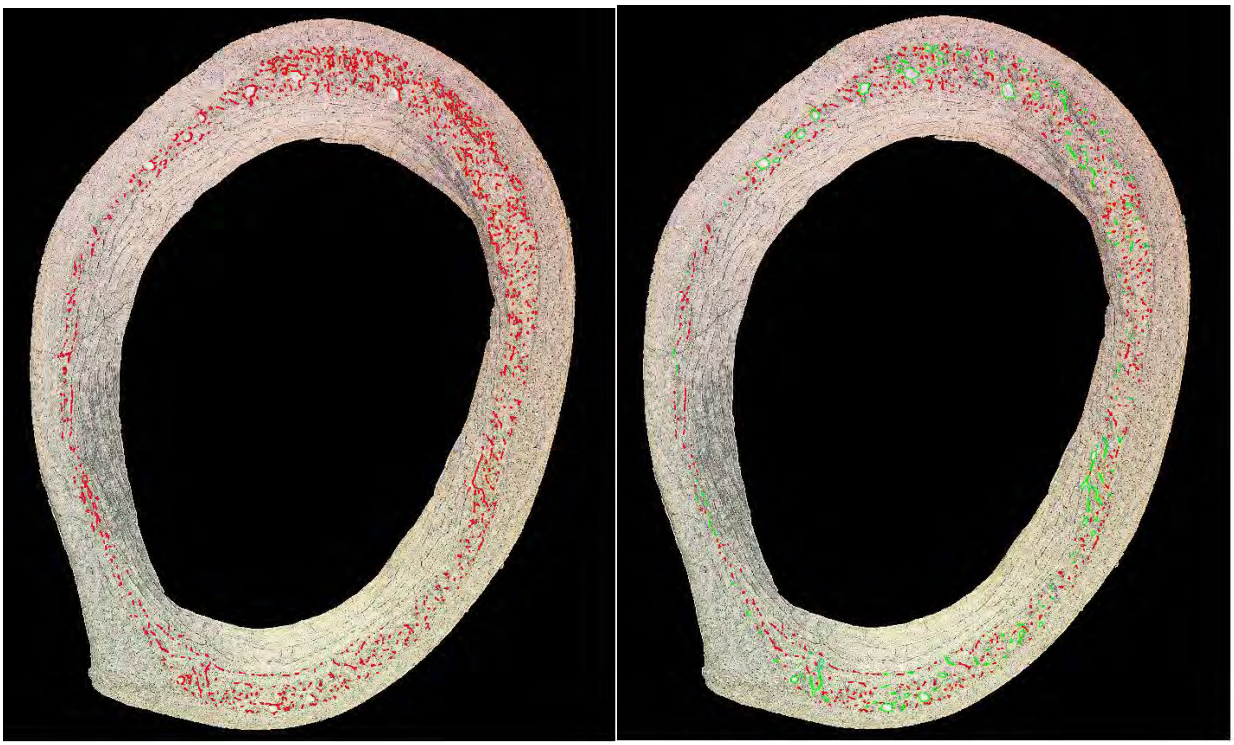


Figure 9: Automatically extracted pores (left, red) exported by Pore Extractor and manually modified pores (right, green) exported by Pore Modifier, Sample 1LF_85µm.

Pore Analyzer (Fully Automated): Anatomical quadrants are defined on each cross-section by extracting the total area (a filled binary mask of the bone inside the periosteal border), finding its centroid in BoneJ, and drawing a line through this centroid to the top and bottom of the image. This line is then rotated 45 degrees, and then 90 degrees, to define the dividing lines between Anterior, Medial, Posterior, and Lateral anatomical quadrants. These anatomical quadrants are extracted as separate ROIs and as binary images from the cortical area image. Finalized pore ROIs located within each anatomical quadrant are assigned to that quadrant. Pore ROIs located on the border between anatomical quadrants are assigned to whichever quadrant contains a larger fraction of that pore area (**Figure 10**).

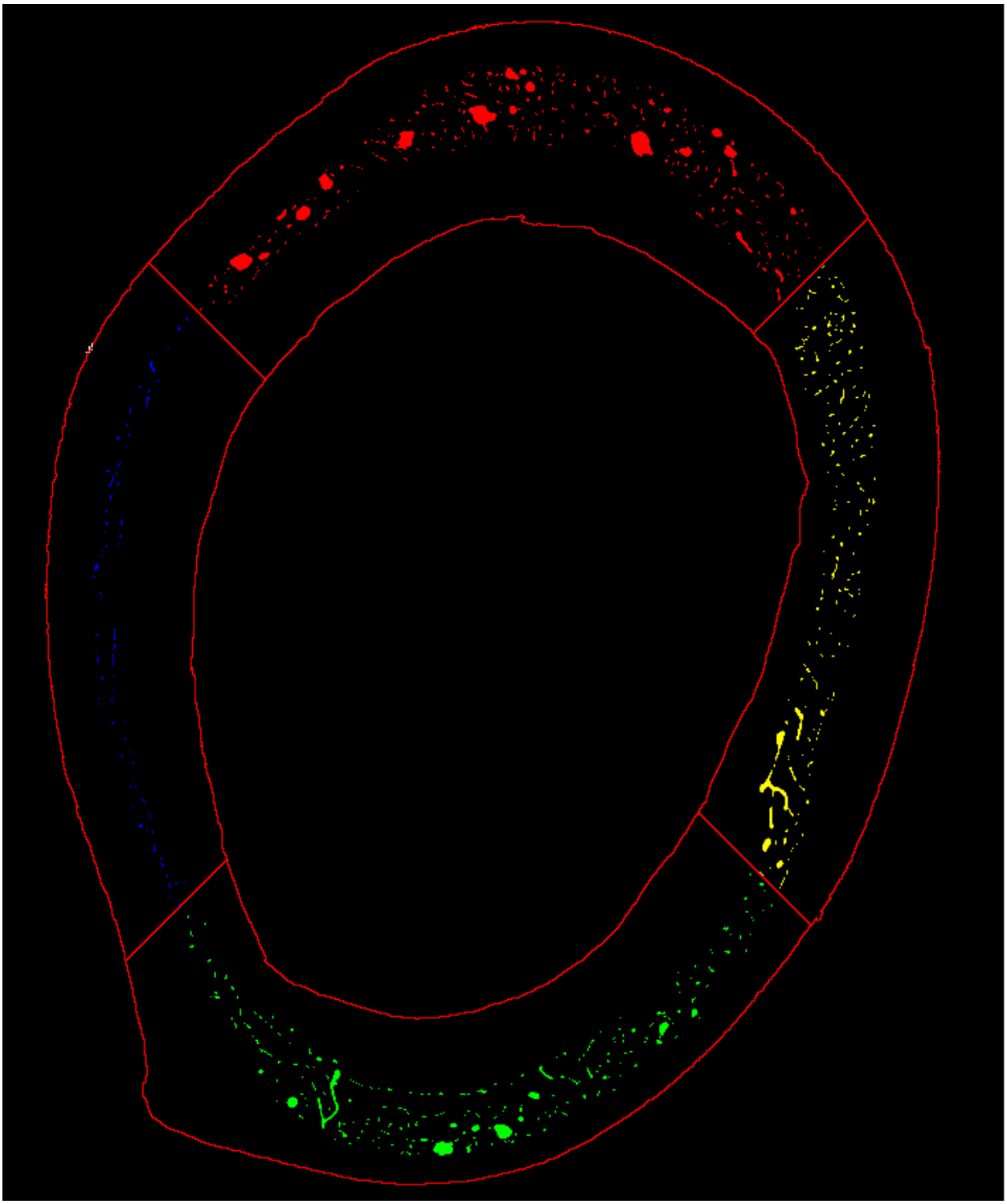


Figure 10: Pores colorized by anatomical quadrant: Anterior (red), Medial (yellow), Posterior (green), Lateral (blue), Sample 1LF_85µm.

Finalized pore ROIs are further classified as cortical or trabecularized based on proximity to the marrow cavity versus minimum diameter. Proximity to the marrow cavity is accomplished by extracting a binary image of the marrow cavity and generating a Euclidean Distance Map (EDM) of this image. Each pixel distance from the marrow cavity is defined as an increasing pixel brightness value, which in a 16-bit system ranges from 0 pixels (absolute black) to 65,536 pixels (absolute white) away from the marrow cavity in any direction. Pore ROIs are superimposed on the EDM image, and the minimum gray value of each pore ROI is automatically measured, which corresponds to their proximity to the marrow cavity in pixel units. Minimum diameter is also automatically measured for each pore ROI as minimum Feret (caliper) diameter. If minimum pore diameter exceeds its proximity to the marrow cavity, then the pore is classified as "trabecularized"; otherwise, the pore is classified as "cortical."

Summary pore morphometry is calculated for all pores in aggregate and for each pore type (total, cortical, and trabecularized) and within each region (Anterior, Medial, Lateral, Posterior), including percent porosity, pore density, and mean pore size and shape descriptors. The macro exports a table of these summary statistics, and a table of measurements for individual pore ROIs, including their regional and pore type classification.

ImageJ macros for osteon analysis included the following:

Osteon Extraction (Fully Automated): The fluorescence image is loaded, along with the ROI for the remodeling area. Local contrast enhancement is applied to emphasize calcein labeling in the fluorescence image (**Figure 11**). Spaces external to the remodeling area are then cleared. Osteon borders are extracted using a global Intermodes threshold, followed by removal of bright outliers less than 10 pixels. Osteon borders are sealed using Euclidean Distance Map opening, through ten cycles, from the BioVoxxel Toolbox plugin for ImageJ. Osteon "rings" representing discrete calcein labels are extracted by subtracting the background, which removes faint fluorescence between rings. Rings are then binarized using an auto local threshold with a Phansalkar algorithm for low-contrast images, and bright outliers less than 5 pixels are removed through four cycles. The enhanced fluorescence image, binarized osteon borders, and binarized osteon rings are exported as separate TIFF images (**Figure 12**). An ROI set of osteon borders is also exported as a zip file.

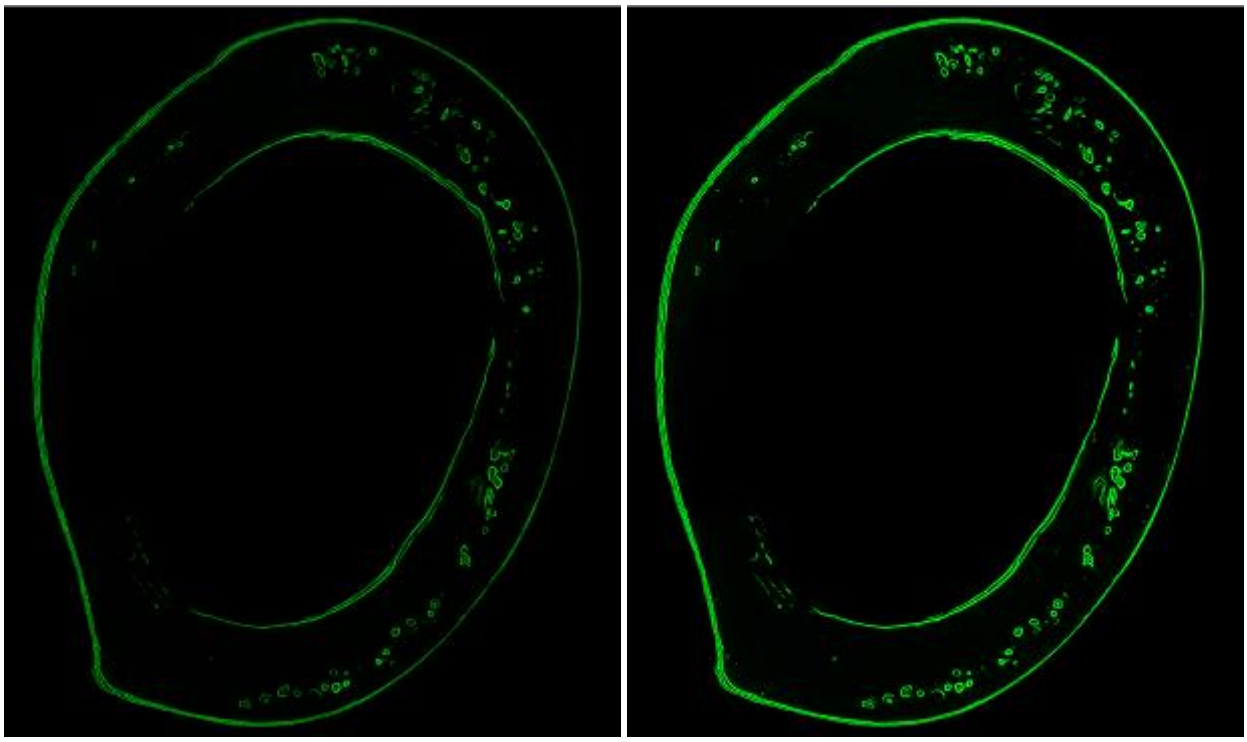


Figure 11: Fluorescence image for Sample 1LF_85µm before (left) and after (right) local contrast enhancement to emphasize calcein labeling.

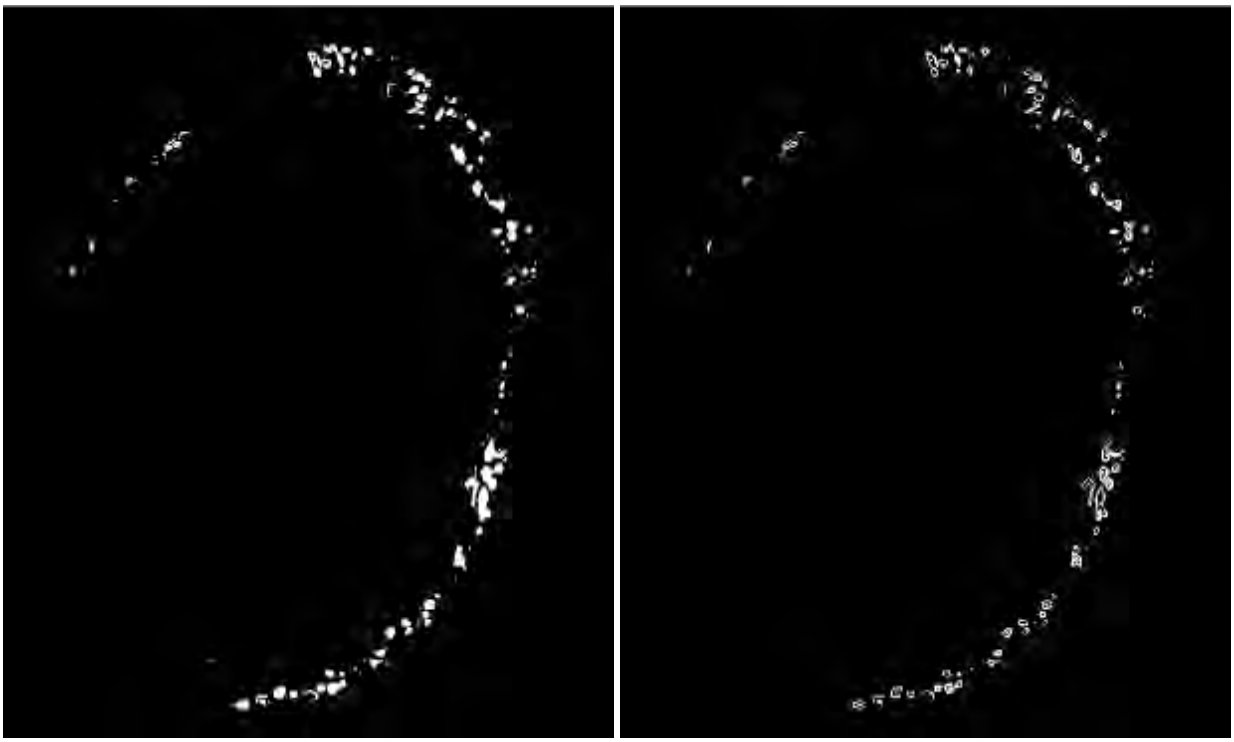


Figure 12: Osteon borders (left) and osteon "rings" (right) representing discrete calcein labels exported for Sample 1LF_85µm.

Osteon Border Correction (Manual): This macro allows the user to split connected osteons, seal open osteon borders, add any missed osteons, and delete any image noise selected by the automated osteon border extraction. The original DIC image, the contrast-enhanced fluorescence image, and the ROI set of unmodified osteon borders are loaded. The DIC image is superimposed on the fluorescence image at 75% opacity so that calcein labels and osteon cement lines can be viewed simultaneously. The osteon border ROI set is then superimposed for the user to inspect and modify (**Figure 13**). The same shortcut utilities in Pore Modifier are available here, including wand selection, selection brush tool modification, freehand selection, ROI label toggling, split/fill/revert functions for ROIs, and auto-saving (**Figure 14**). The macro exports the user-modified ROI set of osteon borders, and a corrected binarized mask of osteon borders **(Figure 15**).

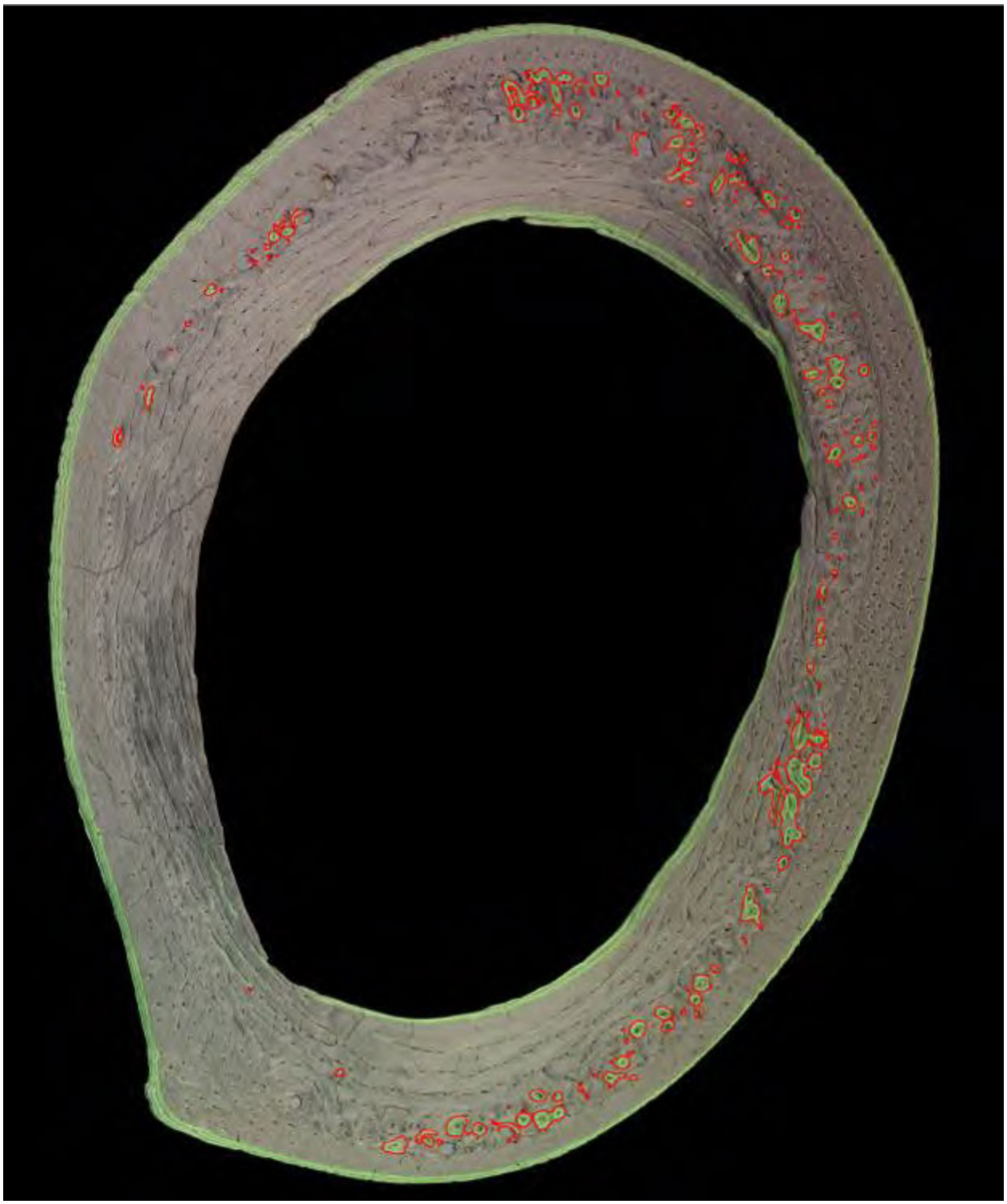


Figure 13: Unmodified osteon borders (red) superimposed on fluorescence image and DIC image at 75% opacity for Sample 1LF_85µm.

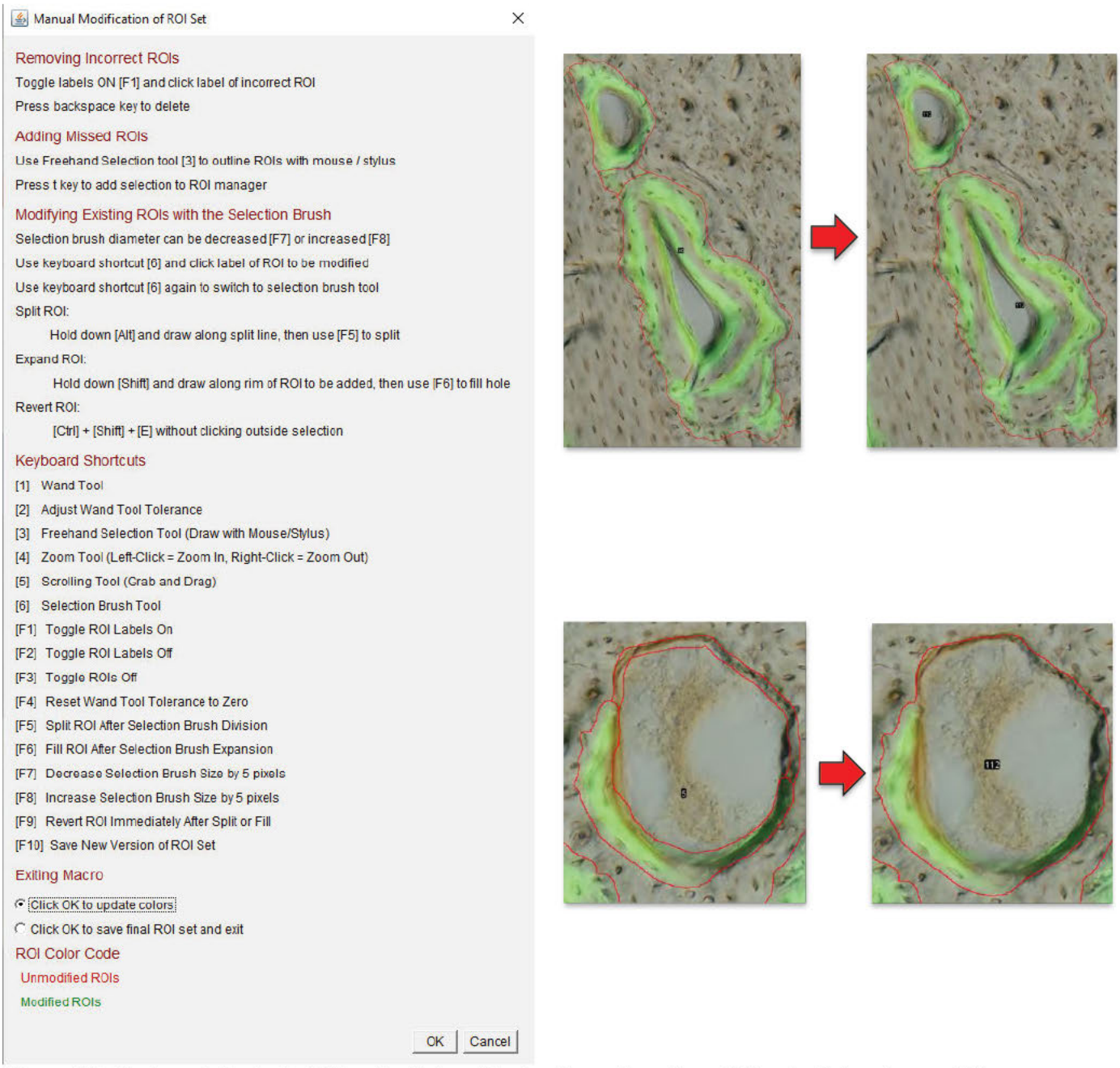


Figure 14: Keyboard shortcut utilities for Osteon Border Correction. Key utilities include osteon splitting (upper right) and osteon filling (lower right) using the selection brush tool.

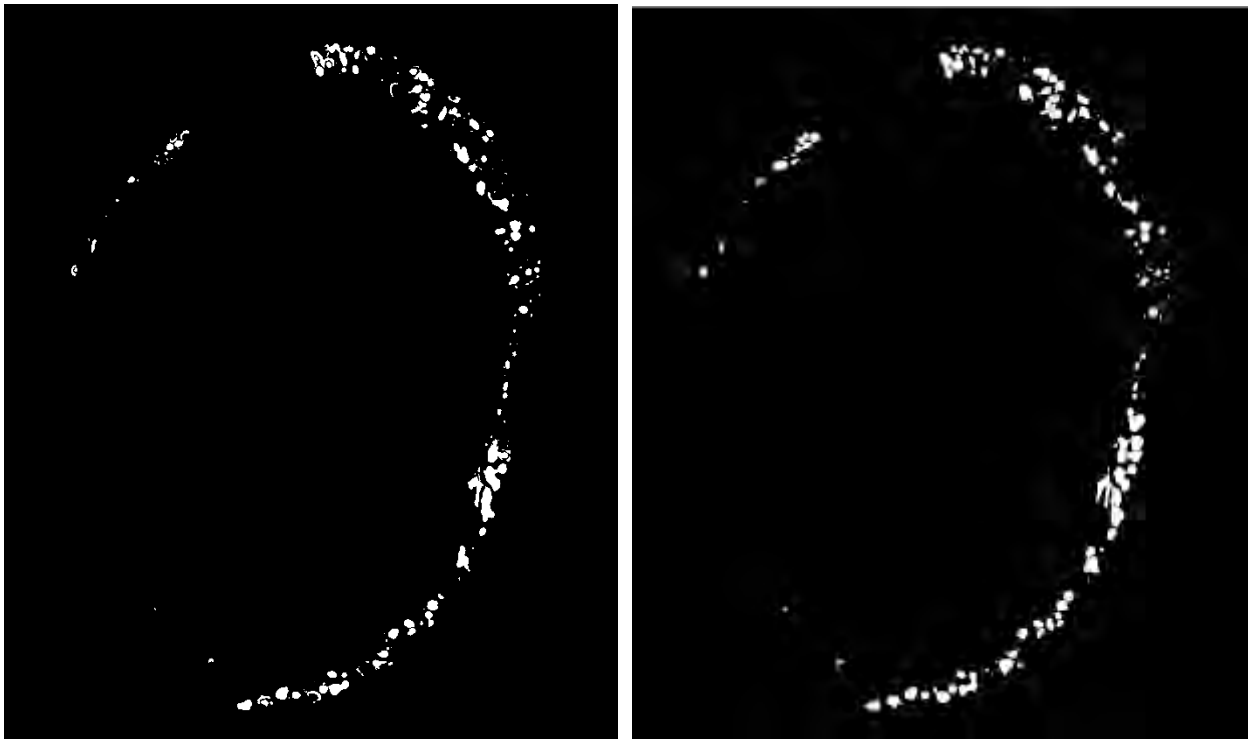
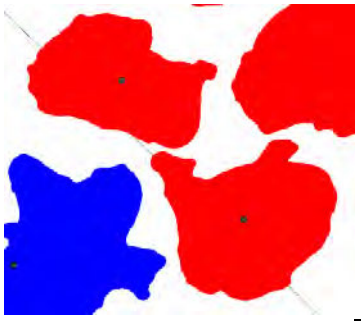


Figure 15: Osteon borders automatically extracted (left) and manually modified (right) for keyboard shortcut utilities for Sample 1LF_85µm.

Osteon Type (Fully Automated): This macro categorizes osteons by anatomical region and calcein label type (partially labeled forming osteon, single/double/triple labeled complete osteon), in addition to detecting the placement of calcein labels and vascular pores to calculate osteon mineral apposition rate (On.MAR) and osteon wall thickness (W.Th), respectively. The preprocessed DIC image, the osteon "rings" image, and corrected osteon border ROIs are loaded. To categorize osteons by anatomical region (Anterior, Medial, Posterior, Lateral), the quadrants are drawn using the same mechanism as Pore Analyzer. Each region is flattened at 255 pixel brightness (absolute white) and extracted separately. For each region, the osteon border ROIs are loaded and their pixel brightness is measured. Osteons located fully within a region will have a mean pixel brightness of 255. Osteons located on the border between regions are classified with the region that has the higher mean pixel brightness, which indicates that a larger fraction of the osteon area falls within that region (**Figure 16**).



Anterior	Medial	Posterior	Lateral	Region
55.946	199.054	0	0	Medial
203.325	0	0	51.675	Anterior
255	0	0	0	Anterior
0	0	0	255	Lateral
161.136	0	0	93.864	Anterior

Figure 16: Pores located on regional borders have an intermediate pixel brightness (1 - 254) in two regions. These pores are classified with the region that has the higher pixel brightness value, indicating that a larger fraction of the pore area is located in that region. Pores located fully within a given region have a pixel brightness of 255 for that region and a pixel brightness of 0 for all other regions.

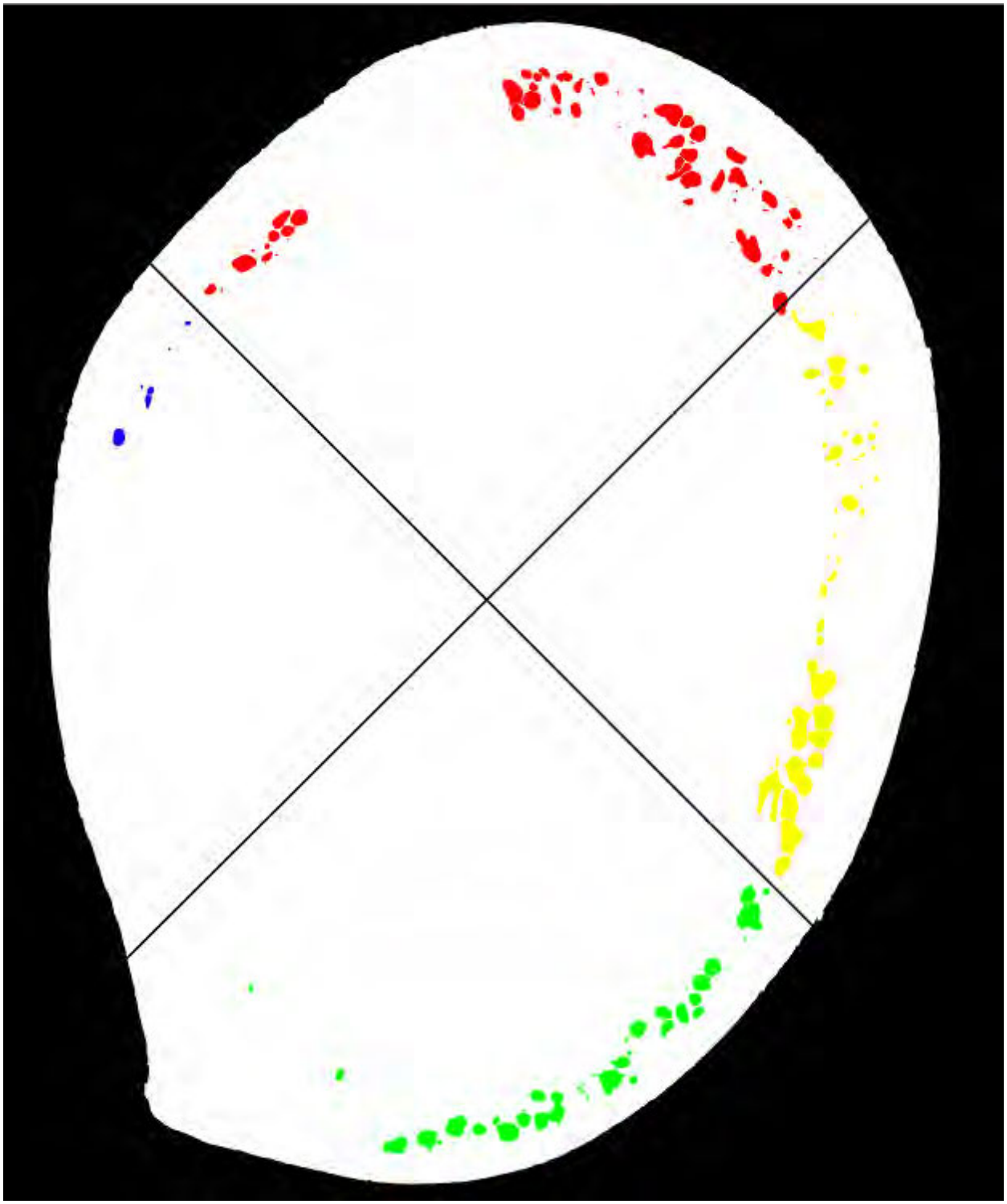


Figure 17: Regional quadrants drawn on the total area binarized image, with the ROI set of osteon borders colorized by region classification superimposed for Sample 1LF_85µm.

The exported objects from this portion of the macro include: ROIs for each anatomical quadrant, a TIFF image with the regional boundaries drawn on the total area binarized image, an ROI set with pores colorized and assigned to ROI groups by region, and a csv file displaying regional pixel brightness values and final regional classification (**Figure 17**). Regional classification for each osteon is saved to an aggregate data table.

Next, the macro classifies osteons and detects label spacing. The corrected osteon border ROI set is superimposed on the osteon "rings" image. An ellipse is fitted to each osteon border ROI, and the major axis is drawn as a line. The pixel brightness intensity profile of the osteon is measured along this line. Labels (white) appear as peaks up to 255 pixel brightness levels, while spaces between labels should be closer to 0 (black) (**Figure 18**). The line may pass through one or both sides of a given "ring" of a label. For forming osteons, the resorption space is partially labeled around its broad circumference, and may have zero peaks or a high number of peaks. The number of peaks corresponds to the osteon label classification. Osteon border ROIs are colorized based on type classification, and the ROI labels are appended to represent this classification (**Figure 19**). Osteon type classification for each osteon is saved to an aggregate data table.

- **Forming Osteon:** 0 or 7+ peaks (color = blue, ROI label = _F)
- **Single-Labeled Osteon:** 2 peaks (color = red, ROI label = _S)
- **Double-Labeled Osteon**: 3 – 4 peaks (color = green, ROI label = _G)
- **Triple-Labeled Osteon:** 5 – 6 peaks (color = yellow, ROI label = _T)

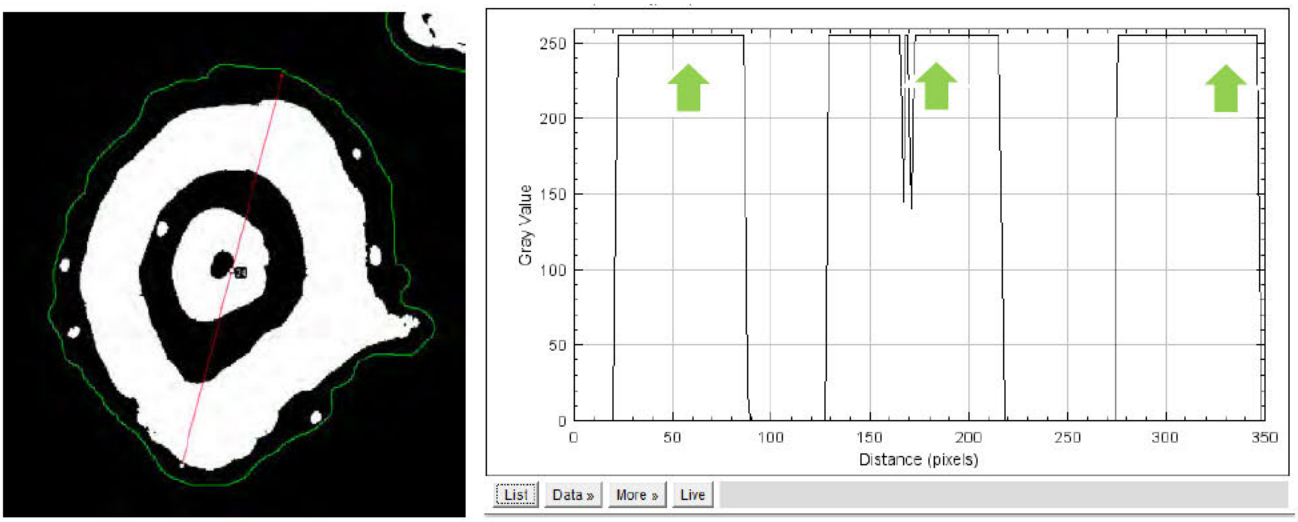


Figure 18: A double-labeled osteon (left) with a red line indicating the major axis of an ellipse fitted to the osteon border (green). The intensity profile of pixel brightness along this line (right) has three peaks (indicated by green arrows), where the outer peaks intersect both sides of the outer ring, and the inner peak intersects both sides of the inner ring without passing through the vascular pore.

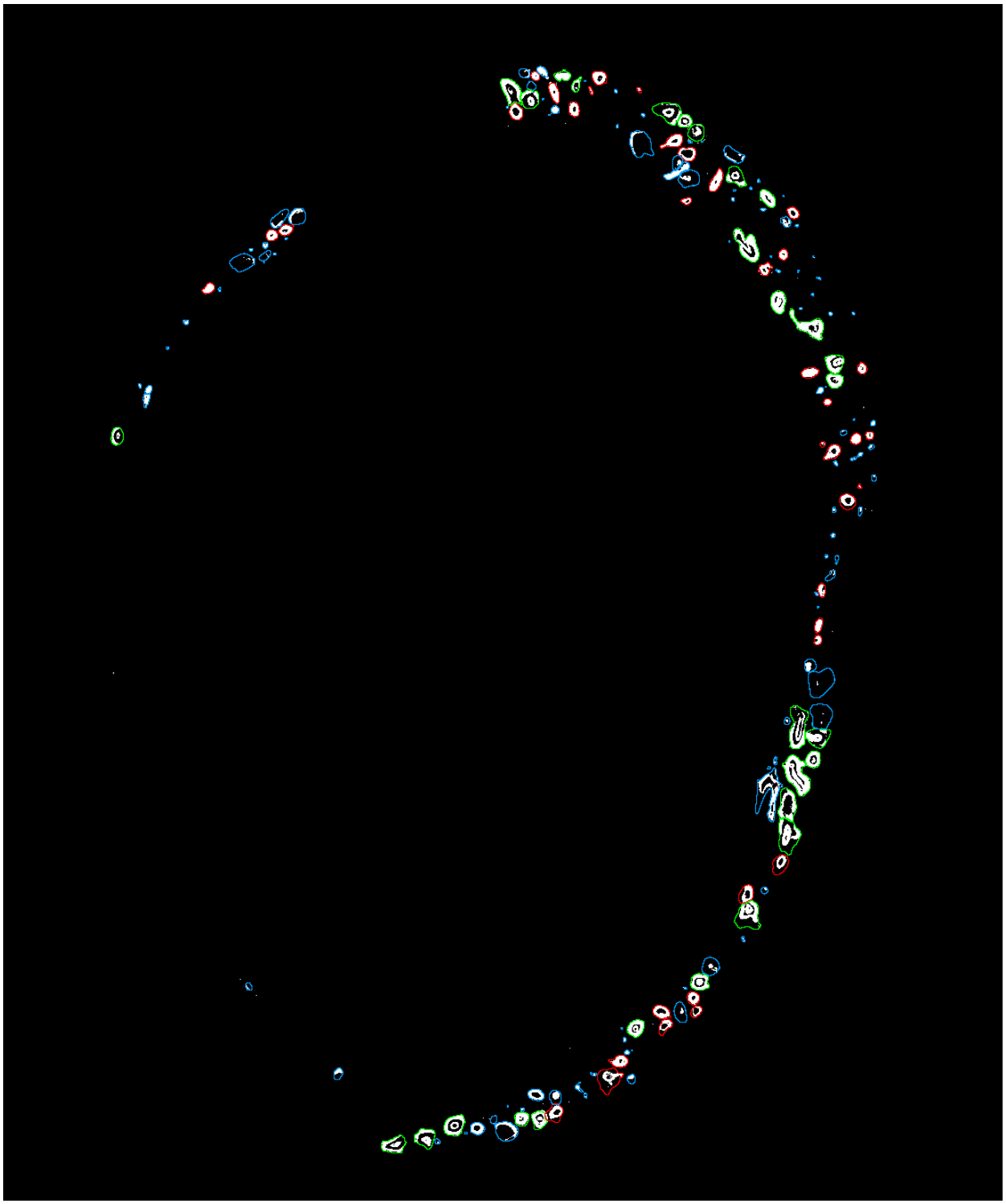


Figure 19: Osteon "ring" image for Sample 1LF_85µm, with an overlay of osteon border ROIs automatically colorized by type as forming (blue), single-labeled (green), double-labeled (red), and triple-labeled (yellow).

Label spacing (osteon mineral apposition rate, or On.MAR) is also measured between the first and second ring for double-labeled osteons, and between both the first and second and the second and third for triple-labeled osteons. X and Y coordinates for each end of the label spacing line are saved to an aggregate data table for each osteon. The distance between these labels is measured as the distance between the peak edges on the intensity profile along the osteon major axis (**Figure 20**). The label spacing in pixels and μm is also saved to this data table.

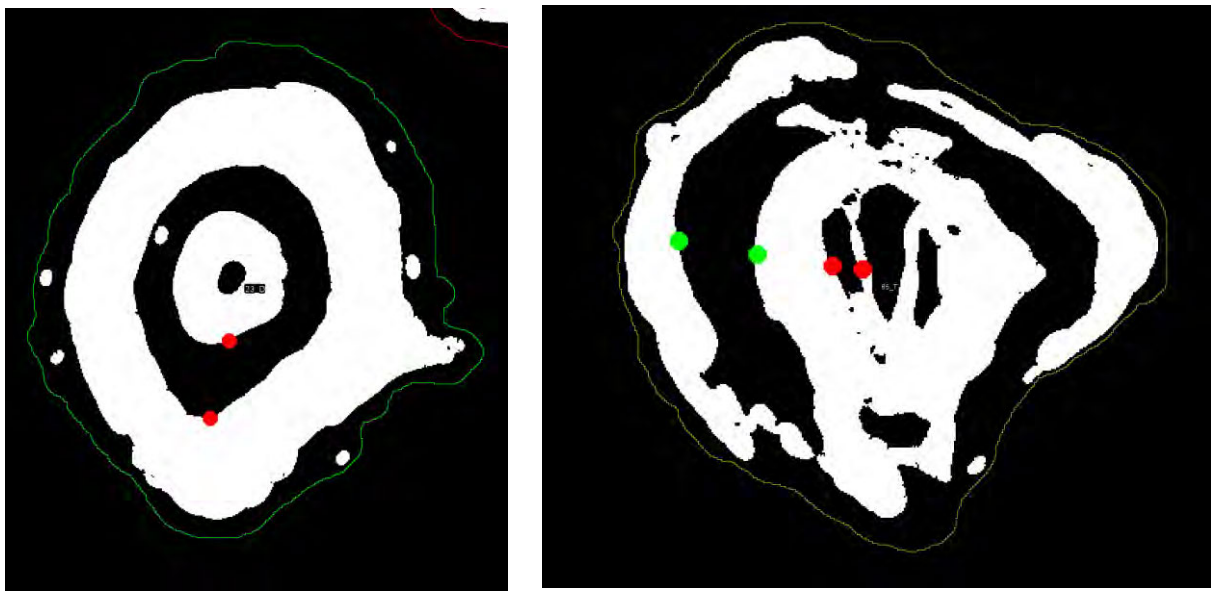


Figure 20: Red dots denote coordinates for label spacing between the first and second rings for both double-labeled (left) and triple-labeled (right) osteons. Green dots denote coordinates for label spacing between the second and third rings for triple labeled (right) osteons.

The macro additionally measures osteon wall thickness as the maximum distance of the osteon's vascular pore from any point on the osteon border. First, a pixel brightness intensity profile is extracted both along the major axis and the minor axis of an ellipse fitted to the osteon border. For both intensity profiles, the two most central peaks (e.g., the innermost label around the pore) are identified, and coordinates at their absolute center are extracted to represent the probable centroid of the pore. If the intensity profile has 0 or 1 peaks, meaning that the ellipse axis passes through a partial label or no labels, then the center of the ellipse axis is used to estimate the pore centroid location. Since the probable pore centroid is extracted for both the minor and major axis of the osteon border ellipse, the coordinates are chosen if they fall closer to the central point of that ellipse (**Figure 21**). The coordinates of the probable pore centroid are saved to an aggregate data table for each osteon.

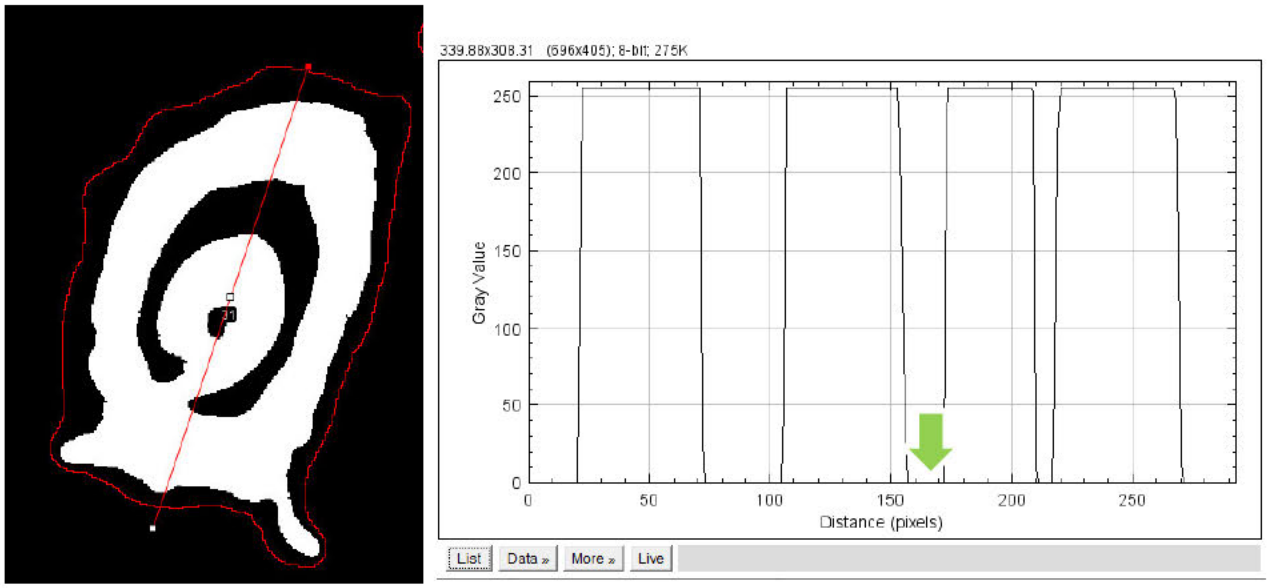


Figure 21: An intensity profile of pixel brightness (right) is extracted along the major axis of the osteon border (red; left). The central black (pixel brightness = 0) point between the innermost peaks is selected as the probable coordinate of the osteon's vascular pore.

Finally, the macro measures osteon wall thickness (W.Th) as the distance from the probable pore centroid to the nearest point on the osteon border. This is accomplished by converting each osteon border into a Euclidean Distance Map (EDM), where the pixel brightness value of any given pixel represents its distance from the nearest point on the osteon border. The pore centroid is superimposed on this EDM, and its pixel brightness value is recorded, representing the distance of the pore centroid from the nearest point on the osteon border (**Figure 22**). This wall thickness value is saved to an aggregate data table for each osteon.

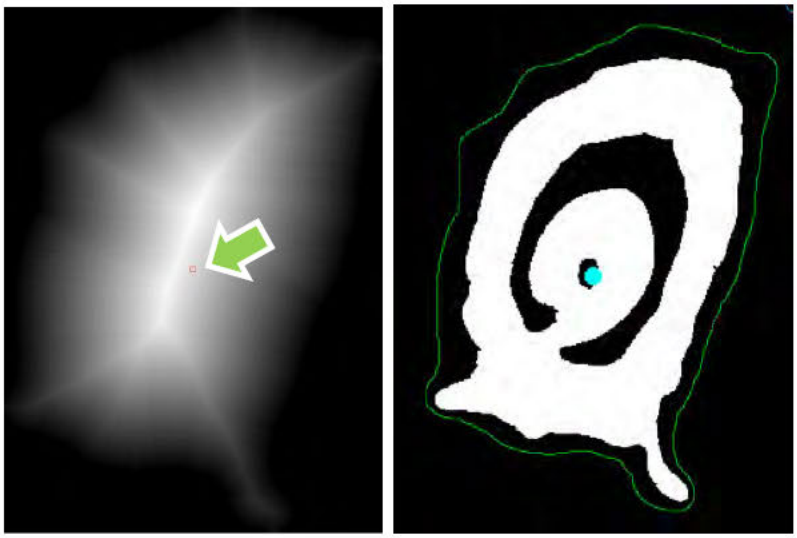


Figure 22: Pore centroid (cyan; right) superimposed on Euclidean Distance Map (left) of the osteon border. The gray value of the pore centroid represents its distance from the nearest point on the osteon border.
An ROI set of osteon borders labeled by osteon type is exported from this macro. The other export is a csv file listing, for each osteon: regional classification, type classification, label spacing coordinates, label spacing (On.MAR) lengths, pore centroid coordinates, wall thickness length, and size/shape morphometric descriptors for each individual osteon.

Osteon Type Correction (Manual): Automated osteon type classification, label identification, and pore centroid identification may be misdirected by noise or by incomplete osteon labeling. The user must manually inspect these components to ensure accuracy. The original DIC image, the contrast-enhanced fluorescence image, and the csv file and ROI set of osteon type classifications are loaded. As in the Osteon Border Correction macro, the DIC image is superimposed on the fluorescence image at 75% opacity so that calcein labels and vascular pores can be viewed simultaneously. The ROI set of osteon borders, colorized by type, is superimposed on this image. Coordinates are also added as point overlays for the label spacing (red = inner labels, green = outer labels) and pore centroids (cyan). Keyboard shortcut utilities were developed for this macro, including an auto-save utility for the ROI set and csv file, and shortcuts to reclassify osteon type and manually place label spacing and pore centroid coordinates. Reclassified osteon types are updated with an asterisk on the label and a color change to the new type designation. Labels and pore centroids are converted from solid dot overlays to hollow dot overlays if they are manually modified (**Figure 24**). This assists the user in tracking ROIs that have already been corrected. Label spacing (On.MAR) and osteon wall thickness (W.Th) are recalculated after manual placement. After the user completes manual correction, the macro exports the final ROI set of osteon borders, colorized by osteon type (**Figure 25**), in addition to the finalized csv file of aggregate osteon morphometry, including region classification, type classification, label spacing, osteon wall thickness, and size and shape descriptors. The macro also exports the DIC image + fluorescence image and the osteon "rings" image with the label and pore centroid coordinates superimposed as overlays, as a record of the final modifications.

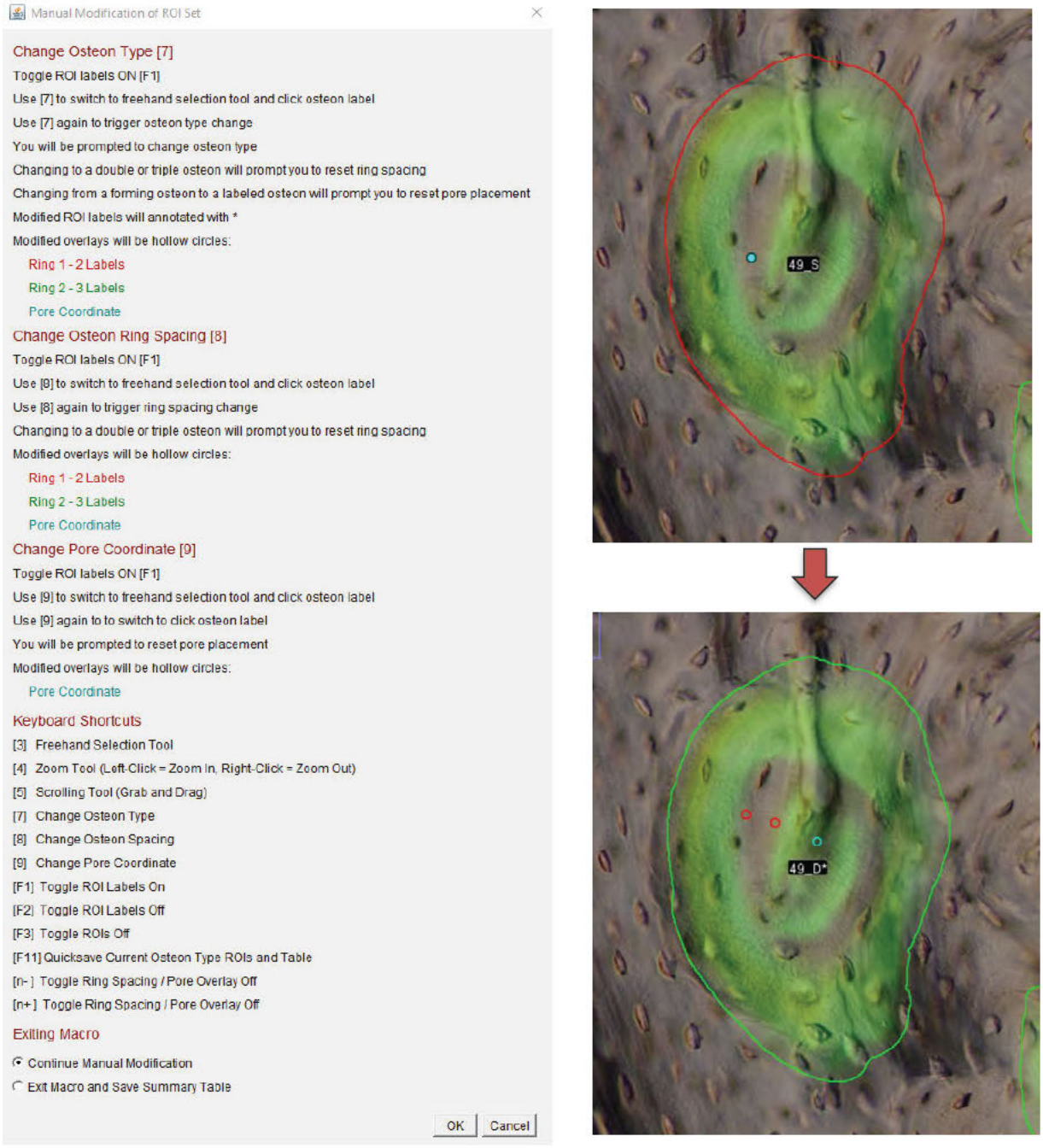


Figure 24: Keyboard utilities (left) are available for quick and easy reclassification of osteon type, label spacing, and pore centroid placement. An osteon automatically classified as single-labeled (top right) is reclassified manually as double-labeled (bottom right). The reclassified osteon type changes color (red → green) and label (_S → _D) to reflect the new osteon type, and the ROI label is appended with an asterisk (_D*). The user is prompted to set label spacing between the first and second rings (red hollow circles). The pore centroid is also manually corrected, changing from a solid cyan circle to a hollow cyan circle.

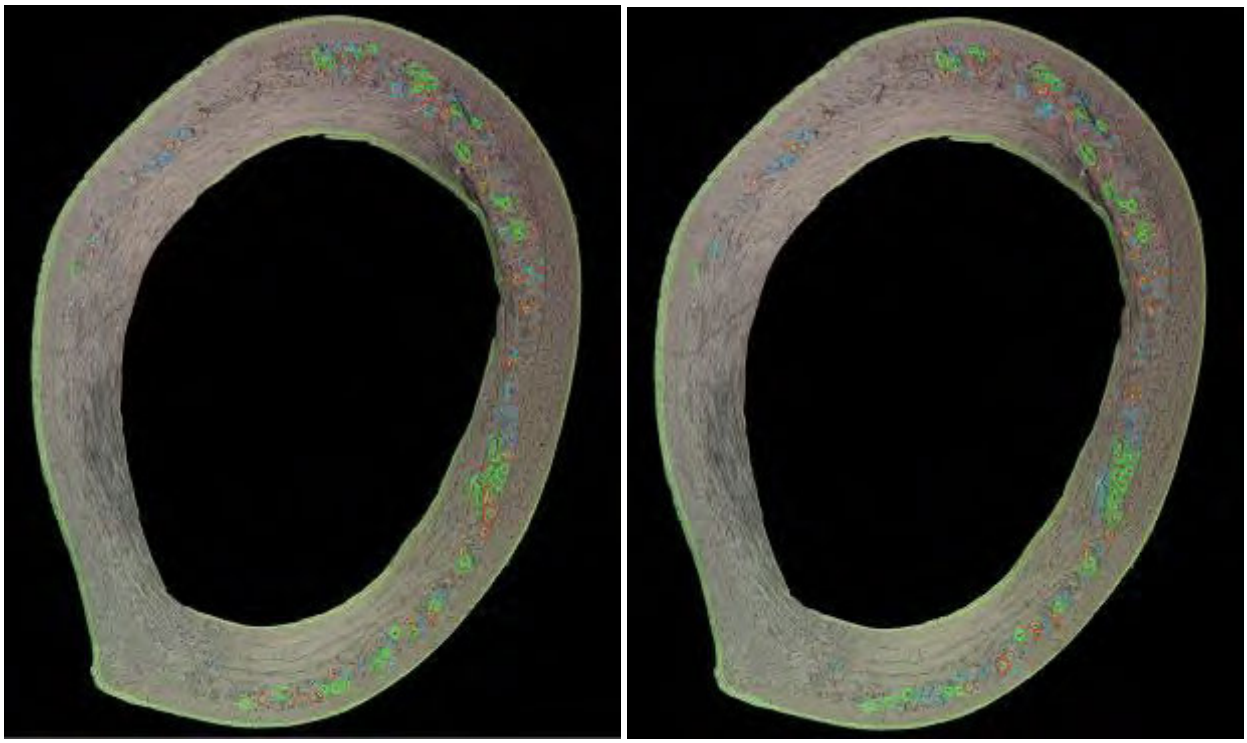


Figure 25: Osteon border ROIs before (left) and after (right) manual osteon type correction, superimposed on fluorescence image and DIC image at 75% opacity for sample 1LF_85um. Osteon ROIs are colorized by type as forming (blue), single-labeled (green), double-labeled (red), and triple-labeled (yellow).

Mineralizing Surface (Fully Automated): This macro calculates the percentage of the total area perimeter (bone surface or BS) that is calcein labeled as a mineralizing surface (MS). Both the periosteal surface (Ps.MS/BS) and the endosteal surface (Es.MS/BS) are assessed. The files loaded include the contrast-enhanced fluorescence image exported by Osteon Border Extraction, the binarized total area and marrow area masks and ROIs exported by Pore Extractor, and the remodeling area ROI. The intracortical region with labeled osteons is subtracted from the fluorescence image by clearing inside the remodeling area ROI. Then, the periosteal and endosteal labels are automatically binarized using a global Huang threshold. To assess Ps.MS/BS, the total area perimeter is extracted from the total area ROI file, and shrunk by a user-selected value (default = 50 pixels) to intersect with the calcein labeled region just below the periosteum. The user may adjust this shrinkage value for the total area ROI until intersection with binarized labels is satisfactory (**Figure 26**). Then, the number of labeled pixels is quantified along this perimeter, and compared to the number of pixels that compose the total area perimeter – the ratio of which is Ps.MS/BS. A similar process is used to assess Es.MS/BS. The marrow area perimeter is extracted from the marrow area ROI file, and expanded by a user-selected value (default = 50 pixels) to intersect with the binarized image of the calcein-labeled endosteum. The number of labeled pixels is quantified along this perimeter, and compared to the number of pixels that compose the marrow area perimeter – the ratio of which is Es.MS/BS. The export from this macro is a csv file containing Ps.MS/BS and Es.MS/BS values.

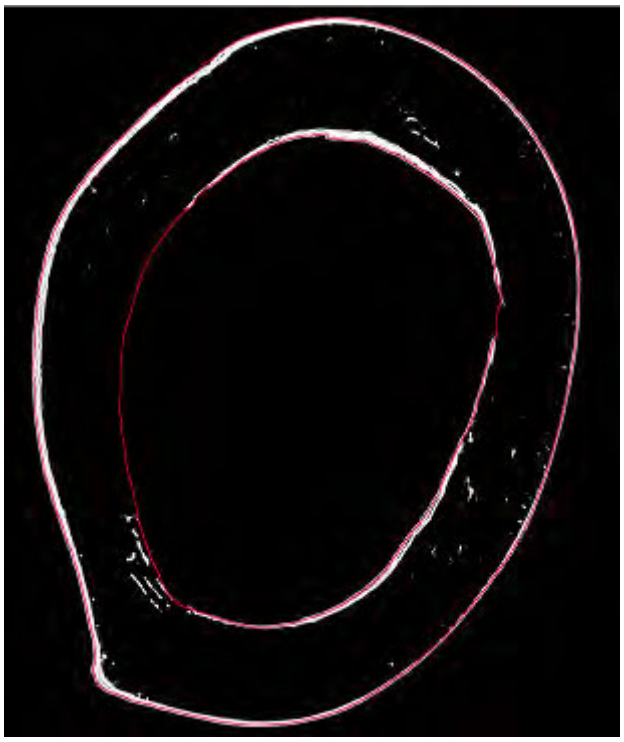


Figure 26: Total area and marrow area ROIs (red) for Sample 1LF_85µm, shrunk and expanded by 50 pixels, respectively, to intersect with the binarized periosteal and endosteal labels. Ps.MS/BS was 98.4%, while Es.MS/BS was 64.6%.

Resorption Cavities: This macro isolates resorption spaces (Rs.N) inside the remodeling area that are not fluorescently labeled, and are therefore not categorized as "forming osteons", but do fall into the aggregate category of active remodeling centers (a.Rm.Cr). Rs.N are also used in the calculation of the ratio of labeled osteons to resorption spaces. Files loaded for this macro include the original DIC image, the contrast-enhanced fluorescence image, the corrected osteon border image, and the corrected ROI set of vascular pores. The table of individual pore measurements exported by Pore Analyzer is also loaded to sort pores by their previously determined regional classification. As in the Osteon Border Correction macro, the DIC image is superimposed on the fluorescence image at 75% opacity so that any fluorescent labeling of vascular pores can be visualized. Pore ROIs are removed if they intersect at least 2/3 of their area with osteon borders (and thus are likely to be fluorescently labeled). Probable resorption spaces are isolated from the remaining pore ROIs by sorting all pore areas into histogram bins. The majority of pores are uniformly small in size and fall into the first histogram bin. These pore ROIs are outlined in blue. All larger pores are identified as probable resorption spaces and are colored red (**Figure 27**). The user inspects these designations and can flip them using a keyboard shortcut. After manual approval, the number of unlabeled resorption spaces (Rs.N) is exported as a csv file, with rows for the whole bone and for each anatomical region.

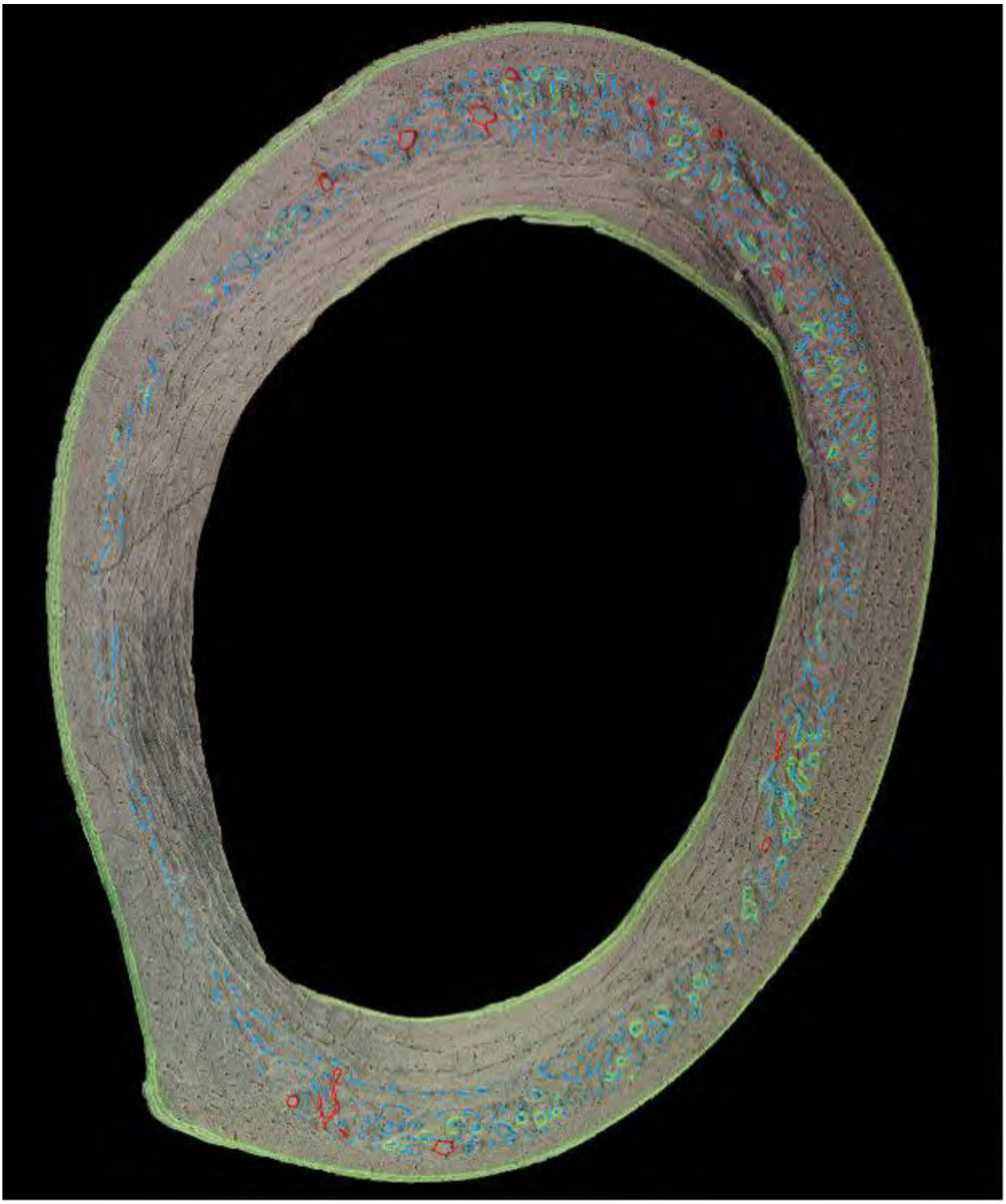


Figure 27: Unlabeled pore spaces for Sample 1LF_85µm, confirmed after user inspection as non-resorption spaces (blue) and resorption spaces (red).

Objective 3: Identify if prolonged opioid use is discernible in cortical bone microstructural features used in histological age-at-death estimation.

Activity 4: Data Analysis
Projected Completion Dates: 02/20 – 06/20
Actual Completion Dates: 07/20 – 12/20
Progress: 100% as per revised timeline

Micro-CT Data Analysis

Statistical Model Design

Pore systems were extracted, and their 3D morphometry analyzed using a custom ImageJ and CTAnalyser workflow. Descriptive statistics of mean, median, and standard deviation were generated for each morphometric variable with package *dplyr*. Descriptive statistics were generated by grouping Bone, Drug Group, and Bone * Drug Group for whole-bone comparisons, and by Region, Drug Group, and Region * Drug Group for anatomical quadrant comparisons (**Appendix XX:** "Micro-CT Descriptive Statistics").

Aggregate pore morphometry variables were assessed in R (The R Foundation, v. 4.1.1) using a Linear Mixed Model (LMM) with the formula *Morphometric Variable ~ Bone Type * Drug Group + (1|Sample)*. Within the femur and tibia individually, regional comparisons between Anterior, Posterior, Medial, and Lateral quadrants were conducted with the formula *Morphometric Variable ~ Bone Region * Drug Group + (1|Sample)*. Morphometric variables were scaled and centered. LMM were tested for each morphometric variable with the *lmer* function within the package *lme4*. The LMM output is an estimate (β) of the mean, standard error (SE), confidence interval, *t* statistic, and a *p*-value for the test statistic computed with Satterthwaite's method for degrees of freedom. Estimates are also reported for the estimate, standard error, percentage of residual variance (ICC), and significant effect attributed to the random effect of Sample. Linear mixed model fixed and random effects, post-hoc tests for all significant fixed effects, and directional trends for all morphometric variables are included for aggregate porosity (**Appendix XXI: "**Micro-CT Linear Mixed Model for Aggregate Porosity"), femoral regions (**Appendix XXII:** "Micro-CT Linear Mixed Model for Femoral Regions"), and tibial regions (**Appendix XXIII:** "Micro-CT Linear Mixed Model for Tibial Regions").

Model fit was assessed with diagnostic plots (fitted vs. residuals plot, residual boxplot, random effect dotplots, QQ plot, scale-location plot) as well as a Shapiro-Wilk test for normality of residuals and a Levene's test for Homogeneity of Variance. If residuals were not normally distributed ($p < 0.05$), morphometric variables were transformed with Tukey's Ladder of Powers with package *rcompanion*. This function loops through lambda (Λ) exponents and transforms the data using the lambda that maximizes the W statistic of the Shapiro-Wilk test. The LMM was then re-run on the transformed morphometric variable with package *lme4*, and diagnostic plots and tests were re-assessed. Following this initial modeling, *p*-values generated by the LMM model were checked against *p*-values generated by a generalized linear mixed model fitted with a Markov Chain Monte Carlo (MCMC) approach, to draw on the high accuracy of Bayesian modeling. We used package *MCMCglmm* with a parameter-expanded prior and 60,000 iterations. To assess LMM model fit, both Akaike information criterion (AIC) and Bayesian information criterion

(BIC) are reported. Additionally, the variance in the morphometric variable explained by the LMM was assessed with a pseudo R^2 from package *MuMIn*. Marginal R^2 is the variance explained by fixed factors, while Conditional R^2 is the variance explained by the entire model, including fixed factors and random effects. Markov Chain Monte Carlo model fit was assessed based on effective sample size and traces of the fixed factors and random effects.

For models that detected significant differences ($p < 0.05$) for fixed factors, post-hoc analyses were carried out to better understand the magnitude and importance of these differences. Package *emmeans* generated estimated marginal means to determine significant differences between factor levels for fixed factors. Effect size of each significant post-hoc comparison was quantified with Cohen's d from function *lme.dscore* within package *EMAtools*. Cohen's d is the absolute value of the difference between group means divided by the average of their standard deviations, such that a Cohen's d of 1 corresponds to a single standard deviation between the means of the compared groups. Effect size thresholds are quantified as small (d = 0.2), medium (d = 0.5), or large (d=0.8). Finally, a retrospective power analysis for each fixed factor was conducted with package *simr*. To examine all directional trends, estimated marginal means were also used to determine the relationship between all levels of each fixed factor, regardless of their significance.

Results: Whole-Bone Analysis

A concise summary of findings from whole-bone and regional analysis of the femur and tibia is available in **Section 2.2.3: Significant Results.**

Opioid exposure inverts the Control group's patterning of higher total porosity in the femur and lower total porosity in the tibia. This suggests that opioid treatment desensitizes skeletal elements to localized mechanical demands, which control intraskeletal variation in pore morphometry in healthy animals.

Whole Bone Analysis: Bone Effects

The rabbit femur has significantly thicker, larger diameter, larger volume, and larger surface area pores, compared to the rabbit tibia. The standard deviations of pore thickness and pore separation are also significantly larger, indicating a wider range of pore sizes. These enlarged pore systems result in significantly higher percentages of open porosity and a larger intersection surface, meaning convergence of pore systems with periosteal or endosteal borders. Skeletonization analysis indicated that the total and mean lengths of individual pore systems are significantly higher in the femur, compared to the tibia. Femoral pore systems are also more highly branched, with a significantly higher mean and maximum number of nodes and number of terminal nodes, and maximum number of segments within pore systems.

The rabbit tibia has significantly smaller, more numerous, and more densely populated pores that are more longitudinally aligned, compared to the rabbit femur. This produces a significantly higher percentage of closed porosity, as pore systems are not as convergent with cortical borders. In terms of cross-sectional geometry, the tibia has a larger relative cortical volume, total and cortical surface area, and average minor axis breadth compared to the femur.

Considering all drug treatment groups, there is no net significant effect on Total Porosity (%) from either the increased size and branching of femoral pore systems, or the increased population density of tibial pore systems. Further analysis of the Bone * Drug Treatment Group interaction, discussed below, indicates that femoral pore patterning produces a significantly higher Total Porosity (%) within Control group rabbits. Drug treatment, however, inverts this intraskeletal patterning, removing this effect from Bone as a fixed factor.

While information on comparative loading in the rabbit femur versus the tibia is sparse[17], a study by Yamada and colleagues[18] suggests that the rabbit tibia experiences higher residual stress than the femur. Residual stress is the stress experienced by bone tissue absent of external force, due to the inherent structure of the tissue and its mechanical properties. The authors found that osteon population density (OPD) was increased under higher residual stress, and that the anterior and posterior tibia had a higher combined OPD than the anterior and posterior femur. High OPD has been associated with a higher frequency of targeted remodeling to repair the microdamage more frequently incurred under high strain[19,20]. However, high strain also inhibits bone resorption, and is known to limit the size of osteons in cross-section[21–23]. The expected pore morphometry for a more highly stressed tibia is an increased density of pores that are smaller in size, which we observed in our study. The rabbit tibia is also known to be stronger than the femur, as demonstrated by the higher force required to fracture the tibia[24]. Pores concentrate stress and are target sites for fracture initiation and propagation[25]. The enlarged pore systems in the rabbit femur may contribute to its observed lower force to fracture, relative to the rabbit tibia.

Whole Bone Analysis: Drug Treatment Group Effects

Drug treatment restricts the overall diameter and volume of aggregate pore systems in both the femur and tibia. Control group rabbits exceed drug treatment group rabbits (Control > Morphine > Fentanyl) in morphometric variables that describe aggregate pore size (Mean and Standard Deviation of Pore Thickness), as well as mean size of individual pore systems (Average Pore Volume, Mean Pore System Total Volume, Pore Segment Mean Radius). Only the Control > Fentanyl comparison reaches significance ($p < 0.05$). Pore Surface : Pore Volume follows the inverse pattern (Fentanyl > Morphine > Control), which may be due to increased truncation. When the femur and tibia are examined individually in the interaction contrast (Bone | Group), both bones retain this trend (Control > Morphine > Fentanyl) for aggregate pore size. When the mean size of individual pore systems is examined, however, Morphine rabbits exceed Control group rabbits (Morphine > Control > Fentanyl) in the tibia, while retaining the original patterning in the femur. Consequently, in the tibia specifically, Morphine treatment enlarges individual pore systems.

*Whole Bone Analysis: Bone * Drug Treatment Group Interaction Effects*

As seen in the fixed factor Bone, the tibia favors small, dense pore systems while the femur favors large, less densely populated, more highly branched pore systems. Neither bone has higher total porosity or aggregate pore volume when drug treatment groups are combined. Intraskeletal differences, however, appear when the femur and tibia are contrasted within each drug group using the interaction contrast Group | Bone. When restricted to Control rabbits, the femur develops significantly higher Total Porosity (%) and Pore Volume, compared to the tibia. The Control femur also displays significantly higher maximum values for pore system branching (Total Length, Number of Nodes, Terminal Nodes). Control rabbits additionally

retain the overall Bone effects of significantly higher Open Porosity (%) and size and branching of individual pore systems (Total Pore Network Volume, Average Pore Volume, Mean Pore System Total Length, Mean Pore System Terminal Nodes), compared to the tibia. Drug treatment, however, silences this significant intraskeletal variability. For Fentanyl and Morphine group rabbits, there is no significant difference between the Femur and Tibia in these same morphometric variables, even though the direction of the trend is the same (Femur > Tibia). Pore Volume goes furthest by inverting this trend, with an insignificant shift to Femur < Tibia in both Fentanyl and Morphine rabbits. Overall, drug treatment appears to equalize the porosity, pore size, and branching complexity of the femur and tibia, removing the significantly increased femoral porosity that exists in Control rabbits.

Drug treatment minimizes this intraskeletal variation both by reducing femoral porosity and increasing tibial porosity. This effect is visible in 3D reconstruction (**Figure 2**). The Bone | Group interaction contrast shows the effects of drug treatment on the femur and tibia individually. As before, in the femur, Control groups have significantly higher Total Porosity (%) and Open Porosity (%), in addition to significantly higher metrics of aggregate volume (Pore Volume, Total Pore Network Volume) and size and branching of individual pore systems (Pore Segment Mean Radius, Mean Pore System Total Length, Mean Pore System Terminal Nodes), compared to drug treatment groups (Control > Morphine > Fentanyl). Descriptive statistics indicate that femoral mean values for each of these variables are decreased in Morphine and Fentanyl rabbits, compared to Controls. Conversely, in the tibia, drug treatment increases these same pore measurements. Morphine is elevated above Control group rabbits (Morphine > Control > Fentanyl) in the tibia for Open Porosity (%), Average Pore Volume, Pore Segment Mean Radius, Total Pore Network Volume, and Mean Pore System Total Length. Both Morphine and Fentanyl are elevated above Control group rabbits (Morphine > Fentanyl > Control) for Total Porosity (%), aggregate Pore Volume, and Mean Pore System Terminal Nodes. Only the femoral trends, however, (Control > Fentanyl and Control > Morphine) reach significance ($p < 0.05$). **Our results confirm that the overall effect of reduced femoral porosity and increased tibial porosity is to equalize intraskeletal porosity under drug treatment.**

Results: Regional Analysis: Femur

Regional Analysis: Femur: Region Effects

In the rabbit femur, the high porosity derived from larger pore size is localized in the Medial and Posterior quadrants, as observed in 3D visualization (**Figure 2**). High pore density is localized in Medial and Lateral quadrants. The relatively smaller pore size in the Lateral quadrant, however, prevents it from accruing high overall porosity.

The Medial quadrant, followed without significant difference by the Posterior quadrant, contains the highest Total Porosity (%), Open Porosity (%), and Connectivity Density. Cortical Fractal Dimension and Pore Fractal Dimension, which signify more patterned spatial distribution, are highest medially, followed with significant difference by the Posterior quadrant. Similarly, the Posterior quadrant, followed without significant difference by the Medial quadrant, contains the largest pores by aggregate and individual measurements (Number of Open Pores, Pore Volume, Pore Surface, Pore Thickness, Connectivity, Average Pore Volume). In all comparisons, Anterior and Lateral quadrants have significantly lower percent porosity and pore size.

The Medial quadrant, followed without significant difference by the Lateral quadrant, displays increased pore density of all pore types (total, open, closed). Closed Porosity (%) is also highest medially, followed with significant difference by the Lateral quadrant. Pore Surface: Pore Volume is highest laterally, followed with significant difference by the Medial quadrant.

The Anterior quadrant shows significantly increased Pore Separation (mean and standard deviation), because it also significantly exceeds all except the Posterior region in Cortical Volume, and thus pore systems are more widespread.

Our findings are consistent with literature on rabbit knee joint biomechanics. During hopping, initial hindlimb contact causes the ground reaction force (GRF) to pass Anterior and Lateral to the knee joint. Intersegmental axial knee force also increases sharply during this initial hindlimb contact, peaking at 40% of body weight between 15-25% of the stance trajectory[17]. The relatively lower mechanical loading experienced by Medial and Posterior regions of the rabbit femur is more permissive to bone resorption[23,26], creating the significantly higher percent porosity and larger pore size that we observed in those regions. We further observed a significantly higher pore density in both Medial and Lateral quadrants of the rabbit femur. High pore density combined with high porosity and large pore size in the Medial quadrant is consistent with stochastic remodeling, which is elevated under lower mechanical strain for routine turnover of old bone[27]. High pore density combined with low porosity and small pore size in the Lateral quadrant is consistent with targeted remodeling, which occurs in a limited radius (~100 μm) for repair of loading-induced microcracks[28].

Regional Analysis: Femur: Drug Treatment Group Effects

The pattern Control > Morphine > Fentanyl is seen for variables related to increased pore size (Pore Thickness Mean and Standard Deviation) and convergence with cortical borders (Open Porosity (%), Intersection Surface), with the Control > Fentanyl comparison reaching significance.

*Regional Analysis: Femur: Region * Drug Treatment Group Effects*

Comparable to the whole bone analysis, drug treatment tends to silence the regional variability observed in the Control group. The Group | Region interaction contrast examines regional comparisons within each drug treatment group. The significant regional differences seen in Control rabbits are insignificant for certain variables in Fentanyl rabbits (Open Porosity %) and for both Fentanyl and Morphine rabbits (Total Porosity %, Average Pore Volume, Intersection Surface). If drug treatment can reduce sensitivity to mechanical control of pore formation at the whole bone level, it further appears to reduce sensitivity to regional mechanical loading within the femoral cross-section.

The reduced porosity associated with drug treatment seen in femoral whole-bone analysis is concentrated in Anterior and Medial regions. The Region | Group contrast examines drug treatment group differences within each region. For Total Porosity (%), the Control group rabbits only significantly exceed drug treatment rabbits in the Medial quadrant, although all quadrants display this pattern (Control > Morphine > Fentanyl). Similarly, for Open Porosity (%), this pattern is significant medially (Control > Fentanyl) and

anteriorly (Control > Fentanyl and Control > Morphine). For the inverse patterning of Pore Surface: Pore Volume (Fentanyl > Morphine > Control), Medial and Anterior quadrants show a significant Fentanyl > Control difference.

Results: Regional Analysis: Tibia

Regional Analysis: Tibia: Region Effects

Regional porosity patterning in the rabbit tibia reflects that of the femur. The Medial and Posterior quadrants again show high percent porosity. Also, as in the femur, both the Medial and Lateral quadrants favor high pore density, but the relatively smaller pore size in the Lateral quadrant prevents it from accruing high overall porosity. Two trends distinguish the tibia from the femur. Unlike the femur, large pore size is restricted to the Posterior quadrant of the tibia, while the Medial quadrant draws its high porosity from high pore density. Also, the tibia shifts the absolute number of pores and connectivity metrics from the Medial and Posterior quadrants to the Anterior quadrant, but there is no net effect on porosity or pore density patterning due to the large Anterior quadrant volume.

The Medial quadrant, followed by the Posterior quadrant, contains the highest metrics of spatial complexity (Cortical Fractal Dimension, Pore Fractal Dimension, Pore Surface : Cortical Volume. Similarly, the Posterior quadrant, followed by the Medial quadrant, contains the highest percentages of all types of porosity (Closed, Open, Total), and several other metrics (Pore Volume, Intersection Surface).

The Posterior quadrant dominates metrics of pore size (Pore Thickness, Pore Thickness Standard Deviation, Average Pore Volume / Surface / Thickness / Major Pore Diameter). It also shares a high Degree of Anisotropy with the Anterior quadrant.

The Medial quadrant, followed by the Lateral quadrant, displays increased open pore density and Pore Tb.N., a proxy of total pore density. The Lateral quadrant, followed by the Medial quadrant, has significantly higher closed and total pore density than other quadrants.

The Anterior quadrant has the highest number of pores (closed, open, total), the highest connectivity and connectivity density, and the highest Pore Surface. These metrics are highest in the Medial and Posterior quadrants in the femur. However, as in the femur, the Anterior quadrant spreads its pore systems through a larger Cortical Volume, maximizing Pore Separation Mean and Standard Deviation. Consequently, the Anterior quadrant does not reach a high percent porosity or pore density.

Our findings are consistent with literature on rabbit knee joint biomechanics. As with the rabbit femur, the Anterior and Lateral regions experience higher mechanical loading by an adjacent ground reaction force (GRF) during initial hindlimb contact while hopping[17]. The higher porosity that we observed in Medial and Posterior quadrants is consistent with their relatively lower mechanical loading. As with the rabbit femur, the high pore density and high porosity of the Medial tibial quadrant is consistent with stochastic remodeling, while the high pore density and low porosity of the Lateral tibial quadrant is consistent with targeted remodeling.

Regional Analysis: Tibia: Drug Treatment Group Effects

Unlike the femur, there are no overall drug treatment group effects on pore morphometry.

Regional Analysis: Tibia: Region Effects

As in the femoral regional analysis and the whole bone analysis, drug treatment tends to silence the regional variability seen in the Control group. A unique tibial effect is the additional elevation of porosity anteriorly (Fentanyl) and pore density laterally (Fentanyl and Morphine). This supports the whole-bone analysis finding that drug treatment increases porosity in the tibia, contributing to the overall loss of intraskeletal variability in porosity.

The Group | Region interaction contrast examines regional comparisons within each drug treatment group. As in the femur, drug treatment removes significant intra-regional comparisons that exist in Control group tibiae for a variety of pore metrics. The loss of regional variability, with no distinct regional elevation to compensate, is generally seen in metrics or proxies of pore size for Fentanyl rabbits (Pore Thickness, Pore Surface : Cortical Volume), Morphine rabbits (Number of Open Pores), or both groups (Average Major Pore Diameter). An additional effect seen in the tibia is that Fentanyl group rabbits tend to increase anterior porosity while diminishing significant differences between other regions for a number of morphometric variables (Open Porosity (%), Total Porosity (%), Pore Volume, Pore Surface, Intersection Surface, Number of Objects, Connectivity Density). This anterior porosity increase can be clearly observed in the 3D visualization of a Fentanyl rabbit tibia (**Figure 2**)**.** Both Morphine and Fentanyl rabbits also elevate lateral pore density (closed and total) at the expense of other regional variability. Finally, drug treatment represses pore system complexity posteriorly for Fentanyl group rabbits (Pore Tb.N, Pore Fractal Dimension) and anteriorly for Morphine group rabbits (Pore Surface : Cortical Volume, Pore Fractal Dimension).

The Region | Group contrast examines drug treatment group differences within each region. Whole bone analysis suggests that drug treatment reduces aggregate Pore Thickness in the femur and tibia, although the size of individual pore systems may be increased in the tibia. In the femur, decreased Pore Thickness is a group level effect that is consistent across all regions (Control > Morphine > Fentanyl). In the tibia, however, only the Posterior quadrant displays this patterning (Control > Morphine > Fentanyl), and only Control > Fentanyl is significant. All other quadrants conversely show elevated Pore Thickness with drug treatment (Morphine > Fentanyl > Control). This may help explain how individual pore systems can be larger, but overall mean Pore Thickness is significantly smaller with drug treatment. Aggregate Pore Volume has a sole significant contrast of Morphine > Fentanyl in the Posterior region.

The sensitivity of Anterior and Lateral quadrants to drug treatment is consistent with the literature. Yamane and colleagues[29] found that osteoporosis induced by parathyroid hormone administration developed porosity preferentially in tibial regions corresponding to our Anterior and Lateral quadrants.

Following only 8 weeks of opioid administration, **our ongoing rabbit study confirms our hypothesis that opioids significantly alter bone porosity and pore morphometry, producing substantial alterations to bone microarchitecture.**

Data analysis of the micro-CT imaging phase of the project proceeded on schedule beginning February 2020. This timeline was not impacted by the COVID-19 pandemic as statistical workflows could be employed remotely. Histological sample processing and imaging were completed on 11/20. The protocol for histological analysis of pore and osteon spaces was developed during the subsequent reporting period. Data analysis of histological images has been completed. Drafting manuscript submissions will follow per the proposed revised timeline.

Femoral Histological Data Analysis

Morphometric Variable Calculations

The OsteoFlo toolkit automatically exports values for each section's cross-sectional geometry and mineralizing surface (Ps.MS/BS and Es.MS/BS). Vascular percent porosity, pore counts, and mean pore size and shape morphometry are also automatically exported. Finally, automatic exports for each osteon type include counts, mean inter-label distance, mean wall thickness, and size and shape morphometry.

Population densities were calculated for vascular pores and for each osteon type, which included total osteons (T.On), complete (calcein-labeled) osteons (C.On), forming osteons (F.On), single-labeled osteons (sL.On), double-labeled osteons (dL.On), triple-labeled osteons (tL.On), unlabeled resorption cavities (Rs.N), and active remodeling centers (T.On + Rs.N) (a.Rm.Cr). Osteon population densities for each osteon type were calculated by dividing the osteon type separately by the cortical area (e.g., T.On.OPD.CA) and by the remodeling area (e.g., T.On.OPD.RA).

Additional values were calculated to characterize remodeling rate, following Dr. Andronowski's previous work published in Harrison et al. 2020[30]. This study had three calcein administrations, while Harrison et al. 2020[30] had two calcein administrations. Therefore, our calculations were modified to accommodate our study's triple-labeled osteons, and to characterize any differences between labeling periods 1-2 and 2-3. Our calculations included:

- Ratio of labeled osteons versus resorption cavities (T.On / Rs.N)
- Osteonal mineral apposition rate (On.MAR, μm/day), calculated as the inter-label distance divided by the labeling period (14 days). Several calculations were explored to determine whether this variable and dependent variables changed over the experimental period.
    - On.MAR.dL – calculated from dL.On only
    - On.MAR.I – calculated from the mean distance of inner labels of dL.On and tL.ON
    - On.MAR.C – calculated from the mean distance of both inner labels (dL.On and tL.On) and outer labels (tL.On)
- Osteon formation time (σ_f, days), calculated as wall thickness divided by On.MAR. We calculated this variable using wall thickness for complete osteons (W.Th.C) divided by each calculation of On.MAR.
    - σ_f dL = W.Th.C / On.MAR.I.dL
    - σ_f I = W.Th.C / On.MAR.I
    - σ_f C = W.Th.C / On.MAR.C

- Activation frequency (Ac.f, #/mm^2/year), calculated as complete (sL.On + dL.On + tL.On) osteon population density (C.On.OPD.CA) divided by σ_f, multiplied by 365 days/year. We calculated this variable for value of osteon formation time derived from each calculation of On.MAR.
    - Ac.F.I.dL ((C.On.OPD.CA/ σ_f dL)*365)
    - Ac.F.I ((C.On.OPD.CA/ σ_f I)*365)
    - Ac.F.I.dL ((C.On.OPD.CA/ σ_f C)*365)

Descriptive statistics of mean, median, and standard deviation were generated for each morphometric variable in R (The R Foundation, v. 4.1.1) with package *dplyr*. Descriptive statistics were generated by Drug Group for whole-bone comparisons, and by Region and Region * Drug Group for anatomical quadrant comparisons (**Appendix XXIV**: "Femoral Histology Descriptive Statistics").

Statistical Model Design*: *Whole Femur

Aggregate variables for cross-sectional geometry, mineralizing surface, vascular pore morphometry, and osteon pore morphometry were assessed in R (The R Foundation, v. 4.1.1) using an ANOVA from the *stats* package with the formula *Morphometric Variable ~ Drug Group*. The ANOVA output is the degrees of freedom (df), sum of squares, mean squares, *F*-statistic, and p-value. Model fit was assessed with diagnostic plots (fitted vs. residuals plot, QQ plot) as well as a Shapiro-Wilk test for normality of residuals and a Levene's test for Homogeneity of Variance. If residuals were not normally distributed or were heteroskedastic ($p < 0.05$), morphometric variables were transformed with Tukey's Ladder of Powers with package *rcompanion*. This function loops through lambda (Λ) exponents and transforms the data using the lambda that maximizes the W statistic of the Shapiro-Wilk test. The ANOVA was then re-run on the transformed morphometric, and diagnostic plots and tests were re-assessed. If the residuals remained non-normal or heteroskedastic, the model was tested using a non-parametric Kruskal-Wallis Rank Sum Test from the *stats* package.

For models that detected significant differences ($p < 0.05$) for Drug Group, post-hoc analyses were carried out to determine which pairwise differences were significant and assess model goodness-of-fit. For morphometric variables assessed with ANOVA, pairwise comparisons were performed using a Tukey Honest Significant Differences (Tukey HSD) test from the *stat* package. The output of this test includes an estimate for the differences in observed means, upper and lower confidence intervals, and a *p*-value adjusted for multiple comparisons. Package *sjstats* gave partial eta squared (η_p^2), which describes the percentage of variance in the morphometric variable derived from the Drug Treatment group, and partial omega squared (ω_p^2), which provides an effect size for Drug Treatment group that is unbiased for small sample sizes (0.01 = Very Small, 0.06 = Small, 0.14 = Medium, >0.14 = Large). A retrospective power analysis was obtained the partial omega squared effect size using package *userfriendlyscience*. For models assessed with Kruskal-Wallis, pairwise comparisons were obtained from a Dunn test with a Bonferroni correction for multiple comparisons from package *rstatix*. Eta-squared was obtained from package *rstatix*, and a retrospective power analysis was carried out using package *MultNonParam*.

The ANOVA tables, post-hoc results, and directional trends are included in **Appendix XXV:** "Histology ANOVA for Whole-Section Femur".

Statistical Model Design*: *Regional Femur

Regional distribution of cross-sectional geometry, mineralizing surface, vascular pore morphometry, and osteon pore morphometry was assessed in R (The R Foundation, v. 4.1.1) using a Linear Mixed Model (LMM). This model was chosen to account for the random effect of sample ID in the repeated measurement of different anatomical regions. The LMM formula was *Morphometric Variable ~ Region * Drug Group + (1|Sample).* We used the same LMM statistical workflow that we developed for regional and drug treatment group comparisons for micro-CT samples.

The LMM tables, post-hoc results, and directional trends are included in **Appendix XXVI:** "Histology Linear Mixed Model for Regional Femur".

Results: Whole Femur Analysis

A concise summary of findings from whole-bone and regional analysis of the femur is available in **Section 2.2.3: Significant Results.**

Whole-section analysis of the femur found that intracortical remodeling was significantly elevated in morphine rabbits and significantly reduced in fentanyl rabbits, compared to controls. Regional analysis of the femur found that population density and mean size were depressed Laterally for vascular pores and osteon types. The interaction between Region and Drug Group demonstrated that drug treatment significantly dysregulates regional patterning, particularly along the Medial-Lateral axis compared to the Anterior-Posterior axis. This regional dysregulation was also observed in micro-CT of vascular pore morphometry in the rabbit femur.

Whole Femur Analysis: Drug Treatment Group Effects

No significant variation between drug treatment groups was observed in cross-sectional geometry, periosteal or endosteal labeling, or any aspect of vascular porosity, including percent porosity, pore density, or pore morphometry. Additionally, no significant differences were observed between drug treatment groups in osteon morphometry, osteon formation time, or activation frequency.

Drug treatment groups did significantly differ in osteon type counts and population densities. This invariably followed the pattern Morphine > Control > Fentanyl, with Morphine > Fentanyl always reaching significance and Morphine > Control sometimes approaching significance. Although the same pattern (Morphine > Control > Fentanyl) was observed for activation frequency, these comparisons do not reach significance for an elevation in Morphine group remodeling rate. However, Morphine group rabbits do reach significance over Fentanyl group rabbits for % Remodeling Area (η_p^2 = 32.2%, power = 60.3%) suggesting that Morphine group elevated osteon counts and population densities are **due to their expansion of the remodeling region within the cortex.**

The Morphine > Fentanyl significant post-hoc comparison was observed for **osteon counts** of total osteons (approaches significance; η_p^2 = 37.7%, power = 98.4%), complete (single/double/triple labeled) osteons (approaches significance; η_p^2 = 35.3%, power = 97.3%), double-labeled osteons (η_p^2 = 56.3%, power =

99.7%), and active remodeling centers (unlabeled Rs.N + total osteons) (Morphine > Fentanyl and Morphine > Control both approach significance; η_p^2 = 35.8%, power = 97.6%).

The Morphine > Fentanyl significant post-hoc comparison was observed for **osteon population densities** of total osteons (approaches significance; η_p^2 = 49.4%, power = 97.9%), complete (single/double/triple labeled) osteons (approaches significance; η_p^2 = 36.5%, power = 97.9%), and active remodeling centers (unlabeled Rs.N + total osteons) (Morphine > Fentanyl and Morphine > Control both approach significance; η_p^2 = 49.1%, power = 97.6%).

Additionally, the mean outer label distance for triple-labeled osteons approached significance for Morphine > Fentanyl and Control > Fentanyl (η_p^2 = 50.8%, power = 98.5%).

Results: Regional Femur Analysis

Regional Femur Analysis: Regional Effects

Regional analyses found that the Lateral quadrant of the femur had significantly lower values for remodeling area fraction, vascular percent porosity and pore density, osteon type counts and population densities, pore and osteon type mean areas, and all calculations of activation frequency. Conversely, the Lateral quadrant of the femur is significantly elevated in aspects of osteon circularity, compared to other quadrants. This suggests that the frequency and extent of secondary remodeling is suppressed in the Lateral quadrant, compared to other quadrants, particularly the Posterior quadrant. This finding is consistent with our micro-CT results, which found significantly reduced porosity and pore size in the Lateral quadrant of the rabbit femur. Again, Lateral repression of remodeling may stem from its high mechanical loading by the adjacent ground reaction force.

While our micro-CT results found significantly elevated pore density in the Medial and Lateral quadrants, our histological results found that the Anterior, Posterior, and Medial quadrants all significantly exceeded the Lateral quadrant in pore density. This difference may stem from measuring pores throughout the cortex in micro-CT, but only within the remodeling area in histological analysis. Significant regional trends included the following patterns:

<u>Significant Lateral Region Depression</u>

Anterior > Lateral

- sL.On/CA (sL.On.OPD.CA)

Posterior > Lateral

- tL.On Count
- sL.On Mean Area

Medial > Lateral

- Mean pore area
- Mean pore perimeter
- Mean Pore Min Feret Diameter

Anterior > Lateral

- Mean pore circularity
- Mean pore roundness

Anterior and Posterior > Lateral

- C.On Count
- sL.On Count
- F.On/CA (F.On.OPD.CA)
- Ac.F.I.dL
- Ac.F.I
- Ac.F.C

Medial, Posterior > Lateral

- T.On Mean Area
- C.On Mean Area

Anterior, Medial, Posterior > Lateral

- Percent porosity
- Pore density CA
- dL.On Count
- F.On Mean Area
- dL.On Mean Area
- a.Rm.Cr/CA (a.Rm.Cr.OPD.CA)
- T.On/CA (T.On.OPD.CA)
- C.On/CA (C.On.OPD.CA)
- dL.On/CA (dL.On.OPD.CA)

Anterior and Posterior > Lateral and Medial

- Cortical Area

Posterior > Anterior, Medial, Lateral + Anterior > Lateral

- Remodeling Area
- RA/CA

Anterior, Medial, Posterior > Lateral + Posterior > Medial

- Total pore number
- Total pore area
- Total osteon count
- Forming osteon count

<u>Significant Lateral Region Elevation</u>

Lateral > Medial, Anterior

- Mean pore aspect ratio

Lateral > Medial, Posterior

- T.On Mean Circularity
- T.On Mean Solidity
- C.On Mean Circularity
- dL.On Mean Circularity

Lateral > Anterior, Medial, Posterior

- C.On Mean Solidity

Regional Femur Analysis: Drug Group Effects

Isolated from the regional effect, Drug Group showed significant Morphine > Fentanyl patterning for a subset of the variables identified in the whole-bone analysis:

- RA/CA
- C.On/CA (C.On.OPD.CA)
- dL.On Count
- dL.On/CA (dL.On.OPD.CA)
- tL.On Mean Outer Label

Regional Femur Analysis: Regional Effects Within Drug Groups

Our post-hoc analyses assessed the regional patterning within each drug treatment group individually. As seen in the micro-CT results, drug treatment significantly dysregulates regional patterning of remodeling-derived microstructures, compared to controls. Morphine and Fentanyl group rabbits lost some significant regional patterns seen in Controls, and gained certain significant regional patterns not seen in Controls. These deviations almost always altered the morphometry along the Anterior-Posterior axis compared to the Medial-Lateral axis. Gain of significant regional variation was more common in Fentanyl group rabbits than Morphine group rabbits.

<u>Regional Differences Lost Compared to Controls</u>

Forming Osteon Count
- Anterior > Medial: lost by Morphine and Fentanyl
- Posterior > Medial: lost by Fentanyl

sL.On Count
- Anterior > Medial: lost by Morphine and Fentanyl
- Posterior > Medial: lost by Fentanyl

C.On Mean Wall Thickness
- Medial > Lateral: lost by Morphine and Fentanyl

dL.On Mean Wall Thickness
- Anterior, Medial, Posterior > Lateral: lost by Morphine and Fentanyl

C.On Mean Area
- Anterior, Medial > Lateral: lost by Morphine and Fentanyl

F.On Mean Aspect Ratio:
- Medial > Anterior: lost by Morphine and Fentanyl

sL.On/CA (sL.On.OPD.CA)
- Anterior > Medial: lost by Morphine and Fentanyl

sL.On/RA (sL.On.OPD.RA)
- Anterior and Lateral > Medial: lost by Morphine and Fentanyl

<u>Regional Differences Gained Compared to Controls</u>

F.On Count
- Medial > Lateral: gained by Fentanyl

sL.On Count

- Medial > Lateral: gained by Morphine and Fentanyl
- Posterior > Lateral: Morphine and Fentanyl

Unlabeled Resorption Space Count (Rs.N)
- Anterior and Posterior > Lateral: gained by Fentanyl
- Posterior > Medial: gained by Fentanyl

C.On Mean Wall Thickness
- Medial > Lateral, Anterior: gained by Morphine

sL.On Mean Wall Thickness
- Posterior, Anterior > Lateral: gained by Fentanyl
- Posterior, Medial > Anterior: gained by Morphine

tL.On Mean Wall Thickness
- Medial > Anterior: gained by Morphine

C.On Mean Aspect Ratio
- Medial > Lateral and Posterior: gained by Fentanyl

F.On Mean Aspect Ratio
- Posterior > Anterior, Lateral: gained by Fentanyl

sL.On Mean Area
- Anterior > Lateral: gained by Fentanyl
- Posterior > Medial and Lateral: gained by Fentanyl
- Medial > Anterior: gained by Morphine

sL.On Mean Solidity
- Anterior > Lateral: gained by Fentanyl

dL.On Mean Aspect Ratio
- Medial > Posterior: gained by Fentanyl

dL.On Mean Roundness
- Posterior > Medial: gained by Fentanyl

dL.On Mean Solidity
- Lateral > Medial and Posterior: gained by Fentanyl

sL.On/CA (sL.On.OPD.CA)
- Anterior and Medial > Lateral: gained by Fentanyl

Rs.N/CA (Rs.N.OPD.CA)
- Posterior > Lateral: gained by Fentanyl

a.Rm.Cr/RA (a.Rm.Cr.OPD.RA)
- Anterior, Medial, Posterior > Lateral: gained by Fentanyl

T.On/RA (T.On.OPD.RA)
- Anterior and Medial > Lateral: gained by Fentanyl

sL.On/RA (sL.On.OPD.RA)
- Anterior and Medial > Lateral: gained by Fentanyl

Rs.N/RA (Rs.N.OPD.RA)
- Anterior and Posterior > Lateral: gained by Fentanyl

Osteon Formation Time (W.Th.C / On.MAR.I.dL)
- Lateral > Medial: gained by Fentanyl
- Medial > Anterior: gained by Morphine

Osteon Formation Time (W.Th.C / On.MAR.I)
- Medial > Anterior: gained by Morphine

Osteon Formation Time (W.Th.C / On.MAR.C)
- Lateral > Anterior and Medial: gained by Fentanyl

Regional Femur Analysis: Drug Group Effects Within Regions

Our post-hoc analyses assessed differences between drug treatment groups within each individual region. Differences between drug treatment groups in osteon type morphometry were concentrated in Medial and Lateral regions. For aspects of osteon morphometry associated with resorption spaces (F.On Count, Rs.N), the Posterior region additionally showed drug treatment group differences. This is consistent with its elevated percent porosity and pore size seen in both micro-CT and histological results.

Regional Drug Treatment Group Patterns

Morphine > Fentanyl

- F.On Count: Lateral and Posterior
- sL.On Mean Wall Thickness: Lateral and Medial
- tL.On Mean Wall Thickness: Medial
- sL.On Mean Solidity: Lateral
- a.Rm.Cr/RA (a.Rm.Cr.OPD.RA): Lateral

Morphine > Control

- F.On Count: Medial and Posterior
- tL.On Mean Wall Thickness: Medial

Fentanyl > Control

- sL.On Count: Medial
- dL.On Mean Wall Thickness: Lateral
- Unlabeled Resorption Space Count (Rs.N): Posterior
- sL.On/CA (sL.On.OPD.CA): Medial
- sL.On/RA (sL.On.OPD.RA): Medial
- Osteon Formation Time (W.Th.C / On.MAR.I): Lateral
- Osteon Formation Time (W.Th.C / On.MAR.C): Lateral

Control > Fentanyl

- sL.On Mean Wall Thickness: Lateral
- C.On Mean Area: Medial
- sL.On Mean Area: Medial
- sL.On Mean Solidity: Lateral
- dL.On Mean Solidity: Anterior and Lateral
- a.Rm.Cr/RA (a.Rm.Cr.OPD.RA): Lateral
- T.On/RA (T.On.OPD.RA): Lateral

Control > Morphine

- C.On Mean Area: Anterior

Activity 5: Manuscript preparation and knowledge dissemination
Projected Completion Dates: 07/20 – 12/20
Revised Completion Dates: 06/21 – 09/21
Progress: 100% as per proposed revised timeline

Planned Scholarly Product

Planned scholarly products include manuscript submissions to competitive peer-reviewed scientific journals. The results of the proposed application and limitations of this research will be published in journals targeting forensic specialists such as forensic anthropologists and archaeologists, missing persons detectives, and crime scene personnel. To reach forensic practitioners and the general forensic science community, scientific journals will include *Journal of Forensic Sciences*, *Forensic Anthropology*, and *Forensic Science International.*

The proposed work is also of interest to the bone biology and biomedical imaging communities. As such, Dr. Andronowski intends to target these groups through discipline specific journals such as the *Journal of Anatomy, Bone, Journal of Bone and Mineral Research, Micron,* and the *Anatomical Record*.

Dissemination Strategy

The ultimate goal of the Andronowski Group is to further understandings of how bone remodeling is related to age-related change using high-resolution imaging modalities (e.g., micro-CT and SR micro-CT), while simultaneously generating and disseminating new scientific knowledge. To broadly disseminate the findings and reach a wide variety of specialists within forensic science and the criminal justice system, law enforcement and legal personnel, medical examiners, forensic anthropologists and forensic archaeologists, initial results will be presented at the annual American Academy of Forensic Sciences Annual Scientific Meeting. For a more targeted audience, results will be presented at the annual Canadian Bone and Joint Conference hosted by the University of Western Ontario's Bone and Joint Institute or the American Society for Bone and Mineral Research Annual Meeting. These conferences bring together experts from across disciplines committed to interdisciplinary and high-impact research related to bone-affecting conditions and their treatment.

In addition, Dr. Andronowski routinely travels to the Canadian Light Source (CLS) synchrotron facility located on The University of Saskatchewan campus to run synchrotron imaging experiments and collaborate on other research projects. She will further disseminate the information learned through this venue and reach a number of bone imaging specialists and musculoskeletal researchers in the Department of Anatomy, Physiology, and Pharmacology. Lab members Dr. Andronowski, Dr. Cole, Reed Davis, and Gina Tubo traveled to the CLS in August 2019 and December 2019 for imaging experiments related to a concurrent project. Due to the COVID-19 pandemic and associated restrictions to international travel, further planned synchrotron experiments were on hold from April 2020 – June 2021.The Andronowski Lab was able to travel to CLS once again in September 2021 for imaging time related to various other research projects.

Additional manuscript preparations and data dissemination continue to be underway.

A manuscript documenting the findings from *Activities 1 and 2* titled **'**Rabbits (*Oryctolagus cuniculus*) as a Model System for Longitudinal Experimental Opioid Treatments: Implications for Orthopedic and Biomedical Research' was recently published in a special issue in the journal *Osteology* (**Appendix X**). All members of the research team contributed. Additional manuscripts describing micro-CT and histology results, and the associated software packages developed for this software, are currently in preparation.

Activity 6: Project management (Andronowski)
Projected Completion Dates: 01/01/19 – Present
Progress: 100% complete as per proposed revised timeline

The study was managed through the Department of Biology at The University of Akron. The Principal Investigator (Dr. Andronowski) provided overall project direction and coordination, contributed to methods and data review. Andronowski prepared quarterly and semi-annual reports to the Office of Justice Programs (OJP), and continues to prepare conference abstracts, and data for journal submissions and other forms of dissemination. She was further responsible for overall project management and coordination. Dr. Andronowski trained and supervised a Post-doctoral Fellow (Dr. Mary Cole), Graduate Research Assistant (Reed Davis), Undergraduate Research Assistant (Adam Schuller), and Tiered Mentoring Undergraduate Students (Gina Tubo, Abigail LaMarca, and Josh Taylor) at The University of Akron, and certified that milestones were met, and ensured the timely submission of quarterly and semi-annual reports to OJP.

Project management proceeded as per the revised schedule with 100% completion as of the end of the no-cost extension, 08/31/21.

2.2.2. Specific Objectives

Nothing to report.

2.2.3. Significant Results, including Major Findings, Developments, or Conclusions

During the experimental dosing period (05/19 – 06/19), our research team developed more specific standard operating procedures for patch application to rabbits. Initially, the fentanyl group and the Tegaderm adhesive patch control rabbits were administered the patch with no additional covering. Following the first few days of treatment, the rabbits were able to remove their experimental manipulations, either by scratching with the hind limb or removal via the teeth. This complication was unanticipated given the prior literature. We procured mesh rabbit jackets from the attending veterinarian to cover the patch application site. In addition, one rabbit was fitted with a cloth-covered foam collar to counter excessive chewing of the jacket. Certain other rabbits further chewed the mesh surrounding the forelimb openings of the jackets, and in these cases, the research team applied veterinary bandage wrap beneath the jacket to reduce friction against the fur/skin of the animals. The adhesive from the fentanyl patches further left a residue on the rabbits' skin. After a thorough literature review and discussions with the attending veterinarian, our team initiated the use of petroleum jelly and/or triple antibiotic ointment to soften the remaining adhesive and

treat any abrasions that resulted from an animal's attempt to remove the restraint jacket, veterinary bandage wrap, or patch with hind limb scratching. This tailored operating protocol will benefit future rabbit research by the Andronowski Lab and other research groups.

During reporting period ending 12/31/19, our research team developed specific standard operating procedures and image processing workflows for the 3D imaging of cortical bone porosity and trabecular architecture (**Appendices I-III**). Drs. Cole and Andronowski further developed custom macros for the image analysis programs ImageJ and CTAnalyser **(Appendices XIV - XV)**. These scripts automatically extract low-contrast cortical pore spaces from micro-CT images, create masks of cortical area and total area to serve as regions of interest, and define morphometric measurements for pore morphometry and cross-sectional geometry. Dr. Cole also developed a macro for ImageJ that divides cortical area masks into Anterior, Posterior, Medial, and Lateral regional quadrants using the section centroid. These data will facilitate analysis of regional variation in pore distribution and geometry, which may be associated with drug treatment group. Drs. Cole and Andronowski further developed statistical workflows for R that fully automate linear mixed model analyses, post-hoc testing, and graphic display.

These tailored operating protocols will benefit future desktop bone imaging research by the Andronowski Lab and other research groups.

For the reporting period (01/01/20 – 06/30/20), micro-CT image processing and statistical analyses related to *Activity 4* were completed according to the revised project timeline. A concise summary of the micro-CT findings from whole-bone and regional analysis of the femur and tibia are outlined below.

For the reporting period (07/01/20 – 12/31/20), tasks related to the histomorphometry experiments of *Activity 3* were completed including methyl methacrylate (MMA) embedding, microscopic slide preparation, digital annotation of thin-sections, and histological analysis protocol development.

During reporting period (01/01/21 – 06/30/21), tasks concerning the analysis of the histomorphometric experiments of *Activity 3* were completed including photomerging, overlaying, and anatomical orientation of the histological images. Protocols were developed and implemented to automate extraction and analysis of cortical pores from histological images and reduce inter-observer error.

During the no-cost extension (06/30/21 – 08/31/21), all histological images were analyzed and statistical results were computed. An initial manuscript was prepared, submitted, and subsequently published (**Appendix X**). Preparation of other manuscripts are underway and will be submitted to peer-reviewed journal articles upon completion.

Major Findings from Micro-CT Analysis:

An 8-week course of Morphine and Fentanyl drug treatment is sufficient to remove the significant intraskeletal variability (Femur > Tibia) in porosity, pore size, and pore system branching complexity seen in Control rabbits. This occurs through both femoral **decreases** in percent porosity (Anterior and Medial) and pore size (all regions), and through tibial **increases** in percent porosity (Anterior) and pore density (Lateral) in response to drug treatment.

Key Findings from Whole-Bone Analysis

Bone Effects: The rabbit femur preferentially develops pore systems that are significantly thicker, larger in diameter and volume, and more highly branched. The rabbit tibia preferentially develops pore systems that are smaller, more densely populated, and more longitudinally oriented.

Drug Treatment Group Effects: Morphine and Fentanyl treatment produces significantly thinner and smaller volume pore systems compared to Controls in both the femur and tibia. There is an elevation of individual pore system mean radius and volume in the Morphine-treated tibia.

Bone * Group Interaction Effects: In Control rabbits, the femur develops significantly higher total percent porosity and pore volume, while retaining the significant elevation of pore size and branching complexity seen in the Bone effect. This significant intraskeletal variation, however, disappears with Morphine and Fentanyl drug treatment. This is due both to decreased femoral mean values and increased tibial mean values of these morphometric measurements. Drug treatment physiologically dysregulates the normal mechanical control that determines the divergent pore patterning of the healthy femur and tibia.

Key Findings from Femoral Regional Analysis

Region Effects: Across all groups, Medial and Posterior quadrants have significantly higher percent porosity and larger pore size. Medial and Lateral quadrants have significantly higher pore density, but Lateral quadrant pores are too small to accrue high percent porosity.

Drug Treatment Group Effects: As seen in the whole bone analysis, Control group rabbits exceed Morphine and Fentanyl rabbits overall in pore size and pore convergence with cortical borders. Reduced femoral porosity observed in the whole-bone analysis is concentrated in anterior and medial regions.

Region * Group Interaction Effects: As seen in the whole bone analysis, drug treatment silences regional variability in aggregate porosity measurements.

Key Findings from Tibial Regional Analysis

Region Effects: The regional patterning in the femur is reflected in the tibia. Across all groups, Medial and Posterior quadrants have significantly higher percent porosity. Posterior quadrants maximize pore size. Medial and Lateral quadrants have significantly higher pore density, but Lateral quadrant pores are too small to accrue high percent porosity. While pore systems are most numerous and highly connected in the Anterior quadrant, the higher pore separation in a larger cortical volume prevents the Anterior quadrant from maximizing porosity or pore density.

Drug Treatment Group Effects: Unlike the femur, the tibia has no overall drug treatment group effects on pore morphometry.

Region * Group Interaction Effects: As seen in the whole bone analysis, drug treatment silences regional variability in metrics of pore size, percent porosity, pore density, and pore system complexity. Additionally, drug treatment significantly increases percent porosity in anterior regions (Fentanyl) and pore density in lateral regions (Fentanyl and Morphine).

Major Findings from Histological Analysis:

Drug treatment significantly dysregulates the density of microstructural products of remodeling and their regional distribution. Osteon population density can be both elevated (Morphine) and reduced (Fentanyl) by drug treatment, compared to controls. Regional dysregulation by drug treatment typically affects distribution of morphometric variables along the Anterior-Posterior axis compared to the Medial-Lateral axis.

Key Findings from Whole-Section Analysis

Osteon type counts and population densities were significantly elevated for Morphine group rabbits and significantly reduced for Fentanyl group rabbits, compared to controls. No significant differences between drug treatment groups were observed for cross-sectional geometry, periosteal or endosteal labeling, or any aspect of vascular porosity.

Key Findings from Regional Analysis

Region Effects: The Lateral quadrant showed a significant reduction in many microstructural products of remodeling, including remodeling area fraction, vascular percent porosity and pore density, osteon type counts and population densities, pore and osteon type mean areas, and all calculations of activation frequency. This may be due to the high loading of the Lateral quadrant by the ground reaction force during hopping.

Drug Treatment Group Effects: Significant elevation of Morphine group rabbits over Fentanyl group rabbits was observed for a subset of the osteon count and population density variables observed in the whole-section analysis.

Region*Group Interaction Effects: Morphine and Fentanyl group rabbits displayed significant dysregulation of regional patterning, with numerous losses and gains of regional patterns compared to Controls. Gain or loss of regional patterning almost always affected Anterior-Posterior axis patterning compared to Medial-Lateral axis patterning. Gain of significant regional patterning was more common in Fentanyl group rabbits.

2.2.4. Key Outcomes or Other Achievements

Nothing to report.

2.3. What opportunities for training and professional development has the project provided?

2.3.1. Professional Communities

Dr. Andronowski strives to provide both her advisees and colleagues with opportunities to share emergent methodological and technological innovations. In particular, starting in July 2018, she drew on her professional network of histologically-focused biological anthropologists to organize an invited poster

symposium at the 88th Annual Meeting of the American Association of Physical Anthropologists (AAPA). The resulting symposium, "Recent Advancements in the Analysis of Bone Microstructure", was accepted and held at the annual meeting on March 28th, 2019 in Cleveland, Ohio, and was chaired by Dr. Andronowski. This session brought together thirteen laboratories from five countries, all focusing on new technological approaches for the 3D visualization of bone tissue histomorphometry.

Dr. Andronowski, Reed Davis and Gina Tubo attended the Annual Meeting of the AAPA in Cleveland, OH, where Davis and Tubo gave a poster presentation along with Dr. Andronowski. This was an invaluable experience for Davis and Tubo as both will be entering career fields where presenting research and professional networking is a requirement. This was Tubo's first academic conference and professional poster presentation. Dr. Cole also gave a poster presentation in this invited symposium, presenting preliminary results from her dissertation research. As the AAPA annual meetings rarely include symposia specifically dedicated to histological techniques, this was a unique opportunity to discuss the future of the discipline as a unified group.

Davis further presented a poster at the Annual Scientific Meeting of the American Academy of Forensic Sciences (AAFS), and a 3-minute flash talk about his research at The University of Akron Integrated Bioscience Retreat, where he received the highest presentation award.

Drs. Andronowski and Cole presented a scientific poster in the Toxicology Section at the AAFS 2020 Annual Scientific Meeting titled 'Longitudinal Transdermal Fentanyl Compared with Morphine Sulfate Treatments in a Rabbit (*Oryctolagus cuniculus*) Model System: Impacts on Behavior and Health'. This work outlined drug administration complications and associated behavioral effects encountered during experimental animal dosing of *Activities 1 and 2*. Additional research team members including Davis, Tubo, LaMarca, and Schuller assisted in the preparation of the poster and associated graphics for presentation. This experience provided both graduate and undergraduate members of the Andronowski Lab with experience in disseminating scientific findings to a diverse group of forensic scientists.

Dr. Andronowski presented a virtual scientific poster and associated recorded talk in the Anthropology Section at the AAFS 2021 Annual Scientific Meeting titled 'Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part I – Intraskeletal Variability and Regional Differences Detected via micro-CT'. This presentation introduced a novel longitudinal model for studying the effects of prolonged opioid exposure on cortical bone remodeling in the rabbit, with a focus on the 3D micro-CT findings related to *Activity 3*. Additional research team members including Cole, Davis, Schuller, Tubo, LaMarca, and Taylor assisted in the preparation of the poster and associated graphics for presentation. This experience provided both graduate and undergraduate members of the Andronowski Lab with experience in disseminating scientific findings to a diverse group of forensic scientists.

Drs. Cole and Andronowski prepared a second virtual scientific poster presentation for dissemination in the Anthropology Section at the AAFS 2021 Annual Scientific Meeting titled 'Automated Techniques for Cortical Bone Histological Variable Segmentation and Image Enhancement'. The presentation described a collection of novel automated techniques utilized to enhance, segment, and analyze micro-computed tomography (μCT), synchrotron radiation-based micro-CT (SRμCT), and confocal microscopy datasets. Additional team members, Davis and Taylor assisted in poster preparation. This experience provided both

graduate and undergraduate members of the Andronowski Lab with experience in disseminating scientific findings to a diverse group of forensic scientists.

In August 2021, Dr. Andronowski submitted an abstract for oral presentation in the Anthropology Section at the AAFS 2022, entitled "Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part II – Intraskeletal Histological Variability and Regional Differences". Additional research team members included Cole, Davis, Schuller, Tubo, and Taylor. This presentation will describe results from the histological analysis portion of this project, including the effect of drug treatment on vascular pore and osteon morphometry, and remodeling activity.

Drs. Cole and Andronowski submitted a second abstract as a poster to the Anthropology Section at the AAFS 2022, entitled "OsteoFlo: A Fiji/ImageJ Toolkit for Semi-Automated Identification and Characterization of Fluorescently Labeled Secondary Osteons". Additional research team members included Taylor and Medhat Hassan. This presentation will describe the OsteoFlo software developed for histological analysis for this project.

Both presentations were accepted for presentation and will be delivered at the AAFS 2022 Annual Scientific Meeting at the Washington State Convention Center in Seattle, Washington from February 21 – 25, 2022.

2.3.2. Training

Through consistent involvement in this project, undergraduate and graduate student team members gained instructional and practical experience in animal experimental treatments, dissection, sample preparation, micro-CT and microscopic imaging, and project management.

Animal handling, husbandry, and experimental treatments

Research team members received technical training and practical experience with animal husbandry, handling, and experimental treatments during *Activities 1 and 2*. All individuals completed CITI training modules including: 1) 'Working with the IACUC', and 2) 'Working with Rabbits in Research Settings' (**Appendix XXVII**). The researchers further completed in-person animal handling training with Dr. Stanley Dannemiller, the attending veterinarian. Animal handling experiences included rabbit transportation, exercise, and provision of enrichment toys and food. Researchers also gained experience in performing animal health checks that included monitoring fecal and urine output, diet, appearance, and behavior. Animal care included the application of veterinary bandage wrapping and mesh rabbit jackets to encourage fentanyl patch adherence (**Appendix VII**), applying antibiotic ointment when prescribed by the veterinarian, and clipping fur to facilitate injection and patch application. Animal experimental treatments included the preparation of injectable reagents (e.g., calcein), transdermal patch administration, and subcutaneous injections. Lastly, members of the research team gained experience in the administration of euthanasia though intraperitoneal injection and bilateral pneumothorax following hands-on training with Dr. Dannemiller.

Rabbit Dissection

Animal dissection, following the experimental portion of the study, allowed researchers to refine their knowledge of vertebrate anatomy, hone their dissection techniques, and reinforce the proper protocol for

sterilizing dissection tools and surfaces. Researchers further gained experience in harvesting bone samples (specifically tibiae, fibulae, and femora) while preserving animal soft tissues. Preparation of samples for micro-CT scanning and traditional microscopy provided experience with these basic laboratory skills for the emerging researchers in the Andronowski Lab.

Bone sample procurement and preparation

As a part of the Andronowski Lab, future health professionals and biomedical researchers gain practical cadaveric dissection skills through bone tissue harvesting trips. Through Dr. Andronowski's professional network, several local medical schools allow sampling of long bones from anatomy lab cadavers for histological analyses. Participating in these experiences provides trainees with early exposure to cadaveric dissection, specifically the appearance of tissues in situ and the unique textures of fresh and embalmed tissues. This exposure has been particularly beneficial to the undergraduate students, many of whom will be attending professional school. Through Dr. Andronowski's connection to The University of Tennessee Knoxville's Forensic Anthropology Center, Dr. Andronowski and former Undergraduate Research Assistant Adam Schuller visited the Anthropology Research Facility (ARF) to collect soil samples for an ongoing research project. This was an invaluable opportunity for students to observe and network with forensic anthropology professionals outside of The University of Akron.

Micro-Computed Tomography Visualization

Three-dimensional visualization of bone microstructure is a significant component of the proposed analyses. Scanning was primarily performed by Dr. Cole, Reed Davis, and Dr. Andronowski. Dr. Andronowski has extensive experience with the SkyScan 1172 micro-CT instrument from prior post-doctoral and faculty research and was additionally trained on protocols specific to the Surface and Optical Analysis Facility within the National Polymer Innovation Center at The University of Akron. Dr. Cole has previous SkyScan 1172 experience from graduate research and was trained for independent operation of the scanner on 6/18/19 and 6/20/19 by the instrumentation scientist, Dr. Andrew Knoll. Reed Davis was trained for independent use on 6/14/19 and 6/19/19.

Three-dimensional visualization software transforms the angular X-ray projections acquired during micro-CT imaging into a stack of (2D) image slices and a 3D reconstruction of the scanned object. Image processing software further extracts features of interest, such as cortical pore networks, and reports their structural geometric measurements. Dr. Andronowski, Dr. Cole, and Reed Davis have significant prior research experience with micro-CT and image processing software such as NRecon, CTAnalyser, ImageJ and AMIRA, including developing automated image processing workflows. They have gained further experience in the development of new workflows through this project, especially through dissemination to other lab members and collaborators. Reed Davis and Gina Tubo were trained in implementation of the image processing protocols (**Appendices XII – XIII**) following development by Drs. Andronowski and Cole. An undergraduate student researcher, Kassidy Wilson, was trained in implementation of the Skeletonization image processing protocol (**Appendix XVI)** for completion of an undergraduate honors thesis.

Davis implemented the devised image processing protocols and expanded his 3D image processing experience by beginning work on a related project examining the effects of morphine and fentanyl on the trabeculae of the proximal tibia. Davis imaged all rabbit proximal tibiae using the SkyScan 1172 housed in

the National Polymer Innovation Center. Davis worked closely with Drs. Andronowski and Cole to determine best practices for 3D imaging, image processing, and data analyses. Davis' previous bone-related research has focused predominately on cortical bone. This project allowed him to expand his knowledge of trabecular bone for a more complete understanding of bone microarchitecture. Davis further adapted workflows for use with trabecular bone in image processing software (e.g., NRecon, CTAnalyser) and is working to train undergraduate members of the Andronowski Lab in the use of these protocols.

Reagent preparation (e.g., Calcein)

Undergraduate and graduate student researchers gained additional practical experience in fluorochrome reagent (calcein) preparation for visualizing longitudinal bone remodeling. Researchers ensured that the calcein was prepared according to protocol (**Appendix XXVIII**) in a dark room and that it exhibited the proper pH level. Preparing the calcein allowed researchers to work with laboratory equipment such as a balance, pH probe, and inverter.

Histological Sample Processing, Imaging, and Analysis

Undergraduate and graduate student researchers were trained on the preparation of bone tissue samples for histological analysis, including Isomet sectioning, dehydration, MMA embedding, diamond wire saw sectioning, and thin-section mounting. The trainees also received instruction on microscopic imaging with brightfield (DIC), circular polarization, and fluorescence using cellSens image capture software. Students further gained experience in photomerge reconstruction of multiple adjacent microscopic images into cross-sectional images using Microsoft Image Composite Editor and Adobe Photoshop, sample orientation in Adobe Photoshop, and image pre-processing for automated image analysis in ImageJ. Dr. Cole further expanded her software development skills in Javascript-based ImageJ macro language through development of the OsteoFlo software package, and in the R language for statistical computing in developing fully automated statistical analysis.

Consumables/equipment ordering/data management

Researchers maintained detailed logs throughout the experimental portion of the project that included information regarding animal care schedules, animal health, enrichment foods, exercise, and experimental treatments (**Appendix XXIX**). The research team refined critical data management skills and were able to communicate information among group members and UARV staff while creating thorough documents to monitor how the project was progressing. The logs were important for project collaboration, resource tracking, and keeping all project members informed on day-to-day tasks.

Dr. Andronowski, Reed Davis, and Dr. Cole maintained the project budget and ordering of consumables. Davis created and maintained an up-to-date interactive spreadsheet to keep track of expenditures and remaining available budget.

2.3.3. Mentoring

Post-Doctoral Mentoring and Collaboration

Dr. Cole joined the Andronowski Lab as a Post-doctoral fellow in part to observe Dr. Andronowski's early career activities. Such enterprises include making professional connections for sample acquisition, start-up and management of a new laboratory, advising graduate and undergraduate educational trajectories, and

performing the unique service activities required of faculty both within and beyond the university. By observing and participating in these activities, Dr. Cole improved the soft skills and management techniques necessary to navigate her own potential career in academic research. Additionally, Dr. Cole had opportunities to pursue research and publish with Andronowski Lab members and collaborators, expanding her immediate professional network beyond her master's and doctoral institution (The Ohio State University). New technical skills acquired by Dr. Cole through this project included animal husbandry, animal experimental treatment and synchrotron radiation micro-computed tomography. These skills have allowed Dr. Cole to extend the methodological scope of her future research. She continues to collaborate with the Andronowski Lab on dissemination of the results of this project in presentations and publications.

Graduate Mentoring and Collaboration

Reed Davis joined the Andronowski Lab as a doctoral student in Fall 2018. In order to be better prepared for post-doctoral employment, Davis gained experience in publishing scientific literature, managing a laboratory, and advising of undergraduate students. Davis also gained experience presenting original research at scientific conferences. From this project, Davis has learned animal husbandry, animal experimental manipulation, and desktop micro-CT imaging and analysis. These skills have allowed Davis to be better equipped for a career in academic research.

Undergraduate Mentoring

Reed Davis acted as graduate student mentor to three undergraduate students involved in this project, specifically Gina Tubo, Abigail LaMarca, and Joshua Taylor as part of The University of Akron's Tiered Mentoring Program (discussed below). This opportunity allowed Davis to gain experience as a mentor and teacher which will be most valuable to him as he prepares for a career in higher education and/or research.

During reporting period 01/01/20 – 06/30/20, skeletonization data collection was performed by undergraduate student Kassidy Wilson for her undergraduate honors thesis. This opportunity allowed Wilson to gain experience with micro-CT image processing, data analysis, and statistical workflows. Her research experience in the Andronowski Lab will better prepare Wilson for a career in biomedical research.

During reporting period 06/30/2020 – 12/31/20, undergraduate student Joshua Taylor was trained in MMA embedding protocols (**Appendix IX**) and microscopic imaging using DIC, polarized light, and fluorescence settings (**Appendix XXX**). These training opportunities allowed Taylor to gain experience with a newly developed MMA embedding protocol developed by our group, microscopic image analysis, and photomerging protocols **(Appendix XXXI)** using software programs such as Microsoft Image Composite Editor, Photoshop 2020, and ImageJ.

During reporting period (01/01/21 – 06/30/21), Joshua Taylor trained two undergraduate students to assist in photomerging histological images. The process provided the undergraduate students with invaluable experience in image processing and employing critical problem-solving skills. With the assistance of Dr. Cole's SOP **(Appendix XXXI)**, Taylor overlayed, anatomically oriented, and subtracted the background of the histological images. This provided Taylor with experience using software programs and employing protocols related to histological analysis. Dr. Cole further trained an additional undergraduate student to isolate the remodeling area of polarized images. This student used multiple image analysis software packages and utilized an SOP (**Appendix XXXII)** created by Dr. Cole to create compatible file types with

each program. This experience provided the trainee with additional experience working with histological samples in various programs that will assist in their future endeavors. Additionally, a macro and SOP **(Appendix XXXIII)** were created by Dr. Cole and utilized by Taylor to extract the cortical area of the histological images.

During the no-cost extension period, Dr. Cole analyzed the histological images utilizing OsteoFlo, a macro toolkit developed for ImageJ. Dr. Cole's macros are detailed in **2.2.1. Major Activities**. Cole computed the statistics for the histological images using R (The R Foundation, v.4.1.1). All team members are currently contributing to manuscript preparation.

Tiered Mentoring

The Tiered Mentoring program at The University of Akron facilitates supervised undergraduate laboratory research. This program was re-implemented in the summer of 2018. The first student employed in this program was Gina Tubo. Tubo formerly worked under the direct supervision of doctoral student Reed Davis. Throughout the summer and fall of 2018, Tubo gained the experience required to handle tasks related to this project. These skills included bone sample processing (maceration, sectioning, grinding, and polishing), the use of fluorescent stains, preparation of slides for microscopy, and the use of computer software for 3D reconstruction of produced images (Image J, NRecon, CTAnalyser, and AMIRA). Further, as a part of this process, Tubo was trained in confocal microscopy and has become a user of a Leica TCS SPE Laser Scanning confocal microscope instrument with motorized galvo-z-stage, 488, 532, and 635nm solid state lasers and LASX software for image acquisition and 3D modeling.

The overall objective of the Tiered Mentoring program is to promote continued undergraduate research. Thus, a new Tiered Mentoring fellow, Abigail LaMarca, joined the Andronowski Lab in January 2019. Under the guidance of both Tubo and Davis, LaMarca learned the required skills to analyze the bone specimens procured from the study. The Tiered Mentoring program has further introduced Abigail to scientific journal writing, grant proposal preparation, and report writing. She has also become more competent in the critical evaluation of scientific articles as a result of the program.

2.4. How were the results disseminated to communities of interest?

The scientific rationale behind the project as described in the approved application was presented at the American Academy of Forensic Sciences 71st Annual Meeting, Baltimore, MD, February 20th, 2019.
Dr. Andronowski presented this invited talk for the Society of Forensic Anthropologists titled 'Investigating the Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling'.

A poster presentation relating to *Activities 1 and 2* titled 'Longitudinal Transdermal Fentanyl Compared with Morphine Sulfate Treatments in a Rabbit (*Oryctolagus cuniculus*) Model System: Impacts on Behavior and Health' was given for the Toxicology section at the American Academy of Forensic Sciences 72nd Annual Meeting on 19 February 2020. The goals of this presentation were to: (1) describe the longitudinal effects of systemic opioids, particularly fentanyl and morphine sulfate, on behavioral and physiologic parameters, and (2) demonstrate the long-term use of transdermal fentanyl patches in the management of analgesia in a rabbit model system.

Dr. Andronowski presented a virtual scientific poster and associated recorded talk in the Anthropology Section at the AAFS 2021 Annual Scientific Meeting titled 'Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part I – Intraskeletal Variability and Regional Differences Detected via micro-CT'. The goals of this presentation were to 1) characterize, for the first time, 3D cortical bone microstructural changes in a rabbit opioid model, and 2) demonstrate intraskeletal and regional effects in the rabbit femur and tibia due to opioid exposure.

Drs. Cole and Andronowski presented a second scientific poster for virtual dissemination in the Anthropology Section at the AAFS 2021 Annual Scientific Meeting titled 'Automated Techniques for Cortical Bone Histological Variable Segmentation and Image Enhancement'. The primary goal of the presentation was to introduce novel automated techniques that produce reliable and repeatable results for various high resolution imaging modalities. Auxiliary goals were to 1) reduce noise and artifacts, 2) standardizing variable analysis, 3) reduce inter-observer error, and 4) use open-source software for macro development and standard operating procedures.

In August 2021, Dr. Andronowski submitted an abstract for oral presentation in the Anthropology Section at the AAFS 2022 Annual Scientific Meeting, titled "Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part II – Intraskeletal Histological Variability and Regional Differences". This presentation will describe histological results from this project. Drs. Cole and Andronowski submitted an additional abstract for poster presentation in the Anthropology Section at the AAFS 2022 Annual Scientific Meeting, titled "OsteoFlo: A Fiji/ImageJ Toolkit for Semi-Automated Identification and Characterization of Fluorescently Labeled Secondary Osteons". This presentation will describe the utilities provided by the OsteoFlo software. Both presentations were accepted for dissemination at the AAFS 2022 Annual Scientific Meeting.

A manuscript documenting the findings from *Activities 1 and 2* titled **'**Rabbits (*Oryctolagus cuniculus*) as a Model System for Longitudinal Experimental Opioid Treatments: Implications for Orthopedic and Biomedical Research' was recently published in a special issue in the journal *Osteology*. All members of the research team contributed.

2.5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

Nothing to report.

2.6. Publications, conference papers, and presentations

Dr. Andronowski presented an invited talk for the Society of Forensic Anthropologists, titled 'Investigating the Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling' at the American Academy of Forensic Sciences Annual Meeting in Baltimore, MD, on 20 February 2019.

This seminar detailed the scientific rationale behind the project as described in the approved application. Dr. Andronowski explained the limitations of employing murine model systems to the study of cortical bone turnover and emphasized the use of rabbits as the smallest traditionally used laboratory animals with well-defined cortical remodeling comparable to humans. The implications of the current work for forensic

practitioners were highlighted, including an emphasis on how life history variables (e.g., drug/alcohol use) can affect bone remodeling and in turn have serious implications for histological age estimation methods in the field of forensic anthropology.

A presentation relating to *Activities 1 and 2* titled 'Longitudinal Transdermal Fentanyl Compared with Morphine Sulfate Treatments in a Rabbit (*Oryctolagus cuniculus*) Model System: Impacts on Behavior and Health' was accepted for the American Academy of Forensic Sciences 72nd Annual Meeting, Anaheim, CA in the Toxicology section. The presentation was given on 19 February 2020. The goals of this presentation were to: (1) describe the longitudinal effects of systemic opioids, particularly fentanyl and morphine sulfate, on behavioral and physiologic parameters, and (2) demonstrate the long-term use of transdermal fentanyl patches in the management of analgesia in a rabbit model system.

A virtual scientific poster and associated recorded talk related to *Activity 3* and *4* titled 'Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part I – Intraskeletal Variability and Regional Differences Detected via micro-CT' was presented in the Anthropology Section at the AAFS 2021 Annual Scientific Meeting. The goals of this presentation were to 1) characterize, for the first time, 3D cortical bone microstructural changes in a rabbit opioid model, and 2) demonstrate intraskeletal and regional effects in the rabbit femur and tibia due to opioid exposure.

A second scientific presentation related to *Activity 4* was accepted for virtual dissemination in the Anthropology Section at the AAFS 2021 Annual Scientific Meeting titled 'Automated Techniques for Cortical Bone Histological Variable Segmentation and Image Enhancement'. The primary goal of the presentation was to introduce novel automated techniques that produce reliable and repeatable results for various high resolution imaging modalities. Auxiliary goals were to 1) reduce noise and artifacts, 2) standardizing variable analysis, 3) reduce inter-observer error, and 4) use open-source software for macro development and standard operating procedures.

In August 2021, Dr. Andronowski submitted an abstract for oral presentation in the Anthropology Section at the AAFS 2022 Annual Scientific Meeting, titled "Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part II – Intraskeletal Histological Variability and Regional Differences". This presentation will describe histological results from this project. Drs. Cole and Andronowski submitted an additional abstract for poster presentation in the Anthropology Section at the AAFS 2022 Annual Scientific Meeting, titled "OsteoFlo: A Fiji/ImageJ Toolkit for Semi-Automated Identification and Characterization of Fluorescently Labeled Secondary Osteons". This presentation will describe the utilities provided by the OsteoFlo software. Both presentations were accepted for dissemination at the AAFS 2022 Annual Scientific Meeting.

A manuscript documenting the findings from *Activities 1 and 2* titled 'Rabbits (*Oryctolagus cuniculus*) as a Model System for Longitudinal Experimental Opioid Treatments: Implications for Orthopedic and Biomedical Research' was submitted and accepted to a special issue in the journal *Osteology*. All members of the research team contributed.

2.7. Website(s) or other Internet site(s)

The following media outlets and Internet sites highlighted the current study and announced the awarding of funding. Results of the research activities or a discussion of the live animal component of the study were not disseminated in any of the below articles.

Feb. 2019 — Press Release: 'New study funded by NIJ research grant to focus on opioid addiction's effects on bones'. Published on Digitaljournal.com. Released: 2/4/2019 URL.

Press Release: 'Skeleton keys: Scientist to study effects of opioid addiction on bone.' published on ForensicMag.com. Released: 2/4/2019.

Article: 'Forensic anthropologist says opioid use may make identifying skeletal remains more difficult' published on Ohio.com. Released: 2/3/2019.

Jan. 2019 — Article: 'Opioid addiction effects on bones is subject of NIJ research grant' published on EurekAlert.org. Released: 1/29/2019. URL.

Press Release: The University of Akron, Media Communications. 'Skeleton Keys: 'Scientist to study effects of opioid addiction on bones'. 1/29/2019. URL.

2.8. Other products

Canadian Light Source Synchrotron Proposal for Experimental Time

Dr. Andronowski submitted a research proposal for experimental time (e.g., 'beam time') for a related project at the Canadian Light Source (CLS) National Synchrotron Facility in February 2020. This follow-up study proposes examining opioid effects on bone at the powerful synchrotron imaging level, where sub-micron resolution can be achieved. Synchrotron imaging can visualize structures within cortical bone that are too small to be detected by desktop micro-CT imaging. For example, the 3D trajectory of osteon cement lines used in histological age-at-death estimation, and the 3D morphometry of cortical pore networks and osteocyte lacunae used as markers of bone fragility can be evaluated using this technology.

Our objectives were to: 1) further identify pathological changes to cortical bone microstructure with prolonged opioid use in a rabbit model, and 2) quantify differences in standard cortical bone microstructural parameters (e.g., osteocyte lacunar volume and density) among treatment and control groups. We hypothesized that: 1) cortical porosity in the experimental groups will exceed that of controls, and 2) a decrease in osteocyte lacunar density will be observed in the experimental opioid groups.

The proposed study supplements our ongoing work and sets the stage for the next phase of the research program, evaluating osteopenia/osteoporosis associated with opioid use in human ribs and femora from a larger sample of modern autopsy cases.

Beam time proposals were reviewed in May 2020 and our submission was scored favorably for the awarding of experimental beam time. Due to the COVID-19 pandemic, however, all synchrotron experiments were on hold from April 2020 to July 2021. The Andronowski Lab was able to travel to CLS once again in September 2021 for imaging time related to various other research projects. During this experimental run, the team imaged 202 bone samples for various ongoing research projects.

Software or NetWare

The following image processing macros were developed by Drs. Cole and Andronowski for extraction and morphometric analysis of cortical pore networks from micro-CT images. The detailed code for these macros is available in **Appendices XIV and XV**.

CTAnalyser Macros

- "TA Extractor" – Extracts a mask of total area from the grayscale micro-CT image for centerline skeletonization and subsequent longitudinal orientation
- "ROI and TA Extractor" – After longitudinal orientation, extracts a mask of total area and a mask of cortical area to serve as regions of interest (ROI) for pore extraction and morphometric analysis
- "Pore Morphometry" – Acquires morphometric measurements from cortical pore networks on whole cross-sections; also generates binary image stacks of despeckled pore networks and grayscale image stacks of pore thickness and pore separation
- "Regional Pore Morphometry" – Same functions as "Pore Morphometry", but limited to a given anatomical region of interest (Anterior, Posterior, Medial, Lateral)
- "Cross-Sectional Geometry" – Extracts cross-sectional geometric measurements from the cortical area image stack, using the total area image stack as a mask

ImageJ Macros

- "Adaptive Thresholding" – Applies a low-contrast, local thresholding Phansalkar algorithm to the grayscale micro-CT image, extracting the cortical pore network as a binary image stack, and excluding external noise by using the cortical area image stack as a mask
- "Skeleton Save" – Automatically runs and saves the Skeletonization 3D plugin on the total area mask, converting it to a centerline skeleton image stack
- "Femur Quadrants / Tibia Quadrants" – Using the cortical area image stack as an input, finds the centroid with the Slice Geometry plugin, and then rotates a line through this centroid to generate image stacks of masks representing the Anterior, Posterior, Medial, and Lateral anatomical regions of each cross-section. A given regional mask can be used as the region of interest (ROI) in the Regional Pore Morphometry macro to restrict morphometric analysis of the pore network to that anatomical region.
- "DIC Preprocessing" – The DIC image is evenly illuminated through application of a high pass filter, contrast enhancement, and background subtraction. Cross-sectional geometry (total, cortical, and marrow areas) are also extracted as binary images and as ROI files.

- "DIC Pore Extractor" – Probable pore ROIs are extracted from the DIC image within the remodeling area ROI using an Intermodes auto-threshold, particle size and circularity thresholding, and binary closing.
- "DIC Pore Modifier" – Pore ROIs are manually edited using custom keyboard shortcuts, including auto-saving.
- "DIC Pore Analyzer" – Pore ROIs are classified as cortical or trabecular based on proximity to the marrow cavity versus minimum diameter. Pores are also regionally subdivided between anatomical quadrants (Anterior, Medial, Lateral, Posterior) calculated using the total area centroid. Summary pore morphometry is calculated for each pore type within each region, including percent porosity, pore density, and mean pore size and shape descriptors.
- "Osteon Extraction" - Probable osteon border ROIs are extracted using local contrast enhancement, histogram equalization, an Intermodes auto-threshold, and binary opening. Fluorescent rings within osteons representing calcein labels are extracted as a binary image using background subtraction and an auto local Phansalkar threshold.
- "Osteon Border Correction" - Osteon border ROIs are manually edited using custom keyboard shortcuts, including osteon splitting and expansion.
- "Osteon Type" - Osteon border ROIs are regionally assigned as in Pore Analyzer. An ellipse is fitted over each ROI, and its major axis is superimposed on the binary image of its calcein labels. An intensity profile along this major axis displays peaks that correspond to the number and spacing of calcein label intersection. This intensity profile is used to guess osteon type (forming, single, double, or triple labeled), mineral apposition rate (On.Mar), which is the distance between two consecutive calcein labels, and osteon wall thickness (W.Th), which is the distance from the pore centroid to the osteon border.
- "Osteon Type Correction" - Osteon border ROIs are colored by guessed osteon type, and coordinates for guessed On.Mar spacing and W.Th pore centroids are superimposed as overlays. Custom keyboard shortcuts quickly change osteon type and re-locate coordinates for On.Mar and W.Th. Summary morphometry is automatically calculated for each region, including osteon type counts, corrected On.Mar and W.Th, and mean size and shape descriptors.
- "Mineralizing Surface" - The ratio of labeled to unlabeled bone on the periosteal (Ps.MS/BS) and endosteal (Es.MS/BS) surfaces is calculated as the number of white pixels intersected by the bone surface perimeter.
- "Resorption Cavities" - Probable unlabeled resorption cavities are identified as pore ROIs that do not intersect with osteon borders and fall above the lowest histogram bin value for pore size. Users can manually change this classification.

III. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS: WHO HAS BEEN INVOLVED?

3.1. What individuals have worked on this project?

Name: Janna M. Andronowski
Project Role: Principal Investigator
Nearest person month worked: 31 (01/01/2019 – 08/30/2021)
Contribution to Project: Conceptualization; Project design; Methodology; Project Administration/Management; Supply/Equipment ordering; Budgeting; Supervision of all trainees; Animal handling; Animal experimental treatments; OJP Reporting; micro-CT protocol development and imaging.

Funding Support: UA Faculty, 9-month salary; 1-month summer salary (2018-DU-BX-0188).
State, U.S. territory, and/or country of residence: Ohio, USA
Collaborated with individual in foreign country: No

Name: Mary E. Cole
Project Role: Post-Doctoral Fellow
Nearest person month worked: 26 (06/01/2019 – 08/30/2021)
Contribution to Project: Animal handling; Animal experimental treatments; Graduate and undergraduate supervision; micro-CT and histological protocol development and imaging; histological sample processing; scripting image analysis, statistical programming.
Funding Support: UA Post-doctoral Fellow, 100% time (2018-DU-BX-0188)
State, U.S. territory, and/or country of residence: Ohio, USA
Collaborated with individual in foreign country: No

Name: Reed A. Davis
Project Role: Graduate Student (Research Assistant)
Nearest person month worked: 31 (01/01/2019 - 08/30/2021)
Contribution to Project: Budgeting; Supervision of undergraduate trainees; Animal handling; Animal experimental treatments; micro-CT protocol development and imaging.
Funding Support: UA Graduate Student, 50% time (2018-DU-BX-0188)
State, U.S. territory, and/or country of residence: Ohio, USA
Collaborated with individual in foreign country: No

Name: Adam J. Schuller
Project Role: Undergraduate Research Assistant
Nearest person month worked: 7 (01/01/2019 - 8/01/2019)
Contribution to Project: Animal handling; Animal experimental treatments; Sample collection.
Funding Support: UA Undergraduate Research Assistant, 20% time (2018-DU-BX-0188)
State, U.S. territory, and/or country of residence: Ohio, USA
Collaborated with individual in foreign country: No

Name: Gina R. Tubo
Project Role: Undergraduate Student
Nearest person month worked: 18 (01/01/2019 - 6/30/2020)
Contribution to Project: Animal handling; Animal experimental treatments; Tiered mentoring supervision.
Funding Support: UA Undergraduate Research Assistant, 20% time (2018-DU-BX-0188)
State, U.S. territory, and/or country of residence: Ohio, USA
Collaborated with individual in foreign country: No

Name: Abigail R. LaMarca
Project Role: Undergraduate Student
Nearest person month worked: 31 (01/01/2019 – 08/30/2021)
Contribution to Project: Animal handling; Animal experimental treatments; Sample processing.

Funding Support: N/A
State, U.S. territory, and/or country of residence: Ohio, USA
Collaborated with individual in foreign country: No

Name: Joshua T. Taylor
Project Role: Undergraduate Student
Nearest person month worked: 15 (07/01/2020 - 08/30/2021)
Contribution to Project: MMA embedding; Microscopic imaging; Photomerging.
Funding Support: UA Undergraduate Research Assistant, 20% time (2018-DU-BX-0188)
State, U.S. territory, and/or country of residence: Ohio, USA
Collaborated with individual in foreign country: No

3.2. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

3.3. What other organizations have been involved as partners?

Organization Name: Surface and Optical Analysis Facility
Location of Organization: Polymer Innovation Center, The University of Akron, Room 212
Partner's contribution to project: Facilities: Access to SkyScan 1172 laboratory micro-CT instrument for bone imaging as described in section 2.2.1.
More detail on partner and contribution: Domestic

Organization Name: The University of Akron Research Vivarium
Location of Organization: Auburn Science and Engineering Center, The University of Akron, Room 212
Partner's contribution to project: Facilities: Animal facility where rabbits were housed for the duration of the study.
More detail on partner and contribution: Domestic

3.4. Have other collaborators or contacts been involved?

Nothing to report.

IV. IMPACT: What was the impact of the project? How has it contributed?

4.1. What was the impact on the development of the principal discipline(s) of the project?

During the reporting period (01/01/19 – 06/30/19), experimental activities were focused on animal habituation, husbandry, and experimental treatment. This is the first study to explore cortical histomorphometry in an opioid rabbit model. Rabbits are the smallest traditionally used laboratory animals with well-defined cortical remodeling comparable to humans[10]. As a result, rabbits have been used as a model system for studying central canal size and the vascular network of cortical bone[31–33]. We optimized

an experimental protocol for dosing rabbits both with injections and transdermal patch application, keeping detailed records of rabbit behavior in response to these treatments. Going forward, forensic anthropologists and skeletal biologists more generally may be encouraged to use rabbit models to study the isolated effects of physiological, mechanical, or pathological treatments on bone tissue histomorphometry. Biomedical researchers may also consider the suitability of rabbit research over the more traditional murine models, who exhibit little to no cortical remodeling over the lifespan[34–38]. The expertise developed by our lab members in these techniques will also facilitate future rabbit model research by the Andronowski Lab and its collaborators.

During the reporting period (06/30/19 – 12/31/19), activities were focused on the bone imaging phase of the project via micro-CT. Our research team developed more specific standard operating procedures and image processing workflows for the three-dimensional imaging of cortical bone porosity. We have developed and optimized protocols for characterizing the cortical pore network using the software packages ImageJ, CTAnalyser, and AMIRA, as described in the approved proposal. Lab members further gained experience with R, the statistical computing software proposed for the analysis, through preliminary projects. The expertise developed by our lab members in these techniques will facilitate future bone imaging research by the Andronowski Lab and its collaborators.

During reporting period (06/30/20 – 12/31/2020), activities were focused on the histomorphometry phase of *Activity 3*. Our research team developed more specific standard operating procedures and data analysis workflows for microscopic imaging and the assessment of opioid-induced changes. We are working to develop and optimize protocols for characterizing microscopic parameters using the software package ImageJ, as described in the approved proposal. Lab members further gained experience with the MMA embedding protocol developed by our group, microscopic imaging, and related photomerging tasks. The expertise developed by our lab members in these techniques will facilitate future bone imaging research by the Andronowski Lab and its collaborators.

During reporting period (01/01/2021 – 06/30/2021), activities focused on histomorphometry and data analysis of *Activity 3* and *4*. Histological image analysis included photomerging, overlaying, and orienting all histological images. The images were further processed by extracting total cortical area, remodeling area. Macros were developed to extract and analyze pores and osteons from the histological images. Lab members gained invaluable experience in image processing and macro development. The macros developed will advance future research in bone imaging by the Andronowski Lab and its collaborators.

During the no-cost extension, activities focused on data analysis of histomorphometric data and manuscript drafts. Our research team implemented the macros developed in the previous period to analyze the histological images. Lab members used R for statistical analysis of the histological data. The research team contributed to manuscript drafts and poster presentation abstracts/talks to disseminate the results. This provided members of the research team with experience in statistical software and knowledge dissemination through manuscripts and poster presentations.

4.2. What was the impact on other disciplines?

This project characterizes bone tissue microstructure by combining traditional 2D histology with 3D visualization through micro-computed tomography (micro-CT). Micro-CT data for this project was collected and Andronowski Lab members developed image processing workflows for refining and analyzing these data. Protocols have been developed for characterizing the cortical pore network using the software packages ImageJ, CTAnalyser, AMIRA, and Dragonfly, as described in the approved proposal. Lab members further gained experience with R, the statistical computing software proposed for the analysis, through preliminary projects.

The devised 3D imaging processing workflows have been applied to desktop micro-CT scans of bear third metacarpals, and synchrotron radiation micro-CT scans of human midshaft femoral cores. The bear metacarpal project was presented at the American Academy of Forensic Sciences 2019 Annual Scientific meeting by Reed Davis and Dr. Andronowski and the manuscript was published in September 2019 in the *Journal of Forensic Radiology and Imaging.*

A manuscript titled 'A Sectioning, Coring, and Image Processing Guide for High-Throughput Cortical Bone Sample Procurement and Analysis for Synchrotron micro-CT' was published in the *Journal of Visualized Experiments* for the femoral coring protocol in June 2020. Researchers applying micro-CT to bone tissue, and to organic or inorganic porous substances more generally, can replicate this image processing workflow in their own analyses.

Drs. Andronowski and Cole published a manuscript titled "Current and emerging histomorphometric and imaging techniques for assessing age-at-death and cortical bone quality" in *Wiley Interdisciplinary Reviews (WIREs) Forensic Science* in October 2020. This manuscript described traditional 2D histological approaches to age-at-death estimation and histomorphometric quantification, along with emerging 3D techniques, such as the micro-CT image analysis workflow developed for this project.

Drs. Andronowski and Cole, along with colleagues from Northeast Ohio Medical University (NEOMED), applied the 3D image processing workflows developed for this project to synchrotron radiation micro-CT scans of femora and humeri from various bat species, finding interspecies and intraskeletal consistency in 3D vascular pore morphometry. They disseminated this research in a manuscript titled "Intraskeletal consistency in patterns of vascularity within bat limb bones", published in *The Anatomical Record* in June 2021.

Drs. Andronowski and Cole and research team members presented conference presentations stemming from this research at the American Academy of Forensic Sciences (AAFS) Annual Scientific Meetings in 2020, 2021, and have abstracts accepted for 2022. These meetings reach a broad audience in the forensic sciences, including pathologists, medical examiners, and other forensic professionals.

A manuscript documenting the findings from *Activities 1 and 2* titled 'Rabbits (*Oryctolagus cuniculus*) as a Model System for Longitudinal Experimental Opioid Treatments: Implications for Orthopedic and Biomedical Research' was published in a special issue in the journal Osteology. All members of the

research team contributed. Additional manuscripts describing micro-CT and histology results, and the associated software packages developed for this software, are currently in preparation.

4.3. What was the impact on the development of human resources?

This project provided fellowship support for a Post-doctoral fellow, a Graduate Research Assistant, and an Undergraduate Research Assistant. Additionally, one undergraduate student participated in a volunteer capacity. Animal research experience increased the competitiveness of medical school applications for the undergraduate volunteers. LaMarca further received two credits related to the summer undergraduate research experience "3100: 497: Biological Problems", quantified as nine research hours per week, per credit.

All researchers involved in the study received formal training in animal research ethics (CITI Training), vivarium use (UARV Training) rabbit handling (NEOMED Training) and working with radiation-generating equipment (RGE Training and SkyScan 1172 micro-CT Training). Informal education in animal handling, experimental dosing, euthanasia, and dissection was afforded by routine involvement of all lab members with daily rabbit care and treatment. Additionally, undergraduate and graduate researchers in the Andronowski Lab routinely engaged in typical histology laboratory tasks, such as handling biohazardous materials, sample cleaning, histological slide preparation, light transmission and confocal microscopy, and image analysis. Familiarity with these fundamental laboratory techniques will prepare junior lab members for future research or medical careers. Lab members Reed Davis and Gina Tubo additionally gained experience with preparing and presenting research results at national conferences. The work done by graduate student Reed Davis on this project will comprise a substantial portion of his dissertation.

For the first reporting period (01/01/2019 – 06/30/2019), research activities were focused on animal husbandry and experimental treatments. The hands-on design of this study required these researchers to perform daily activities commonly relegated to UARV staff, including animal enrichment (daily feeding and thrice-weekly exercise), health checks, experimental dosing through injection and patch placement, and completion of associated documentation of these activities. By engaging with the animals every day, researchers were able to quickly notice and resolve experimental difficulties, such as specific animals removing patches, or exhibiting the early stages of health issues. Additionally, researchers became familiar with the behavioral habits of each animal, providing environmental insight that may help explain individual differences in bone microstructure during the data analysis phase. This experimental familiarity will also be essential in accurate communication of methodology during formal and informal data dissemination, facilitating replication of future research.

Near the end of the first reporting period, participants gained technical experience in the euthanasia of research animals under the direction of Dr. Stanley Dannemiller, followed by gross dissection for bone tissue procurement. Additionally, researchers employed by this project gained lab management and project management experience, such as ethics training for animal research, purchasing and budgeting supplies, experimental troubleshooting and modification, and experimental documentation. As all experimental treatments were performed in groups of two or more researchers, participants also gained team-building and communicative skills related to caring for and experimentally dosing the animals.

For reporting period (06/30/19 – 12/31/19), research activities were focused on the 3D bone imaging phase (*Activity 3*). Dr. Andronowski, Dr. Cole, and Reed Davis optimized micro-CT imaging settings for the midshaft femur and tibia and the proximal tibio-fibula by testing a range of imaging resolutions. Dr. Cole and Reed Davis gained substantial experience with sample mounting and micro-CT imaging of 63 discrete bone regions, comprised of the midshaft femora, tibiae, and proximal tibio-fibulae from 21 rabbits. Dr. Cole expanded her coding experience through the development of image processing workflows in ImageJ and CTAnalyser, and automated statistical workflows in R. Reed Davis and Gina Tubo were also trained in the implementation of these image processing workflows.

Dr. Andronowski, Dr. Cole, and Taylor optimized microscopic imaging settings for thin-sections of the mid-shaft femur by troubleshooting a range of settings. Dr. Cole expanded her coding experience through the development of image processing workflows in ImageJ, and automated statistical workflows in R. Reed Davis and Joshua Taylor were further trained in the implementation of these image processing workflows.

For the previous reporting period (01/01/21 – 06/30/21), Dr. Cole and Taylor applied histological analysis techniques to femoral thin-sections. Taylor learned different techniques and applications of various programs including Microsoft Image Composite Editor (ICE), ImageJ, and Photoshop for histological analysis. Dr. Cole further expanded her coding experience through numerous macro developments for image processing in ImageJ. Taylor was trained to apply these workflows to the histological images.

During the no-cost extension, activities focused on data analysis of histomorphometric data and manuscript drafts. Dr. Cole implemented the macros developed in the previous period to analyze the histological images. Dr. Cole further used R for analysis of the histological data. The research team contributed to manuscript drafts and poster presentations to disseminate the results. This provided Dr. Cole with further experience in macro development and statistical computing. All research members gained increased experience in knowledge dissemination through manuscript preparation and poster presentations.

All research participants are at varying stages of an educational trajectory leading either to advanced health professions or academic/industrial biological research. By gaining experience with all levels of experimental design, from planning through implementation, this project improved the performance and retention of these researchers in their future health or research careers. Additionally, Dr. Andronowski was well suited to advise the three female members of her research team on unique challenges and opportunities they may face as women in early career STEM fields.

4.4. What was the impact on teaching and educational experiences?

Dr. Andronowski is the founder and campus advisor for The University of Akron's Biological Anthropology and Human Anatomy Student Organization (BAHA). BAHA membership is open to both undergraduate and graduate students in any discipline, and provides members with anatomical and anthropological educational experiences, outreach opportunities, and career guidance. Previous educational experiences include participating in human cadaveric dissections in Dr. Andronowski's Human Anatomy laboratory and visiting the Cuyahoga County Medical Examiner's Office for an autopsy demonstration. Outreach events hosted by BAHA have included cadaver experiences for Barberton High School Advancement to Nursing program students and the UA Neuroscience Club. Additionally, BAHA members

have provided hands-on forensic anthropology demonstrations during three Cleveland Museum of Natural History's "Think and Drink with the Extinct" events. The Andronowski Lab is committed to continuing public outreach both within and beyond the university.

4.5. What was the impact on physical, institutional, and information resources that form infrastructure?

4.5.1. Improvements to Physical Facilities in the UARV

In preparation for the live animal portion of the project (*Activities 1 and 2*), improvements to the physical facilities in the UARV were needed. The following jobs were completed in Room 212 (or affected Room 212) where the rabbits were housed:

1. Installed temporary air conditioner in Room 212.
2. Calibrated all of the Metastats (Metasys thermostats) in the entire UARV.
3. Repaired the chiller located on the roof servicing the UARV air handler.
4. Verified air flow and adjusted dampers servicing Room 212.
5. Verified operation of the humidifier control valve servicing Room 212.
6. Replaced the sink fixture in Room 212 (former sink was rusty).
7. Sealed around the edges of all of electrical outlets as these were leaking air.
8. Installed door sweeps in Room 212 to minimize air infiltration.
9. Installed a temporary humidity, temperature, and flood monitoring system (AVTECH) in the room to alert researchers of potential fluctuations in housing conditions.

There were additional facilities issues in the UARV facility that required attention prior to the arrival of the rabbits. These included:

1. Ceiling repairs were completed in the cage washer room (Room 112).
2. Replaced the door seals on the cage washer.
3. Installed a cover on a communication/electrical box in the cage washer storage room (Room 114).
4. Sealed around all of the floor level return grills on the second floor.
5. Installed an exhaust diffuser in the potable water storage room (Room 210).
6. Upgraded the electrical outlets to eliminate extension cords in two other rooms in the UARV
7. Improved door seals around the elevator to reduce air infiltration in the UARV.

By spearheading the above maintenance to the UARV, this project improved experimental conditions both for its own animals and for concurrent and future UARV users.

4.6. What was the impact on technology transfer?

Nothing significant occurred during this reporting period. Study results and methodological innovations will be transferred through peer-reviewed journal publications, and through presentations at conferences

such as the annual meetings of the American Academy of Forensic Sciences, the Canadian Bone and Joint Conference, and/or the American Society for Bone and Mineral Research.

4.7. What was the impact on society beyond science and technology?

Nothing significant occurred during this reporting period. Given the widespread and accelerating rate of opioid overdoses in the United States, it is critical to tailor forensic techniques to accurately build a biological profile. This project is working to adjust traditional histological aging methods to accommodate chronic opioid users and will seek to identify unique bone microstructural presentations that may signify opioid use. By refining the image processing and analysis methods required for this analysis, this project will also increase accessibility of these methods to forensic and academic institutions.

4.8. What was the impact on society beyond science and technology?

Nothing significant occurred during this reporting period. Given the widespread and accelerating rate of opioid overdoses in the United States, it is critical to tailor forensic techniques to accurately build a biological profile. This project is working to adjust traditional histological aging methods to accommodate chronic opioid users and will seek to identify unique bone microstructural presentations that may signify opioid use. By refining the image processing and analysis methods required for this analysis, this project will also increase accessibility of these methods to forensic and academic institutions.

4.9. What percentage of the award's budget was spent in foreign country(ies)?

Nothing to report.

V. CHANGES/PROBLEMS

5.1. Changes in approach and reasons for change

A proposed change affecting the delivery of fentanyl from subcutaneous injection to transdermal patch administration was proposed by Dr. Andronowski and a VVC Modification was approved by the IACUC on 04/07/2019 (**Appendix VI**). The details of this change in experimental vehicle administration are outlined in section 2.1.

A second proposed change affecting the fentanyl experimental group was initiated that differed from the agency approved plan. A VVC Modification (**Appendix VII**) was put forward to cover the drug eluting transdermal patches (fentanyl) and control patches (Tegaderm) in order to prevent the animals from chewing, removing, and/or ingesting these from the subscapular region. The details of this change related to jackets and other patch coverings is outlined in section 2.1.

5.2. Actual or anticipated problems or delays and actions or plans to resolve them

5.2.1. Actual Delays

Due to a delay in the creation of the Post-doctoral Fellow position by The University of Akron's Human Resources department, the prospective candidates were unable to interview until late January/February 2019. Dr. Andronowski's NIJ-appointed grant manager, Mr. Theodore Robinson, was notified of the hiring delay on 02/08/2019.

Dr. Andronowski recruited a most suitable Post-doctoral Fellow candidate, Dr. Mary Cole. Dr. Cole has graduate experience in dissection and preparation of human and animal bone tissue samples for traditional brightfield microscopy, confocal microscopy, and micro-CT. She is also proficient with the analytical software proposed for the data generated by this project, including CTAnalyser, ImageJ, R, SPSS, and SAS. The recruitment process involved an informal interview at The Ohio State University (1/4/19) and a formal interview with a research presentation by Dr. Cole at the University of Akron (1/25/19). Dr. Cole accepted the position on 2/14/19. Dr. Cole was working towards completion of her doctoral degree in Anthropology (Biological) at The Ohio State University, with a dissertation defense date of 05/15/2019. Her dissertation was funded by the National Institute of Justice Graduate Research Fellowship in Science, Technology, Engineering, and Math (Award #2017-MU-CX-0009). In order to complete the data collection and analysis proposed in this grant, as well as the dissertation defense and revisions, Dr. Cole requested that her official start-date be adjusted to 06/03/2019 as per her contract.

To expedite Dr. Cole's capacity to fully participate in the project, Dr. Andronowski arranged for several training sessions ahead of the start date. This included completion of a UARV Packet and Health Assessment to acquire UARV access (3/4/19), CITI Lab Safety Training (3/6/19), NEOMED rabbit handling training (3/11/19), UARV training (3/11/19), diamond wire saw installation and training (4/3/19), and all preliminary Human Resources documentation (3/11/19). Due to this advanced training, Dr. Cole was able to access UARV and Andronowski Lab facilities and participate in animal handling and experimental treatments beginning on the start-date of the position.

Regardless of the later start-date of Dr. Cole, the project timeline proceeded as scheduled without delay.

On 03/11/2020, the World Health Organization declared the novel coronavirus outbreak (COVID-19) a global pandemic. As a result, The University of Akron cancelled in-person classes that day and in-person lab work was ordered to cease immediately. As per our project timeline, our research team was preparing rabbit femoral bone thin-sections for dynamic histomorphometry (*Activity 3.4*). This process involved daily lab work that included bone dehydration, methyl methacrylate (MMA) infiltration and curing, sectioning via the in-house Well Diamond Wire Saw, and slide mounting. The stalling of in-lab operations and the shutdown of The University of Akron halted the progress of *Activity 3.4* indefinitely.

On 05/21/2020, The University of Akron administration announced a 'Researcher Return to Work' initiative and Principal Investigators were invited to create individual Return to Work plans for their groups for review by administrators and unit heads. The document Dr. Andronowski created (**Appendix XVII**) outlined the guidelines and expectations of Andronowski Lab members for a safe return to lab activities during the ongoing COVID-19 pandemic. On 8 June 2020, the plan was approved with restrictions related to limits on laboratory personnel numbers and office use and occupancy, the implementation of shift work, and personal protective equipment requirements. Due to current social/physical distancing guidelines

recommending separation distances of 6 feet or more, no more than 2-3 individuals are permitted in Dr. Andronowski's main lab space at any given time. Lab procedures requiring more than one person in close proximity are to be minimized as much as possible. As a result, our bone histomorphometry specimen preparation and the subsequent analyses were anticipated to be slowed. A detailed timeline narrative and summary table (**Appendix XVIII**) have been prepared that outline the revised trajectory for *Activities 3.4* and *4.2* in line with our lab safety guidelines. As of 06/30/20, we were on schedule with the revised timeline.

The planned in-lab training for junior research team members could no longer be carried out face-to-face with the social/physical distancing requirements. Drs. Andronowski and Cole filmed a video of the MMA embedding protocol and step-by-step guides for the imaging and analysis of bone thin-sections to virtually train the junior research team members.

5.2.2. Anticipated Delays

In using a complex instrument such as the SkyScan 1172 micro-CT laboratory system, there was a chance that the X-ray source could die, or other instrument issues may arise that delay machine use. Luckily, no required maintenance or equipment failure occurred during our study.

5.3. Changes that had a significant impact on expenditures

Delay in hiring Dr. Cole due to finishing her doctoral dissertation as described in section 5.2.1. Approximately $23,000 of her salary needs to be redistributed, though no Budget Modification GANs were submitted during this reporting period.

Due to the hiring delay and COVID-19 pandemic, submitted an eight-month no-cost extension to complete the delayed histomorphometry portion of the project and allow Dr. Cole to complete her full two-year post-doctoral term. The unused grant funds associated with the post-doctoral fellowship from 01/01/19 to 06/02/19 were available for this extension. This extension allowed Dr. Cole to remain fully engaged with manuscript submission and review stemming from this project.

5.4. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

The details of two changes pertaining to vertebrate animal care are outlined in sections 2.1. and 5.1.

5.5. Change of primary performance site location from that originally proposed

Nothing to report.

VI. BUDGETARY INFORMATION

Supplies and consumables associated with *Activities 1 and 2* were purchased during the first reporting period (01/01/2019 – 06/30/2019). Certain costs proposed in the approved application were deemed unnecessary due to more cost-effective alternatives being available from other sources. For example, cage

battery housing for the animals was rented from a local university (Cleveland State University) instead of purchasing new batteries from a laboratory supply company.

Supplies and consumables associated with *Activity 3* were purchased during this reporting period. Certain costs proposed in the approved application were deemed unnecessary due to more cost-effective alternatives being available from other sources. No Budget Modification GANs were submitted over the course of the project.

VII. PROJECT OUTCOMES

7.1. What were the outcomes of the award?

The ultimate goal of this project for forensic anthropology is the refinement of histological age-at-death estimation in the context of widespread chronic opioid use. This project will further contribute to our understanding of basic bone biology more generally by describing changes in bone tissue microstructure both over time (longitudinally) and in 3D space. Due to historical reliance on 2D imaging, the 3D geometry of bone microstructure is not well defined, especially in relation to aging, modified physiology, and pathology[39]. Tracking these changes and individual variations in bone microstructure is also biomedically important for understanding what makes bones weak, when they are at risk of fracture, and how these complications of poor bone quality may be prevented.

Forensic anthropologists typically develop histological methods using human cadaveric samples. Biological human variation, differences in life history, and limitation to a single time point can introduce uncertainty into the accuracy and applicability of these methods. The design of the current project, employing a rabbit model as an animal proxy of human cortical remodeling, allowed us to control more tightly both for individual variation and the parameters of drug use. Examining these more isolated effects of chronic opioid use should aid forensic anthropologists in discerning which histological pathologies in humans result from drug use, and which may be related to life history co-morbidities (e.g., poor nutrition, exposure to communicable disease through needle sharing, and/or chronic stress)[40–42]. Given the rising prescription of opioids for chronic pain relief, it is essential to determine how chronic drug use on its own modifies forensic techniques, even when these lifestyle co-morbidities are absent.

VIII. DEMOGRAPHIC INFORMATION FOR SIGNIFICANT CONTRIBUTORS

Name: Janna M. Andronowski
Project Role: Principal Investigator
Gender: Female
Ethnicity: Not-Hispanic or not-Latino
Race: White
Disability Status: No

Name: Mary E. Cole
Project Role: Post-Doctoral Fellow
Gender: Female

Ethnicity: Not-Hispanic or not-Latino
Race: White
Disability Status: No

Name: Reed A. Davis
Project Role: Graduate Student (Research Assistant)
Gender: Male
Ethnicity: Not-Hispanic or not-Latino
Race: White
Disability Status: No

Name: Adam J. Schuller
Project Role: Undergraduate Research Assistant
Gender: Male
Ethnicity: Not-Hispanic or not-Latino
Race: White
Disability Status: No

Name: Abigail R. LaMarca
Project Role: Undergraduate Research Assistant
Gender: Female
Ethnicity: Not-Hispanic or not-Latino
Race: White
Disability Status: No

Name: Gina R. Tubo
Project Role: Undergraduate Research Assistant
Gender: Female
Ethnicity: Not-Hispanic or not-Latino
Race: White
Disability Status: No

Name: Joshua T. Taylor
Project Role: Undergraduate Research Assistant
Gender: Male
Ethnicity: Not-Hispanic or not-Latino
Race: White
Disability Status: No

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THE UNIVERSITY OF AKRON

MEMORANDUM

TO: Janna Andronowski

CC: IACUC@uakron.edu

FROM: Beth Kenaga, IACUC Administrator

DATE: December 10, 2018

SUBJECT: APPROVAL NOTICE
Protocol: 18-11-12 ARC
Titled: Longitudinal effects of prolonged opioid use on cortical bone remodeling in a rabbit model

Your Protocol was approved by The University of Akron's Institutional Animal Care and Use Committee (IACUC) designated member review process on **December 7, 2018.**

PLEASE NOTE: Your study cannot begin until EH&S/Occupational Health reviews/provides input/approves the "Hazardous Substance Form."

A copy of this form will be filed with your protocol in the Research Office. If you have any questions, please contact Beth Kenaga, IACAUC Administrator, at 330-972-5845 or via email at bkenaga@uakron.edu.

Beth Kenaga

Beth A Kenaga

IACUC Administrator

The University of Akron operates under the Public Health Service (PHS) Assurance number A3870-01.

REQUEST TO USE ANIMALS

1. PROTOCOL SUMMARY

1. A. Protocol Title:

Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model

1. B. Principal Investigator's Institution:

The University of Akron

1. C. Facility(ies) where animals will be housed: *If animals will be housed in a facility not listed below, please identify the location under "OTHER LOCATION".*

UNIVERSITY OF AKRON	☒	SUMMA HEALTH SYSTEM	☐
KENT STATE UNIVERSITY		YOUNGSTOWN STATE UNIVERSITY	
Cunningham	☐	Cushwa	☐
Kent	☐	DeBartolo	☐
Tuscawaras	☐	Ward Beecher	☐
NEOMED	☐	OTHER	☐

OTHER LOCATION (Institution, building & room OR geographic location for field studies): N/A

1. D. Source of funding for the project:

INTERNAL ☐
EXTERNAL ☒

For external awards, identify the agency(ies) and award number(s).

National Institute of Justice, Award Number 2018-DU-BX-0188 (0)

1. E. Anticipated start date:

January 1, 2019

1. F. Expected animal use over the three year approval period: *Summarize all animal use by species.*

Species	Number	Source
Oryctolagus cuniculus	21	Charles River Laboratories
(New Zealand White Rabbit)		

1. G. If this protocol is a continuation of a previously approved protocol, indicate the protocol number and provide a brief summary of the progress made to date. *Your response is limited to the space provided.* **N/A:**

Previous protocol number: N/A

Brief summary of progress/results: N/A

1. H. Project overview. *The response MUST be in lay terminology and understandable to a person with no scientific background.*

(1) Describe the medical condition, scientific question, or teaching value that is being addressed and its importance.

The misuse and addiction to opioids (and synthetic opioids) is a serious public health crisis nationwide that has become an epidemic. The overall yearly cost to the United States government for opioid abuse-related healthcare, law enforcement, and education/prevention programs has been estimated at $78.5 billion. This crisis afflicts millions of Americans and costs our healthcare system billions. Current evidence suggests that opioids upset the balance of bone remodeling towards more destruction and less formation of bone. Experimental studies have been limited by the fact that small laboratory animals traditionally used in bone research (mice and rats) do not exhibit spontaneous cortical bone remodeling, making them a poor choice of animal model for this subject. The current project seeks to develop a longitudinal model for studying the effects of prolonged opioid exposure on cortical bone remodeling in an animal which remodels its cortical bone in a manner comparable to humans, the rabbit. Given the limited data available related to the longitudinal impact of opioid abuse on bone remodeling, we must further understandings of the underlying biological processes to improve the applicability of histological age-estimation methods and scientific standards within the field of forensic anthropology.

Two central research questions will be addressed: 1) What are the effects of prolonged morphine and fentanyl use on cortical bone remodeling? Will cortical bone microstructure vary randomly with respect to opioid use, or will it be correlated? And 2) are the effects of prolonged opioid use discernable in cortical bone microstructural features used in histological age-at-death estimation?

(2) List the goals of the project.

The ultimate goal of this project is to describe how analgesic drugs, particularly morphine and fentanyl, affect microscopic structures of cortical bone used in histological age estimation methods in forensic anthropology.

(3) Provide a chronological <u>summary</u> of the animal use from the beginning of the project through its end. *A lay description of the experimental design can be used as the response IF it addresses the intent of the question. <u>Do not</u> provide detailed descriptions of the procedures here.*

Skeletally mature, 6-month old (3.7-3.9 kg), male New Zealand White rabbits will be divided into three groups of 7 animals each: morphine, fentanyl, and control (saline vehicle). Male animals were selected to avoid the potential influence of female hormone cycles on bone physiology. All narcotics will be supplied by Henry Schein, a worldwide distributor of medical, dental, and veterinary supplies (including pharmaceuticals).

A small pilot study with experimental groups of 3 animals each will be carried out prior to the start of the full-scale experiment. This will ensure that the proposed opioid dosings are appropriate and do not induce distress in either the morphine or fentanyl group.

The rabbits will be floor-housed in individual wire pens to allow for some interaction, while keeping the animals lodged separately to prevent potential aggressive encounters. The rabbits will further be habituated to the testing conditions for two weeks following their arrival at the University of Akron Research Vivarium (UARV). After the acclimation period, the opioid groups (morphine and fentanyl) and controls will each be dosed by subcutaneous injection every other day for 8 weeks. The morphine hydrochloride group will receive a dose of 3 mg/kg/day, and the fentanyl group will receive 0.5 mcg/kg/day. The control group will be administered saline at a dose of 3 mg/kg/day. The proposed opioid dosing levels are consistent with clinical recommendations for analgesia in rabbits and were finalized with the former UARV Attending Vveterinarian. The PI and designated project staff (including the Post-doctoral fellow, a UA employee) will be responsible for administering the opioids. Those injecting the substances will wear cut-resistant gloves in addition to latex/nitrile gloves. The designated project staff will receive training regarding the safe handling of opioids and an emergency-response exposure protocol will be in place prior to the start of the project.

All animals will undergo subcutaneous injection with a bone-labelling fluorochrome, calcein, to facilitate *ex vivo* dynamic histomorphometry following euthanasia. Calcein will be administered at a level 10/mg/kg (65) after two weeks (days 13 and 14), four weeks (days 27 and 28), six weeks (days 41 and 42), and eight weeks (days 55 and 56) of the opioid regimens. The fourth calcein dosing will be just prior to intravenous injections containing a euthanasia solution, pentobarbital sodium (Fatal-Plus or equivalent). The euthanasia solution will be delivered in the UARV necropsy suite at a level of 125 mg/kg per animal.

2. DESCRIPTION OF PROCEDURES INVOLVING LIVE ANIMALS

Please review all parts of this section before answering because there are separate parts for specific types of animal use. Each part will expand to accommodate the response. Mark N/A for sections that do not apply.

2. A. Animal Identification:

Indicate how animals will be identified. Multiple methods may be selected.

CAGE CARD	☒	COLLAR/TAG	☐
EAR PUNCH/NOTCH	☐	EAR TAG	☐
INDELIBLE MARKER	☐	MICROCHIP	☐
TATTOO	☒	OTHER (describe below)	☐

Describe the identification procedure if it involves penetration of the skin. Toe clipping is discouraged and, if it is used, a justification must be provided.

All rabbits will be tattooed at Charles River Laboratories with an identification number prior to their delivery. This way, if an animal escapes a floor pen, they will be easily identified.

2. B. Breeding: N/A: X

Describe the breeding scheme that will be used. Indicate weaning age of offspring.

2. C. *Genotyping:* N/A: X

Describe the method used to genotype the animals. Include the amount of tissue taken, age of animals, method of analgesia, and method of instrument sterilization.

2. D. Experimental manipulations: N/A:

List and describe in detail all nonsurgical experimental manipulations carried out on live animals. Euthanasia is to be described in 2.F. The response must include a statement of the known or expected impact of each procedure on animal well-being.

The opioid groups (morphine and fentanyl) and controls will each be dosed by subcutaneous injection every other day for 8 weeks. The morphine hydrochloride group will receive a dose of 3 mg/kg/day, and the fentanyl group will receive 0.5 mcg/kg/day. The control group will be administered saline at a dose of 3 mg/kg/day. The opioids (morphine and fentanyl) will be pharmaceutical grade.

Two individuals from the research team will be responsible for dosing the animals every second day using manual restraint and a laboratory table for support. The individual handling the morphine/fentanyl will wear cut-resistant gloves during injections as an extra safety precaution. The proposed opioid dosing levels are consistent with clinical recommendations for analgesia in rabbits and were finalized with the former UARV Attending Veterinarian. Possible side effects of opioid use in rabbits may include: bradycardia, respiratory depression, nausea, ileus, and excessive sedation. All animals will be monitored closely by our research group, the UARV staff, and UARV Attending Veterinarian during the experimental period to ensure the continued health and well-being of all animals.

All animals will undergo subcutaneous injection with a bone-labelling fluorochrome, calcein, to facilitate *ex*

vivo dynamic histomorphometry following euthanasia. Calcein will be administered at a level 10/mg/kg (65) after two weeks (days 13 and 14), four weeks (days 27 and 28), six weeks (days 41 and 42), and eight weeks (days 55 and 56) of the opioid regimens. The fourth calcein dosing will be just prior to intravenous injections containing a euthanasia solution, pentobarbital sodium (Fatal-Plus or equivalent). It is important to note that the injection of calcein at this dose level is not associated with any undesirable side effects.

The main source of discomfort for the rabbits will be the injections of morphine, fentanyl, saline, and bone labeling fluorochromes. From our previous work however, we found that a subcutaneous (SC) injection in the tent of skin located between the shoulder blades, as opposed to an intramuscular (IM) injection, was better tolerated by the rabbits and thus we will continue with SC injections.

2. E Surgical manipulations. N/A: X

2.E.(1) Description of surgical procedures:

Describe each surgical procedure under a separate heading. Procedures that are performed on the same animal at the same time may be described as one procedure. IF more than four different surgeries are planned, then similar ones may be combined into a single response.

Surgical procedure #1:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Describe the procedure in detail. Include the pre-operative preparation of the animal, a description of the aseptic technique and how instruments and implantable devices are sterilized. The response must include a statement of the known or expected impact of the procedure on animal well-being.

Surgical procedure #2:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

Surgical procedure #3:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

Surgical procedure #4:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

2.E.(2) Multiple major survival surgery:

Does this project involve multiple major survival surgeries in the same animal? **YES: NO:**

If so, provide a justification.

2. F. Anesthesia/Sedation. N/A:

List the procedures that require anesthesia or sedation individually below and describe the anesthetic regimen used for each. If multiple procedures use the same anesthetic regimen, then they can be combined into one response.

Anesthesia/sedation procedure #1:

Identify the procedure requiring anesthesia or sedation. List all drugs (including neuromuscular blocking agents) used as part of the anesthetic/sedative regimen; include the dose, route of administration and indicate the frequency of repeat dosing. If animals will be anesthetized with inhalants, indicate the percentage of anesthetic gas, any auxiliary gases used, oxygen flow rate and ventilatory parameters (for mechanically ventilated animals).

Euthanasia will be performed via intravenous injections (ear vein) containing a pentobarbital sodium solution (Fatal-Plus or equivalent) following the 8-week experimental period. The animals will be restrained (short-term) in an acrylic/plexiglass rabbit restrainer while the euthanasia solution is administered.

Describe the procedures and equipment used to monitor the depth of anesthesia and animal well-being. If neuromuscular blocking agents are used, include techniques that are reliable in paralyzed animals. N/A

Describe the supportive measures to assure animal well-being while under anesthesia.

The animals will be kept comfortable, secure, and calm during the euthanasia procedure. Two individuals from the research team will be present to ensure this.

Anesthesia/sedation procedure #2:

Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above. N/A

Procedures and equipment used to monitor the depth of anesthesia and animal well-being: N/A

Supportive measures: N/A

Anesthesia/sedation procedure #3: N/A

Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above.

Procedures and equipment used to monitor the depth of anesthesia and animal well-being:

Supportive measures:

Anesthesia/sedation procedure #4: N/A

Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above.

Procedures and equipment used to monitor the depth of anesthesia and animal well-being:

Supportive measures:

2. G. Building(s) and room number(s) where the procedures will take place:

Nonsurgical Procedures:

Animal housing: UARV, Room 212
Animal euthanasia: UARV Necropsy Suite
Carcass Storage: Andronowski Lab, ASEC B228

Surgical Procedures: N/A

2. H. Postprocedural care and monitoring:

1) Describe the post-procedural care and monitoring for both surgical (after recovery from anesthesia) and nonsurgical procedures. Identify the parameters being monitored and the frequency and duration of monitoring for each study related procedure. Include how records of the care will be maintained and their location.

Animals will be monitored once daily (including weekends/holidays) by the PI and designated project staff. The rabbits will be housed individually and receive enrichment toys in UARVs suite 212 with continuous radio play during daylight hours to provide environmental stimulation. The PI and UARV manager will order new rabbit pens which will be used for housing purposes. During daily acclimatization visits and injection schedules, each rabbit will receive positive reinforcement through food items such as high fiber rabbit chow and additional food (greens, carrots, shredded wheat, raisins, alfalfa, etc.). The research team will create and post a behavioral sheet in the rabbit room to track observed behaviors during both the acclimation and experimental periods. Each animal will have their own behavioral/observation sheet to note information during surveillance checks.

2) Identify by title who will conduct the care and monitoring.

Principal Investigator, Graduate student, Undergraduate student, Post-doctoral Fellow.

*3) List any analgesics or other medically related pharmaceutical agents that animals may receive. Include **a)** dose, **b)** route of administration **c)** frequency of administration, and **d)** duration of therapy.*

The opioid groups (morphine and fentanyl) and controls will each be dosed by subcutaneous injection every other day for 8 weeks. The morphine hydrochloride group will receive a dose of 3 mg/kg/day, and the fentanyl group will receive 0.5 mcg/kg/day. The control group will be administered saline at a dose of 3 mg/kg/day. The proposed opioid dosing levels are consistent with clinical recommendations for analgesia in rabbits and were finalized with the former UARV Attending Vveterinarian.

4) List the criteria that will be used to determine that relief from pain or distress is needed and how the adequacy of that relief will be assessed. N/A

5) List the humane endpoints that will be used to euthanize an animal or otherwise remove an animal from a study.

Weight loss (of 20% or more) due to opioid injections, changes in fecal production, especially it decreases or stops completely as this is an indication of gastrointestinal stasis which can be lethal. If this was to occur, the animal would be placed on a hand fed diet based on the recommendation of the Attending Veterinarian until the stasis cleared. Other signs of distress in any animal such as hair loss, lethargy, hyperactivity, excessive or there-lack of grooming would be closely monitored in accordance with the UARV Manager and Attending Veterinarian.

2. I. Disposition of animals:

Describe the method of euthanasia including the name, dose, and route of administration of any pharmaceutical agents used. Describe the method(s) that will be used to confirm death. Animals euthanized by an overdose of carbon dioxide must undergo a secondary method of euthanasia to confirm death. If animals will not be euthanized, describe their disposition.

Intravenous injections containing a euthanasia solution, pentobarbital sodium (Fatal-Plus or equivalent). The euthanasia solution will be delivered in the UARV necropsy suite at a level of 125 mg/kg per animal.

Post-euthanasia, rabbit carcasses will be transported discretely and in accordance with UARV policy to the PI's laboratory located in the Auburn Science and Engineering Center, room B228. All carcasses will be stored in a -80 freezer in the laboratory prior to harvesting of the long bones, which can only be accessed by research group members.

2.J. Chemical/compound administration to live animals

Are all of the chemicals (e.g., test compounds, receptor agonists/antagonists, labeling compounds, anesthetics, analgesics, euthanasia agents, etc.) administered to live animals commercially available pharmaceutical preparations intended for animal or human use?

Yes: X **No:**

If not, then complete the following for each product.
Identify the chemical/compound and describe how it is prepared and stored to assure appropriate purity, sterility and suitability for administration to animals. Indicate the shelf life of the prepared product.

Are all of the chemicals/compounds listed above pharmaceutical grade? **Yes:** **No:**
If not, then list them and provide a justification for not using a pharmaceutical grade preparation.

3. SPECIAL CONSIDERATIONS

Mark N/A for sections that do not apply.

3.A. Food/ fluid restriction: N/A: X

*If the study involves scheduling access to food or fluid OR restricting food or fluid intake beyond that associated with a routine overnight pre-procedural fast or weight control, then describe **a)** the amount and time of the restriction, **b)** expected impact on animal well-being, and **c)** criteria for removal of the restriction.*

Describe the record-keeping associated with ongoing restrictions. Indicate where the records will be maintained. At a minimum animal weights must be documented once weekly and food/water consumption noted daily.

3. B. Prolonged restraint: **N/A: X**

*If the project involves more than routine restraint of conscious animals for brief periods, then describe: **a)** the restraint, **b)** its duration and frequency, **c)** how animals will be conditioned to it, and **d)** how frequently animals will be observed while restrained.*

Provide a justification for the restraint.

3. C. Immunologic adjuvants: **N/A: X**

If the project involves the use of immunologic adjuvants (e.g., Freund's adjuvant, RIBI adjuvant) complete the following.

	First Injection	*Second Injection*	*Subsequent Injections*
Adjuvant			
Anatomic site of injection & route			
Number of sites			
Volume per site			
Time interval between injections			

3. D. Dog exercise: **N/A: X**

If the project involves the use of dogs, indicate if any animals will be exempted from the dog exercise program and include the duration of the exemption and a justification for it. If there are no exemptions, enter "no exemptions".

3. E. Environmental enrichment for primates: **N/A: X**

If the project involves the use of nonhuman primates, indicate if any animals will be exempted from the environmental enrichment program for primates and include the duration of the exemption and a justification for it. If there are no exemptions, enter "no exemptions".

3. F. Housing or enrichment restrictions: **N/A:**

If the project involves the single housing of animals of a social species OR exemption from normal environmental enrichment, then describe and provide a justification for the restriction.

The rabbits will be floor-housed in individual wire pens to allow for some interaction, while keeping the animals lodged separately. As the proposed animals are males, they will be housed separately to avoid any

potentially aggressive interactions.

3. G. Hazardous material use: N/A:

If the project involves the administration of any potentially hazardous materials to live animals, complete the following for each material and attach the appropriate hazardous material form(s) required by the institution at which the work will take place.

Name of hazardous agent(s):

Morphine, fentanyl

Select the appropriate classification of hazard(s)

CARCINOGEN	☐	INFECTIOUS AGENT	☐
RADIOACTIVE ISOTOPE	☐	RECOMBINANT NUCLEIC ACID	☐
TOXIN	☐	HUMAN TISSUE/CELLS	☐
OTHER	☒		

Describe the potential health effects of the hazard and list the possible routes of exposure hazard:

The risks associated with this study include accidental subcutaneous needle sticks and accidental dosing with narcotics. Cut-resistant gloves will be worn whenever handling the rabbits or needles in order to prevent any accidental needle sticks and/or animal scratches.

The standard therapeutic dose of fentanyl for humans is 50-100 mcg. The doses we will be administering for the study are well below this range (1.8 mcg maximum). The standard starting therapeutic dose of morphine for adults greater than or equal to 50kg is 4-10 mg every 3-4 hours. Our maximum dose for this study is 11.7mg, posing a possible risk of exposure to the researchers. In the case of accidental exposure, provisions will be handled by EH&S/Occ Health.

Number of animals receiving material:

7 animals to receive morphine; 7 animals to receive fentanyl

3. H. Genetically modified animals: N/A: X

If the project involves the use, breeding, or creation of genetically modified animals, complete the following for each genotype.

List the animals by genotype and describe the known or expected impact of the associated phenotype on animal well-being:

Describe the measures to relieve or manage pain or distress related to each phenotype that is associated with an adverse impact on animal well-being:

Will any new genetically modified animals be created in the project? **Yes:** **No: X**

If so, describe the monitoring associated with the new line to assure adequate provision of humane animal care. Previously undescribed phenotypic conditions that negatively impact animal well-being must be reported to the IACUC:

3.I. Animal housing outside of main animal facility: **N/A: X**

If animals will be maintained outside of the main animal facility longer than 12 hours for USDA covered species or longer than 24 hours for all others, then complete the following.

Identify the building, room number, species, and number of animals to be housed. Indicate the duration of housing.

Provide a justification for the extramural housing.

Has the IACUC previously approved the location? **YES:** **NO:**

3.J. Field studies: **N/A: X**

If the project involves the use of animals in a field setting, complete the following.

Identify the occupational health and safety issues associated with studying the species in the wild.

Describe the potential impact of the study on native populations of the species being studied and others that may be affected by the study.

List and attach the permits and other necessary permission documents that are needed to carry out the study.

3.K. Procedures performed at a supplier location: **N/A: X**

If animals will undergo experimental or surgical procedures at a supplier's location, complete the following and attach a statement from the supplier confirming IACUC approval of the procedure.

Identify the procedure and supplier's Public Health Service Animal Welfare Assurance number and USDA registration number (as applicable).

4. CLASSIFICATION OF PROCEDURES ACCORDING TO LEVEL OF PAIN AND/OR DISTRESS

Mark the appropriate category for each animal procedure and identify the procedure(s) in the spaces provided. List the number of animals in each pain category in the box provided. If individual animals will undergo procedures in multiple pain categories, then include them in the tabulation for the highest pain category.

☒ - **Category C** - Procedures that involve no more than momentary or slight pain or distress.

List procedures:

The main source of discomfort for the rabbits will be the injections of morphine, fentanyl, saline, and bone labeling fluorochromes. From our previous work (at The University of Saskatchewan), we found that a subcutaneous (SC) injection in the tent of skin located between the shoulder blades, as opposed to an intramuscular (IM) injection, was better tolerated by the rabbits and thus we will continue with SC injections.

Number of animals in category C:

21

☐ - **Category D** - Procedures that may cause more than momentary or slight pain or distress for which appropriate analgesia, anesthesia or tranquilization is provided.

List procedures: N/A

Number of animals in category D:

☐ - **Category E** - Procedures that may cause pain or distress which are not relieved by analgesia, anesthesia, or tranquilization.
List procedures:

Number of animals in category E:

For Category E procedures: Provide a detailed scientific justification for withholding analgesia, anesthesia, and tranquilization. N/A

5. ALTERNATIVES TO THE USE OF ANIMALS AND PAIN OR DISTRESS PRODUCING PROCEDURES

Provide a written narrative description of the methods and sources that were used to determine that suitable alternatives to the use of animals and to the pain or distress producing procedures described in the protocol are not available. Provide an explanation for alternatives that were identified but deemed unsuitable. Literature searches must include a) databases searched, b) the date of the search, c) the years covered by the search (minimum 10 years), and d) the search strategy including keywords used. At least two acceptable information sources must be used. The response must address the three R's: Replacement models, Refinements in technique, and Reduction in animal numbers. Information sources that are commonly used include http://www.pubmed.gov, http://agricola.nal.usda.gov, http://www.nal.usda.gov/awic, and specifically for teaching activities, http://oslovet.veths.no.

Overall, we strongly believe that the proposed experimental protocol will not lead to pain and/or distress of the animals.

Two literature searches (Pubmed.gov and Web of Science) were performed on Oct. 19, 2018 with results spanning 1972-2018 (Pubmed) and 1991-2018 (Web of Science) using the keywords "opioid", "animal", and "bone". We have determined that the most common replacement models for the New Zealand White rabbits are mice or rats. These are unsuitable for our study as neither mice nor rats undergo spontaneous remodeling of bone and maintain primary osteons throughout life. This study proposes to look at how bone remodeling is impacted over time by prolonged opioid use. This cannot be done with animal models that do not spontaneously remodel bone. Rabbits are the smallest common laboratory animal that have spontaneous bone remodeling similar to humans. The suggested number of animals per experimental/control group (n=7) is below the average of comparable studies which range between 10-15 animals per group and is in line with previous similar studies (see 6.C). As the proposed experiment is aiming to examine the changes in bone remodeling over time with prolonged use of opioids, living animals must be used in lieu of

computer simulations or chip assays to examine the changes seen in the whole organism. Common routes of animal dosing include injection via intraosseous (IO), intravenous (IV) intramuscular (IM), subcutaneous (SC), or intrathecal and, alternatively, transdermal patch. Of these common routes, transdermal patches have the potential to fall off the animal, skewing results if the animal did not receive the full dose of opioid drug prior to the patch falling off. There is also more accurate control of the dose of drug if an injectable form is used. Of the injectable methods, SC in the tent of skin between the shoulder blades is the least invasive and minimally painful mode of administration as determined by proof-of-principle experiments at the University of Saskatchewan.

Literature Search Strategy:

1. Database used: Pubmed.gov
Range covered: 1972-2018
Date of search: 10-19-18
Keywords: bone AND animal AND opioid

Reasoning for animal of choice: NZW rabbits are a well-established animal model for bone studies since their bone turnover process is comparable to that of humans.

Mice and rats do not undergo spontaneous remodeling but are used to examine remodeling in a post-fracture callus, a confounding variable, or bone cancer pain.

Example papers:
https://www.ncbi.nlm.nih.gov/pubmed/23955193
https://www.ncbi.nlm.nih.gov/pubmed/29680509

2. Database used: Web of Science
Range covered: 1991-2018
Date of search: 10-19-18
Keywords: bone AND animal AND opioid abuse

Reasoning for animal of choice: Animal numbers are already rather low. From the studies surveyed, 10-15 is average per experimental group.

Intrathecal injection is common, as is IO or IV injection, however, SC injection is done with a smaller needle and causes less pain.

Example papers:
http://apps.webofknowledge.com/full_record.do?product=WOS&search_mode=GeneralSearch&qid=2&SID=6BfZC7SymiMhCjfEpRg&page=1&doc=2
https://www.sciencedirect.com/science/article/pii/S0304395902001021#aep-section-id23

Following our thorough evaluation of the available literature, we did not identify a less sentient species who will provide the results we are expecting.

6. JUSTIFICATION FOR THE USE OF ANIMALS

6. A. Provide a rationale for involving animals.

Animal models provide essential platforms for experimental studies which investigate the factors affecting the regulation of bone remodeling. Inter-species variation in cortical microarchitecture, however, can limit the utility of particular models for specific questions. The cortical bone of larger vertebrates, including

humans, is dominated by Haversian systems while smaller species, including mice and rats exhibit little to no cortical remodeling and retain primary canals throughout their lives (Sietsema, 1995; Turner et al., 2001; Jee & Yao, 2001; Pearce et al., 2007; Reinwald & Burr, 2008). Since the cortical bone of adult rodents displays no ambient Haversian bone remodeling, mature rodents are best used as a model for cancellous bone remodeling (54, 55). Thus, to study remodeling, larger animal models such as sheep, goats, rabbits, dogs, or swine have been utilized (Sietsema, 1995; Turner et al., 2001, Pearce et al., 2007; Reinwald & Burr, 2008). Rabbits are the smallest traditionally used laboratory animals with well-defined cortical remodeling comparable to humans (Recker et al., 2011). As a result, rabbits have been used as a model system for studying central canal size and the vascular network of cortical bone (Pazzaglia et al., 2007; 2009; 2010).

6. B. What is the basis for selecting the species that you have chosen?

New Zealand White rabbits will be used as these are the smallest laboratory animals to exhibit cortical bone remodeling comparable to humans. Thus, these animals have been recommended by the American Food and Drug Administration for bone-loss related studies (Thompson et al., 1995). Through collaborative proof-of-principle micro-CT experiments, bone remodeling events were successfully observed within the cortices of rabbit tibiae. Thus, transitioning to this larger animal model which naturally exhibits cortical remodeling has been proven and will serve as a novel platform for studying bone microstructural changes associated with prolonged opioid exposure.

6. C. Number of animals requested:

Provide a justification for the number of animals requested. Identify the species, genotypes, strains, and/or stocks of animals. Include other descriptors as relevant (e.g., age or weight, gender, timed pregnant). For research protocols, list the experimental and control groups and indicate the number of animals in each. Include the statistical justification, or other basis, for selecting the number requested. If a research protocol includes the use of animals solely for training (i.e., the training does not occur as part of the experimental use of animals), then include the expected number of animals to be used for training. Animals used for training can be justified by documenting the expected number of persons to be trained and the number that can be trained per animal.

Skeletally mature, 6-month old (3.7-3.9 kg), male New Zealand White rabbits will be divided into three groups of 7 animals each: morphine, fentanyl, and control (saline vehicle). The group sizes that we chose are based on the mean number of animals used in previous related studies focusing on trabecular or cortical geometry/density (Castaneda et al., 2008; Baofeng et al., 2010; Castaneda et al., 2006; Wen, et al., 2015; Liu et al., 2012). Male animals were selected to avoid the potential influence of female hormone cycles on bone physiology.

6. D. Provide written assurance that the use of animals described in this protocol does not unnecessarily duplicate previous experiments.

Current research offers an incomplete picture of the extent that advancing age explains the variability in histological structures used in microscopic methods. Investigating the influences of extrinsic and intrinsic life history variables such as biomechanical stressors, drug and alcohol use, disease, trauma, diet and nutrition, and hormones (e.g. vitamin D, estrogen) provide avenues for future research as their influences are not fully understood. Previous studies have shown that pathological conditions can affect bone remodeling and therefore age estimations. Work by Karinen (2009), for example, revealed that individuals who abuse methamphetamine were more likely to have their age underestimated when evaluated using histological criteria. He found that known users were under aged an average of 11.57 years compared to nonusers. The recognition that low osteon counts are associated with substance abuse has serious implications for anthropologists attempting to employ age at death analyses based on

the products of bone remodeling. **This project aims to fill a critical knowledge gap and address the effects of substance abuse on bone turnover, with a specific focus on prolonged opioid use.**

Individual remodeling events have never been followed over time, or directly observed *in situ*. To address this limitation, we proposed this project to combine animal models of cortical bone remodeling with *ex vivo* high-resolution phase contrast micro-computed tomography imaging. Initial experiments focused on rats and are now transitioning to rabbits which have cortical microarchitecture which more closely approximates that of humans.

7. HOUSING AND HUSBANDRY

7. A. Indicate the approximate number of animals to be housed at one time and approximate duration of housing.

21 animals housed for 10 weeks. The rabbits will be quarantined for up to two weeks, and habituated to the testing conditions following their arrival at the UARV. After the acclimation period, the opioid groups (morphine and fentanyl) will each be dosed by subcutaneous injection every other day for 8 weeks.

7. B. If rodents are to be housed, is there a preference as to the type of caging (i.e., plastic, wire-bottom, microisolator or other) OR the number of animals per cage?

YES: NO: X

If yes, please specify. Note that the use of wire-bottom cages or single housing of animals requires a justification.

7. C. Will a light cycle other than the standard 12 hours light/12 hours dark be necessary for any of the animals on this protocol? **YES: NO: X**

If yes, please specify the light cycle(s) and indicate the group(s) of animals that will require it.

7. D. Will the animals on this protocol have any special temperature or humidity requirements? **YES: X NO:**

If yes, please describe.

Low humidity is preferred since rabbits are temperature sensitive. The UARV Manager decided on UARV Room 212 to house the animals as it typically has more stable temperature/humidity levels than others.

7. E. Will the animals on this protocol require a special diet or special water?

YES: NO: X

If yes, please identify the product, the number of animals receiving it, and who will prepare and administer it.

7. F. Will the animals on this protocol require any other special housing, care, environmental conditions, or other considerations? **YES: NO: X**

If yes, please describe.

The opioids administered to the experimental rabbits may have GI tract stasis as a side effect. Therefore, rabbits may be given a variety of high-fiber nutritional supplements such as High-fiber rabbit chow as their normal feed, grass hays and cubes, fruits and vegetables, etc. to stimulate GI mobility.

7. G. Will it be necessary to house animals after they have received any hazardous materials (refer to Part 3.G.)? **YES:** **NO: X**

If yes, please identify the material, the number of animals, and the duration of housing. Describe how the housing cages and room will be identified to alert personnel that a hazard is present.

8. PROTOCOL APPROVAL

Click "Choose Institution" to select the institution to which the protocol will be submitted.

Protocol approval is indicated by the signatures of the institution-specific individuals identified below. The individuals signing confirm that they have reviewed the protocol and find it to be in compliance with applicable animal care and use regulations and institutional policies.

University of Akron

Approval Signatures:

_____ Date _____
Facility Director

_____ Date _____
Department Chair/Research Director

_____ Date _____
IACUC Member

_____ Date 12-18-18
Attending Veterinarian

_____ Date 1-9-19
IACUC Chairperson

For IACUC Use Only
Category: C Special Considerations: USDA SPECIES Protocol #: 18-11-12 ARC

INVESTIGATOR ASSURANCE

Commented [IACUC55]: Complete the blanks below for the principal investigator and up to two co-investigators. Additional co-investigators can simply be identified as such on a "Participant Qualifications" page. A signed copy of this page must be submitted to the IACUC coordinator before IACUC approval of the protocol can be granted.

By signing below I/we agree to:

A. Employ procedures that will avoid or minimize discomfort, distress, and pain to animals, consistent with sound research design.

B. Comply with the protocol as approved by the Institutional Animal Care and Use Committee (IACUC) and to obtain the consent of the IACUC before implementing any changes to the protocol.

C. Comply with the policies of the IACUC of the institution at which this work is conducted, the National Research Council Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the regulations of the Animal Welfare Act and other applicable federal, state and local regulations governing the use of animals in research, teaching, and testing.

D. Maintain adequate records of all animal experimentation procedures.

E. The provision of emergency veterinary care including euthanasia by the attending veterinarian or his/her designee for animals showing evidence of unbearable pain, distress, or illness with the understanding that an effort will be made to contact me or my designee prior to the initiation of any treatment.

Principal Investigator:

Name: Janna M. Andronowski Department: Biology

Commented [IACUC56]: For non-departmental affiliations, identify the name of the organization.

Email address: jandronowski@uakron.edu Telephone number: 3309725419

Signature _____ Date: Dec. 10/18

Co-Investigator:

Name: Department:

Commented [IACUC57]: For non-departmental affiliations, identify the name of the organization.

Email address: Telephone number:

Signature _____ Date: _____

Co-Investigator:

Name: Department:

Commented [IACUC58]: For non-departmental affiliations, identify the name of the organization.

Email address: Telephone number:

Signature _____ Date: _____

For IACUC Use Only
Category: C Special Considerations: USDA SPECIES Protocol #: 18-11-12 ARC

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Janna M. Andronowski, Ph.D.

TITLE: Principal Investigator

List the participant's responsibilities on the protocol.

Dr. Andronowski's role will span the full project. She will bring her related experience — both in research and imaging — to the team and interface closely with Davis, Schuller, and the proposed Post-doctoral Fellow to execute the research protocol. Dr. Andronowski will direct the team and assist with rabbit care, handling, monitoring, and administering injections.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☐ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Dr. Andronowski completed online courses in biosafety training and ethical care, and received animal handling for New Zealand White Rabbits and anaesthesia training at the University of Saskatchewan in 2017. Dr. Andronowski will receive the training specific to the University of Akron (both online and animal handling at NEOMED) prior to the beginning of this study, as per the recommendations of Dr. Dannemiller. NEOMED-specific training with Dr. Dannemiller will include rabbit handling, subQ and IV injections, and restraint.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
University of Saskatchewan UACC Animal Ethics course	University of Saskatchewan, SK, Canada	March 2017
University of Saskatchewan UACC Practical Skills: Rodent Handling	University of Saskatchewan, SK, Canada	April 2017
University of Saskatchewan UACC Practical Skills: Euthanasia (Mammal)	University of Saskatchewan, SK, Canada	April 2017

PARTICIPANT SIGNATURE

Dec. 10/18
DATE

Consortial protocol 0114.doc Version 01/14

Commented [IACUC59]: A completed and signed copy of this form for each person listed or working on the protocol must be submitted to the IACUC coordinator before IACUC approval of the protocol can be granted. Six copies of this page are included in this "Request to Use Animals" form. If more than four persons will work on the project, then additional copies of the page are available in the document identified with the file name "Participant qualifications 2".

Commented [IACUC60]: For example, Principal Investigator, Co-Investigator, Graduate Student, Technical Staff, or other position title or description.

Commented [IACUC61]: For example, anesthesia, behavioral testing, breeding, euthanasia, post-operative care, surgery.

Commented [IACUC62]: The experience/qualifications must be specific to the activities that the individual will perform (e.g., surgical implantation of microdialysis probes, rat anesthesia, mouse euthanasia, rat ovariectomy). A reference to the number of years of experience with the procedures and/or number of procedures performed or the person who provided the training can be used. For those individuals who do not have the necessary training or experience, it is acceptable to indicate that they will be trained by the experienced staff listed on the project, by the attending veterinarian, or by members of the animal facility staff who are experienced in the procedure. For teaching activities, only the training and experience of the instructors must be provided. It is not necessary to describe it for the students or trainee participants in the course.

Commented [IACUC63]: The experience/qualifications should be specific to the activities that the individual will perform (e.g., surgical implantation of microdialysis probes, rat anesthesia, mouse euthanasia, rat ovariectomy). A reference to the number of years of experience with and/or number of procedures performed or the person who provided the training is helpful. For those individuals who do not have the necessary training or experience, it is acceptable to indicate that they will be trained by the experienced staff listed on the project, by the attending veterinarian, or by members of the animal facility staff who are experienced in the procedure. For teaching activities, only the training and experience of the instructors must be provided. It is not necessary to describe it for the students or trainee participants in the course.

Commented [IACUC64]: List any formal course work taken or presentations attended relevant to the use of animals in research, teaching, or testing. Include the date and location. If the participant has had none, enter "None". For those who have more than three entries, enter only the three most recent or relevant activities AND include any that are required by the institution. Requirements for formal training vary across the consortium. Contact the IACUC Coordinator at the institution to which the protocol will be submitted with questions concerning specific requirements.

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Category: C Special Considerations: USDA SPECIES Protocol #: 18-11-12 ARC

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Reed Davis

TITLE: Graduate Research Assistant, Ph.D. Student

List the participant's responsibilities on the protocol.

Dosing of rabbits with morphine, fentanyl, and/or saline, calcein, as well as the euthanasia solution.
Dissection of rabbits to procure femora and tibiae for micro-CT and confocal imaging.
Data collection and analysis.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☐ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Previous work in the UA vivarium using zebrafish under Dr. Qin Liu (IACUC # 15-07-08-LFD) for my Master's thesis including animal handling and feeding, anaesthesia, surgery to crush the optic nerve, euthanasia, and subsequent removal of brains (2 yrs.)

Training for rabbit handling will be completed at NEOMED as well as the CITI training . NEOMED-specific training with Dr. Dannemiller will include rabbit handling, subQ and IV injections, and restraint.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
CITI Program: Investigators, Staff, and Students	Online (U Akron)	8/24/2016
CITI Program: Working with Zebrafish (Danio rerio) in Research Settings	Online (U Akron)	8/24/2016
NEOMED-specific rabbit training	NEOMED	TBD

Reed Davis
PARTICIPANT SIGNATURE

12-10-18
DATE

Consortial protocol 0114.doc Version 01/14

Commented [IACUC65]: A completed and signed copy of this form for each person listed or working on the protocol must be submitted to the IACUC coordinator before IACUC approval of the protocol can be granted. Six copies of this page are included in this "Request to Use Animals" form. If more than four persons will work on the project, then additional copies of the page are available in the document identified with the file name "Participant qualifications 2".

Commented [IACUC66]: For example, Principal Investigator, Co-Investigator, Graduate Student, Technical Staff, or other position title or description.

Commented [IACUC67]: For example, anesthesia, behavioral testing, breeding, euthanasia, post-operative care, surgery.

Commented [IACUC68]: The experience/qualifications must be specific to the activities that the individual will perform (e.g., surgical implantation of microdialysis probes, rat anesthesia, mouse euthanasia, rat ovariectomy). A reference to the number of years of experience with the procedures and/or number of procedures performed or the person who provided the training can be used. For those individuals who do not have the necessary training or experience, it is acceptable to indicate that they will be trained by the experienced staff listed on the project, by the attending veterinarian, or by members of the animal facility staff who are experienced in the procedure. For teaching activities, only the training and experience of the instructors must be provided. It is not necessary to describe it for the students or trainee participants in the course.

Commented [IACUC69]: The experience/qualifications should be specific to the activities that the individual will perform (e.g., surgical implantation of microdialysis probes, rat anesthesia, mouse euthanasia, rat ovariectomy). A reference to the number of years of experience with and/or number of procedures performed or the person who provided the training is helpful. For those individuals who do not have the necessary training or experience, it is acceptable to indicate that they will be trained by the experienced staff listed on the project, by the attending veterinarian, or by members of the animal facility staff who are experienced in the procedure. For teaching activities, only the training and experience of the instructors must be provided. It is not necessary to describe it for the students or trainee participants in the course.

Commented [IACUC70]: List any formal course work taken or presentations attended relevant to the use of animals in research, teaching, or testing. Include the date and location. If the participant has had none, enter "None". For those who have more than three entries, enter only the three most recent or relevant activities AND include any that are required by the institution. Requirements for formal training vary across the consortium. Contact the IACUC Coordinator at the institution to which the protocol will be submitted with questions concerning specific requirements.

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Category: C Special Considerations: USDA SPECIES Protocol #: 18-11-12 ARC

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Adam Schuller

TITLE: Undergraduate Research Assistant

List the participant's responsibilities on the protocol.

To aid with care and husbandry management of the rabbits prior to and while receiving test article administration. Facilitate dosing via SubQ injection of experimental agent or vehicle control. Assist with restraint of rabbits and delivery of euthanizing agent at end of dosing period, in compliance with all guidelines regarding humane care and use of animals as applied in this experimental protocol.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☐ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Three years of formal comparative medicine research including the use of mice, rats, naked mole rats, rabbits, and dogs. He has undergone CITI training and has 6 additioanal months experience at a GLP pre-clinical safety assessment sight. The participant additionally has raised rabbits for 10 years commercially and has extensive experience handling them as demonstrated by a rabbit judging licensure.

Will undergo facility protocol training and CITI Renewal in conjunction with the CMU at NEOMED. NEOMED-specific training with Dr. Dannemiller will include rabbit handling, subQ and IV injections, and restraint.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
CITI Training: Aseptic Technique	NEOMED	01March2016
CITI Training: Reducing Pain in Lab Mice and Rats, Working with Mice and Rats in Research, and Working with the IACUC	NEOMED	31May2016
NEOMED-specific rabbit training	NEOMED	TBD

Adam Schuller
PARTICIPANT SIGNATURE

12-10-2018
DATE

Commented [IACUC71]: A completed and signed copy of this form for each person listed or working on the protocol must be submitted to the IACUC coordinator before IACUC approval of the protocol can be granted. Six copies of this page are included in this "Request to Use Animals" form. If more than four persons will work on the project, then additional copies of the page are available in the document identified with the file name "Participant qualifications 2".

Commented [IACUC72]: For example, Principal Investigator, Co-Investigator, Graduate Student, Technical Staff, or other position title or description.

Commented [IACUC73]: For example, anesthesia, behavioral testing, breeding, euthanasia, post-operative care, surgery.

Commented [IACUC74]: The experience/qualifications must be specific to the activities that the individual will perform (e.g., surgical implantation of microdialysis probes, rat anesthesia, mouse euthanasia, rat ovariectomy). A reference to the number of years of experience with the procedures and/or number of procedures performed or the person who provided the training can be used. For those individuals who do not have the necessary training or experience, it is acceptable to indicate that they will be trained by the experienced staff listed on the project, by the attending veterinarian, or by members of the animal facility staff who are experienced in the procedure. For teaching activities, only the training and experience of the instructors must be provided. It is not necessary to describe it for the students or trainee participants in the course.

Commented [IACUC75]: The experience/qualifications should be specific to the activities that the individual will perform (e.g., surgical implantation of microdialysis probes, rat anesthesia, mouse euthanasia, rat ovariectomy). A reference to the number of years of experience with and/or number of procedures performed or the person who provided the training is helpful. For those individuals who do not have the necessary training or experience, it is acceptable to indicate that they will be trained by the experienced staff listed on the project, by the attending veterinarian, or by members of the animal facility staff who are experienced in the procedure. For teaching activities, only the training and experience of the instructors must be provided. It is not necessary to describe it for the students or trainee participants in the course.

Commented [IACUC76]: List any formal course work taken or presentations attended relevant to the use of animals in research, teaching, or testing. Include the date and location. If the participant has had none, enter "None". For those who have more than three entries, enter only the three most recent or relevant activities AND include any that are required by the institution. Requirements for formal training vary across the consortium. Contact the IACUC Coordinator at the institution to which the protocol will be submitted with questions concerning specific requirements.

Northeast Ohio MEDICAL UNIVERSITY

Appendix II: NEOMED Training Confirmation

Form # CMU.001.00
Effective Date: 09/30/11

MEETING ATTENDANCE LIST

Date of Meeting:	03/11/19	**Duration:**	1.00 Hours
Title/Subject:	Rabbit Training		
Trainer (Print Name):	Dr. Stanley Dannemiller	**Organization:**	NEOMED-CMU (for The University of Akron)
Description of Training:	• Handling and Restraint • Injection Sites and Performing Injections		

List of Attendees

List names of all persons attending meeting.
Cross out all unused lines.

Print Name	Institution	Signature/Date
Reed Davis	Akron U	[signature] 3/11/19
Adam Schuller	Akron U	[signature] 3/11/19
Gina Tubo	Akron U	[signature] 3/11/19
Abigail LaMarca	Akron U	[signature] 3/11/19
Janna Andronowski	Akron U	[signature] 3/11/19
Mary Cole	Akron U	[signature] 3/11/19

Management Signature/Date: [signature] 3-11-19

Appendix III: Covance Rabbit Order Receipt

COVANCE
SOLUTIONS MADE REAL

Covance Research Products Inc.
310 Swamp Bridge Road
Denver PA 17517
Tax ID: 23-1886521

INVOICE

Invoice:	**1080076475**
Invoice Date:	**Apr/18/2019**
Payment Terms:	**NET 30**
Due Date:	**May/18/2019**
Page:	**1 of 1**
Billing Currency:	**USD**
Order Number:	**RB00018057**
Ship Date:	**Apr/12/2019**

Bill To: 1007320
UNIVERSITY OF AKRON
ATTN: CHARLOTTE LABELLE
302 BUCHTEL COMMON
AKRON OH 44325-6214
UNITED STATES

Ship To: 1007320
UNIVERSITY OF AKRON
CONTACT: CHARLOTTE LABELLE
235 CARROLL ST
AKRON OH 44325
UNITED STATES

Shipping Information:	CRP TRUCK
Customer PO:	CREDIT CARD
Billing Contact Name:	Jill Garant
Billing Inquiry Phone:	608-310-2957

Original Invoice

Line	Quantity	Unit Amt	Gross Amount	Net Amount
1	21.00	267.67	5,621.07	5,621.07
NZ-5060MM		5.0-6.0 mos-Male-NZW		
21 Male NZW Rabbits DOB Lot 10/20/18 tattoos provided by client write DOB on container and provide a neat D DOB and WT list with shipment				
2	21.00	0.00	0.00	0.00
GEL		Gelled Water		
3	21.00	11.51	241.71	241.71
TATTOO-RB		Tattoo-Rabbit		
4	21.00	18.76	393.96	393.96
BOX-RABBIT-SPACE		Rabbit Shipping Space		
5	1.00	529.36	529.36	529.36
ODS		Shipping Charge-Truck		

Total Pretax Amount:	6,786.10	
Amount Due:	**6,786.10**	**USD**

Invoice Notes
Morning Delivery -Deliver 4/16/19, Beth Kenaga 330-972-5845, Quote 00063365-2, PO 181112, CREDIT CARD Ending (0480)

Order Entered By: Gitke,Scott John

Please remit with invoice number to:

Primary Remittance Address:
Covance Research Products Inc.
PO Box 2485
Burlington, NC 27216

Wire Account Details:
Wells Fargo Bank N.A.
ABA. No. 121000248
Acct. No. 4244842209

Courier Address:
LabCorp - Covance
Lockbox Operations
1225 Jay Lane
Graham, NC 27253

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

Appendix IV: Revised Project Timeline and Expected Milestones

Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model

Plan | Ongoing | Milestone | Completed | Mandatory Shutdown

		Planned Start (mm/yy)	Planned Duration (# quarters)	QUARTER - 3 months Start Date (01/2019)											
				Year 1				Year 2				Year 3			
				1	2	3	4	5	6	7	8	9	10	11	12
Activity 1: Ethics application for IACUC, animal handling training, animal ordering and acclimation															
1.1	Prepare ethics application for the University of Akron IACUC; await approval	01/19	1												
1.2	Order project supplies, develop database, refine data collection protocols	02/19	1												
1.3	Animal ethics and handling training at UARV for all project members	02/19	1												
1.4	Order and shipping of New Zealand White Rabbits from Charles River Laboratories	03/19	1												
1.5	Rabbit acclimation period at University of Akron Vivarium (UARV); animals prepared for Activity 2	04/19	1												
Activity 2: Animal dosing															
2.1	Rabbits will be dosed every other day with either saline (control), morphine, or fentanyl at a level of 1.5/mg/kg/day	05/19	1												
2.2	Calcein, a fluorochrome labeler, will be administered to all animals after two, four, six, and eight weeks	05/19	1												
2.3	Submit semi-annual report to DOJ	06/19	1												
2.4	Euthanasia of animals by intravenous injection of Euthanyl	07/19	1												
Activity 3: Micro-CT imaging and histomorphometry															
3.1	Dissection of rabbit femora and fixation for imaging and histomorphometry	08/19	1												
3.2	Micro-CT imaging will be executed and completed at the University of Akron National Polymer Innovation Center	09/19	3												

3.3	Submit semi-annual report to DOJ	12/19	1													
3.4	Bone thin-sections will be prepared for dynamic histomorphometry in Dr. Andronowski's laboratory	06/20	1													
Activity 4: Data analysis																
4.1	Reconstructing and processing micro-CT datasets	02/20	2													
4.2	Imaging of prepared thin-sections	07/20	2													
4.3	Analysis of histomorphometric variables from prepared thin-sections	01/21	2													
4.4	Statistical analysis of data	05/21	2													
4.5	Submit semi-annual report to DOJ	07/20	1													
Activity 5: Manuscript preparation and knowledge dissemination																
5.1	Preparation of manuscript(s) for submission to peer-reviewed journals	07/20	5													
5.2	Presentation of results at national and international conferences	07/21	1													
5.3	Submit data to the NACJD	08/21	1													
Activity 6: Project Management																
6.1	Project Management (Andronowski)	01/19	11													

THE UNIVERSITY OF AKRON

MEMORANDUM

TO: Janna Andronowski

CC: IACUC@uakron.edu

FROM: Beth Kenaga, IACUC Administrator

DATE: February 7, 2019

SUBJECT: APPROVAL NOTICE FOR MR – Change rabbit housing
Protocol: 18-11-12 ARC
Titled: Longitudinal effects of prolonged opioid use on cortical bone remodeling in a rabbit model

Your significant modification to the above protocol was reviewed and approved by The University of Akron's Institutional Animal Care and Use Committee (IACUC) designated member review process on **February 5, 2019.**

A copy of this form will be filed with your protocol in the Research Office. If you have any questions, please contact Beth Kenaga, IACAUC Administrator, at 330-972-5845 or via email at bkenaga@uakron.edu.

Beth Kenaga

Beth A Kenaga

IACUC Administrator

The University of Akron operates under the Public Health Service (PHS) Assurance number A3870-01.

For IACUC Use Only		
Category: C	Special Considerations: Change housing	Protocol Number: 18-11-12 ARC

MODIFICATION - REQUEST TO USE ANIMALS

Protocol Number: 18-11-12 ARC

Protocol Title: Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model

1. ADDITIONAL ANIMALS **N/A:**

Indicate the number of additional animals that will be needed & provide a justification for the request. If no new animals are requested, then mark N/A and move to Section 2.

2. PROCEDURAL ADDITIONS or MODIFICATIONS **N/A:**

Describe any proposed procedural changes or additions involving living animals & include a justification for the change. If none are proposed, then mark N/A and move to Section 3.

Rabbit Housing:

The rabbits will be individually housed in rabbit batteries (with 5 ft^2 floor space by 16" height cages) alongside one another which will allow them to visualize other rabbits, while keeping the animals lodged separately to prevent potential aggressive encounters. The rabbits will further be habituated to the testing conditions for two weeks following their arrival at the University of Akron Research Vivarium (UARV).

Exercise in floor-pens will be encouraged daily for all animals. This will allow for normal postural changes (sitting on hind legs, stretching, running), and provide additional socialization for the rabbits. Enrichment items in the pen will include food, novel objects to gnaw, toss, nudge, and carry (huts, dumbbells, Pex pipe, jingle balls, flexi-keys, etc.). Rabbits will be tattooed and cage cards will be transferred with the animal for identification purposes. When bedding is used it will be changed out after all rabbits are returned to their home cages.

2.A. Describe the training/experience of each protocol participant as it relates to the new procedure(s) listed above.

All research team members will be trained in proper rabbit handling/transportation techniques to safely remove/return rabbits from their cages, and transport them to and from the exercise pens.

To remove/return the rabbits to the cage, the scruff of the neck will be firmly grasped with one hand, while the hindquarters are supported with the other hand.

To transport the rabbits, the scruff of the neck will be firmly grasped with one hand, while placing the head under the arm/elbow (ensuring that breathing is not impeded). The rabbit's hindquarters will be supported with the other hand.

2. B. Postprocedural care and monitoring:

If the Modification Request includes any procedural additions or modifications, then complete the following.

1) Describe the post-procedural care and monitoring for both surgical (after recovery from anesthesia) and nonsurgical procedures. Identify the parameters being monitored and the frequency and duration of monitoring for each study related procedure. Include how records of the care will be maintained and their location.

Time and frequency in the exercise pen will not interfere with the proposed protocol procedures and will be encouraged daily.

Animals will be monitored once daily (including weekends/holidays) by the PI and designated project staff. The rabbits will be housed individually and receive enrichment toys in UARVs suite 212 with continuous radio play during daylight hours to provide environmental stimulation. During daily acclimatization visits and injection schedules, each rabbit will receive positive reinforcement through food items such as high fiber rabbit chow and additional food (greens, carrots, shredded wheat, raisins, alfalfa, etc.). The research team will create and post a behavioral sheet in the rabbit room to track observed behaviors during both the acclimation and experimental periods. Each animal will have their own behavioral/observation sheet to note information during surveillance checks.

2) Identify by title who will conduct the care and monitoring.

Principal Investigator, Graduate student, Undergraduate student, Post-doctoral Fellow.

3) List any analgesics or other medically related pharmaceutical agents that animals may receive. Include **a)** dose, **b)** route of administration **c)** frequency of administration, and **d)** duration of therapy.

The opioid groups (morphine and fentanyl) and controls will each be dosed by subcutaneous injection every other day for 8 weeks. The morphine hydrochloride group will receive a dose of 3 mg/kg/day, and the fentanyl group will receive 0.5 mcg/kg/day. The control group will be administered saline at a dose of 3 mg/kg/day. The proposed opioid dosing levels are consistent with clinical recommendations for analgesia in rabbits and were finalized with the former UARV Attending Veterinarian.

4) List the criteria that will be used to determine that relief from pain or distress is needed and how the adequacy of that relief will be assessed. N/A

5) List the humane endpoints that will be used to euthanize an animal or otherwise remove an animal from a study.

Weight loss (of 20% or more) due to opioid injections, changes in fecal production, which can be lethal, especially if it decreases or stops completely as this is an indication of gastrointestinal stasis. If this was to occur, the animal would be placed on a hand fed diet and/or given GI motility enhancing drugs based on the recommendation of the Attending Veterinarian until the stasis cleared. Other signs of distress in any animal such as hair loss, lethargy, hyperactivity, excessive or there-lack of grooming would be closely monitored in accordance with the UARV Manager and Attending Veterinarian.

2.C. *According to the NIH Grants and Policy Statement, Part II, section 8.1.2.5., NIH grantees must obtain prior approval from the NIH awarding Institute or Center for animal use protocol*

modifications that result in a change in scope of a funded project. Potential indicators of a change in scope can be viewed at:
http://grants.nih.gov/grants/policy/nihgps_2010/nihgps_ch8.htm

Will the new procedure(s) described above change the scope of the funded project? YES: NO:
If YES, please contact your Office of Research and Sponsored Programs.

2.D. Chemical/compound administration to live animals N/A
If the Modification Request involves the administration of any chemicals to animals that were not described in the original protocol, then complete the following.

Are all of the chemicals (e.g., test compounds, receptor agonists/antagonists, labeling compounds, anesthetics, analgesics, euthanasia agents, etc.) administered to live animals commercially available pharmaceutical preparations intended for animal or human use?
Yes: No:

If not, then complete the following for each product.
Identify the chemical/compound and describe how it is prepared and stored to assure appropriate purity, sterility and suitability for administration to animals. Indicate the shelf life of the prepared product.

*Are all of the chemicals/compounds listed above pharmaceutical grade?***Yes: No:**
If not, then list them and provide a justification for not using a pharmaceutical grade preparation.

3. New or revised pain/distress classification N/A:
For any protocol modification that includes a new or revised pain/distress producing procedure, place an "X" in front of the appropriate category(ies) and identify the procedure(s). Otherwise mark N/A and move to Section 3.

☐ - **Category C** - Procedures that involve no more than momentary or slight pain or distress.
List procedures:

Number of animals in category C:

☐ - **Category D** - Procedures that may cause more than momentary or slight pain or distress for which appropriate analgesia, anesthesia or tranquilization is provided.

List procedures:

Number of animals in category D:

☐ - **Category E** - Procedures that may cause pain or distress which are not relieved by analgesia, anesthesia, or tranquilization.
List procedures:

Number of animals in category E:

For Category E procedures: Provide a detailed scientific justification for withholding analgesia, anesthesia, and tranquilization.

4. ALTERNATIVES TO THE USE OF ANIMALS AND PAIN OR DISTRESS PRODUCING PROCEDURES N/A

Provide a written narrative description of the methods and sources that were used to determine that suitable alternatives to the use of animals and to the pain or distress producing procedures described in the protocol are not available. Provide an explanation for alternatives that were identified but deemed unsuitable. Literature searches must include a) databases searched, b) the date of the search, c) the years covered by the search (minimum 10 years), and d) the search strategy including keywords used. At least two acceptable information sources must be used. The response must address the three R's: Replacement models, Refinements in technique, and Reduction in animal numbers. Information sources that are commonly used include http://www.pubmed.gov, http://agricola.nal.usda.gov, http://www.nal.usda.gov/awic, and specifically for teaching activities, http://oslovet.veths.no.

5. OTHER CHANGES **N/A:**

Describe any other proposed changes to the protocol & include a justification for the change.

For IACUC Use Only
Category: C Special Considerations: Change housing Protocol Number: 18-11-12 ARC

For IACUC Use Only
Category: C Special Considerations: Change housing Protocol Number: 18-11-12 ARC

4. MODIFICATION APPROVAL

Select the institution to which the Modification Request will be submitted from the drop down menu below.

Approval of the protocol modification is indicated by the signatures of the institution-specific individuals identified below. The individuals signing confirm that they have reviewed the modification and find it to be in compliance with applicable animal care and use regulations and institutional policies.

University of Akron

Approval Signatures:

_____ Date _____
Facility Director

_____ Date _____
IACUC Member

Tom Connerville Date 2-14-19
Attending Veterinarian

Date 3/20/19
IACUC Chairperson

For IACUC Use Only
Category: C Special Considerations: Change housing Protocol Number: 18-11-12 ARC

INVESTIGATOR SIGNATURE

I request the above described modifications to my previously approved "Request to Use Animals". I acknowledge that all assurances listed in the original "Request to Use Animals" remain in effect.

Principal Investigator:

Name: Janna M. Andronowski

Signature JAndronowski Date: 01/23/19

OR

Co-Investigator:

Name:

Signature _____ Date: _____

THE UNIVERSITY OF AKRON

MEMORANDUM

TO: Janna Andronowski

CC: IACUC@uakron.edu

FROM: Beth Kenaga, IACUC Administrator

DATE: April 7, 2019

SUBJECT: APPROVAL NOTICE OF VVC MODIFICATION – ADD Fentanyl Patches
Protocol: 18-11-12 ARC
Titled: Longitudinal effect of prolonged opioid use on cortical bone remodeling in a ribbit model

Your modification to the above protocol was reviewed and approved by The University of Akron's Institutional Animal Care and Use Committee (IACUC) VVC review process on **April 5, 2019.**

A copy of this form will be filed with your protocol in the Research Office. If you have any questions, please contact Beth Kenaga, IACAUC Administrator, at 330-972-5845 or via email at bkenaga@uakron.edu.

Beth Kenaga

IACUC Administrator

The University of Akron operates under the Public Health Service (PHS) Assurance number A3870-01.

VVC MODIFICATION - REQUEST TO USE ANIMALS

Date of Veterinary Consultation: 4/5/2019

Protocol Number: #18-11-12 ARC

Protocol Title: Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model

1. CHANGES IN DURATION, FREQUENCY, TYPE, OR NUMBER OF PROCEDURES PERFORMED ON AN ANIMAL **N/A:**

Describe any proposed procedural changes or additions involving living animals & include a justification for the change. If none are proposed, then mark N/A and move to next section.

1A. Describe the training/experience of each protocol participant as it relates to the new procedure(s) listed above. N/A

2. CHANGES IN ANESTHESIA, ANALGESIA, SEDATION OR EXPERIMENTAL SUBSTANCES **N/A:**

Describe any proposed procedural changes or additions involving living animals & include a justification for the change. If none are proposed, then mark N/A and move to next section.

The proposed change will affect the method of delivery of the experimental pharmacologic agents (morphine and fentanyl). According to literature published by Foley et al. (2001) and Jain et al. (2018) there is demonstrable evidence that transdermal patch delivery of fentanyl resulted in a detectable change in bone metrics, which we are seeking to measure. We have proposed this modification to eliminate the lack of consensus in recommended fentanyl dosing from various professional consultants, and to reduce risk of accidental exposure during administration of the narcotics. Further, this change will address the concerns of the IACUC regarding injections being handled by trainees (undergraduate and graduate students) for the more dangerous of the two pharmacologic agents while the Principal Investigator is attending conferences, or if she should be sick, or otherwise unable to attend dosing periodically throughout the duration of the study.

The transdermal patch administration will also reduce the need to dose the animals every other day and will instead act as a slow-release delivery agent and allow for the discarding of the patches and novel administration every third day.

Hair will be removed via clipping prior to the application of the transdermal patches. The rabbits will be contained in a European style rat housing tub with a pad on the flooring to prevent the rabbits from slipping. This will prevent injury to rabbit or experimenter while performing the clipping, which will take place against the way the fur normally lays. A 25-ug/h slow release fentanyl patch will be placed over the intrascapular region. on the animals in the experimental groups. This size patch and specific dosage was chosen based on data obtained by Foley et al. (2001) and Jain et al. (2018). The patches will be adhered via adhesives associated with the patch manufacturing. Patches will further be secured by medical-grade adhesive tape (Tegaderm) around the patch edges. A placebo patch will be placed on the control animals with an adhesive and medical-grade tape comparable to the patches applied to the experimental animals. All animals will be checked daily for any skin irritation associated with hair clipping/patch and tape adhesives. The research team will also ensure the patches are adhering to the animals daily. If a patch is coming loose, new medical-grade tape will be applied to secure any loose edges. In the event that there is a patch discovered to have come completely loose from one of the rabbits, UARV staff should alert Dr. Andronowski or someone listed as a point of contact for the study from her lab group so that the deviation from the protocol can be logged and a new patch can be administered. If the patch is within a distance which may be reached by the rabbit, such as on the cage floor, the UARV staff may remove this from the cage wearing gloves and place in a location away from the rabbits where this event can be documented by the research team who will dispose of the patch accordingly.

No fewer than two researchers will be present when dosing the rabbits in order to prevent any risk of misuse of the narcotics. In the unlikely event that only one researcher is available at a dosing time due to illness or unforseen circumstance, they must be supervised by either the PI or Post-doctoral Fellow when dosing the animals. There will be a log book associated with each drug and doses/wastes (e.g., folding of a patch) will be recorded as a form of inventory control. These log sheets will be routinely inspected by the PI and/or UARV supervisor.

The researchers will wear cotton lab coats (to provide an extra layer of protection from scratches), disposable plastic gowns, and cut-proof gloves when handling the rabbits as well as wearing examination gloves while applying/disposing of the patches.

Patches will be disposed of in an approved biohazard container immediately after dosing or if there is premature loss of adhesive property during the dosing period.

The secondary proposed change will be the return of the original route of administration for the morphine treatment. After extensive review of the literature and exhaustive search of pharmaceutical ordering companies, there does not appear to be a commercially available slow-release patch for the delivery of morphine. The return to this dosing method would mean dosing by subcutaneous injection in the tent of the skin located between the shoulder blades every other day for 8 weeks. The morphine sulfate group will receive a dose of 3mg/kg/day and the control group will experience a saline dose of 3mg/kg/day. The morphine agent will be pharmaceutical grade.

Two individuals from the lab team will be tasked with dosing by injection every second day using safe manual restraint and a laboratory table which will provide support. Those individuals handling the pharmacologic agent will wear cut-resistant gloves during injections as a secondary safety precaution. The proposed dosing level is consistent with clinical recommendations and this was finalized with the former UARV veterinarian, Dr. Walter Horne.

3. CHANGES IN METHODS OF EUTHANASIA **N/A:**

Describe any proposed procedural changes or additions involving living animals & include a justification for the change. If none are proposed, then mark N/A and move to next section.

To clarify the protocol, the secondary means of confirmation of successful euthanasia event shall be with the use of bilateral thoracotomy. This will be performed after the test for remaining consciousness with toe/tail pinches to observe any flinch reflex reaction as well as after the primary means of euthanasia (administration of Fatal-Plus or equivalent) has been successfully completed as described in the original protocol.

4. CHEMICAL/COMPOUND ADMINISTRATION TO LIVE ANIMALS

If the Modification Request involves the administration of any chemicals to animals that were not described in the original protocol, then complete the following.

Are all of the chemicals (e.g., test compounds, receptor agonists/antagonists, labeling compounds, anesthetics, analgesics, euthanasia agents, etc.) administered to live animals commercially available pharmaceutical preparations intended for animal or human use?

Yes: **No:**

If not, then complete the following for each product.
Identify the chemical/compound and describe how it is prepared and stored to assure appropriate purity, sterility and suitability for administration to animals. Indicate the shelf life of the prepared product.

N/A

Are all of the chemicals/compounds listed above pharmaceutical grade? **Yes:** **No:**
If not, then list them and provide a justification for not using a pharmaceutical grade preparation.

N/A

5. VVC MODIFICATION APPROVAL

Approval of the protocol modification is indicated by the signature of the institution-specific individual identified below. The individual signing confirms that he/she has reviewed the modification and finds it to be in compliance with applicable animal care and use regulations and institutional policies.

Approval Signature:

*See Attachment Date 4/5/2019

Attending Veterinarian

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

INVESTIGATOR SIGNATURE

I request the above described modifications to my previously approved "Request to Use Animals". I acknowledge that all assurances listed in the original "Request to Use Animals" remain in effect.

Principal Investigator:

Name: Janna M. Andronowski

Signature _____ Date: _____

OR

Co-Investigator:

Name:

Signature _____ Date: _____

Kenaga,Beth A

From: Stanley Dannemiller <sdannemiller@neomed.edu>
Sent: Friday, April 5, 2019 12:03 PM
To: Kenaga,Beth A; Andronowski,Janna Michelle
Subject: RE: Revised VVC and issues with morphine ordering

Hi Beth & Janna:

I have reviewed the VVC modification for protocol 18-11-12 ARC and approve it as re-submitted.

Stan

From: Kenaga,Beth A <bkenaga@uakron.edu>
Sent: Friday, April 5, 2019 11:45 AM
To: Stanley Dannemiller <sdannemiller@neomed.edu>
Subject: FW: Revised VVC and issues with morphine ordering

fyi

From: Andronowski,Janna Michelle
Sent: Thursday, April 4, 2019 3:39 PM
To: 'Stanley Dannemiller' <sdannemiller@neomed.edu>
Cc: Adam Schuller <ajs289@zips.uakron.edu>; rad115@zips.uakron.edu; Kenaga,Beth A <bkenaga@uakron.edu>; Gina Tubo <grt13@zips.uakron.edu>
Subject: Revised VVC and issues with morphine ordering

Hi Stan,

I wanted to reach out with some updates as we have been having issues relating to the drug ordering for morphine.

Firstly, after extensive review of the literature and calling/corresponding with numerous pharmaceutical companies, there does not appear to be a commercially available slow-release patch for the delivery of morphine. As such, we have revised the VVC to reflect the original drug administration plan. The return to this dosing method would mean dosing by subcutaneous injection in the tent of the skin located between the shoulder blades every other day for the 8 weeks. Can you review the attached VVC and let me know if you have suggestions/amendments to make?

MEMORANDUM

Appendix VII: VVC for Jacketing

TO: Janna Andronowski

CC: IACUC@uakron.edu

FROM: Beth Kenaga, IACUC Administrator

DATE: May 29, 2019

SUBJECT: APPROVAL NOTICE OF VVC MODIFICATION – Rabbit jackets/covers
Protocol: 18-11-12 ARC
Titled: Longitudinal effects of prolonged opioid use on cortical bone remodeling in a rabbit model

Your modification to the above protocol was reviewed and approved by The University of Akron's Institutional Animal Care and Use Committee (IACUC) VVC review process on **May 23, 2019.**

A copy of this form will be filed with your protocol in the Research Office. If you have any questions, please contact Beth Kenaga, IACAUC Administrator, at 330-972-5845 or via email at bkenaga@uakron.edu.

Beth Kenaga

Beth A Kenaga

IACUC Administrator

Date of Veterinary Consultation: 05/03/2019

Protocol Number: #18-11-12 ARC

Protocol Title: Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model

1. **CHANGES IN DURATION, FREQUENCY, TYPE, OR NUMBER OF PROCEDURES PERFORMED ON AN ANIMAL** **N/A:**
Describe any proposed procedural changes or additions involving living animals & include a justification for the change. If none are proposed, then mark N/A and move to next section.

The proposed modification will attempt to cover the drug eluting transdermal patches (fentanyl) and control patches (Tegaderm) to prevent the animals from chewing, removing, and/or ingesting these from the subscapular region. Following consultation with the primary veterinarian, Dr. Stan Dannemiller, he provided a prescription via email on 05/03/2019 that stated:

'This e-mail is to document my prescription of the use of jackets and other wraps in an attempt to cover the drug eluting patches for Dr. Andronowski's rabbit study. Methods that may be used to keep the patches on the rabbits include the following:

- Jackets
- Vet wrap
- Rabbit booties (made from human baby socks) for the rear feet that maybe taped on or held on with vet wrap
- Elizabethan collars
- Neck towel rolls

Please document in the individual rabbit record any of the above, or other, methodologies used to keep the rabbits from dislodging the adhesive patches. Different methodologies may be needed for different rabbits. This prescription is good until Dr. Andronowski is able to determine what works best for this study and submit a protocol amendment to the IACUC for their evaluation and approval.

Stan Dannemiller, DVM, MS, DACLAM
Attending Veterinarian, University of Akron'

After further conversations with Dr. Dannemiller and Michelle Chapman, the Andronowski research team were loaned rabbit jackets from Dr. Dannemiller at NEOMED and purchased additional jackets from the vendor (Lomir) for placement on the experimental and control patch rabbits. For the majority of rabbits, the jackets work well to prevent dislodging/removal of the patches. However, rabbit #2 (fentanyl group) consistently chews his jackets, resulting in regular (every second day) jacket changes. He has also chewed his fur around the forelimb openings and was able to remove the patch from beneath the jacket (without removing the jacket itself). We further applied a vet wrap in attempt to keep the patch on, but he is able to shift the jacket with [illegible]

collar for this animal. We ask that we can use this methodology, and explore other possible methodologies, for other rabbits who may become sufficient at removing their treatment patches in the future. Thus, our research team would like to request formal permission from the IACUC to explore additional methodologies (such as those mentioned in Dr. Dannemiller's email above) for keeping the patches secure on the animals, in case other issues arise during the remainder of the study and the jackets/collars become problematic.

As such, the proposed modification of rabbit jackets on all treatment and control transdermal patch animals and collars on rabbit #2/future patch removing animals, will reduce the likelihood of these rabbits chewing, removing, and/or ingesting their patches. This change will also address the concerns of the Andronowski research team and UARV staff pertaining to fentanyl patch ingestion, which we fear will harm rabbit #2, specifically.

1A. Describe the training/experience of each protocol participant as it relates to the new procedure(s) listed above.

No fewer than two researchers will be present when placing or changing the rabbit jackets or collar, in order to safely secure the animal during jacket/collar placement. In the unlikely event that only one researcher is available due to illness or unforseen circumstance, they must be supervised by either the PI or Post-doctoral Fellow.

The researchers will wear cotton lab coats (to provide an extra layer of protection from scratches), disposable plastic gowns, and cut-proof gloves when handling the rabbits as well as wearing examination gloves while handling the rabbits.

SUBSTANCES **N/A:**

Describe any proposed procedural changes or additions involving living animals & include a justification for the change. If none are proposed, then mark N/A and move to next section.

3. **CHANGES IN METHODS OF EUTHANASIA** **N/A:**

Describe any proposed procedural changes or additions involving living animals & include a justification for the change. If none are proposed, then mark N/A and move to next section.

4. CHEMICAL/COMPOUND ADMINISTRATION TO LIVE ANIMALS

If the Modification Request involves the administration of any chemicals to animals that were not described in the original protocol, then complete the following.

Are all of the chemicals (e.g., test compounds, receptor agonists/antagonists, labeling compounds, anesthetics, analgesics, euthanasia agents, etc.) administered to live animals commercially available pharmaceutical preparations intended for animal or human use?

Yes: **No:**

If not, then complete the following for each product.

Identify the chemical/compound and describe how it is prepared and stored to assure appropriate purity, sterility and suitability for administration to animals. Indicate the shelf life of the prepared product.

N/A

5. VVC MODIFICATION APPROVAL

Approval of the protocol modification is indicated by the signature of the institution-specific individual identified below. The individual signing confirms that he/she has reviewed the modification and finds it to be in compliance with applicable animal care and use regulations and institutional policies.

Approval Signature:

[signature], DVM

Attending Veterinarian

Date 5-23-2019

INVESTIGATOR SIGNATURE

I request the above described modifications to my previously approved "Request to Use Animals". I acknowledge that all assurances listed in the original "Request to Use Animals" remain in effect.

Principal Investigator:

Name: Janna M. Andronowski

Signature [signature]

Date: 06/03/2019

OR

Co-Investigator:

Name:

Signature _____

Date: _____

Appendix VIII: Euthanasia
Training Confirmation

From: Stanley Dannemiller <sdannemiller@neomed.edu>
Sent: Friday, June 28, 2019 11:46 AM
To: Andronowski,Janna Michelle <jandronowski@uakron.edu>
Cc: Kenaga,Beth A <bkenaga@uakron.edu>
Subject: Training of Dr. Andronowski and staff for rabbit euthanasia (Protocol #18-11-12 ARC)

Dear Dr Andronowski & Beth:

On June 27, 2019 I performed training for staff listed below on IP injection of Fatal Plus for euthanasia and creation of bilateral pneumothorax to assure death in rabbits on Protocol # 18-11-12 ARC. The rabbits demonstrated no signs of pain or distress when the drug was administered. Staff provided training were:

Janna Andronowski, Ph.D. – PI for protocol
Mary Cole, Ph.D. - Post-doctoral fellow
Reed Davis, - Ph.D. graduate student
Adam Schuller, undergraduate student
Abigail LaMarca, undergraduate student

All of the staff trained demonstrated their capability to perform the IP injection and create a pneumothorax to my satisfaction. Please contact me if there are any questions regarding this training.

Stan

Stanley D. Dannemiller, DVM, MS, DACLAM
Director, Comparative Medicine Unit

Northeast Ohio Medical University
4209 St. Rt. 44 | PO Box 95 | Rootstown, Ohio 44272
v 330.325.6558 | f 330.325.5918 | e sdannemiller@neomed.edu

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

Appendix IX: MMA Embedding SOP

Monday (Day 1) – 70% EtOH on tube rotator
Batch 1: 10 mL/tube x 10 samples = 100 mL of 70% ethanol
Batch 2: 10 mL/tube x 11 samples = 110 mL of 70% ethanol

Tuesday (Day 2) – 95% EtOH on tube rotator
Batch 1: 10 mL/tube x 10 samples = 100 mL
95 mL of 100% ethanol + 5 mL distilled water
Batch 2: 10 mL/tube x 11 samples = 110 mL
104.5 mL of 100% ethanol + 5.5 mL distilled water

Wednesday (Day 3) – 95% EtOH on tube rotator
Batch 1: 10 mL/tube x 10 samples = 100 mL
95 mL of 100% ethanol + 5 mL distilled water
Batch 2: 10 mL/tube x 11 samples = 110 mL
104.5 mL of 100% ethanol + 5.5 mL distilled water

Thursday (Day 4) – 100% EtOH on tube rotator
Batch 1: 10 mL/tube x 10 samples = 100 mL of 100% ethanol
Batch 2: 10 mL/tube x 11 samples = 110 mL of 100% ethanol

Friday (Day 5) – 100% EtOH – Leave Weekend on tube rotator
Batch 1: 10 mL/tube x 10 samples = 100 mL of 100% ethanol
Batch 2: 10 mL/tube x 11 samples = 110 mL of 100% ethanol
Dehydrate all MMA in calcium chloride pellets
Batch 1: 490.8 mL = **491 mL MMA**
245.5 mg Ca = 0.246 g Ca
Batch 2: 539.4 mL = **540 mL MMA**
270 mg Ca = 0.270 g Ca

Monday (Day 6) – 100% EtOH on tube rotator + Make MMA III bases
Batch 1: 10 mL/tube x 10 samples = 100 mL of 100% ethanol
Batch 2: 10 mL/tube x 11 samples = 110 mL of 100% ethanol
Make **MMA I** and set on stirrer overnight (100% MMA and 1% BP)
Batch 1: 15 mL/tube x 10 samples = 150 mL MMA I
150 mL MMA + 1.5 g BP
Batch 2: 15 mL/tube x 11 samples = 165 mL MMA I
165 mL MMA + 1.65 g BP
Make **MMA II** and set on stirrer overnight (96% MMA + 4% DP + 1.5% BP)
Batch 1: 15 mL x 10 samples = 150 mL MMA II
144 mL MMA + 6 mL DP + 2.25 g BP
Batch 2: 15 mL x 10 samples = 165 mL MMA I
158.4 mL MMA + 6.6 mL DP + 2.475 g BP
Make **MMA III** for bases and set on stirrer overnight (96% MMA + 4% DP + 2.5% BP)
Batch 1 Bases: 10 samples at 5 mL per base + extra safety base = 55 mL MMA III + **InFill =** 15 mL x 10 samples = 150 mL MMA II = **205 mL MMA III**

196.8 mL MMA + 8.2 mL DP + 5.125 g BP

Batch 2 Bases: 11 samples at 5 mL per base + extra safety base = 60 mL MMA III + **InFill** = 15 mL x 11 samples = 165 mL MMA I = **225 mL MMA III**

216 mL MMA + 9 mL DP + 5.625 g BP

Tuesday (Day 7) – Make MMA III Bases in film canisters

Pour bases (one per sample + one extra) of 5 mL each of **MMA III**

Vacuum at 25 Hg for 2 hours with foil caps

Cap and put in 37 C waterbath (put in and then turn on waterbath) for two days

Bones remain in ethanol

Wednesday (Day 8) – Put bones in MMA I in film canisters

Drain ethanol and put bones in film canisters with 15 mL each of **MMA I**

Vacuum at 25 Hg for 24 hours with foil caps

MMA III bases remain in waterbath (do not open!)

Thursday (Day 9) – Put bones in MMA II in film canisters

Take **MMA III** bases out of waterbath after 48 hours and set on bench to cure one day

Drain **MMA I** and put bone in film canisters with 15 mL each of **MMA II**

Vacuum at 25 Hg for 24 hours with foil caps

Friday (Day 10): Put bones in MMA III in film canisters on cured bases

Drain **MMA II** and put bones in cured base film canisters with 15 mL each of **MMA III**

Vacuum at 25 Hg for 2 hours with foil caps

Cap and put in 37 C waterbath (put in and then turn on waterbath)

Saturday – Sunday (Days 10 – 11):

MMA III curing in waterbath

Monday (Day 12):

Take **MMA III** out of waterbath and set on bench to cure one day

Tuesday (Day 13):

Samples should be cured and ready for sectioning

osteology

MDPI

Article

Rabbits (*Oryctolagus cuniculus*) as a Model System for Longitudinal Experimental Opioid Treatments: Implications for Orthopedic and Biomedical Research

Janna M. Andronowski [1,*], Adam J. Schuller [2], Mary E. Cole [3], Abigail R. LaMarca [4], Reed A. Davis [4] and Gina R. Tubo [5]

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5 College of Medicine, Northeast Ohio Medical University, 4209 State Route 44, Rootstown, OH 44272, USA; gtubo@neomed.edu
* Correspondence: jandronowski@mun.ca

check for updates

Citation: Andronowski, J M.; Schuller, A.J.; Cole, M.E.; LaMarca, A.R.; Davis, R.A.; Tubo, G.R. Rabbits (*Oryctolagus cuniculus*) as a Model System for Longitudinal Experimental Opioid Treatments: Implications for Orthopedic and Biomedical Research. *Osteology* **2021**, *1*, 225–237. https://doi.org/10.3390/osteology1040021

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Abstract: Due to the high prevalence of opioid prescription following orthopedic procedures, there is a growing need to establish an animal model system to evaluate the effects of opioids on bone remodeling. Rabbits have been employed as model organisms in orthopedic research as they exhibit well-defined cortical bone remodeling similar to humans. Existing research in rabbits has been limited to modes of opioid administration that are short-acting and require repeated application. Here, we present data from a proof-of-principle longitudinal study employing two opioid analgesic administration routes (subcutaneous injection and transdermal patch) to evaluate the efficacy of studying chronic opioid exposure in a rabbit model. Skeletally mature male New Zealand White rabbits (*Oryctolagus cuniculus*) were divided into three groups of seven animals: morphine, fentanyl, and control. Experimental treatments were conducted for eight weeks. Preparation of the skin at the fentanyl patch site and subsequent patch removal presented experimental difficulties including consistent skin erythema. Though noninvasive, the patches further caused acute stress in fentanyl animals. We conclude that though transdermal fentanyl patches may be preferred in an acute clinical setting, this method is not feasible as a means of long-term pain relief or opioid delivery in a laboratory context.

Keywords: transdermal fentanyl patch; opioid; rabbit; analgesia; bone remodeling

1. Introduction

Each year, over 70% of the 500,000 drug-related deaths worldwide are categorized as opioid-related [1]. In the United States, drug overdoses remain the leading cause of death for Americans under age 50 [2] with 21.7 deaths recorded per 100,000 people in 2017 [3]. In 2019, a record-high spike of opioid overdose deaths in the United States was recorded at nearly 50,000, with 73 percent involving synthetic opioids [4].

The prevalence of opioid use is due, in part, to developed dependence following legal prescription. Orthopedic surgeons are the third highest prescribers of opioids in the United States [5]. Orthopedic surgeons further account for an estimated 8.8% of acquired chronic opioid dependence following surgery [5]. In this sector of healthcare, morphine is a common treatment for post-operative pain following total joint arthroplasty [6]. In addition to morphine, transdermal fentanyl application has been shown to provide substantial levels

for chronic pain management. Three meta-analyses demonstrated reduced side effects with transdermal fentanyl compared to sustained release oral-morphine [7–9]. Although efficacious in providing post-operative analgesia, a more recent review highlighted the detrimental effects with prolonged opioid exposure [10]. Pre-operative opioid use has been linked to negative post-operative outcomes, including the need for early total knee arthroplasty revision [11]. Not only is premature revision often required, but pre-operative opioid abuse may further lead to reduced effectiveness of opioid-induced analgesia following total joint arthroplasty [12].

Despite being commonly prescribed following orthopedic procedures, the overall effect of opioids at the cellular level of cortical bone remains understudied. Characterization of subcellular events requires the application of histological techniques that prevent analyses in living patients. This necessitates the use of animal models which mimic human bone remodeling in a controlled, reproducible environment. Previous researchers have explored the effects of opioid administration in small rodents, primarily to elucidate the impact of opioids on central neural pathways [13–16]. Domestic New Zealand White (NZW) rabbits (*Oryctolagus cuniculus*), however, demonstrate the potential to more accurately model human cortical bone dynamics [17]. Comparable to humans, rabbits display spontaneous cortical bone remodeling, whereas smaller laboratory animals (e.g., rodents) retain primary canals throughout their lives and exhibit little to no cortical bone remodeling [17–20]. As such, rabbits have been employed as a model organism in orthopedic research, including post-operative infection [21–23], orthopedic implants [24–27], and joint injury [28–33].

Despite well-documented use of opioids for post-operative pain in orthopedic procedures, and the extensive use of rabbits in orthopedic relevant research, the potential impact of opioid exposure on rabbit behavior and health remains understudied. Previous work has examined the effect of observer presence on rabbit behavior in a post-operative setting using morphine and tramadol hydrochloride as analgesics [34]. Other research has evaluated the use of fentanyl transdermal patch application in post-operative pain management in rabbits [35,36]. These studies, however, do not assess rabbit behavior and the efficacy of the transdermal delivery system over time. To that end, we present data from a longitudinal proof-of-principle study assessing the practicality of transdermal patches for rabbits in long-term opioid exposure experiments. Our overarching objectives were to (1) demonstrate the long-term use of injectable morphine sulfate and transdermal fentanyl patches in a rabbit model system, (2) examine the behavior of rabbits exposed to extended opioid regimens, and (3) test the hypothesis that NZW rabbits are an appropriate animal model system for studying the prolonged effects of opioid exposure on bone turnover for use in orthopedic comparative medicine.

2. Materials and Methods

A detailed animal protocol (18-11-12 ARC) was approved by The University of Akron Institutional Animal Care and Use Committee (IACUC). All research team members completed in-person training with The University of Akron Research Vivarium (UARV) attending veterinarian in proper ethical care, handling, euthanasia, and use of laboratory animals.

2.1. Animals

Skeletally mature, healthy, 6-month-old (2.3–3.0 kg), male NZW rabbits (*Oryctolagus cuniculus*; $n = 21$) were acquired from Covance Research Products Inc. (Denver, PA, USA). Rabbits were individually housed in stainless steel rabbit batteries with perforated plastic floor inserts that allowed for limited visual interaction between animals, while keeping the animals lodged separately. Rabbits were fed Harlan Teklad Global High Fiber Rabbit Diet (Envigo, Madison, WI, USA) (150 g/day) and water was provided ad libitum by way of hard plastic water bottles. Enrichment foods (e.g., spinach, dried fruits, papaya tablets) were provided daily, and enrichment devices (e.g., rattles, jingle balls, flexi-keys) were provided in rabbit batteries and exercise pens and changed weekly. Rabbits were placed in floor-based exercise pens three times weekly for a 45 min period to allow for normal

postural changes (e.g., hindlimb stretching, running). The housing room was maintained at 61 °F to 70 °F (16 °C to 21 °C), at 30% to 70% humidity, and on a 12:12/h light:dark cycle. The rabbits were quarantined and habituated to the testing conditions for a two-week period prior to experimental treatments.

2.2. Experimental Design

Using a random number generator, the rabbits were divided into three groups of seven animals each: morphine, fentanyl, and controls. The control group was further randomly divided into saline vehicle (n = 3) and transdermal sham patch groups (n = 4). These group sizes were based on the mean numbers employed in previous characterizations of this model for cancellous bone or cortical geometry/density [37–41]. After the acclimation period, experimental treatments were applied to control and opioid groups (morphine and fentanyl) for eight weeks. The proposed opioid dosing levels are consistent with clinical recommendations for analgesia in rabbits and were finalized with the UARV veterinarian. Drug dose was calculated based on individual rabbit weight (kg), measured at the start of each week. The morphine sulfate group received a dose of 3 mg/kg/day via subcutaneous bolus injection. The saline vehicle control group was administered saline at a dose of 3 mg/kg/day also via subcutaneous injection.

Transdermal fentanyl patches acted as a slow-release delivery agent and reduced the dosing frequency from daily to every third day. The dorsum of each fentanyl-group rabbit was shaved to remove fur, and a 25 µg/h slow-release transdermal fentanyl patch (Henry Schein Inc., Melville, NY, USA) was placed on the interscapular region, according to the manufacturer's instructions. Patch size and specific dosage were selected based on data obtained by Foley et al. [42] and Jain et al. [35] The patches were affixed via adhesives associated with the patch manufacturing. Patches were further secured by applying a medical-grade Tegaderm™ (3M, Maplewood, MN, USA) transparent film dressing, used in clinical settings to cover IV insertion sites and burns. A 2% isopropyl myristate softening solution was applied to the skin of control patch animals and placebo patches (Tegaderm™ transparent film dressing) [43]. Rabbit jackets (Lomir Biomedical, Malone, NY, USA) were used to cover fentanyl and control transdermal patches (Tegaderm™) to prevent the animals from chewing, removing, or ingesting these from the interscapular region.

2.3. Data Collection

Daily observations were recorded and scored at consistent time points throughout the experiment. These records detailed normalcy, or acute changes, in fecal output, food consumption, appearance, and behavior. All animals were weighed weekly.

2.4. Statistical Analysis

Statistical analyses were performed in R version 3.6.1 (5 July 2019) (The R Foundation for Statistical Computing). To analyze statistical differences between drug treatment groups, fecal output (normal, slightly low, low, very low) and food consumption levels (full, moderate, low) were coded numerically. Jacket use (yes/no) was also coded numerically for use as a covariate. Analysis was restricted to the experimental drug treatment period, following acclimation.

3. Results

Fecal output was numerically coded as Normal = 1, Slightly Low = 2, Low = 3, and Very Low = 4 following comparable fecal output scoring descriptions as outlined in Weaver et al. [44]. Food consumption was numerically coded as Full = 1, Moderate = 2, and Low = 3. Mean fecal output per animal decreased from fentanyl (mean = 1.24, SD = 0.305) to control (mean = 1.26, SD = 0.216) to morphine groups (mean = 1.33, SD = 0.151). Mean food consumption per animal decreased from control (mean = 1.32, SD = 0.204) to fentanyl (mean = 1.49, SD = 0.362) to morphine groups (mean = 1.81, SD = 0.251).

Non-jacketed animals included all morphine group rabbits (n = 7) and saline control group rabbits (n = 3). Non-jacketed animals had lower mean fecal output (mean = 1.33 SD = 0.283) than jacketed animals (mean = 1.23, SD = 0.152). Similarly, non-jacketed animals had lower mean food consumption (mean = 1.68, SD = 0.382) than jacketed animals (mean = 1.41, SD = 0.249). These trends may reflect the lowest fecal output and food consumption of morphine group rabbits, which comprise 70% of non-jacketed animals.

Graphic representation of food consumption and fecal output (Figure 1) suggested that these two variables were correlated. Pearson's correlations were used, as a Shapiro-Wilk test indicated that all drug groups were normally distributed in both mean fecal output (Control: W = 0.928, p = 0.532; Morphine: W = 0.865, p = 0.167; Fentanyl: W = 0.905, p = 0.365) and mean food consumption (Control: W = 0.910, p = 0.394; Morphine: W = 0.927, p = 0.527; Fentanyl: W = 0.913, p = 0.420). Fecal output and food consumption were strongly (r = 0.5–1.0) and significantly ($p < 0.05$) correlated for all drug groups (Control: r = 0.821, p = 0.034; Morphine: r = 0.901, p = 0.006; Fentanyl: r = 0.857, p = 0.024).

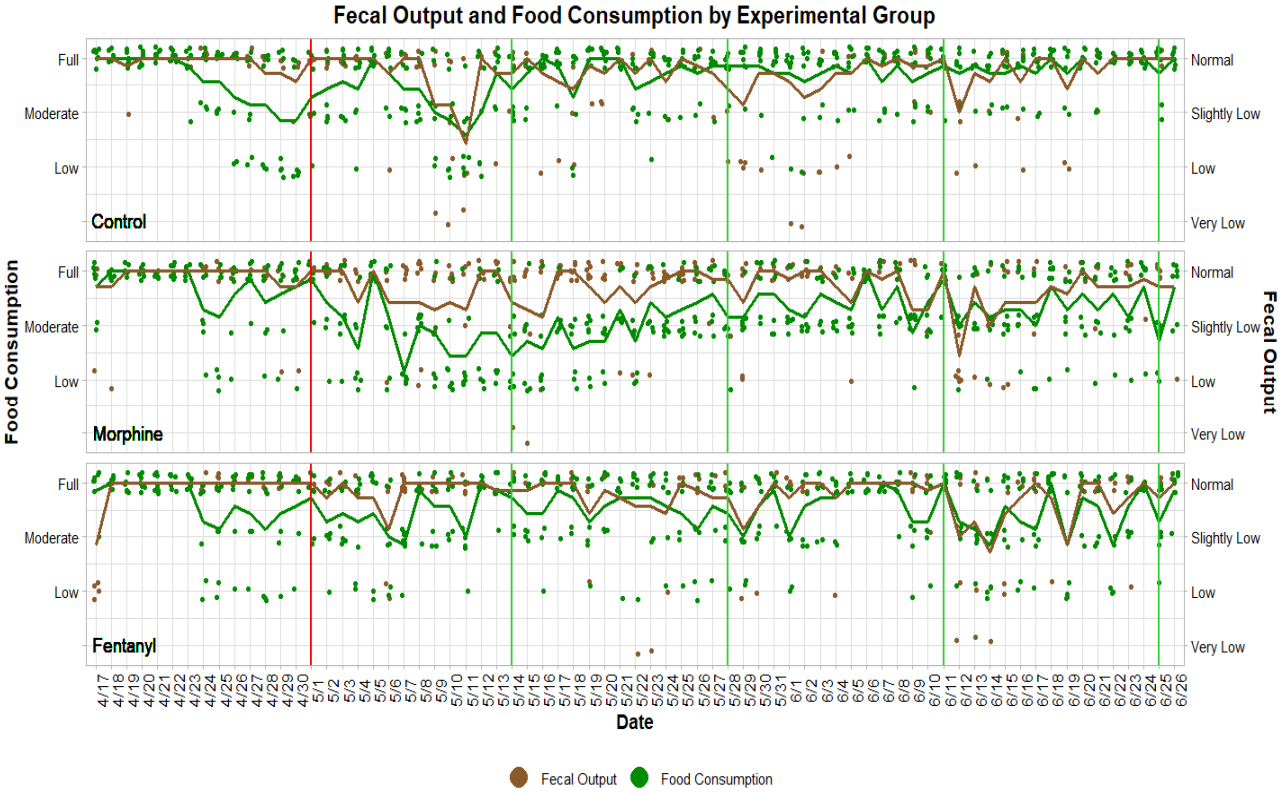

Figure 1. Correlation between mean food consumption (solid green line) and mean fecal output (solid brown line). Data points indicate food consumption (green) and fecal output (brown) for individual animals. Study timepoints include drug treatment initiation (red vertical line) and subsequent calcein injections (green vertical lines). For morphine rabbits, mean food consumption is depressed relative to mean fecal output between the drug treatment start date and the third injection.

A one-way ANCOVA indicated that fecal output was not significantly impacted by either the main effect of drug treatment group (F(2, 17) = 0.286, p = 0.755, ηp2 = 0.033, power = 0.095) or the co-variate of jacketing (F(1, 17) = 0.451, p = 0.767, ηp2 = 0.511, power = 0.103). ANCOVA was suitable for this analysis as a Shapiro-Wilk test indicated that residuals were normally distributed (W = 0.930, p = 0.211). A Levene's test confirmed homogeneity of variances (F = 0.877, p = 0.433).

The main effect of drug treatment group did have a significant effect on food consumption, as indicated by a one-way ANCOVA (F(2, 17) = 5.441, p = 0.0149, ηp2 = 0.390, power = 0.841). A retrospective power analysis confirmed that our drug treatment group size (n = 7) exceeds the per-group sample size (n = 6.14) needed to obtain the observed

effect size (Cohen's f = 0.8) at the recommended 0.80 power Mean food consumption followed the pattern Control > Fentanyl > Morphine. Post-hoc analyses with Tukey's HSD indicated that differences in mean food consumption reached statistical significance only for the Control > Morphine pairwise comparison (p = 0.012). Food consumption did not significantly differ between control and fentanyl groups (p = 0.501) or fentanyl and morphine groups (p = 0.118). The co-variate of jacketing had no significant effect on food consumption (F(1, 17) = 0.177, p = 0.680, ηp2 = 0.010, power = 0.07) (Figure 2).

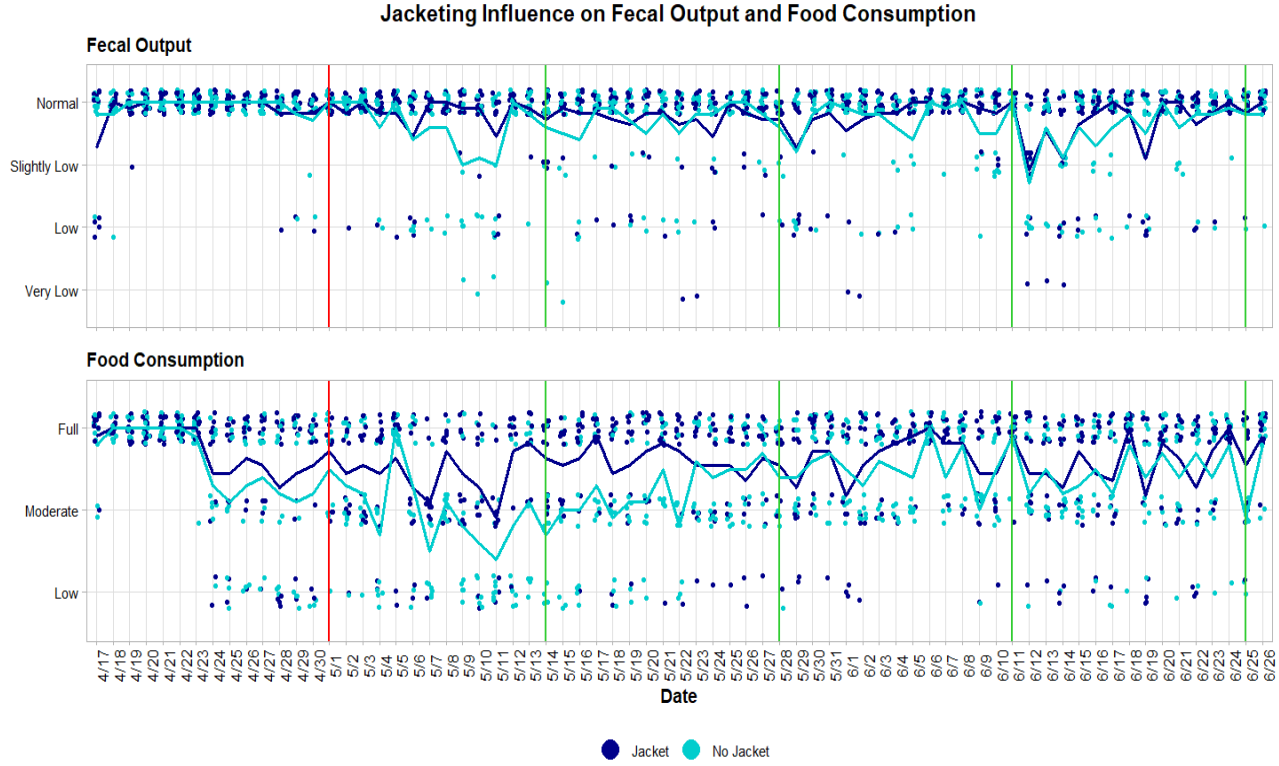

Figure 2. Non-significant differences between jacketed (dark blue) and non-jacketed (light blue) animals in both fecal output and food consumption. Study timepoints include drug treatment initiation (red vertical line) and subsequent calcein injections (green vertical lines).

ANCOVA was suitable for this analysis as a Shapiro-Wilk test indicated that residuals were normally distributed (W = 0.958, p = 0.474) and a Levene's test confirmed homogeneity of variances (F = 1.611, p = 0.227). The injection route of analgesic administration further appeared less stressful for the animals qualitatively when compared to fentanyl rabbits. Yet, the morphine sulfate injections consistently resulted in severe sedation within the first hour following dosing.

4. Discussion

Here, we provide a novel longitudinal perspective on opioid delivery in a rabbit animal model via transdermal fentanyl patch application for experimental research. Prior studies have reported limited concomitant effects of short-term use. Foley and colleagues [42] evaluated the efficacy of transdermal fentanyl patch administration in NZW rabbits. Animals were treated with fentanyl patches for one patch application cycle (72-h). Skin irritation was noted by the authors [42]; however, it was largely attributed to shaving the animals' fur for patch application. More recently, Mirschberger and colleagues [36] examined the use of transdermal fentanyl patches in rabbits on three different patch locations in the context of post-operative pain management. Their group reported that the neck and outer ear surface were the best options for transdermal patch placement. Their experimental period was 120-h and the authors reported associated erythema and animals attempting patch

removal. Ultimately, the authors concluded that transdermal patches are an acute and effective delivery route for pharmacologic analgesics. Additionally, Jain and colleagues [35] examined how opioid administration delays recovery and bone healing following spinal fusion surgery in a rabbit model. In this study, animals were treated with transdermal fentanyl patches for 10 weeks (four weeks pre-operatively and six weeks post-operatively). No information was reported regarding subject skin condition in relation to treatment with transdermal fentanyl patches. Although transdermal fentanyl patches have been documented as an effective pain relief agent in such clinical settings, we report novel side effects correlated with prolonged use which have implications for efficacy in the laboratory setting.

4.1. Skin Irritation Resulting from Prolonged Patch Application

Long-term transdermal fentanyl patch treatment presented several qualitative challenges for overall rabbit health and experimental facilitation by lab personnel. Specifically, rabbit chewing, and removal of transdermal patches had to be countered with patch re-application, telemetry jackets and VetWrap™ (3M, Maplewood, MN, USA; Figure 3). Grooming restrictions and excessive chewing behaviors were imposed by the coverings. These limitations, combined with a strong adhesive associated with the fentanyl patches, resulted in notably irritated, flaky, and bruised patch application sites compared to the other groups (Figure 4). Surrounding fur was further matted and compressed (Figure 5).

While transdermal patches did not require daily application, this mode of drug delivery incited the need for daily patch checks and preventative measures to counter chewing behavior and skin irritation. Initially following patch application, certain rabbits began chewing the patch and dressings, and exhibited marked grooming surrounding the associated skin and fur. Fentanyl group rabbits were more commonly observed displaying such behaviors, compared to control group rabbits with only Tegaderm™ patch treatment. This difference may be due to the increased adhesive residue left behind by the fentanyl patch or a differential behavioral response associated with opioid delivery.

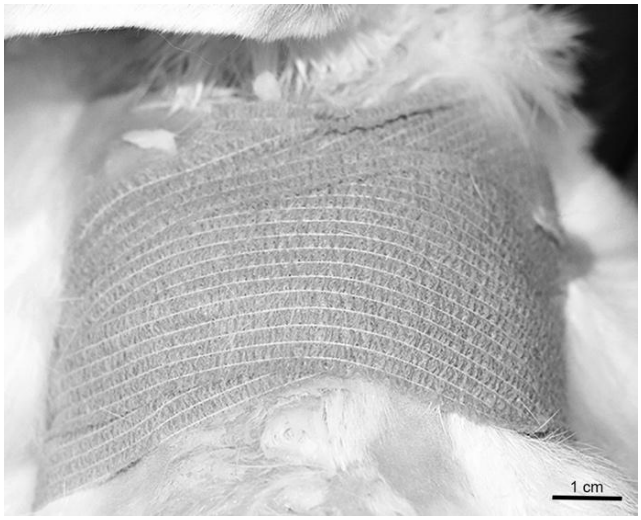

Figure 3. Fentanyl patch rabbit with applied VetWrap™ to prevent treatment patch chewing and manipulation.

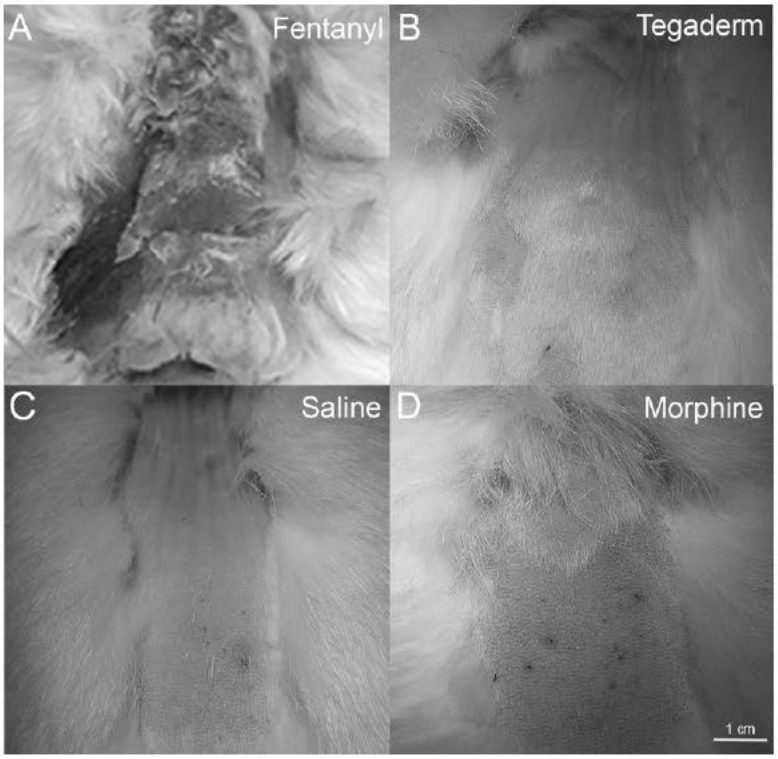

Figure 4. Representative images of skin irritation in each of the treatment groups. Transdermal fentanyl patch rabbit (**A**) displaying a contusion at a previous patch removal site, fur regrowth with adhesive residue, dry skin, and poor fur condition. Tegaderm™ control patch rabbit (**B**) showing fur regrowth and marked difference in quality of skin and fur condition. Saline control injection rabbit (**C**) with little fur regrowth, few injection site blemishes, and healthy fur condition. Morphine sulfate injection rabbit (**D**) displaying characteristic 'V' shaped fur regrowth, injection site blemishes, and good overall fur condition.

Figure 5. Fentanyl patch rabbit displaying poor overall fur quality resulting from grooming restrictions and excessive chewing behaviors from the placement of VetWrap™ and Lomir jackets to prevent the chewing and removal of the fentanyl patches.

To prevent chewing, all fentanyl and patch control rabbits were fitted with jacket coverings (Lomir Biomedical Inc.). The jackets fully covered the chest and back of the rabbit, and were secured with collars at the neck, shoulders, and hips. One rabbit in the fentanyl treatment group removed the jacket and ingested the opioid-containing patch from underneath the jacket. No long-term consequences were documented beyond an acute sedative effect. To prevent subsequent patch ingestion events, this rabbit was fitted with a pillow collar in addition to the jacket. Several other rabbits were able to partially

unzip and remove the jackets on multiple occasions but did not attempt to remove or ingest the patch.

Jackets that rubbed against the patch application sites were observed to lift the Tegaderm™ dressings and loosen the patches' adhesive edges. When jacket removal or patch loosening was observed, the back and chest of the rabbit were wrapped with VetWrap™ to prevent patch manipulation. Rabbits commonly attempted to pull the VetWrap™ from underneath the jacket and chew it open, with fentanyl rabbits spending significant portions of their observation time continually chewing/pulling the jackets and underlying VetWrap™. These behaviors required repeated replacement of jackets that were chewed beyond repair. By the study's end, patch presence and adherence needed to be checked daily, negating the time-saving qualities of applying a slow-release patch versus daily subcutaneous injection.

Additionally, fentanyl group rabbits commonly developed contusions or abrasions after a series of patch removal events (Figure 6). Compared to the Tegaderm™ dressing, the fentanyl patch adhered very tightly to the skin and was difficult to remove when associated with adjacent fur or areas of new fur growth. Over the 72-h patch cycle, adhesive residue became tightly bound to the skin. These adhesive deposits could not be easily removed from bare skin and required the underlying hair follicles to grow out enough to allow for removal with clippers. Consequently, we typically adjusted patch placement immediately laterally, rostrally, or caudally to the original application site to avoid existing bruising, erythema, or adhesive buildup Restraining an increasingly irritated rabbit was difficult and time-consuming during the process of patch removal, fur clipping, patch re-application, antibacterial ointment application to contusions, VetWrap™ application, and jacketing.

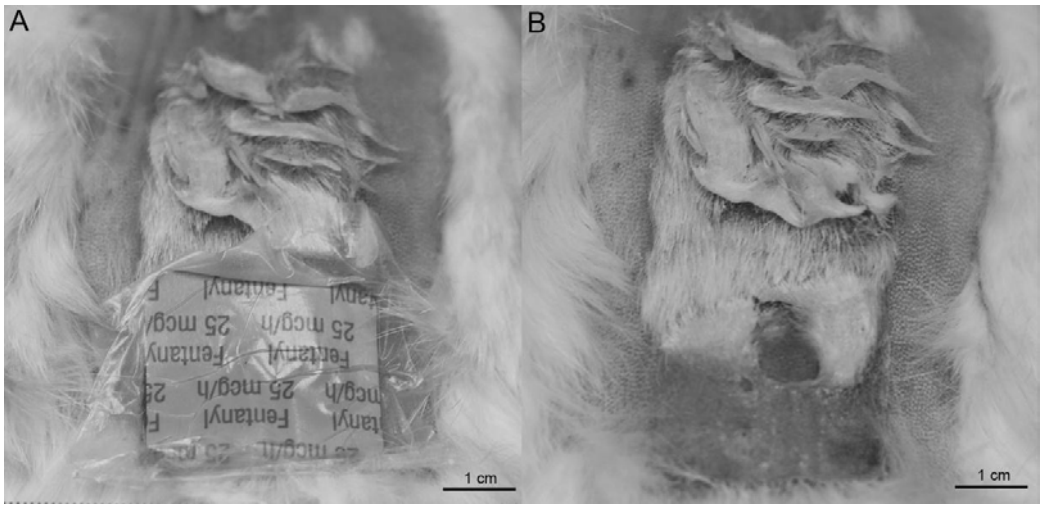

Figure 6. A representative example of skin irritation and contusions from prolonged use of transdermal fentanyl patch placement. (**A**) displays patch placement with Tegaderm™ covering applied. (**B**) shows the same animal with the fentanyl patch removed. Note the adhesive residue and matted fur from previous patch placement in the superior portion of both figures.

4.2. Opioid Effects on Fecal Output and Food Consumption

Analysis of fecal output revealed no significant influences from drug treatment or jacket use. The common side-effect of constipation from chronic opioid use [45] was not observed in morphine or fentanyl treatment groups, compared to controls. Food consumption was significantly impacted by the drug treatment, with morphine group rabbits consuming significantly less food on average than control or fentanyl group rabbits. Morphine group rabbits experienced a qualitative change in activity within the first hour after dosing, appearing sedated and sedentary, which was attributed to the subcutaneous delivery of the bolus. Since all rabbits were fed daily with base and enrichment foods following dosing, sedation during the initial delivery of new food may have reduced overall food consumption for morphine group rabbits. Fecal output and food consumption were

strongly and significantly correlated. Control group and fentanyl group rabbits maintained this correlation throughout the study. Graphic visualization (Figure 1) indicated that morphine group rabbits dropped in mean food consumption, relative to mean fecal output, after the drug treatment start date. Mean food consumption, however, increased over this depressed period, suggesting that morphine group rabbits became acclimated to the sedative effect over the course of the study. Mean fecal output and food consumption are aligned for morphine group rabbits.

Although fentanyl group rabbits were acutely agitated by the application of jackets, collars, VetWrap™ (3M), and tightly adhered patches, these devices did not significantly affect their food consumption or fecal output in comparison to controls. Notably, jacket use also had no significant effect on fecal output or food consumption across jacketed control and fentanyl group rabbits.

Certain animals across each opioid group presented with acute anorexia, which was associated with abnormally low fecal output. No direct causes of anorexia were identified apart from one rabbit who sustained a laceration of the lower gingiva and was treated with a chlorohexidine rinse. All animals exhibiting this trend regained normal gastrointestinal motility within one week of onset.

4.3. Limitations

Longitudinal blood collection was not conducted to assess plasma opioid concentration at different timepoints throughout the dosing period. Regular blood draws would have increased stress for the animals due to the employment of rabbit restrainers and were discouraged by attending veterinarians given the stress behaviors witnessed. This exclusion presents challenges for determining the circulating level of opioid in the blood of the animal. Consequently, the experimental dose was based on literature from pre-existing studies where acute opioid delivery was assessed in similar rabbit models with established success [42]. In addition, our study did not employ quantitative measures for determining food consumption or fecal output (e.g., obtaining weights of these variables). Daily observations were performed and recorded, however, by a consistent technician team with extensive rabbit husbandry experience. These observations were further consistent with previously published scoring descriptions provided by Weaver et al. [44]. It is also important to note that animals may have obtained higher doses of opioids via subcutaneous injection due to difficulties with jacket compliance in the fentanyl group. As such, a promising area for future experimentation may involve the administration of opioids via osmotic pumps [46]. While likely not capable of spanning the longitudinal duration of this study, a revised administration route may prevent a low-maintenance alternative to slow-release transdermal patch application.

4.4. Bone Remodeling and Transdermal Fentanyl Patches

To better characterize the effects of chronic opioid use on bone remodeling, rabbits offer a promising model system as they are the smallest traditionally used laboratory animals with well-defined cortical remodeling [17]. As a result, rabbits have been used as experimental animals for studying central canal size and the vascular network of cortical bone [18,19]. Bone remodeling can be highly influenced by mechanical loading properties (including force strength, frequency, duration), resulting in increases in bone deposition [47]. While there are documented differences in mechanical loading properties following chronic opioid administration [48], it is critical to note that restrictions imposed via jackets, VetWrap™, and pillow collar can influence bone remodeling, as evidenced by behavioral changes in canine studies [49]. Thus, researchers must remain consistent in strategic interventions to prevent patch removal across treatment and control groups. It is possible that the extrapolation of these data to the context of the greater literature may result in inconsistencies for jacketed, patch administration animals. This evidence further supports our recommendation to refrain from using transdermal patches for longitudinal opioid delivery in a rabbit model in the context of bone research.

5. Conclusions

Rabbits have served as model organisms to test the efficacy of prophylactic antibiotics following surgery [21–23], new biocompatible implants and hardware [24–28], and to mimic bone and tendon pathological states [28–32]. These studies highlight the benefit of the use of rabbits in comparative research to explore novel therapeutic methods in the orthopedic field. The literature, however, fails to consider the practical use of rabbits as a model organism when administered opioid analgesics. Although the use of transdermal fentanyl patches for rabbit analgesia may be preferred in an acute clinical setting, this delivery method is not feasible for long-term pain relief or opioid delivery in a laboratory context. In the current study, all animals subjected to transdermal fentanyl patch application consistently displayed erythema and continuously attempted to remove patches, which resulted in acute animal stress and an increased time commitment for lab personnel. In contrast, the morphine sulfate injection group did not demonstrate detrimental changes in fur appearance or overall health, though these animals consumed less food throughout the study. There were no significant effects of opioid administration on fecal output, with morphine group rabbits regaining normal correlation with food and fecal output for the last fourth of the experimental period. The observed sedative side-effects of the subcutaneous delivery of a larger opioid dose could have implications for individuals hoping to assess metabolic performance or other time-sensitive metrics. With the drawbacks associated with transdermal patch opioid administration, we suggest that orthopedic researchers employ an alternative administration route when conducting longitudinal opioid studies. In future work, we plan to evaluate the prolonged impact of longitudinal fentanyl administration via subcutaneous injection on bone turnover in a rabbit model system, while controlling for indirect confounding effects of opioid exposure including caloric intake, hormonal effects, and exercise regimens.

Author Contributions: Author contributions are as follows: (1) J.M.A.: Conceptualization; Project Design; Methodology; Acquisition of Data; Data Visualization; Data Analysis/Interpretation; Project Administration; Supervision; Writing—original draft; Critical Revision of Manuscript; Writing—review and editing; Approving Final Version of Manuscript. (2) A.J.S.: Methodology; Acquisition of Data; Writing—original draft; Critical Revision of Manuscript; Writing—review and editing; Approving Final Version of Manuscript. (3) M.E.C.: Acquisition of Data; Data Visualization; Figure Preparation; Data Analysis/Interpretation; Writing—original draft; Critical Revision of Manuscript; Writing—review and editing; Approving Final Version of Manuscript. (4) A.R.L.: Acquisition of Data; Writing—original draft; Writing—review and editing; Approving Final Version of Manuscript. (5) R.A.D.: Acquisition of Data; Data Visualization; Figure Preparation; Writing—original draft; Writing—review and editing; Approving Final Version of Manuscript. (6) G.R.T.: Acquisition of Data; Writing—original draft; Writing—review and editing; Approving Final Version of Manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Akron (18-11-12 ARC, 7 December 2018).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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Appendix XI: Rabbit Opioid SkyScan µCT SOP

Cutting Tibia

1. Thaw the tibia inside the tube the day before the scan by moving it from the freezer to the refrigerator. Alternatively, set out the tibia in the chemical hood for approximately 30 minutes.

2. Put on proper PPE – double gloves, lab coat, goggles. You may want a face shield or mask during the waterpik portion to prevent splashback.

3. Unwrap tibia

4. Use the calipers to measure **87** from proximal end to the distal shaft. Mark this circumference on the bone

5. Set up the isomet and sharpen the larger 8 inch blade if this has not been done recently

6. Clamp bone in the small chuck and cut off the distal end at this line.

7. Wash out the inside of the bone with the waterpik inside the sink. A tube of fat will likely fall out of the marrow cavity

8. Wrap bone as indicated below

9. Rewrap the cut distal end in the saline soaked gauze and replace in the tube

10. Spray the outside of the tube, isomet surface, chuck, isomet blade, water pik, inside of the sink, and your working surface with 10% bleach and dry with a paper towel, or use a Clorox wipe to sterilize.

11. Place wrapped tibia in a plastic sample bag with the tube alongside to maintain provenience.

12. If you are scanning the next day, place tibia in the refrigerator. If you are scanning in several days, freeze the tibia.

Wrapping and Orientation Markers

1. Pre-cut your parafilm
    a. You will need approximately four squares and two double-squares of parafilm per bone
    b. Stretch the parafilm thin between your fingers before wrapping. As you wrap, pull the parafilm taut and press it down onto the bone to adhere it to previous layers.

2. Wrap the proximal end using a single square of parafilm. Repeat for the cut distal end.

3. Wrap the shaft using a double-square of stretched parafilm. Wrap one end of the bone, and then proceed in a diagonal wrapping manner to reach and wrap the other end of the bone. Repeat this process with the second double-square of stretched parafilm.

4. Make sure there are no exposed tissue regions or leakage – if water is drawn out of the bone during scanning through a leak, the bone may move.

5. Spray the outside of the parafilm with 10% bleach (in the spray bottle by the grinder sink) and dry with paper towels. Or use Clorox wipes to sterilize the external surface of the parafilm-wrapped bone.

    a. You also need to sterilize the outside of the tube, your working surface, and any tools used on this unfixed tissue
    b. Change your gloves before proceeding

6. Select a foam "A" and place it upside down just under the tibial crest. Fill the triangular hole in the "A" with modeling clay or sticky tack.

7. Select a foam "M" and place it upside down on the medial side of the bone, directly adjacent to and at the same level as the foam "A". Fill the triangular divot in the "M" with modeling clay or sticky tack. You will need to squish the sides of the "M" to get it to fit on the medial side.

8. Cover the letters with a half-square of stretched parafilm to hold them in place.

9. It is recommended to draw the outline of the tibial diaphysis on the outside of the parafilm with Sharpie, so that it is easier to position.

10. Place the wrapped bone and the tube in a plastic sample bag to transport to the microCT

Turning On the Micro-CT

1. Turn on SkyScan 1172 machine using the 'ignition' key on the right side. Turn the key to the middle then to the right.

2. Open the software on the computer, Skyscan 1172 shortcut.
    - Select yes

3. Select the hazard/radiation symbol (yellow) and wait 15 minutes for the X-ray tube to warm up

4. While the tube is warming up, prepare sample on the machine platform with dental wax and wrap the bottom in parafilm.

    a. Use dental wax to fully plug the smaller stage. The tibia needs to sit **just above** the metal rim on a platform made of dental wax.
    b. You will need to invert the tibia so that the proximal end is on the stage and the distal (cut) end points up
    c. Cover the proximal end of the tibia with dental wax and press the dental wax down onto and around the edges of the stage.

d. **For proximal end scans:** Center the entire proximal end on the stage over the central peg below the stage
e. **For diaphyseal scans:** Center the diaphysis on the stage over the central peg below the stage. The fibular region will likely **hang somewhat off the stage,** so reinforce it with dental wax underneath. The entire bone will also be tilted off its central axis so that the **tibial diaphysis alone** is vertically oriented over the central peg.
f. Wrap the dental wax base in a stretched square of parafilm. Check and re-position the tibial orientation after wrapping as it may have moved.

Alignment (Once Per Day)

1. Select Options tab→Select Ctrl+Alt+Shift+S to make grayed out options (Acquisition Mode/Alignment) appear

2. Make sure no filters applied

3. Select open door button and insert taller calibration peg, then close the door

4. Click the TV button to make the alignment peg appear on screen

5. Options→ Alignment → Yes
   - Machine will run the alignment automatically. It may say "Not Responding". When responding, the scanner will place hash marks on the alignment peg's anatomical center and rotate the stage 360°

6. If the scanner asks if you want to compensate for misalignment by camera movement, click Yes

7. Remove alignment pin

Flatfield Correction

1. Options → Preferences → Uncheck the two boxes to the right of flatfield
   a. The two boxes to the right of median filter should be checked

2. Click voltage symbol (Δ) to set at **74 kV**. Source current should be **134 uA**.
   a. To achieve this combination, uncheck the box to keep power maximized
   b. You can use the arrow keys to adjust voltage/current stepwise if you click on the button next to the voltage/current

3. TV button (Live View)

4. Right click to bring up average current measurement on screen

5. Adjust filters at the bottom of the screen
   a. **Filter: Al 0.5 mm ← Will auto-change voltage, so check it**
   b. **Image pixel size:**
      i. **5.5 um for diaphysis (will change to 5.49)**
      ii. **11 um for proximal end (will change to 10.99)**

c. **Camera size: Medium Camera, 2000 columns x 1300 rows**
d. The vertical stage position does not need to be constant

6. Let current average drop to ~60% +/- 2
5. Options→Acquisition Modes
    - Acquire bright + dark for current mode = OK
    - Use central positioning
    - Turn off xrays after acquiring
    - Don't modify any other settings

Sample Positioning

1. Put in sample

2. TV button (Live View)

3. Options → Preferences → Check two boxes to the right of flatfield again

4. Double click on height at the bottom tab to raise the sample into the field of view (FOV)
    a. Place the clay markers at the **bottom** of the FOV for the tibia scans
    b. Can increase resolution to zoom out and see if centered
    c. Check orientation by rotating to 90° and 180°
        i. Recommended 10° increments if you are unsure whether it is out of the FOV
        ii. You may need to move the sample down slightly to a slimmer region, but keep majority of clay markers in the FOV
    d. Open door and reorient sample if its longest width does not fit within the FOV
    e. Check voltage and resolution each time you open the door – the system may reset it

Scan Setup

1. Options → Preferences: Make sure the two boxes to the right of **Flatfield** and to the right of **Median Filter** are checked

2. Options→Acquisition (from Cooper Lab SOP)
    - **Rotation Step (deg)=0.200**
    - **Frame Averaging=ON (4)**
    - **Random Movement=OFF (0)**
    - **Use 360 Rotation=NO**
    - **Camera Offset=OFF**

3. From lab SOP:
    - Modify file path and prefix - Browse→Select file→new folder→rename file
    - Xray off after scanning
    - Uncheck open door after scan
    - Uncheck partial width

4. Check **voltage, pixel size, filter, camera size** to see if they changed

5. Right click the screen to show gray levels. Wait a minute for them to stabilize. Stabilization may take long if the door was open for a long time.

6. Select blue arrow “recycle” button and begin scan

7. **Log out manually or remotely on FOM near end of start time!**

Appendix XII: Rabbit Opioid μCT Image Processing SOP

NRecon

NRecon converts the microCT acquisitions (projections) into cross-sectional slices called reconstructions.

1. Click on the NRecon shortcut on the Desktop
    a. The GPUReconServer will pop up. You can minimize this window.
2. Open dataset… should pop up. If not, click the folder at the upper left hand corner of dataviewer.
3. Click on the first projection from your micro-CT acquisition
4. Under Output:
    a. Uncheck Use ROI
    b. Uncheck Scales ON
    c. Destination: Browse and create a new folder called "Recon"
    d. File format: BMP(8)
5. Under Advanced: No changes needed. Defaults should be:
    a. Smoothing kernel: Gaussian
    b. Uncheck Undersample
    c. Uncheck Defect pixel masking
6. Under Settings
    a. Check Smoothing: 1
    b. Check Misalignment Compensation
        i. Typically this estimation is close to correct. You can manually adjust the arrows up and down to move the image so that the right and left edges align as closely as possible.
    c. Uncheck Object larger than field of view
    d. Check Ring artifacts: 10
    e. Check Beam Hardening: 30%
    f. CS rotation (deg): 0.00
7. Under Start → Fine Tuning
    a. Select Post-Alignment
    b. Number of Trials = 5
    c. Parameter Step = 0.5
    d. Click Start and five images will be generated under Output. You can switch between them using the arrow keys on the top toolbar, and zoom in and out using the magnifying glasses on the top toolbar.
        i. Select the image that **minimizes** overlapping or shadowing at the edges of larger pores. Try to **maximize** the number of small, round pores that are visible.
        ii. If you cannot see the porosity, click Output. Click Auto. Move the right-side bar closer to the histogram peaks. This will increase brightness.
    e. The Alignment for your chosen image appears in the very bottom border of NRecon under "Fine-tune(PA:)" Make sure that this is also selected under Settings: Alignment.
        i. Copy the Alignment value into the Google Sheets document under NRecon Alignment
8. Adjust the histogram under Output by clicking Auto. The resulting image will be dim! We will adjust this more accurately in ImageJ later.

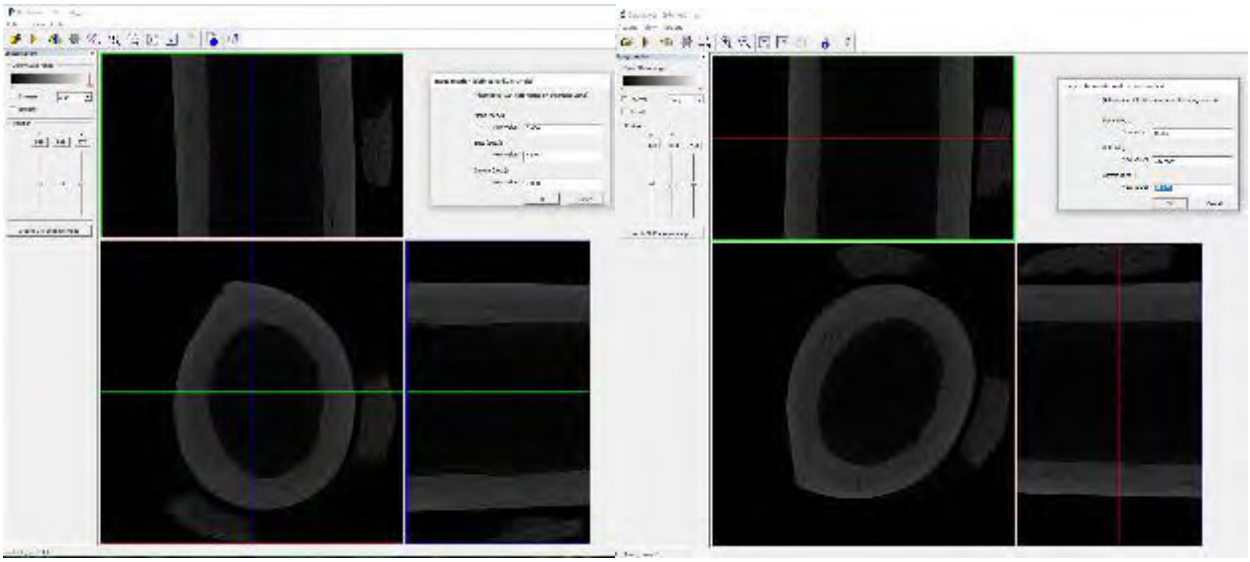
9. Under Start, click Start to begin processing.

Anatomical Tomo Rotation

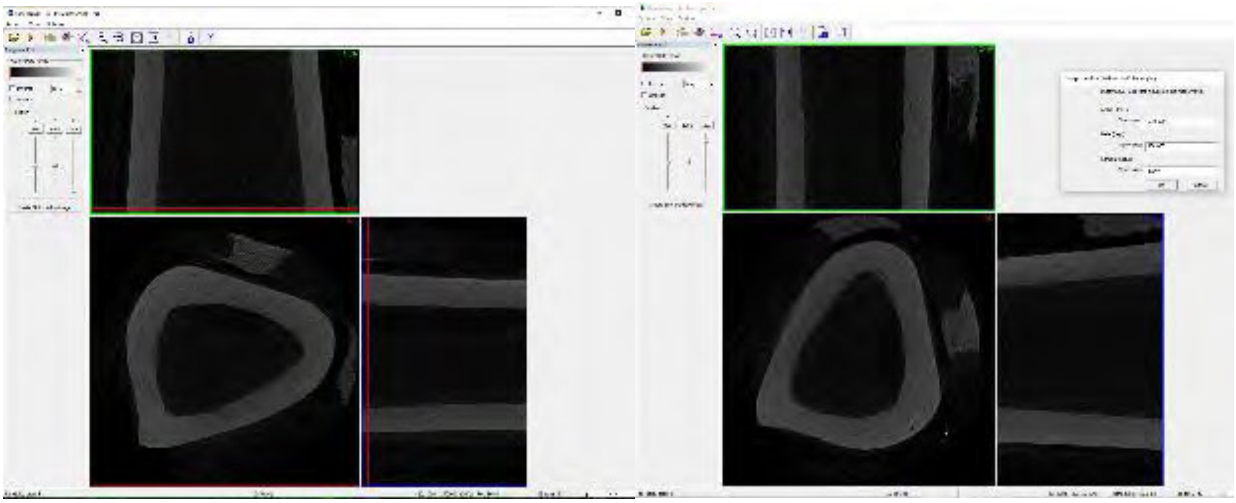
The reconstructed images will be referred to as "tomo" (tomographic) images throughout this guide. We want to orient the image so that anterior is up and medial to the right of each image slice. Clay markers have been included with the micro-CT scan to indicate anterior and medial orientation. We will rotate the image in DataViewer.

1. In File Explorer, open the new Recon folder
    a. Move the spr projection and the text file to a new subfolder "Files" so that they do not disrupt image processing
2. Load the tomo files from the reconstruction in Dataviewer
    a. Click the DataViewer shortcut on the desktop
    b. Click the folder in the top left corner
    c. In the popup window, navigate to the "Recon" folder and click the first image file in the folder
    d. Change "Open As" dropdown box to "3D View"
    e. Do not check the Resize box
    f. Click Open
3. Click View → Rotate/Shift Operations → Rotate 180 degrees
    a. Wait a moment for the image to rotate. On the transaxial (lower left) cross-sectional image, one clay marker should be approximately on the top and the other clay marker should be approximately on the right.
4. Click in the approximate center of the marrow cavity on the transaxial (lower left) image to move the crosshairs to that location
5. Rotate the transaxial (lower left) cross-sectional image by holding down Ctrl and clicking and dragging the mouse cursor
    a. To get a better view of the clay markers, move the Z-level of the transaxial cross-section.
        i. Or click in the coronal (top) or sagittal (lower right) windows to reposition the cross-hairs on a thicker part of the clay marker
    b. For the tibia, the anterior clay marker is on the pointed end (tibial crest). Rotate the transaxial image until the middle of the anterior clay marker intersects with the vertical line of the crosshairs.
    c. You may need to reposition the crosshairs in the center of the marrow cavity by clicking.
    d. The horizontal line of the crosshairs should approximately intersect the medial clay marker on the right side. However, it may not be in the center of the medial clay marker.
6. Click View → Rotate/Shift Operations → Rotate to Any Orientation
    a. The Image Rotation window will pop up
    b. Copy the values for Alpha, Beta, and Gamma into the Google Sheets file under Tomo Alpha, Tomo Beta, and Tomo Gamma, respectively
        i. Tomo Alpha should always be 270
        ii. Tomo Beta should always be 180
        iii. Tomo Gamma will be variable as it is the transaxial rotation value
    c. Click Cancel in the Image Rotation window
7. Save the rotated image stack using Actions → Save → Transaxial (X-Y) Images as Dataset

a. Two popups will warn you that the image is rotated and that output will be 8-bit. Click OK for both.
b. Save in a new folder “Anatomical Tomo” as type (8bit)BMP(*.bmp)



Femur: Tomo file before (left) and after (right) anatomical rotation.
Note that the linea aspera often points more to the left. Anterior is at the top of the transaxial image, and medial is at the right.

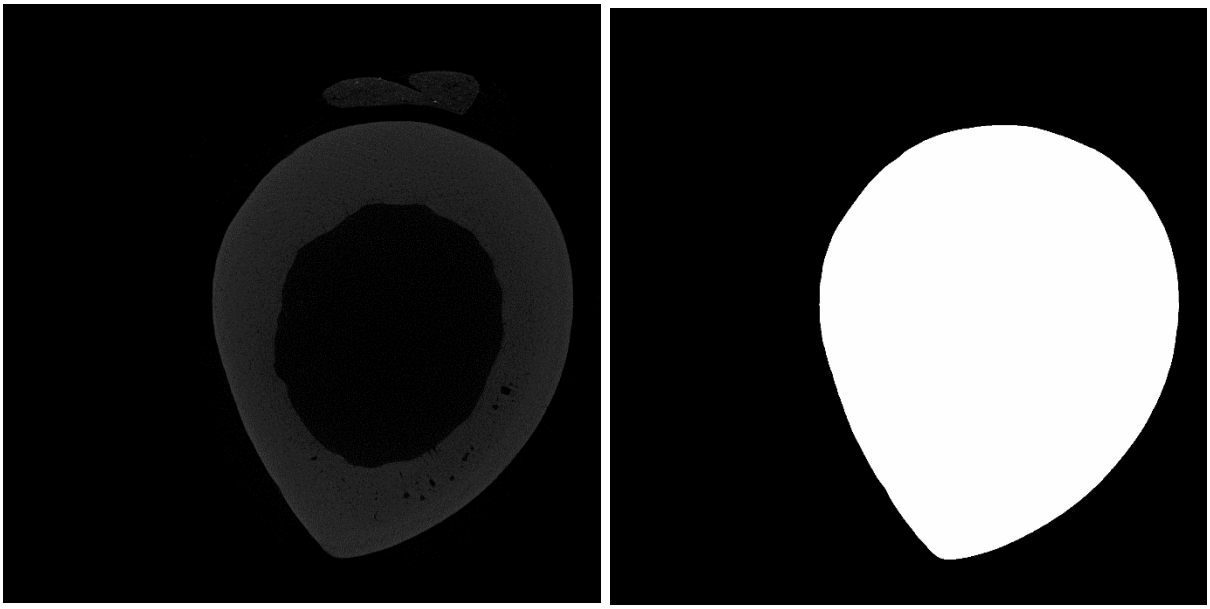


Tibia: Tomo file before (left) and after (right) anatomical rotation.
Note that the anterior clay marker (top) sits on the tibial crest. Anterior is at the top of the transaxial image, and medial is at the right.

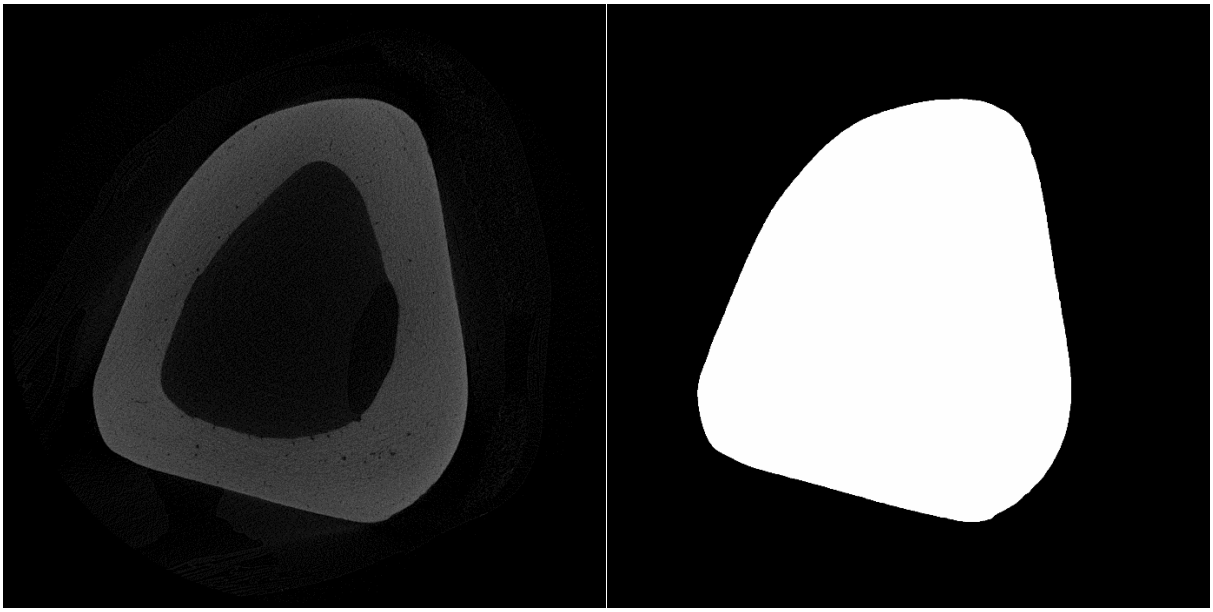
TA Extraction

Now that the tomo files are oriented anatomically in a cross-sectional sense, they must also be adjusted vertically. We will orient the filled total area (TA) mask of the tomo image stack, which we first extract in Batman, which is the batch manager for CTAnalyser. This uses a custom macro I made for this purpose.

1. Navigate to the new Anatomical Tomo folder. The first and (sometimes) last image file are partial cross-sections due to the slight rotation. Move the partial cross-sections and the text file to a new subfolder called “Partial”
2. Click the Batman shortcut on the Desktop
3. Click Import. Navigate to Local Disk (D:) → Rabbit Opioid Scans Backup → CTan Macros → TAExtractor.ctt. Click Open and the macro will load.
4. Click Add. Open the Anatomical Tomo folder. Click the first image. Click Open.
    a. Do not check the box to resize the image
    b. You can load multiple Anatomical Tomo image stacks if you have several ready to go. Click Add for each image stack and they will line up to batch process.
5. Click the arrow next to Properties → Change Pixel Size. Make sure the pixel size is 5.49426 um for All Datasets.
6. Make sure the boxes are checked next to all of the loaded Anatomical Tomo files
7. Click Start and the macro will run on its own.
    c. The Anatomical Tomo folder will receive a subfolder labeled TA



Femur: Anatomical Tomo file (left) and TA mask (right) created by CTan macro



Tibia: Anatomical Tomo file (left) and TA mask (right) created by CTan macro

Skeletonization

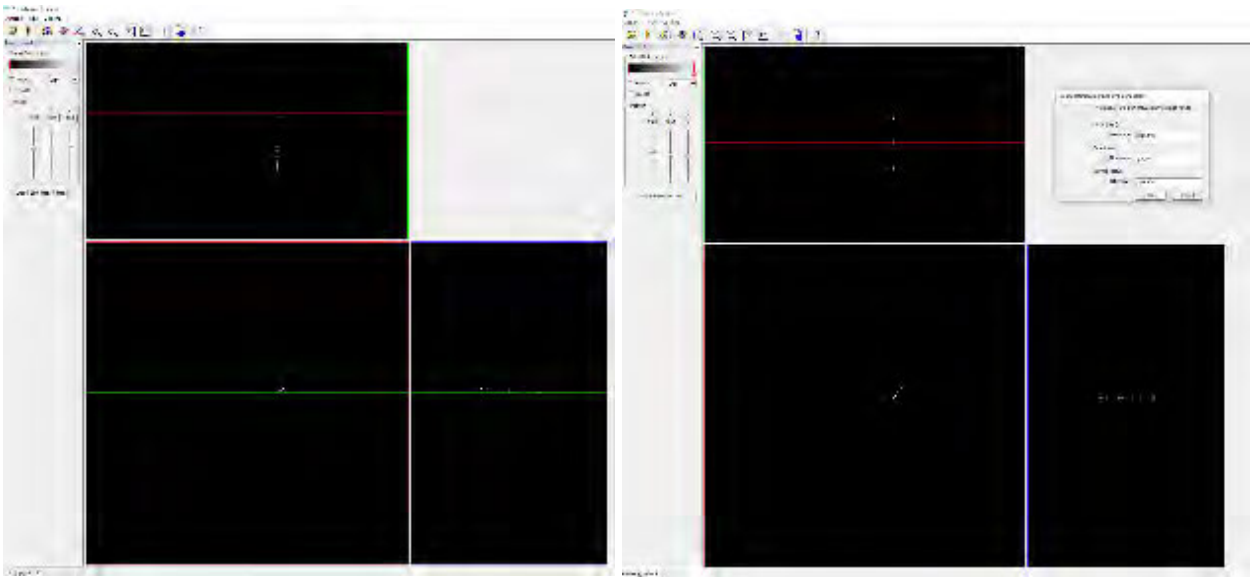
Due to the curved nature of the femoral shaft and the imperfect manual positioning in the micro-CT, the shaft is not completely vertical within the field of view. We want to rotate the shaft segment to its most vertical orientation so that pore orientations relative to the longitudinal axis will be correct. We do this by skeletonizing the TA image stack, vertically orienting the skeleton, and then using the same vertical orientation for the anatomical tomo image stack. This procedure is based on Behrooz et al. 2017. This protocol runs through a macro that prompts the user to select the output folder and open the image stack. The protocol automatically skeletonizes TA, dilates it three times for visibility, and saves it in the user-selected output folder.

1. Open ImageJ
2. Install the Skeleton Save macro
    a. Open Plugins → Macros → Install, which will pop up the macros filter
    b. Click Skeleton Save.ijm then Open
3. Click Plugins → Macros → Skeleton Save
4. In the popup Select_Output, navigate to the Anatomical Tomo folder
    a. Create a new subfolder “Skeleton”
    b. Enter the Skeleton folder
    c. Click Select
5. You will be prompted to open Image Sequence
    a. Navigate to the TA folder, and enter it so that the TA images appear.
    b. Click the first TA image
    c. Click Open
6. The Sequence Options window will open.
    a. Make sure Sort Names Numerically is checked
    b. Don’t check any other boxes, and make sure Use Virtual Stack is not checked

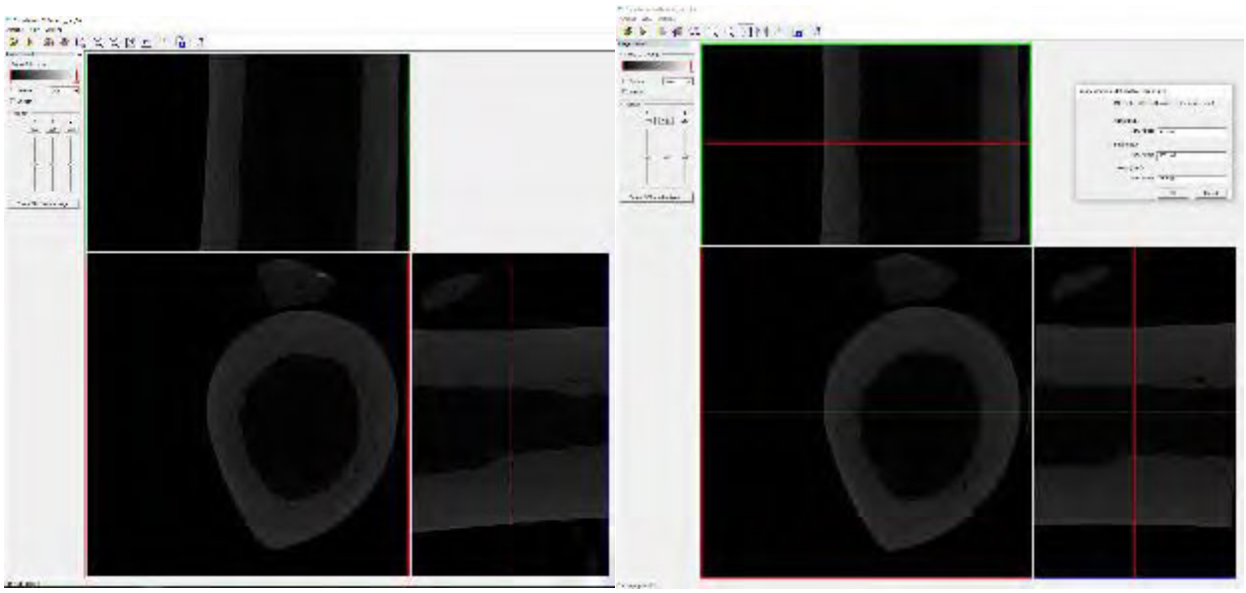
    c. Click OK
7. Allow the macro to run on its own
    a. **Warning:** This procedure has many thinning iterations and will take approximately half a day to run. You can run multiple skeletonizations in separate ImageJ windows. However, the more you have open, the longer each procedure will take.
8. Load the completed skeleton in DataViewer
    a. Click the DataViewer shortcut on the desktop
    b. Click the folder in the top left corner
    c. In the popup window, navigate to the "Skeleton" folder and click the first image file in the folder
    d. Change "Open As" dropdown box to "3D View"
    e. Do not check the Resize box
    f. Click Open
9. Enter pixel sizes in the User Input popup window: 5.49426 um for both X and Y pixel size
10. Re-orient the skeleton longitudinally
    a. In the transaxial window (lower left), use the cursor to click on a bright dot. You may have to scroll through the slices using the "Z" position on the toolbar to the left
    b. The skeleton should appear in the coronal (top) and sagittal (lower right) windows
    c. Hold down Ctrl on the keyboard and click in the coronal (top) window.
        i. Still holding down Ctrl, move the mouse to rotate the skeleton until it is aligned with the vertical bar. You can click inside the coronal window to reposition the vertical bar.
    d. Hold down Ctrl on the keyboard and click in the sagittal (bottom right) window.
        i. Still holding down Ctrl, move the mouse to rotate the skeleton until it is aligned with the vertical bar. You can click inside the sagittal window to reposition the vertical bar.
11. Find the revised coordinates of the rotated skeleton
    a. In DataViewer, click View → Rotate/Shift Operations → Rotate to Any Orientation
        i. The Image Rotation window will pop up
    b. Copy the values for Alpha, Beta, and Gamma into the Google Sheets file under TA Alpha, TA Beta, and TA Gamma, respectively
    c. Click Cancel in the Image Rotation window
12. Rotate the anatomical tomo files to match the skeleton orientation
    a. In DataViewer, click the folder in the top left corner
    b. In the popup window, navigate to the "Anatomical Tomo" folder and click the first image file of the tomo sequence in the folder
    c. Change "Open As" dropdown box to "3D View"
    d. Do not check the Resize box
    e. Click Open
    f. In DataViewer, click View → Rotate/Shift Operations → Rotate to Any Orientation
        i. The Image Rotation window will pop up
    g. Enter the values for TA Alpha, TA Beta, and TA Gamma that you copied from the skeleton
        i. A popup will warn you that 3D values will be loaded from the memory buffer. Click OK.
    h. Click OK and the Anatomical Tomo image will rotate to its most vertical orientation

i. Save the rotated image stack using Actions → Save → Transaxial (X-Y) Images as Dataset
    i. Two popups will warn you that the image is rotated and that output will be 8-bit. Click OK for both.
    ii. Save in a new folder "Centerline Anatomical Tomo" as type (8bit)BMP(*.bmp)

13. Remove partial anatomical tomo files
    a. Near the top and bottom of the anatomical tomo file stack, cross-sections will be cut off by the image rotation. Move these to a folder called "Partial"
        i. Also move the text file created by DataViewer to this folder
    b. If you cannot clearly see if a file is cut off, click and drag the Centerline Anatomical Tomo folder from File Explorer into ImageJ. Or click File → Open → Image Sequence and click on the first image file in the Centerline Anatomical Tomo folder.
        i. Check the box "Use Virtual Stack" and click Yes.
        ii. Scroll through the image stack to see which files are partial. Note their file numbers so you now which images to move.
    c. Record the start and end file numbers in the Google Sheets document under Tomo Start and Tomo End. Number of slices will calculate automatically.



TA Skeleton Before (Left) and After (Right) Vertical Rotation

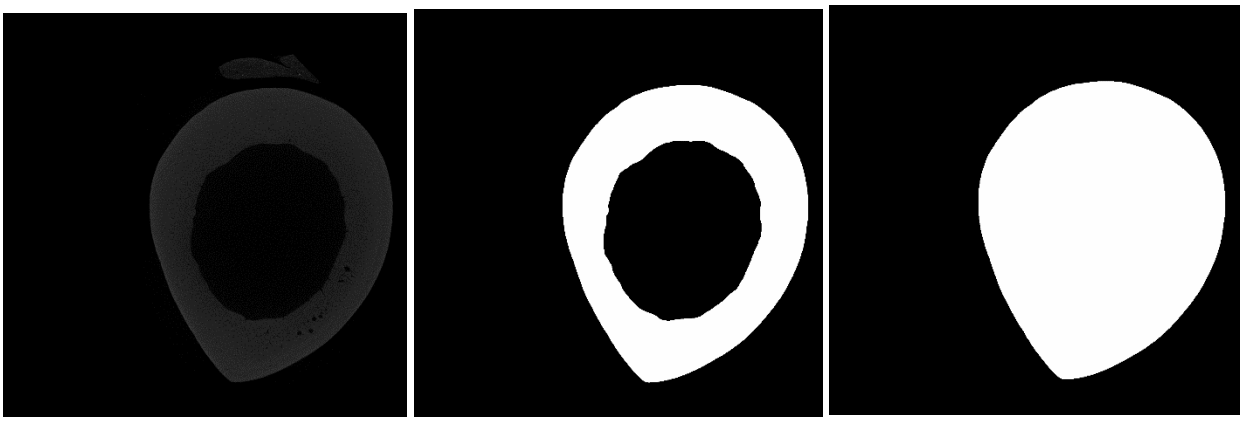


Anatomical Tomo Before (Left) and After (Right) Vertical Rotation

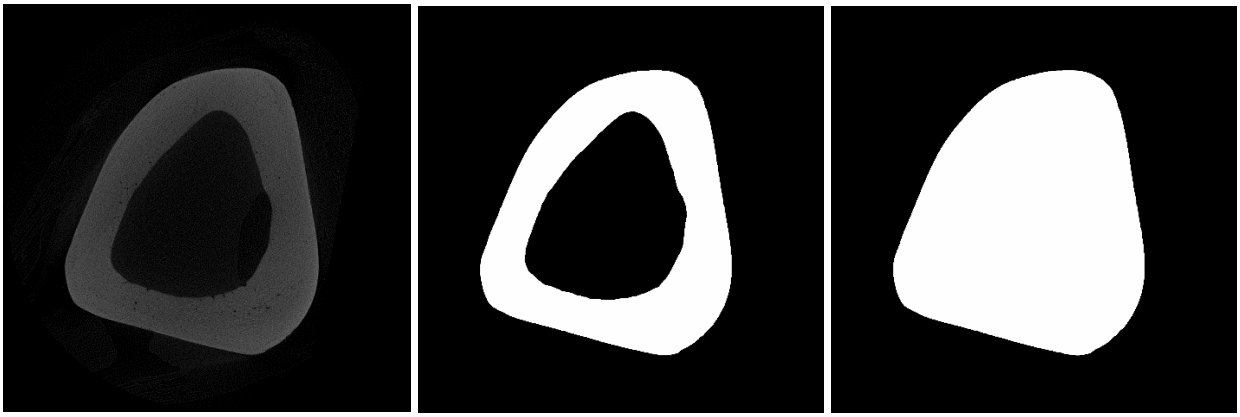
ROI and TA Extraction

In order to calculate percent porosity, we need a region of interest (ROI) mask of the cortex. We will extract this from the centerline anatomical tomo image in Batman. This macro will also extract the correctly oriented TA, which we will use to calculate relative cortical area later.

1. Click the Batman shortcut on the Desktop
2. Click Import. Navigate to Local Disk (D:) → Rabbit Opioid Scans Backup → CTan Macros → ROIandTAExtractor.ctt. Click Open and the macro will load.
3. Click Add. Open the Centerline Anatomical Tomo folder. Click the first image. Click Open.
    a. Do not check the box to resize the image
    b. You can load multiple Centerline Anatomical Tomo image stacks if you have several ready to go. Click Add for each image stack and they will line up to batch process.
4. Click the arrow next to Properties → Change Pixel Size. Make sure the pixel size is 5.49426 um for All Datasets.
5. Make sure the boxes are checked next to all of the loaded Centerline Anatomical Tomo files
6. Click Start and the macro will run on its own.
    a. The Centerline Anatomical Tomo folder will receive subfolders labeled ROI and TA



Femur: The centerline anatomical tomo file (left) and the cortical area ROI (center) and TA mask (left) created by the custom CTan macro



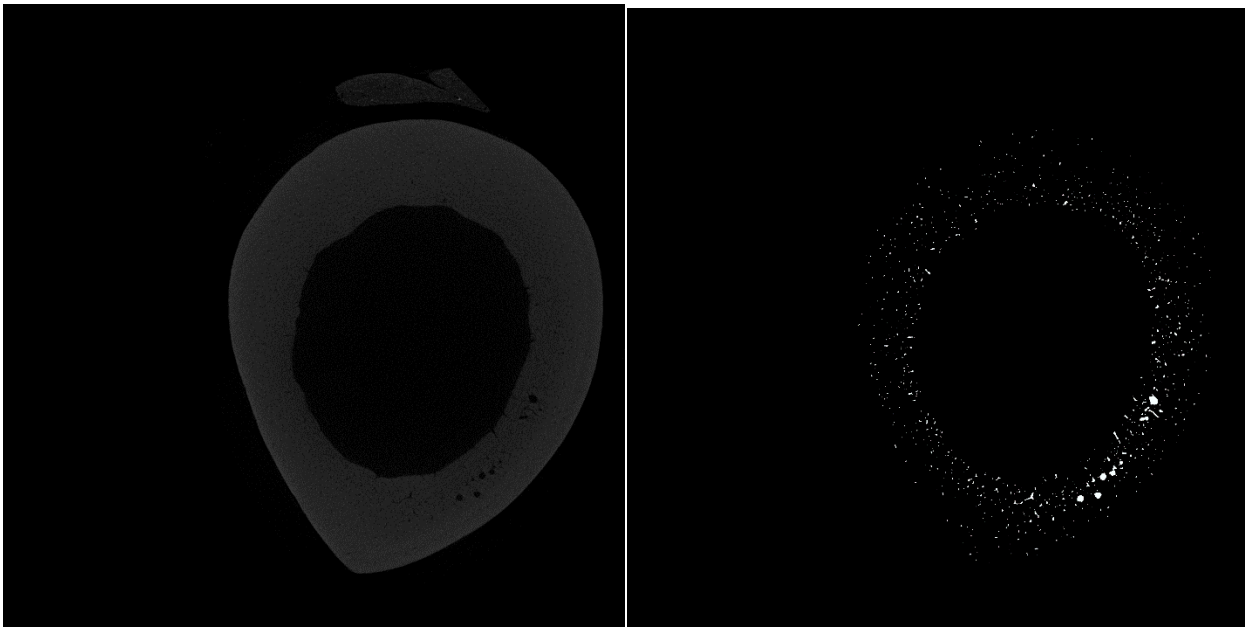
Tibia: The centerline anatomical tomo file (left) and the cortical area ROI (center) and TA mask (left) created by the custom CTan macro

Porosity Extraction

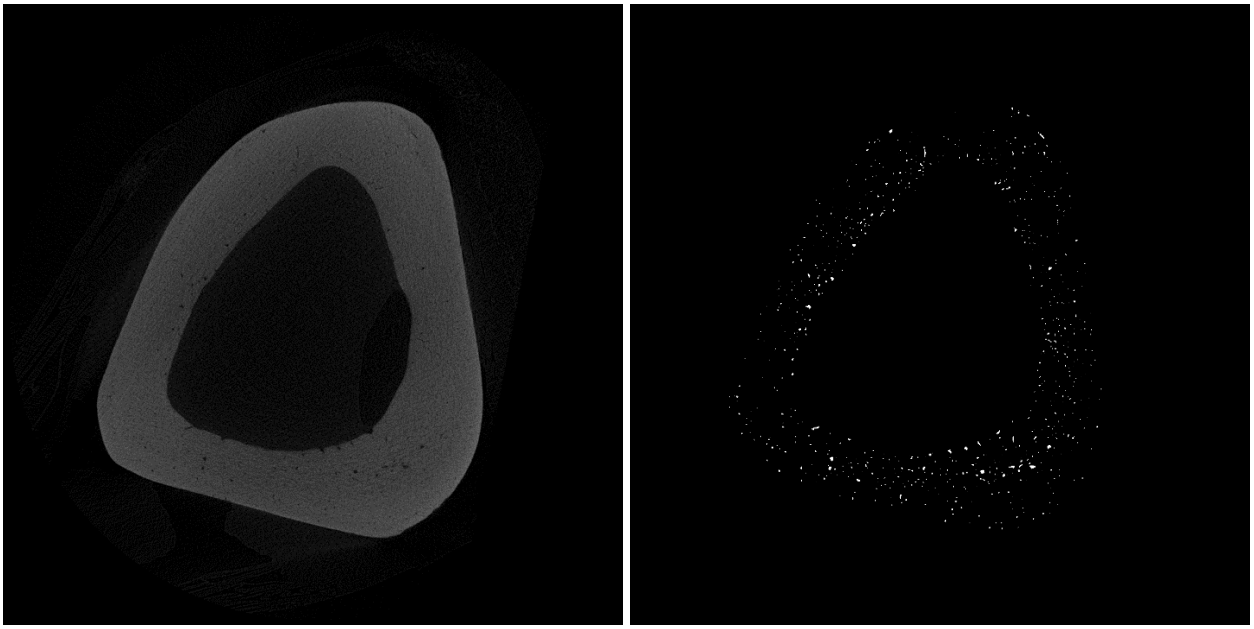
We extract the pores from the centerline anatomical tomo image using histogram enhancement followed by local (neighborhood) thresholding with a low-contrast Phasalkar filter. This is a custom macro I made that runs automatically. The required inputs are the centerline anatomical tomo image and its ROI mask, which is used to eliminate noise outside of the cortex.

1. Within the Centerline Anatomical Tomo folder, move the tomo images to a subfolder "Tomo". This will make them easier to find during the macro prompts. Exclude the Batman output files.
2. Open ImageJ from the Desktop shortcut.
3. Open Plugins → Macros → Install, which will pop up the macros filter
4. Click Adaptive Thresholding.ijm then Open
5. Click Plugins → Macros → Adaptive Thresholding
6. In the popup Select_Tomo_Images, navigate to your Centerline Anatomical Tomo folder and enter the Tomo subfolder.
    a. Click Select
    b. Note: You will not see any images appear

7. In the popup Select_ROIs, navigate to your Centerline Anatomical Tomo folder and enter the ROI subfolder.
    a. Click Select
    b. Note: You will not see any images appear
8. In the popup Select_Output, navigate to your Centerline Anatomical Tomo folder and click the New Folder icon (folder with a starburst).
    a. Create a new subfolder “Pores”
    b. Enter the Pores folder
    c. Click Select
9. The macro will run automatically. It will pop up a threshold window. You can view progress by looking at the Pores folder in File Explorer, where the extracted pore images will appear.
10. You can run multiple pore extractions simultaneously, but they must be in separate ImageJ windows.



Femur: The centerline anatomical tomo file (left) and the extracted porosity (right)



Tibia: The centerline anatomical tomo file (left) and the extracted porosity (right)

Pore Morphometry

A custom macro for Batman runs 3D pore morphometry on the porous cortex (bone = white, pores = black) to extract porosity, and on the isolated pores (bone = black, pores = white) to extract summed and individual object pore morphometry. The output is a summary morphometry .csv file, and individual object morphometry .csv file, and folders containing grayscale images representing trabecular thickness, trabecular separation, and individual object size.

1. Click the Batman shortcut on the Desktop
2. Click Import. Navigate to Local Disk (D:) → Rabbit Opioid Scans Backup → CTan Macros → PoreMorphometry8-27-2019.ctt. Click Open and the macro will load.
3. Click Add. Open the Pores folder. Click the first image. Click Open.
    a. Do not check the box to resize the image
4. Click the down arrow next to Add. In the popup menu, select Load ROI.
    a. Navigate to the ROI subfolder of Centerline Anatomical Tomo
    b. On the lower right dropdown menu, change ROI files (.roi) to Bitmap files (.bmp). The list of ROIs should appear.
    c. Click the first image on the list, then Open
    d. You will be warned that the ROI will be monochrome. Click OK.
5. You can load multiple Pores and matched ROIs in this manner.
6. Click the arrow next to Properties → Change Pixel Size. Make sure the pixel size is 5.49426 um for All Datasets. **Note that pixel size must typically be changed at this point, because the resolution data is lost through ImageJ processing.**
7. Make sure the boxes are checked next to all loaded Pores files
8. Click Start and the macro will run on its own.
9. The macro should output:
    a. A _.batman file containing summary pore data

b. A _i3d file containing individual pore data
c. TBTH: Image stack of trabecular thickness
d. TBSP: Image stack of trabecular separation
e. Individual Objects by Size: Image stack
f. Note: The three image stacks use grayscale coding of the lowest values (approximate to black) so they will not be very visible on the actual image. However, they can be seen clearly in Dragonfly or Amira.

Pore Morphometry Data Transfer

Open the spreadsheet file in D:/Rabbit Opioid Scans Backup/Statistics/Excel Spreadsheets - Colored/Total Pore Statistics – 100 um Despeckling

In the _.batman file, the first set of results represents porosity measurements of black pores on white bone.

Copy the following data from the first "Morphometry Results" vertical column to the boxes listed under "From First Vertical Column". These headers are highlighted in **blue.**

- Fractal dimension
- Number of closed pores
- Closed porosity (percent)
- Open porosity (percent)
- Total porosity (percent)

The second set of results represents individual pore measurements of white pores on black space.

Copy the following data from the second "3D Analysis Summary" horizontal row to the boxes listed under "From Second Horizontal Row". These headers are highlighted in **yellow.**

- Tissue volume
- Bone volume
- Percent bone volume
- Tissue surface
- Bone surface
- Intersection surface
- Bone surface / volume ratio
- Bone surface density
- Trabecular thickness
- Trabecular separation
- Trabecular number
- Degree of anisotropy (note – divide the numbers into DA1 and DA2 (parenthetical))
- Fractal dimension
- Number of objects
- Euler number
- Connectivity
- Connectivity density
- Standard deviation of trabecular thickness
- Standard deviation of trabecular separation

All other morphometry measurements (under the "Calculated" heading in **green**) are automatically calculated based on the data entered. Pull down the corner of the above columns to continue this calculation.

Cross-Sectional Geometry

A custom macro for Batman runs 2D and 3D morphometry on the cortical area (ROI) with the total area (TA) as a mask to extract cross-sectional geometry (2D morphometry) and relative cortical volume (BV/TV from 3D morphometry)

1. Click the Batman shortcut on the Desktop
2. Click Import. Navigate to Local Disk (D:) → Rabbit Opioid Scans Backup → CTan Macros → CrossSectionalGeometry9-9-2019.ctt. Click Open and the macro will load.
3. Click Add. Open the ROI folder from within Centerline Anatomical Tomo. Click the first image. Click Open.
    a. Do not check the box to resize the image
4. Click the down arrow next to Add. In the popup menu, select Load ROI.
    a. Navigate to the TA subfolder of Centerline Anatomical Tomo
    b. On the lower right dropdown menu, change ROI files (.roi) to Bitmap files (.bmp). The list of TA images should appear.
    c. Click the first image on the list, then Open
    d. You will be warned that the ROI will be monochrome. Click OK.
5. You can load multiple ROIs and matched TAs in this manner.
6. Click the arrow next to Properties → Change Pixel Size. Make sure the pixel size is 5.49426 um for All Datasets.
7. Make sure the boxes are checked next to all loaded ROI files
8. Click Start and the macro will run on its own.
9. The macro should output:
    a. A _.batman file containing summary cross-sectional data for 2D and 3D morphometry
    b. A 2D analysis summary line in the Cross-Sectional Geometry .csv folder under the Statistics folder in Rabbit Opioid Scans Backup

Appendix XIII: Femur and Tibia Quadrant Regional Processing SOP

Region Extraction

1. Open ImageJ from the Desktop shortcut.
2. **First time you use that session of ImageJ Only:** BoneJ always opens an orientation window that apparently cannot be closed from inside a macro. This will crash all subsequent attempts to open BoneJ from a macro. When you open a new ImageJ window, you must "burn" a BoneJ session to prevent this
    a. Open a single random ROI image in ImageJ by dragging and dropping it only ImageJ from File Explorer, or by using File → Open
    b. Click Plugins → BoneJ → Slice Geometry
    c. Immediately Cancel the Options Window
    d. Close the Orientation window
    e. Close the Image
    f. Now you are free of the orientation bug! You can skip this step if you run a subsequent region extraction in this same ImageJ session.
3. Open Plugins → Macros → Install, which will pop up the macros filter
4. Click Femur Quadrants.ijm (for the femur) or Tibia Quadrants.ijm (for the tibia) then Open
5. Click Plugins → Macros → Femur Quadrants (or Tibia Quadrants)
6. In the BoneJ Usage popup, click OK
7. In the popup Select_Cortical_ROI, navigate to:Local Disk (D:) → Rabbit Opioid Scans Backup → [Choose the next femur to run] → Anatomical Rotation → Centerline Anatomical Tomo → ROI
    a. Click Select
    b. Note: You will not see any images appear
8. In the popup Select_Output_Directory, navigate to your Centerline Anatomical Tomo folder and click the New Folder icon (folder with a starburst).
    a. Create a new subfolder "Region ROI"
    b. Enter the Region ROI folder
    c. Click Select
9. The macro will run automatically. It will pop up a threshold window and a results window – you can move these around but **do not close them**. You can view progress by looking at the Region ROI folder in File Explorer, where the extracted ROI regions will appear.
10. The outputs into the new Region ROI folder are ROI masks:
    a. Anterior ROI
    b. Lateral ROI
    c. Medial ROI
    d. Posterior ROI
    e. Drawn Octants – shows the drawn lines of the four quadrants

Pore Morphometry

10. Click the Batman shortcut on the Desktop

11. Click Import. Navigate to: Local Disk (D:) → Rabbit Opioid Scans Backup → CTan Macros → RegionalPoreMorphometry10-7-2019.ctt.
    a. Click Open and the macro will load.
12. Click Add. Navigate to an existing pore output in: Local Disk (D:) → Rabbit Opioid Scans Backup → [Choose the next femur to run] → Anatomical Rotation → Centerline Anatomical Tomo → Pores
    a. Click the first image. Click Open.
    b. Do not check the box to resize the image
13. Click the down arrow next to Add. In the popup menu, select Load ROI. Navigate to:

Local Disk (D:) → Rabbit Opioid Scans Backup → [Choose the next femur to run] → Anatomical Rotation → Centerline Anatomical Tomo → Region ROI

    a. Select **one** of the Regional ROI subfolders (e.g. Anterior ROI) and click Open
    b. On the lower right dropdown menu, change ROI files (.roi) to Bitmap files (.bmp). The list of ROIs should appear.
    c. Click the first image on the list, then Open
    d. You will be warned that the ROI will be monochrome. Click OK.
14. You can load multiple Pores and Regional ROIs in this manner. For example, load the same Pores set four times. Then load Anterior, Posterior, Medial, and Lateral ROIs separately as ROIs
15. Click the arrow next to Properties → Change Pixel Size. Make sure the pixel size is 5.49426 um for **All Datasets**. **Note that pixel size must typically be changed at this point, because the resolution data is lost through ImageJ processing.**
16. Make sure the boxes are checked next to all loaded Pores files
17. Click Start and the macro will run on its own.
18. The macro should output the following inside the Pores folder:
    a. A _.batman file containing summary pore data
    b. A _i3d file containing individual pore data
    c. TBTH: Image stack of trabecular thickness
    d. TBSP: Image stack of trabecular separation
    e. Individual Objects by Size: Image stack
    f. Note: The three image stacks use grayscale coding of the lowest values (approximate to black) so they will not be very visible on the actual image. However, they can be seen clearly in Dragonfly or Amira.
19. **If you loaded more than one ROI for the same pore set:** Each output will be numbered according to the order of the runs [e.g. TBTH, TBTH(1), TBTH(2)].
    a. If you are not sure which is which, check the order of the runs associated text file that ends in .batman. The name of the ROI will be listed in the first line.
        i. Alternatively open a despeckled pore image in that numbered folder and check whether it is Anterior (top), Medial (right), Posterior (bottom), or Lateral (left)
    b. Move each set of outputs into its own subfolder (e.g. Anterior Pores)

Appendix XIV: CTAnalyser Macros

TA Extractor

Description: Extracts a mask of total area from the grayscale micro-CT image for centerline skeletonization and subsequent longitudinal orientation

Workflow:

Thresholding (2D space)
Mode Two-dimensional (Otsu method)
Kernel Round
Radius 1
Background Dark

Despeckle
Type: Sweep (3D space)
Remove: all exept the largest object
Apply to: Image

ROI shrink-wrap
Mode : Shrink-wrap (3D space)

Morphological operations
Type: Erosion (3D space)
Kernel: Round
Radius: 2
Apply to: Region of Interest

Morphological operations
Type: Dilation (3D space)
Kernel: Round
Radius: 2
Apply to: Region of Interest

Morphological operations
Type: Closing (2D space)
Kernel: Round
Radius: 30
Apply to: Region of Interest

Morphological operations
Type: Erosion (3D space)
Kernel: Round

Radius: 2
Apply to: Region of Interest

Save bitmaps (only ROI)
File format: bmp

ROI and TA Extractor

Description: After longitudinal orientation, extracts a mask of total area and a mask of cortical area to serve as regions of interest (ROI) for pore extraction and morphometric analysis

Workflow:

Thresholding (2D space)
Mode Two-dimensional (Otsu method)
Kernel Round
Radius 1
Background Dark

Despeckle
Type: Sweep (3D space)
Remove: all exept the largest object
Apply to: Image

ROI shrink-wrap
Mode : Shrink-wrap (3D space)

Morphological operations
Type: Erosion (3D space)
Kernel: Round
Radius: 2
Apply to: Region of Interest

Morphological operations
Type: Dilation (3D space)
Kernel: Round
Radius: 2
Apply to: Region of Interest

Morphological operations
Type: Closing (2D space)
Kernel: Round
Radius: 30

Apply to: Region of Interest

Morphological operations
Type: Erosion (3D space)
Kernel: Round
Radius: 2
Apply to: Region of Interest

Save bitmaps (only ROI)
File format: bmp

Despeckle
Type: Remove pores (2D space)
Detected by: by image borders
Apply to: Region of Interest

Save bitmaps (only ROI)
File format: bmp

Pore Morphometry

Pore Morphometry Description: Acquires morphometric measurements from cortical pore networks on whole cross-sections; also generates binary image stacks of despeckled pore networks and grayscale image stacks of pore thickness and pore separation

Regional Pore Morphometry Description: Same functions as "Pore Morphometry", but limited to a given anatomical region of interest (Anterior, Posterior, Medial, Lateral)

Workflow:

Thresholding	
Mode	Global
Lower grey threshold	1
Upper grey threshold	255
Despeckle	
Type: Remove white speckles (3D space)	
Volume : less than 100 voxels	
Apply to: Image	
Thresholding	
Mode	Global

Lower grey threshold	0
Upper grey threshold	254

3D analysis
MORPHOMETRY RESULTS

Description	Abbreviation	Unit
Fractal dimension	FD	
Number of closed pores	Po.N(cl)	
Closed porosity (percent)	Po(cl)	%
Open porosity (percent)	Po(op)	%
Total porosity (percent)	Po(tot)	%

Thresholding

Mode	Global
Lower grey threshold	0
Upper grey threshold	254

Save bitmaps (only image)
File format: bmp

3D analysis
MORPHOMETRY RESULTS

Description	Abbreviation	Unit
Tissue volume	TV	U^3
Bone volume	BV	U^3
Percent bone volume	BV/TV	%
Tissue surface	TS	U^2
Bone surface	BS	U^2
Intersection surface	i.S	U^2
Bone surface / volume ratio	BS/BV	1/U
Bone surface density	BS/TV	1/U
Trabecular thickness	Tb.Th	U
Trabecular separation	Tb.Sp	U
Trabecular number	Tb.N	1/U
Degree of anisotropy	DA	
Fractal dimension	FD	
Number of objects	Obj.N	
Euler number	Eu.N	
Connectivity	Conn	
Connectivity density	Conn.Dn	1/U^3
Standard deviation of trabecular thickness	SD(Tb.Th)	U

Standard deviation of trabecular separation	SD(Tb.Sp)	U

Individual object analysis (3D space)

Description	Abbreviation	Unit
Object volume	Obj.V	um^3
Object surface	Obj.S	um^2
Volume of pores	Po.V	um^3
Surface of pores	Po.S	um^2
Porosity	Po	%
Number of pores	Po.N	
Centroid x	Crd.X	um
Centroid y	Crd.Y	um
Centroid z	Crd.Z	um
Moment of inertia (x)	MMI(x)	um^5
Moment of inertia (y)	MMI(y)	um^5
Moment of inertia (z)	MMI(z)	um^5
Polar Moment of inertia	MMI(polar)	um^5
Radius of gyration (x)	Gr.R(x)	um
Radius of gyration (y)	Gr.R(y)	um
Radius of gyration (z)	Gr.R(z)	um
Polar Radius of gyration	Gr.R(polar)	um
Product of inertia (xy)	Pr.In(xy)	um^5
Product of inertia (xz)	Pr.In(xz)	um^5
Product of inertia (yz)	Pr.In(yz)	um^5
Orientation theta	Or(theta)	°
Orientation phi	Or(phi)	°
Structure model index	SMI	
Structure thickness	St.Th	um
Equivalent rod length	ERL	um
Major diameter	Maj.Dm	um
Volume-equivalent sphere diameter	ESDv	um
Surface-equivalent sphere diameter	ESDs	um
Sauter diameter	Sau.Dm	um
Sphericity	Sph	
Mean density	Dens	Index
Maximum density	Dens(max)	Index
Surface convexity index	SCv.I	1/um
Euler number	Eu.N	
Connectivity	Conn	

Legend of color-coded images:
Index
ESDv (um)
Save bitmaps (clipboard)

Cross-Sectional Geometry

Description: Extracts cross-sectional geometric measurements from the cortical area image stack, using the total area image stack as a mask

Workflow:

Thresholding	
Mode	Global
Lower grey threshold	1
Upper grey threshold	255

2D analysis
Summary 2D data

Description	Abbreviation	Unit
Tissue volume	TV	um^3
Bone volume	BV	um^3
Percent bone volume	BV/TV	%
Tissue surface	TS	um^2
Peripheral tissue surface	TS(per)	um^2
Bone surface	BS	um^2
Peripheral bone surface	BS(per)	um^2
Bone surface / volume ratio	BS/BV	1/um
Mean total crossectional tissue area	T.Ar	um^2
Mean total crossectional tissue perimeter	T.Pm	um
Mean total crossectional bone area	B.Ar	um^2
Mean total crossectional bone perimeter	B.Pm	um
Average moment of inertia (x)	Av.MMI(x)	um^4
Average moment of inertia (y)	Av.MMI(y)	um^4
Mean polar moment of inertia	MMI(polar)	um^4
Average principal moment of inertia (max)	Av.MMI(max)	um^4

Average principal moment of inertia (min)	Av.MMI(min)	um^4
Mean eccentricity	Ecc	
Crossectional thickness	Cs.Th	um
Centroid (x)	Crd.X	um
Centroid (y)	Crd.Y	um
Centroid (z)	Crd.Z	um
Mean fractal dimension	FD	
Total intersection surface	i.S	um^2
Percent intersection surface	i.S/TS(per)	%

Appendix XV: ImageJ Macros

Adaptive Thresholding

Description: Applies a low-contrast, local thresholding Phansalkar algorithm to the grayscale micro-CT image, extracting the cortical pore network as a binary image stack, and excluding external noise by using the cortical area image stack as a mask

Workflow:

```
macro "Adaptive Thresholding"{
setBatchMode(true);

dir1= getDirectory("Select_Tomo_Images");
list1= getFileList(dir1);

dir2= getDirectory("Select_ROIs");
list2= getFileList(dir2);

dir3= getDirectory("Select_Output");

while (nImages>0) {
        selectImage(nImages);
        close(); }

//Clear any past results

run("Clear Results");

//Image loop

for (i=0; i<lengthOf(list1); i++){

//Open the pore image and threshold

open(dir1+list1[i]);

tomo=getTitle();

selectImage(tomo);

run("8-bit");
run("Enhance Contrast...", "saturated=0.3 normalize");
```

```
run("8-bit");
run("Gaussian Blur...", "sigma=1");

run("8-bit");
run("Auto Local Threshold", "method=Phansalkar radius=15 parameter_1=0 parameter_2=0 white");

//Invert tomo

selectImage(tomo);
run("8-bit");
run("Invert");
tomo=getTitle();

//Open ROI and use as mask

open(dir2+list2[i]);

roi=getTitle();

imageCalculator("AND create", tomo,roi);

out=getTitle();

selectImage(out);

setAutoThreshold("Default dark");
run("Threshold...");
setThreshold(1, 255);
setOption("BlackBackground", true);
run("Convert to Mask");

selectImage(out);

run("8-bit");
run("Close-");
run("Fill Holes");

saveAs("bmp", dir3 + tomo);

//Close all open images

while (nImages>0) {
        selectImage(nImages);
        close(); }}}
```

Skeleton Save

Description: Automatically runs and saves the Skeletonization 3D plugin on the total area mask, converting it to a centerline skeleton image stack

Workflow:

```
macro "Skeleton Save"{
setBatchMode(true);

out= getDirectory("Select_Output");

run("Image Sequence...");
orig=getTitle();

selectWindow(orig);

run("Skeletonise");

selectWindow(orig);
close();

skel=getTitle();

selectWindow(skel);

//3x Dilate

run("Dilate", "stack");

run("Dilate", "stack");

run("Dilate", "stack");

selectImage(skel);

run("Image Sequence... ", "format=BMP name=Skeleton start=0001 save=out");

while (nImages>0) {
        selectImage(nImages);
        close();
}}
```

Femur Quadrants / Tibia Quadrants

Description: Using the cortical area image stack as an input, finds the centroid with the Slice Geometry plugin, and then rotates a line through this centroid to generate image stacks of masks representing the Anterior, Posterior, Medial, and Lateral anatomical regions of each cross-section. A given regional mask can be used as the region of interest (ROI) in the Regional Pore Morphometry macro to restrict morphometric analysis of the pore network to that anatomical region.

Workflow:

```
macro "Femur Quadrants"{
setBatchMode(true);

//Pop up BoneJ Usage Legacy and prompt user for OK

run("BoneJ Usage (Legacy)");

//Clear any past results

run("Clear Results");

//Set background color to black for clearing regions

setBackgroundColor(0, 0, 0);

//Set input directories to prompt user to load cortical ROI stack

dir1= getDirectory("Select_Cortical_ROI");
list1= getFileList(dir1);

//Make an output directory

dir2= getDirectory("Select_Output_Directory");

//Make output subdirectories

regiondraw=dir2+"/Drawn Octants/";
File.makeDirectory(regiondraw);

regionA=dir2+"/Anterior ROI/";
File.makeDirectory(regionA);

regionM=dir2+"/Medial ROI/";
File.makeDirectory(regionM);
```

```
regionP=dir2+"/Posterior ROI/";
File.makeDirectory(regionP);

regionL=dir2+"/Lateral ROI/";
File.makeDirectory(regionL);

//Burner Slice Geometry

open(dir1+list1[1]);
run("Slice Geometry", "bone=femur bone_min=1 bone_max=255 slope=0.0000 y_intercept=0");
close();
run("Clear Results");

//Loop Start

for (i=0; i<lengthOf(list1); i++){

//Open the cortical mask image

	open(dir1+list1[i]);
	orig=list1[i];

	imgname=getTitle();

//Remove scale

run("Set Scale...", "distance=0");

//Clear results
run("Clear Results");

//Run BoneJ Slice Geometry

run("Slice Geometry", "bone=femur bone_min=1 bone_max=255 slope=0.0000 y_intercept=0");

//Save slice geometry centroid X value

cX=getResult("X cent. (pixels)",0);
cY=getResult("Y cent. (pixels)",0);

//Draw a line through the centroid to the top and bottom of the image

w = getWidth();
```

```
h = getHeight();

selectImage(orig);

//Coordinates for centroid to top of image
//makeLine(cX, 0, cX, h);

//Rotation code based off of: https://stackoverflow.com/questions/14842090/rotate-line-around-center-point-given-two-vertices
//Starting coordinates are as follows
//x = cX
//y = 0
//Add -1000 to Y to extend beyond borders
//cx = cX
//cy = cY

//Rotate 45 degrees counterclockwise to AL line

a = 45 * PI / 180;

xAL = (  (cX - cX) * cos(a) + (-1000 - cY) * sin(a) ) + cX;
yAL = ( -(cX - cX) * sin(a) + (-1000 - cY) * cos(a) ) + cY;

//Rotate 135 degrees counterclockwise to PL line

a = 135 * PI / 180;

xPL = (  (cX - cX) * cos(a) + (-1000 - cY) * sin(a) ) + cX;
yPL = ( -(cX - cX) * sin(a) + (-1000 - cY) * cos(a) ) + cY;

//Rotate 225 degrees counterclockwise to PM line

a = 225 * PI / 180;

xPM = (  (cX - cX) * cos(a) + (-1000 - cY) * sin(a) ) + cX;
yPM = ( -(cX - cX) * sin(a) + (-1000 - cY) * cos(a) ) + cY;

//Rotate 315 degrees counterclockwise to AM line

a = 315 * PI / 180;

xAM = (  (cX - cX) * cos(a) + (-1000 - cY) * sin(a) ) + cX;
yAM = ( -(cX - cX) * sin(a) + (-1000 - cY) * cos(a) ) + cY;
```

```
//Duplicate image and draw lines

selectImage(orig); run("Duplicate...", "title=[Drawn]");
drawoct="Drawn";

selectImage(drawoct);

makeLine(cX, cY, xAL, yAL);
run("Draw");
makeLine(cX, cY, xPL, yPL);
run("Draw");
makeLine(cX, cY, xPM, yPM);
run("Draw");
makeLine(cX, cY, xAM, yAM);
run("Draw");

saveAs("bmp", regiondraw + imgname);
close();

//Duplicate image and draw anterior quadrant

selectImage(orig);
run("Duplicate...", "title=[Temp Octant]");
tempoct="Temp Octant";

makePolygon(xAM,yAM,xAL,yAL,cX,cY);

setBackgroundColor(0, 0, 0);
run("Clear Outside");

setAutoThreshold("Default dark");
run("Threshold...");
setThreshold(1,255);
setOption("BlackBackground", true);
run("Convert to Mask");

saveAs("bmp", regionA + imgname);

close();


//Duplicate image and draw lateral quadrant

selectImage(orig);
```

```
run("Duplicate...", "title=[Temp Octant]");
tempoct="Temp Octant";

makePolygon(xAL,yAL,xPL,yPL,cX,cY);

setBackgroundColor(0, 0, 0);
run("Clear Outside");

setAutoThreshold("Default dark");
run("Threshold...");
setThreshold(1,255);
setOption("BlackBackground", true);
run("Convert to Mask");

saveAs("bmp", regionL + imgname);

close();


//Duplicate image and draw posterior quadrant

selectImage(orig);
run("Duplicate...", "title=[Temp Octant]");
tempoct="Temp Octant";

makePolygon(xPL,yPL,xPM,yPM,cX,cY);

setBackgroundColor(0, 0, 0);
run("Clear Outside");

setAutoThreshold("Default dark");
run("Threshold...");
setThreshold(1,255);
setOption("BlackBackground", true);
run("Convert to Mask");

saveAs("bmp", regionP + imgname);

close();

//Duplicate image and draw posterior quadrant

selectImage(orig);
run("Duplicate...", "title=[Temp Octant]");
```

```
tempoct="Temp Octant";

makePolygon(xPM,yPM,xAM,yAM,cX,cY);

setBackgroundColor(0, 0, 0);
run("Clear Outside");

setAutoThreshold("Default dark");
run("Threshold...");
setThreshold(1,255);
setOption("BlackBackground", true);
run("Convert to Mask");

saveAs("bmp", regionM + imgname);

close();

//Close original image

selectImage(orig);
close();

}
}
```

Appendix XVI: Skeletonization SOP

General Avizo Tips

You can save your project view using File → Save Project As → Minimize Project Size → OK → Save as type Amira Project (*.hx). When you open this file in Amira, all of your code will re-run.
Or choose Minimize Project Computation to save all of the backup files so you do not have to rerun code (requires more space)
To delete an object in the Project View field, drag it to the Trash Can, or click Delete on the keyboard
To organize your Project View nicely, click the Reorder Project View  button at the top

Extract Despeckled Pore File

Navigate to Desktop → Kassidy Skeletonization → Rabbit Femora
Select one of the 7zip Pore Files. Right-click → 7zip → Open Archive
In the opened 7zip archive, navigate to Pores → Total Pores
Click on the Despeckled Pores Folder (it should turn blue)
Click "Extract" in the top toolbar
In the Copy popup, click OK
Despeckled Pores will extract to the main Rabbit Femora Folder
Right click → Rename
Rename it with Number_Femur_Despeckled
E.g. 01_Femur_Despeckled

Load File in Amira

Double-click the Amira 6.4.0. icon on the Desktop
Open Blank Project
Open Data (Green button in upper left hand corner)
Navigate to Desktop → Kassidy Skeletonization → Rabbit Femora → The extracted despeckled pore file
In the popup, click the top file in the folder. Scroll to the bottom. Hold down shift and click the bottom file in the folder. All of the files in the folder should now be highlighted. Click Open.
Amira should pop up an "Out of Core Data" popup. If not, check the Amira icon on the bottom of the window – it may be hidden behind Amira.
Select "Read complete volume into memory"
Do not check Run Batch
Click OK
Image Read Parameters should pop up.
Channel conversion: Channel 1
You can change the object name if you like, or leave it as default
Original Coordinate Units: micrometer
Define: Voxel Size
Min coord: 0 0 0
Voxel size: 5.494265.494265.49426
Uncheck Read as multiple files
Click OK

Sometimes you accidentally load only one slice. To make sure the volume loaded in 3D, you can click the green object file that popped up when you loaded the image, then select "Volren"
Use the hand tool at the top of the toolbar to rotate the 3D image
Use the four-way arrow tool to move the 3D image laterally or up and down within the field of view
Use the magnifying glass to zoom in and out

Setting Up Auto-Skeleton

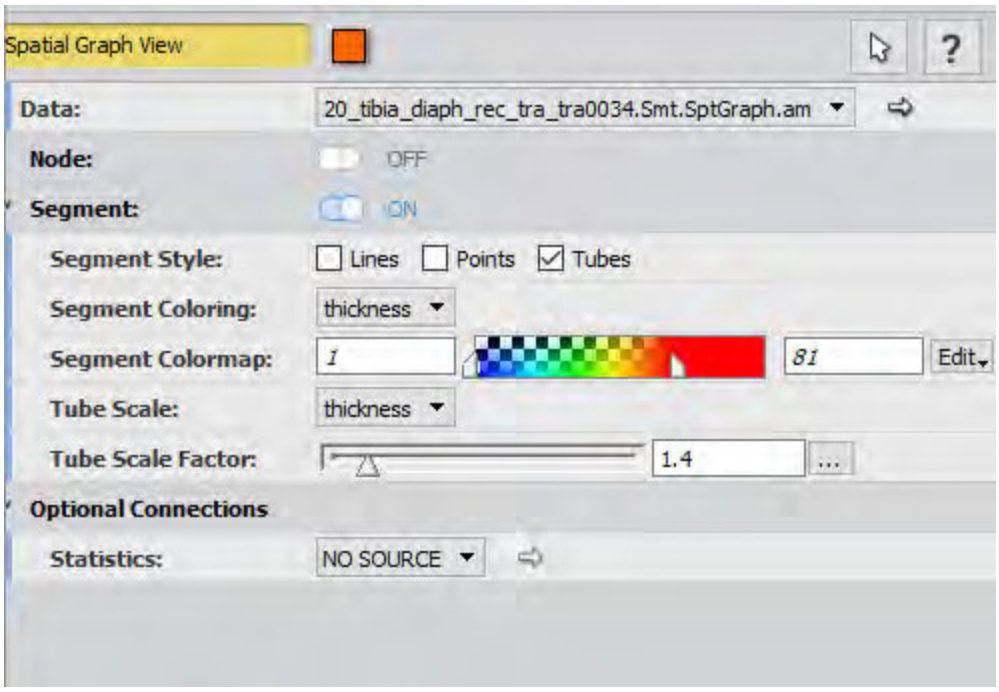
Right-click the green object file that popped up when you loaded the image. If you changed the file name, it will have your chosen name.
Right-click will pop up a menu of functions.
Navigate to Image Processing → Skeletonization → Auto-Skeleton → Create
OR you can search for Auto Skeleton in the toolbar on the popup
Auto-Skeleton Properties
Data = Name of your loaded image stack
Filament type = Bright on Dark
Threshold = 1 (or can leave as default, since all pixels in this image are 254 pixel brightness)
Preview = Off (saves time)
Smoothing = ON
Smooth = 0.5
Attach to Data = 0.25
Number of Iterations = 10
Click on the arrow next to Output Options
Create Objects: **Uncheck** All
Show Spatial Graph: **Check**
Click Apply and the Auto-Skeleton will run. It may take several hours to run.
Try to not touch Amira during this time or run any other programs. It may say it is Not Responding, but it is still running. If it pops up an error, choose "Wait for Program to Respond". The screen may become grayed out.

Exporting LineSet and Statistics

Saving Spatial Graph: Right click the .SptGraph object created by Auto Skeleton
Click on Export Data As… which looks like a folder with a yellow arrow
In the popup Export Data As, change Save As Type to "AmiraMesh Spatial Graph (*am)"
Navigate to your Number_Femur_Despeckled folder and click New Folder, which you will name "Skeletonization Files"
Save the SptGraph ibject in your new Skeletonization Files folder
We will use the LineSet data to run Isaac Pratt's code eventually
Creating LineSet: Right click the .SptGraph object
In the functions menu, select Convert → Spatial Graph to Line Set → Create → Apply
Saving LineSet: Right click on the .SptGraph-LS object created
Click on Export Data As… which looks like a folder with a yellow arrow
In the popup Export Data As, change Save As Type to "LineSet(*lineset)
Change the save location to your Number_Femur_Despeckled → Skeletonization Files folder and click Save
We will use the LineSet data to run Isaac Pratt's code eventually

Creating Statistics: To export statistics, right click the .SptGraph object created by Auto Skeleton
In the functions menu, select Measure and Analyze → Spatial Graph Statistics Create
In Spatial Graph Statistics Properties:
Data: Your SptGraph object
Output: Spreadsheet
Click Apply
This creates a .statistics object
Saving Statistics: Right click on the .statistics object
Click on Export Data As… which looks like a folder with a yellow arrow
In the popup Export Data As, change Save As Type to "CSV (*csv)"
Change the save location to your Number_Femur_Despeckled Skeletonization Files folder and click Save
You can open the .csv file in Excel to get summary statistics and individual segment statistics

Adjusting Visualization
Click on the Amira 6.4.0 icon to open Amira
File → Open Project
Open your Amira Spatial Graph Image Template
File → Open Data → Open the SptGraph object you want to visualize
Right-click on the SptGraph green object Display Spatial Graph View Create
Click the Spatial Graph View so its Properties appear
Data: Spt.Graph object
Node = OFF
Segment = ON
Segment Style
Uncheck Lines and Points
Check Tubes
Tube Scale = Thickness
Tube Scale Factor = 1.4
Segment Coloring = Thickness
Segment Colormap
Edit → physics_VolRend_1to81.am
Make sure the left value is 1 and the right value is 81. **If this alters the colormap, you will need to adjust the annotation as seen below.**
Edit → Options → Uncheck everything by clicking on it



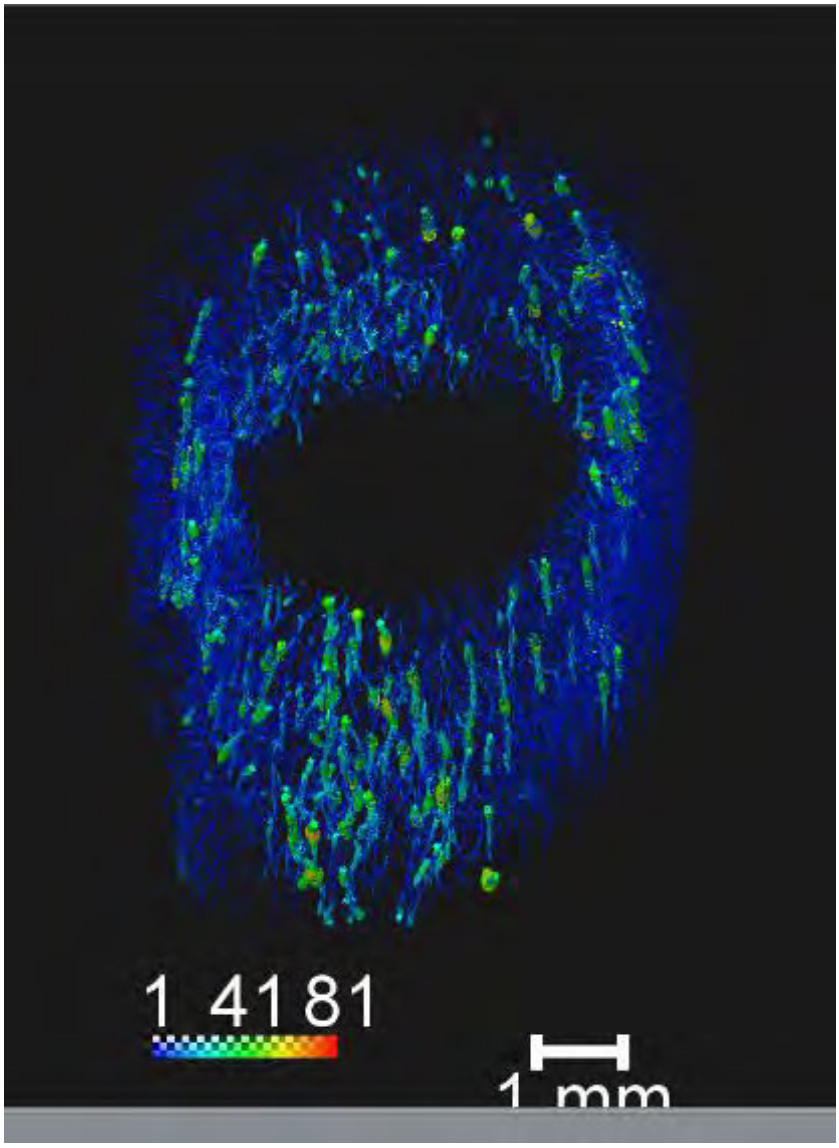
Orient the object as you like using the top toolbar. I like to do a ½ tilt view so you can see both the sides and the top.

Click the Orthographic projection button so the eye is staring straight lines:  This makes the scale bar accurate.

Click the XY button at the top toolbar to view the image from above: 

Use the hand tool to grab the bottom of the skeletonized image and tilt it up slightly so that the side can be seen: 

Use the arrow tool to move the image laterally within the field so it is centered if needed: 



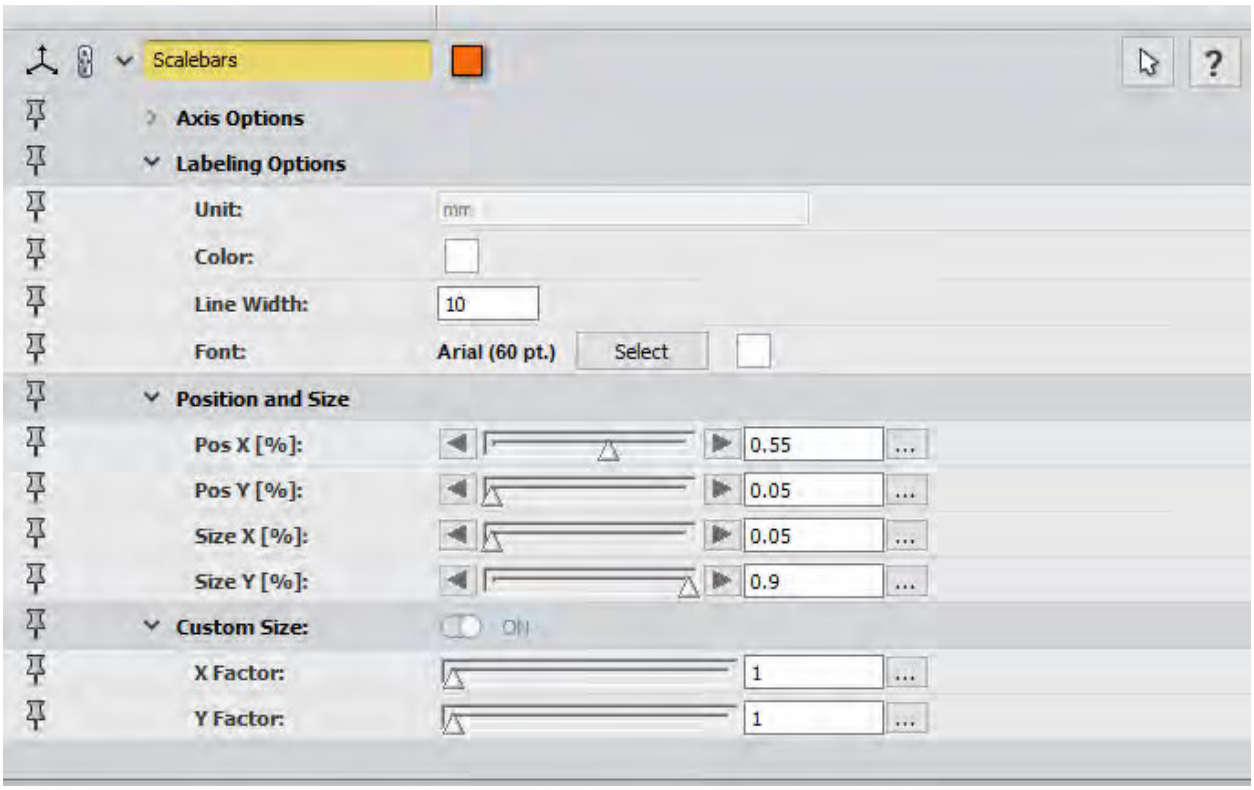
A ½ tilt view of a femur. Note the enlarged colormap and scale bar to allow tiling of the image.

<u>If You Need to Adjust or Create a Scale</u>

To create a NEW scalebar, right click in the Project View area (not on a particular box)

Create Object → Scalebars → Create from the drop down menu

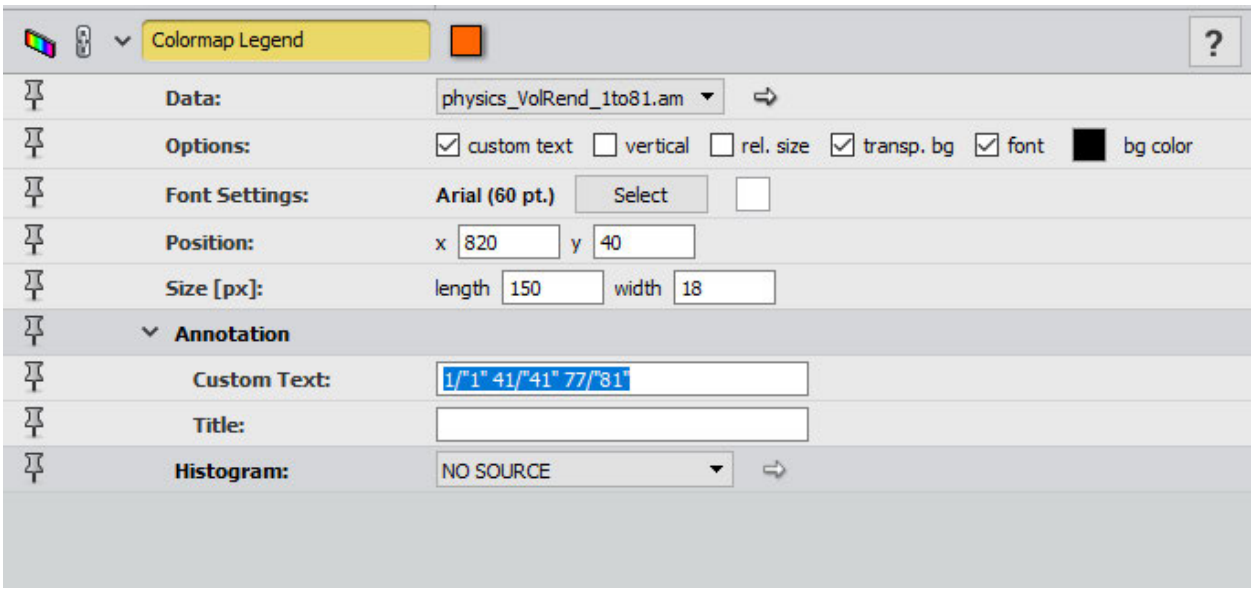
Click the yellow Scalebars object and replicate these settings. Change Font using the Select button. **Note that the scale bar will look unusually big – this is because we are taking an enlarged tiled image.**



If You Need to Adjust or Create a Colormap

To create a NEW colormap, right click on the physics_VolRend_1to81.am green object. Select Annotate → Colormap Legend → Create
Click the yellow Scalebars object and replicate these settings. **Note that the colormap will look unusually big – this is because we are taking an enlarged tiled image.**
The most likely scenario is that adjusting the values for the colormap will alter the custom text. In that case, copy the following into the Custom Text box:

1/"1" 41/"41" 77/"81"



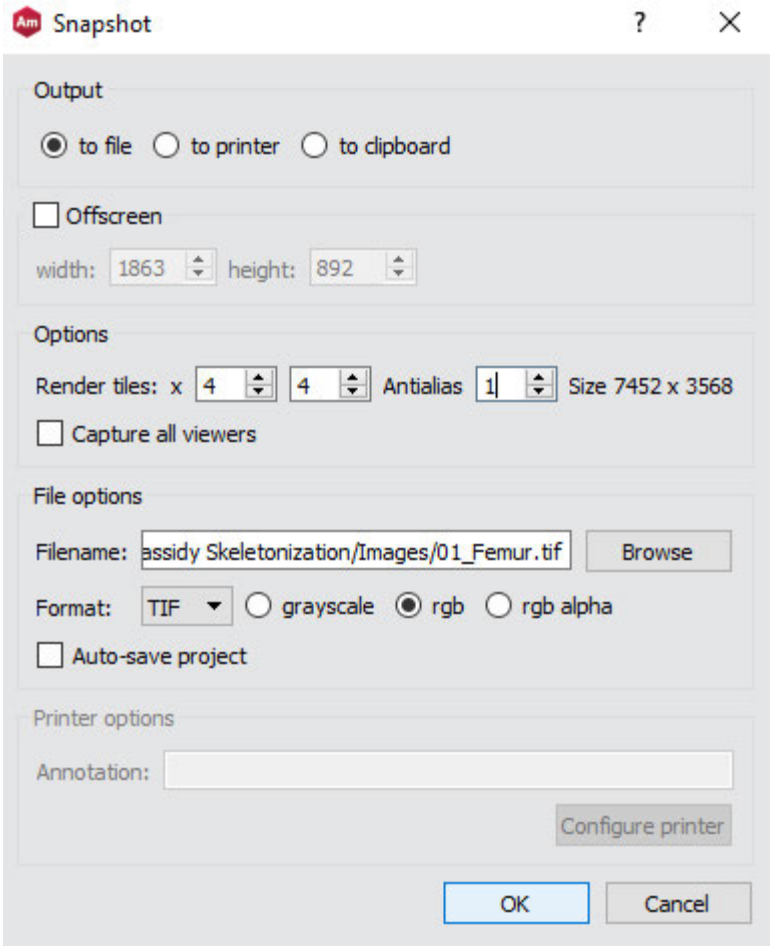
Taking the Image

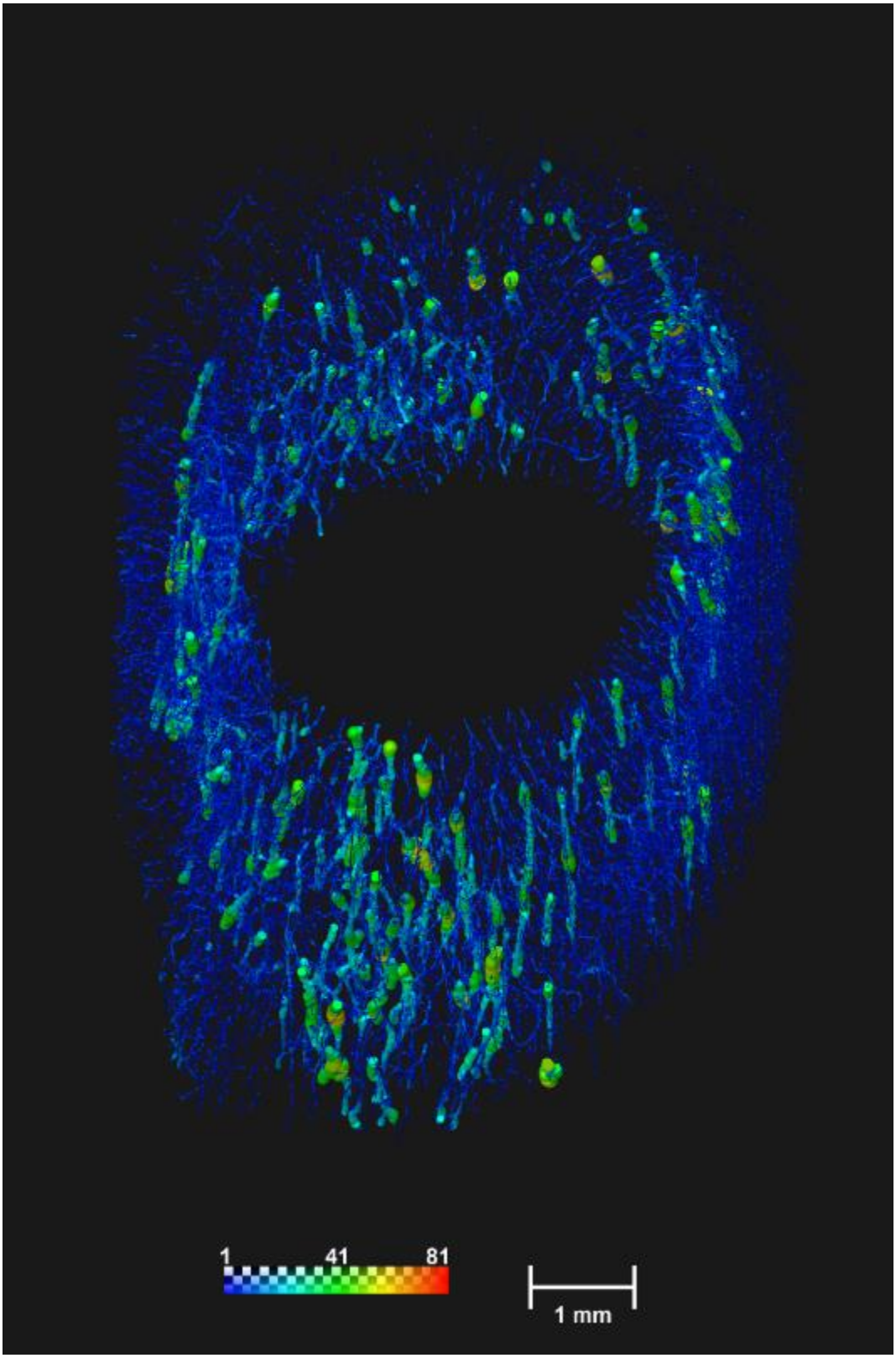
Click on the camera icon on the top toolbar: 

In the Snapshot popup:

Output: to File
Uncheck Offscreen
Options: Render tiles 4 x 4
Antialias 1
Uncheck Capture All viewers
File options:
Browse to change the Filename to the folder where you want to output the images. I have put them into C:\Users\FROST\Desktop\Kassidy Skeletonization\Images
Add an image name like Number_Bone
Change Save as type to TIF (*tif)
Format: TIF, rgb
Uncheck Auto-Save Project

Click OK and the image will render. Check the image using ImageJ





Final Image (Cropped)

Cropping and Color Bar Title in ImageJ

Note: You can also just do this in Microsoft Word

The color bar is based on the maximum pore radius (81 um) of the sample with the largest pore thickness, 20 Tibia. We need to add a title to the color bar in ImageJ or in Word. It cannot be done in Amira because the tiling mechanism of the screenshot causes the color bar title to overlap with the colorbar itself.

Cropping:

Open ImageJ from the desktop.
Open your image with File Open
Use the rectangle tool to draw a box of the desired size around the image
Image Crop

Adding Color Bar Title

Modify the font using Edit Options Fonts
- Font = Arial
- Style = Bold
- Size = 60
- Click Close

Select the Text tool (looks like a letter A)
Draw a box under the color bar. Type **Pore Radius (um)**
After typing, you can move the box around by clicking and dragging to position it better
To merge the text with the image, type Ctrl + D on the keyboard, or click Edit Draw
Save the image using File Save As Tiff. I recommend you save it using a new name so you also have the original image.

Aggregating Data in Excel

Note: Descriptions of each statistics can be found in Amira under Help User's Guide and search for Spatial Graph Statistics
Open the .statistics csv file in Excel
Copy the line of numbers under Graph Summary to your Master Skeletonization Spreadsheet
Right click → Insert four times to add four lines above the Graph Statistics section.
- Note: These are the summed values of the

Type Mean, Median, Max, and Min in the added four rows in the first column (A)
In the second column, to the right of Mean, type **=AVERAGE(**
- Click the first numeric box (to the right of Graph0), under Number of Segments
- On the keyboard, press Ctrl + Shift + Down Arrow. This will skip to the bottom of the column
- Type the close parentheses **)**
- This should move you back up to the top of the page and calculate the average for Number of Segments
- To get the means of the rest of the Graph Statistics, click the Mean numeric box you just filled in. A dot should appear in the lower right corner. Click on the dot and drag it to the last Graph Statistics column (Isolated Nodes). This should fill in the means for all the Graph Statistics

Repeat step 5 for the other statistics:
- **=MEDIAN(**
- **=MAX(**

=MIN(

Important: The Graph Summary "mean length" and "mean radius" are calculated from the individual segments (you can see this if you perform the statistics on the Segment Statistics section at the bottom of the page). The "mean length" and "mean radius" you will calculate are based on the individual pore systems, which are composed of multiple segments. Therefore, these variables will not match. I have clarified "Segment Mean Length" and "Pore System Mean Length" on the master spreadsheet.

I have also modified the names of the other columns a bit to be clearer. You can change the variable names in the master spreadsheet to whatever makes sense to you, or whatever fits best on your statistics readout. You might need to make up abbreviations for a nice statistics graph readout.

Some of the means will also be applied to means. For example, the Mean Pore System Mean Length is the average of all pore system mean lengths. The Mean Pore System Total Length is the average of all pore system total lengths.

To copy a strip of values (e.g. means) to the master spreadsheet, click and drag to highlight the row, then right click → Copy or Ctrl+C on the keyboard

On the master spreadsheet, right click in the first mean value box and click the image of the clipboard with 123. This will paste the numeric values. If you copy and paste with Ctrl+V, you will get an error because it is trying to copy the formula.

Note: As there are many fragments, mean and median values of some numeric quantities (e.g. intermediate nodes, terminal nodes) may be zero. Minimum values may also be 1 or 0. If all individuals have the same number (e.g. intermediate nodes = 0), don't bother running stats on this variable. You may be able to toss out of lot of these types of variables, but calculate them for now to see if there is any variation between individuals.

Appendix XVII: Researcher Return to Work

Andronowski Research Group: Researcher Return to Work Plan – COVID-19
Dr. Janna M. Andronowski – Office: ASEC 306, Laboratory: ASEC B228

This document is designed to outline the guidelines and expectations of Andronowski Lab members for a safe return to lab activities during the ongoing COVID-19 pandemic. All group members must understand the information in this manual regarding the guidelines that have been mandated by The University of Akron to prevent the spread of the Coronavirus (COVID-19) both on campus and in the greater community. Guidelines regarding how we will employ a safe return to lab work are outlined below.

Remote and On-Campus Work

Laboratory members must voluntarily agree to support Dr. Andronowski and her research on a "need to be there" basis. ***This guidance is not intended to replace work that can be done remotely.*** All group meetings, individual meetings, and project discussions will continue to be conducted remotely. Face masks must be worn when in the same room as others, daily disinfecting must be conducted, and social/physical distancing must be maintained. Laboratory and office occupancy should be held only to the necessary minimum.

Limits on Laboratory Personnel Numbers

Current social/physical distancing guidelines recommend separation distances of 6 feet or more. No more than 2-3 individuals are permitted to be in the B228 main lab space at any given time. Any work in the back rooms is limited to one person at a time. Lab procedures requiring more than one person in close proximity will be minimized as much as possible. If more than one person is required in less than a 100 square foot area, the individuals involved must wear PPE (masks, gloves, and eye protection) and treat each other as if they have COVID-19 but are asymptomatic.

Signage will be posted on the lab doors (outside doors to the hallway) identifying all approved personnel for B228.

Implementation of Shifts

Lab shift schedules will be utilized in order to ensure social distancing requirements. We will employ the TimeTree app to schedule and change shifts. Our weekly schedule must be shared with Dr. Weeks each Monday morning.

Randomized 'spot checks' of the lab will be performed once or twice per week to ensure that the safety guidelines identified herein are being followed.

Documentation of Biology lab checks can be found here: https://docs.google.com/spreadsheets/d/11__BdDCQlQ2TtDhLEOnjk_bi0pFtkDZkP1XOo__QbLI/edit?usp=sharing

Limits on Office Use and Occupancy

As noted above, office work should continue to take place at remote locations (e.g., at home). For those engaged in laboratory work, and for rare research tasks requiring access to individual offices, office occupation should be limited to one person. Doors must remain closed when offices are occupied. For office spaces larger than 200 sq.ft., exceptions may be possible, but need to be pre-approved by the Provost in conjunction with Vice Presidents, Deans or Department Chairs.

Personal Protective Equipment (PPE) Requirements

Safety is of utmost importance to the Andronowski Lab. Proper safety protocols are an extremely important aspect of working in a biology lab, especially in this modified research environment. Face masks are required in all common areas (hallways, restrooms, break areas). Face masks must be worn in public and any time there is more than one person in a given space, including times of brief interaction between co-workers or friends. This rule is applicable anytime another person is within six feet. Masks should also be worn in the main lab space, even if working alone, to minimize contamination. Employees working alone in their offices, however, do not need to wear a mask. No one-on-one meetings or group meetings are to be held in individual offices, and the door should remain closed.

All shared work areas (desktop, keyboards, chairs, etc.) and equipment needs to be cleaned and disinfected before and after each use.

Keep the lab tidy – clean up any messes or spills (use the spill kit if necessary) and put any items not in use back in storage. Do not let cardboard accumulate in the lab – place it in the hallway outside the lab and label with 'Trash'. It will be picked up by custodial staff.

Recommended PPE and Sanitizing Practices

Required PPE: Face masks are required in common spaces. Gloves and eye protection to be worn in the lab if within 100 feet of others. Normal required PPE for lab experiments (e.g., lab coats).

Sanitizing Practices: Wash hands upon arrival and prior to departure. Clean and disinfect all shared work surfaces and equipment (e.g., with disinfectant wipes or equivalent) before and after each visit.

Minimize contamination by removing gloves and lab coats before entering office areas, break rooms, or the hallway. If you are transporting chemicals, use a secondary container and the lab cart rather than wearing gloves. There are grey pails for large (4 L) bottles or even small bottles, and a green lab cart for other items.

Your used glassware should be thoroughly washed as soon as possible after use – plan to do dishes on a daily basis. All dishes should first be scrubbed with soap and water and then soaked in the base bath (if necessary).

No ***food or drink*** in the lab. This includes water bottles/coffee to avoid contamination when masks are removed. Beverages and snacks can be enjoyed in the large common area on B level, etc.

Working Alone

You should not be working alone in the laboratory after hours. There are some cases, however, where you have to conduct research on evenings and weekends. Please fill in the TimeTree schedule accordingly. If you have to work on evenings and weekends, ask another designated lab member if they will be in as well. If you have to work alone, please do not use certain laboratory equipment (e.g., mill-drill press, Well Diamond Wire Saw, Grinder/Polisher, IsoMet saw) or conduct potentially dangerous procedures (e.g., using harsh chemicals). You also must be in contact with Dr. Andronowski or a senior and competent member of the group by texting or telephone every hour. When you leave the lab, let your contact person know that you have safely left the lab and are finished with your experiments. This is to ensure that emergency response personnel or campus security can be requested in the event of an accident.

Note: Certain aspects of this protocol is based on The University of Akron and NEOMED's policies for safe return to work.

_____

*By signing, I _____ **(print name)** certify that I understand the safety information provided in this document by Dr. Andronowski and The University of Akron.*

_____ **(Signature)**

_____ **(Date)**

Appendix XVIII: Projected MMA Timeline

Week	Isomet	Dehydrate	MMA	Diamond Saw	Microscope Imaging	Image Analysis	Other
6/1 – 6/5		Test					
6/8 – 6/12			Test				
6/15 – 6/19	Batch 1			Test			
6/22 – 6/26		Batch 1					
6/29 – 7/3			Batch 1A				
7/6 – 7/10			Batch 1B				
7/13 – 7/17	Batch 2A	Batch 2A		Batch 1A			
7/20 – 7/24	Batch 2B	Batch 2B	Batch 2A	Batch 1B			Microscopic Imaging Training
7/27 – 7/31	Batch 2C	Batch 2C	Batch 2B	Batch 2A			Microscopic Imaging Training
8/3 – 8/7			Batch 2C	Batch 2B	Batch 1		Analysis Protocol Development
8/10 – 8/14				Batch 2C	Batch 1		Analysis Protocol Development
8/17 – 8/21					Batch 1		Analysis Protocol Development
8/24 – 8/28					Batch 1		
8/31 – 9/4					Batch 1		
9/7 – 9/11					Batch 1		
9/14 – 9/18					Batch 1		
9/21 – 9/25					Batch 1		
9/28 – 10/2					Batch 1		
10/5 – 19/9					Batch 1		
10/12 – 10/16					Batch 1		
10/19 – 10/23					Batch 1		
10/26 – 10/30					Batch 2		
11/2 – 11/6					Batch 2		
11/9 – 11/13					Batch 2		
11/16 – 11/20					Batch 2		
11/23 – 11/27							Thanksgiving Break
11/30 – 12/4					Batch 2		
12/7 – 12/11					Batch 2		
12/14 – 12/18					Batch 2		
12/21 – 12/24					Batch 2		
12/25 – 1/1							Christmas / New Years
1/4 – 1/8					Batch 2		
1/11 – 1/15					Batch 2		Digital Image Analysis Training

1/18 – 1/22					Batch 2	Batch 1	
1/25 – 1/29					Batch 2	Batch 1	
2/1 – 2/5						Batch 1	
2/8 – 2/12						Batch 1	
2/15 – 2/19						Batch 1	
2/22 – 2/26						Batch 1	
3/1 – 3/5						Batch 1	
3/8 – 3/12						Batch 1	
3/15 – 3/19						Batch 2	
3/22 – 3/26						Batch 2	
3/29 – 4/2						Batch 2	
4/5 – 4/9						Batch 2	
4/12 – 4/16						Batch 2	
4/19 – 4/23						Batch 2	
4/26 – 4/30						Batch 2	
5/3 – 5/7						Batch 2	
May 2021							Statistics
June 2021							Statistics; Draft Publications; AAFS 2021 Meeting
July 2021							Finalize and Submit Publications
August 2021							Prepare and Submit Final NIJ Report

MMA Timeline Narrative 2020 -2021

General Parameters for Batches of 10-11 Samples:

Slidemaking: 9 weeks (4-weeks dehydration, 5-weeks MMA infiltration and curing, 5-weeks Diamond Wire Saw sectioning and mounting, overlapping steps in each week).

Imaging: Two slides per individual * Three views per slide (brightfield, polarized, fluorescence) = 6 cross-sections imaged per individual. Assume 2 hours imaging and photomerging per slide at 200x or 12 hours for all six cross-sections per individual. For a batch of 10 individuals, assume 120 hours total. If an individual uses the scope two hours per day, five days per week, this will take 12 weeks for a single batch of 10 individuals.

Analysis: Most likely, people will analyze one sample per day depending on density of features. This will include point counts, manual circling of osteons, and potentially some manual or automated component to fluorescence imaging. Since each individual has two slides, assume four days per sample for analysis, or 8 weeks per batch of 10 samples.

June 2020: Preliminary Slidemaking Test with Mini Rex Rabbits

Week 1 (6/1 – 6/5): Test rabbit dehydration sequence

Week 2 (6/8 – 6/12): Test rabbit MMA infiltration and waterbath curing

Week 3 (6/15 – 6/19): Test rabbit diamond wire saw testing; Batch 1 (#1 – #10) isomet sectioning

Week 4 (6/22 – 6/26): Batch 1 dehydration sequence

July 2020: Batch 1 and 2 Processing

Week 1 (6/29 – 7/3): Batch 1A MMA infiltration and waterbath curing

Week 2 (7/6 – 7/10): Batch 1B MMA infiltration and waterbath curing

Week 3 (7/13 – 7/17): Batch 1 diamond wire saw and mounting begins; Batch 2A (#11 – 14) isomet sectioning; Batch 2A dehydration sequence; Batch 1A diamond wire saw

Week 4 (7/20 – 7/24): Batch 2B (#15 – 18) isomet sectioning; Batch 2B dehydration sequence; Batch 2A MMA infiltration and waterbath curing; Batch 1B diamond wire saw; Microscopic imaging training

Week 5 (7/27 – 7/31): Batch 2C (#19 – 21) isomet sectioning; Batch 2C dehydration sequence; Batch 2B MMA infiltration and waterbath curing; Batch 2A diamond wire saw; Microscopic imaging training

August 2020: Batch 2 Processing and Batch 1 Imaging

Week 1 (8/3 – 8/7): Batch 2C MMA infiltration and waterbath curing; Batch 2B diamond wire saw; Analysis protocol development; Microscopic imaging of Batch 1 begins

Week 2 (8/10 – 8/14): Batch 2C diamond wire saw; Analysis protocol development; Microscopic imaging of Batch 1 continues

Week 3 (8/17 – 8/21): Analysis protocol development; Microscopic imaging of Batch 1 continues

Week 4 (8/24 – 8/28): Microscopic imaging of Batch 1 continues

September 2020: Batch 1 Imaging

Week 1 (8/31 – 9/4): Microscopic imaging of Batch 1 continues

Week 2 (9/7 – 9/11): Microscopic imaging of Batch 1 continues

Week 3 (9/14 – 9/18): Microscopic imaging of Batch 1 continues

Week 4(9/21 – 9/25): Microscopic imaging of Batch 1 continues

October 2020: Batch 1 and Batch 2 Imaging

Week 1 (9/28 – 10/2): Microscopic imaging of Batch 1 continues

Week 2 (10/5 – 19/9): Microscopic imaging of Batch 1 continues

Week 3 (10/12 – 10/16): Microscopic imaging of Batch 1 continues

Week 4 (10/19 – 10/23): Microscopic imaging of Batch 1 completed

Week 5 (10/26 – 10/30): Microscopic imaging of Batch 2 begins

November 2020: Batch 2 Imaging

Week 1 (11/2 – 11/6): Microscopic imaging of Batch 2 continues

Week 2 (11/9 – 11/13): Microscopic imaging of Batch 2 continues

Week 3 (11/16 – 11/20): Microscopic imaging of Batch 2 continues

Week 4 (11/23 – 11/27): Thanksgiving Break

December 2020: Batch 2 Imaging

Week 1 (11/30 – 12/4): Microscopic imaging of Batch 2 continues

Week 2 (12/7 – 12/11): Microscopic imaging of Batch 2 continues

Week 3 (12/14 – 12/18): Microscopic imaging of Batch 2 continues

Week 4 (12/21 – 12/24): Microscopic imaging of Batch 2 continues

Week 4.5 - 5 (12/25 – 1/1): Christmas / New Years

January 2021: Batch 2 Imaging

Week 1 (1/4 – 1/8): Microscopic imaging of Batch 2 continues

Week 2 (1/11 – 1/15): Microscopic imaging of Batch 2 continues

Week 3 (1/18 – 1/22): Microscopic imaging of Batch 2 continues; Image analysis of Batch 1 begins

Week 4 (1/25 – 1/29): Microscopic imaging of Batch 2 continues; Image analysis of Batch 1 continues

February 2021:

Week 1 (2/1 – 2/5): Image analysis of Batch 1 continues

Week 2 (2/8 – 2/12): Image analysis of Batch 1 continues

Week 3 (2/15 – 2/19): Image analysis of Batch 1 continues

Week 4 (2/22 – 2/26): Image analysis of Batch 1 continues

March 2021:

Week 1 (3/1 – 3/5): Image analysis of Batch 1 continues

Week 2 (3/8 – 3/12): Image analysis of Batch 1 completed

Week 3 (3/15 – 3/19): Image analysis of Batch 2 begins

Week 4 (3/22 – 3/26): Image analysis of Batch 2 continues

Week 5 (3/29 – 4/2): Image analysis of Batch 2 continues

April 2021:

Week 1 (4/5 – 4/9): Image analysis of Batch 2 continues

Week 2 (4/12 – 4/16): Image analysis of Batch 2 continues

Week 3 (4/19 – 4/23): Image analysis of Batch 2 continues

Week 4 (4/26 – 4/30): Image analysis of Batch 2 continues

May 2021: Batch 2 Imaging; Statistics

Week 1 (5/3 – 5/7): Image analysis of Batch 2 completed

June 2021: Statistics; Draft Publications; AAFS 2021 Meeting

July 2021: Finalize and Submit Publications

August 2021: Prepare and Submit Final NIJ Report

Appendix XIX: Histomorphometric Variables

- **Periosteal / Endosteal Measurements**
  - Mineralizing surface per bone surface calculated as (dLS + sLS/2)/BS on the endosteal (Es.MS/BS, %) and periosteal (Ps.MS/BS, %) surfaces.
- **Cross-sectional geometry measurements**
  - Uncorrected Area Measurements
    - Cortical Area (CA)
    - Marrow Area (MA)
    - Total Area (TA)
    - Remodeling Area (RA)
  - Relative Area Measurements
    - %Ct.Ar (CA/TA)
    - %Ma.Ar (MA/TA)
    - %RA.Ar (RA/TA)
  - Regional % RA.Ar (RA in each region / Regional Area)
  - Estimates of maximal and minimal bending (second moments of area, Imax, Imin, mm4)
  - Torsional stiffness (torsional section modulus, Zpol, mm3)
- **Means for all porosity and pore shape descriptor variables**
  - Total and Regional
  - All pores, cortical pores, and trabecularized pores
    - % Porosity
    - Pore Density
    - Mean Pore Area
    - Mean Pore Perimeter
    - Mean Pore Circularity
    - Mean Pore Aspect Ratio
    - Mean Pore Roundness
    - Mean Pore Solidity
- **Means for osteon area and shape descriptors**
  - Total, total by type, regional, region by type means
    - Mean Osteon Area
    - Mean Osteon Perimeter
    - Mean Osteon Circularity
    - Mean Osteon Aspect Ratio
    - Mean Osteon Roundness
    - Mean Osteon Solidity
- **Intracortical remodeling activity** – **Counts** of single labeled osteons (sL.On), double labeled osteons (dL.On), and resorption cavities (Rs.N)
    - Total and Regional
  - **OPD:** Normalize to cortical area (sL.On/Ct.Ar, dL.On/Ct.Ar, and Rs.N/Ct.Ar, mm^{-2})
    - Total and Regional
  - Ratio of labeled osteons versus resorption cavities ((sL.On+dL.On)/Rs.N)
    - In cross-sections where Rs.N was zero, a denominator of 1 was used to maximize the inclusion of data

        - Total and Regional
- **Activation frequency** - calculated as Ac.f = ((sL.On+dL.On)/Ct.Ar)/σf (#/mm2/year), where σf, the osteon formation time, was calculated as W.Th/On.MAR
    - **W**.**Th** = osteon wall thickness
    - **On**.**Mar** = mineral apposition rate – the distance between two consecutive osteonal calcein labels, divided by the labeling period
- **Overall remodeling activity (not normalized to osteon formation time):**
    - Active remodeling centers (a.Rm.Cr, mm^{-2}) as the sum of resorption spaces and labeled osteons normalized to Ct.Ar: (sL.On+dL.On+Rs.N)/Ct.Ar.

Appendix XX: Micro-CT Descriptive Statistics

Aggregate Porosity by Bone

Statistic	Femur			Tibia		
	Mean	Median	SD	Mean	Median	SD
Avg.Ecc	0.635	0.637	0.0326	0.621	0.621	0.0342
Avg.Imax	1.6E+14	1.61E+14	2.42E+13	1.65E+14	1.64E+14	2.31E+13
Avg.Imin	9.43E+13	9.4E+13	1.07E+13	1.01E+14	1.02E+14	1.28E+13
Avg.Maj.Por.D	133	133	13.1	132	130	9.18
Avg.Orient.Phi	173	174	6.67	194	196	6.2
Avg.Orient.Theta	38.9	38.6	3.85	41.2	41.8	3.2
Avg.Pol.MI	2.54E+14	2.55E+14	3.41E+13	2.66E+14	2.62E+14	3.49E+13
Avg.Por.Sph	0.6	0.6	0.00585	0.597	0.596	0.00521
Avg.Por.Surf	21000	20400	4620	17700	17500	2370
Avg.Por.Th	18.2	18.2	0.331	17.9	17.9	0.208
Avg.Por.Vol	158000	154000	63000	108000	100000	26600
Bone Surface	4.46E+08	4.16E+08	1.33E+08	4.68E+08	4.97E+08	1.41E+08
CA	1.57E+11	1.59E+11	1.15E+10	1.62E+11	1.63E+11	1.42E+10
CA.Surf	3.46E+08	3.46E+08	1.51E+07	3.57E+08	3.65E+08	1.86E+07
Closed Pore Density	118	117	30.4	149	138	45.9
Closed Porosity (%)	0.587	0.602	0.132	0.836	0.856	0.237
Connectivity	4920	4900	2930	5820	5040	2730
Connectivity density	30.5	30	18.6	36.7	30	16.5
Cortical Fractal	2.41	2.39	0.0695	2.43	2.44	0.0741
Cortical Surface	3.26E+08	3.26E+08	1.39E+07	3.32E+08	3.39E+08	1.70E+07
Cortical Volume	1.57E+11	1.59E+11	1.15E+10	1.62E+11	1.63E+11	1.41E+10
CS.Th	1180	1190	81	1200	1200	77.7
Degree of Anisotropy	0.388	0.378	0.055	0.42	0.418	0.0419
Euler number	16600	15300	5220	21000	20100	6530
Intersection Surface	2.96E+06	3.06E+06	1.15E+06	1.72E+06	1.62E+06	5.79E+05
Max Pore System Branching Nodes	1830	1050	2090	793	631	829
Max Pore System Mean Length	1360	1210	473	1750	1560	577
Max Pore System Mean Radius	32.4	32.4	8.03	34.4	36.2	9.95
Max Pore System Number of Nodes	3090	1860	3520	1280	954	1370
Max Pore System Number of Segments	3480	2030	3970	1470	1150	1560
Max Pore System Terminal Nodes	1250	750	1440	492	322	547
Max Pore System Total Length	3.47E+05	2.49E+05	3.93E+05	1.37E+05	9.93E+04	1.32E+05
Max Pore System Total Volume	4.02E+08	1.64E+08	6.51E+08	6.45E+07	4.17E+07	5.22E+07
Mean Pore System Branching Nodes	0.662	0.623	0.282	0.52	0.541	0.122

Mean Pore System Mean Length	98.7	97.4	12.2	93.1	90.9	6.69
Mean Pore System Mean Radius	3.61	3.6	0.0877	3.53	3.53	0.0716
Mean Pore System Number of Nodes	3.13	3.03	0.429	2.88	2.91	0.19
Mean Pore System Number of Segments	2.26	2.15	0.516	1.98	2.02	0.223
Mean Pore System Terminal Nodes	2.47	2.44	0.152	2.36	2.37	0.0716
Mean Pore System Total Length	226	222	41.5	194	190	21
Mean Pore System Total Volume	72700	57000	56200	31100	29600	12700
Med.Maj.Por.D	93.5	92.5	5.96	89.5	89.2	2.22
Med.Orient.Phi	163	169	13.9	218	221	9.63
Med.Orient.Theta	33.6	33.2	6.06	37.6	38.4	4.79
Med.Por.Vol	27300	27200	1330	26800	26600	932
Med.Pore.Sph	0.604	0.604	0.00539	0.602	0.601	0.00591
Med.Pore.Surf	7110	7100	272	7050	7020	210
Med.Pore.Th	18.1	18.1	0.253	17.8	17.9	0.26
Median Pore System Mean Length	79.1	78.3	6.77	74.7	73.9	2.59
Median Pore System Mean Radius	3.29	3.29	0.0619	3.24	3.26	0.0479
Median Pore System Total Length	84.9	84.2	6.01	81.3	80.9	2.39
Median Pore System Total Volume	3060	3110	315	2840	2820	163
Min Pore System Mean Length	24.2	24.5	1.66	23.2	23.4	2.39
Min Pore System Total Length	25.1	25.1	1.13	24.5	24.6	1.04
Min Pore System Total Volume	618	620	24.7	616	618	21.2
Number Closed Pores	18700	17700	5500	24200	22300	8190
Number of Total Pores	21500	20400	5950	26800	25000	8860
Number Open Pores	2740	2510	527	2580	2550	717
Open Pore Density	17.4	16.4	2.83	15.9	14.8	4.18
Open Porosity (%)	1.5	1.33	0.763	0.9	0.781	0.298
Perif.CA.Surf	2.66E+08	2.67E+08	1.17E+07	2.68E+08	2.69E+08	1.37E+07
Perif.TA.Surf	1.61E+08	1.62E+08	6.05E+06	1.64E+08	1.67E+08	7.83E+06
Pore Density	136	135	32.7	165	153	49.8
Pore Fractal	2.19	2.17	0.101	2.21	2.24	0.114
Pore Segment Mean Length	102	98.6	15.9	98.9	94.8	11.4
Pore Segment Mean Radius	6.13	5.84	1.1	4.76	4.72	0.467
Pore Separation	316	323	51.7	287	288	41.7
Pore Surface:Cortical Volume	0.00281	0.00263	0.000745	0.00288	0.00292	0.000791
Pore Surface:PoreVolume	0.142	0.141	0.025	0.168	0.165	0.0194
Pore Tb.N	0.000452	0.000463	0.00012	0.000529	0.000512	0.00015
Pore Thickness	46.9	42.3	16.1	33.2	33.5	6.02
Pore Volume	3.30E+09	3.23E+09	1.35E+09	2.81E+09	2.81E+09	8.56E+08
RCS	0.00221	0.00218	0.000133	0.00222	0.00223	0.000123
RCV	57.4	57.9	3.29	59.6	59.2	3.01
SD Pore Separation	131	138	31.2	107	107	15.7

SD Pore Thickness	36.2	30.7	17	22.5	22.7	5.6
TA	2.75E+11	2.77E+11	1.83E+10	2.71E+11	2.74E+11	2.16E+10
TA.Surf	2.59E+08	2.61E+08	1.22E+07	2.71E+08	2.74E+08	1.37E+07
Total Number of Pore Segments	39300	37900	13700	42800	39000	16100
Total Number of Pore Systems	17400	16100	4780	21300	19700	6990
Total Pore Network Length	3.90E+06	3.66E+06	1.15E+06	4.12E+06	4.40E+06	1.28E+06
Total Pore Network Volume	1.21E+09	8.61E+08	9.55E+08	6.33E+08	5.23E+08	2.56E+08
Total Porosity (%)	2.08	1.96	0.797	1.73	1.73	0.475

Aggregate Porosity by Drug Treatment Group

Statistic	Control			Fentanyl			Morphine		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Avg.Ecc	0.636148	0.638978	0.036712	0.623715	0.625973	0.034284	0.623968	0.620252	0.031233
Avg.Imax	1.59E+14	1.62E+14	2.14E+13	1.62E+14	1.63E+14	2.35E+13	1.67E+14	1.67E+14	2.64E+13
Avg.Imin	9.38E+13	9.87E+13	1.24E+13	9.79E+13	9.50E+13	1.17E+13	1.01E+14	1.04E+14	1.21E+13
Avg.Maj.Por.D	135.4328	134.665	10.437	130.4114	126.5861	12.20651	131.5644	131.3765	11.03398
Avg.Orient.Phi	183.8362	182.326	11.92037	182.1381	182.3352	15.80249	184.1632	183.8948	10.11979
Avg.Orient.Theta	39.91016	39.60321	4.160933	40.43178	41.31279	3.712449	39.87882	39.02917	3.367299
Avg.Pol.MI	2.52E+14	2.55E+14	3.23E+13	2.6E+14	2.60E+14	3.41E+13	2.67E+14	2.74E+14	3.8E+13
Avg.Por.Sph	0.598092	0.598827	0.006276	0.598056	0.594784	0.006484	0.599018	0.599582	0.004301
Avg.Por.Surf	20937.35	20711.07	3994.201	17762.61	16787.98	2410.062	19355.17	17740.05	4798.839
Avg.Por.Th	18.09654	18.07341	0.319948	17.97126	18.02239	0.230789	18.04158	18.00837	0.3627
Avg.Por.Vol	156550.8	151502.2	58212.53	109531.4	100465.4	30369.36	133002.9	107812.5	60677.18
Bone Surface	4.56E+08	4.77E+08	1.44E+08	4.56E+08	4.65E+08	1.22E+08	4.58E+08	4.22E+08	1.5E+08
CA	1.56E+11	1.58E+11	1.33E+10	1.61E+11	1.61E+11	1.47E+10	1.62E+11	1.64E+11	1.08E+10
CA.Surf	3.49E+08	3.47E+08	1.59E+07	3.51E+08	3.54E+08	1.73E+07	3.56E+08	3.61E+08	2.04E+07
Closed Pore Density	124.6836	122.795	42.87304	143.8457	129.675	40.01202	131.9557	124.345	42.03771
Closed Porosity (%)	0.66682	0.644957	0.238742	0.728133	0.709141	0.212048	0.739053	0.698785	0.24225
Connectivity	5200.357	4526	3377.933	5553.357	5347	2794.798	5359.5	4879	2447.602
Connectivity density	32.85714	30	22.33609	34.28571	30	16.50841	33.57143	30	14.46861
Cortical Fractal	2.416269	2.419051	0.078361	2.423845	2.416498	0.061199	2.412575	2.391277	0.080192
Cortical Surface	3.26E+08	3.26E+08	14497503	3.28E+08	3.33E+08	14725227	3.33E+08	3.39E+08	18135630
Cortical Volume	1.56E+11	1.57E+11	1.32E+10	1.61E+11	1.61E+11	1.46E+10	1.62E+11	1.64E+11	1.07E+10
CS.Th	1175.823	1166.327	97.61926	1206.311	1196.355	89.14783	1198.696	1198.413	41.74714
Degree of Anisotropy	0.407107	0.41482	0.047927	0.396555	0.393718	0.056547	0.40917	0.415283	0.050911
Euler number	16753.79	16847	5136.923	20688.64	17979.5	6941.721	18828.86	16727	6349.512

Intersection Surface	2687337	2373810	1392119	2147167	1858801	904014	2188131	1819857	922043.4
Max Pore System Branching Nodes	1631.786	1223.5	1934.832	902.9286	658.5	847.388	1402.357	817.5	1974.698
Max Pore System Mean Length	1549.013	1428.842	466.9438	1491.793	1497.542	493.4139	1622.046	1418.72	711.827
Max Pore System Mean Radius	34.54153	32.51605	7.123643	30.16035	29.9185	9.613187	35.49835	36.08188	9.697697
Max Pore System Number of Nodes	2731.643	2103	3179.629	1467.143	1009	1287.163	2352.786	1337.5	3454.648
Max Pore System Number of Segments	3084.429	2340	3643.944	1685.429	1197.5	1554.636	2654.357	1552	3800.931
Max Pore System Terminal Nodes	1099.857	725.5	1254.426	570.0714	389	447.6864	950.9286	445.5	1484.673
Max Pore System Total Length	306714.1	230394	324946.6	158814.5	105540.8	117934.7	259546.2	148867.4	409792.8
Max Pore System Total Volume	4.07E+08	1.40E+08	7.56E+08	1.13E+08	4.46E+07	1.53E+08	1.80E+08	9.57E+07	3.18E+08
Mean Pore System Branching Nodes	0.641197	0.58316	0.304911	0.55514	0.530713	0.192876	0.576814	0.634032	0.16527
Mean Pore System Mean Length	97.7021	93.48934	10.51914	95.20872	90.57769	11.38345	94.82954	93.63808	8.711479
Mean Pore System Mean Radius	3.585289	3.585042	0.078087	3.543849	3.544988	0.07705	3.583227	3.566061	0.104218
Mean Pore System Number of Nodes	3.0947	3.002794	0.470774	2.94567	2.891761	0.287912	2.974822	3.048027	0.268017
Mean Pore System Number of Segments	2.213731	2.1168	0.560052	2.05056	2.000784	0.35283	2.08572	2.182725	0.306565
Mean Pore System Terminal Nodes	2.453503	2.433137	0.171278	2.39053	2.361278	0.098166	2.398008	2.399619	0.109597
Mean Pore System Total Length	224.475	218.3498	40.24087	198.9967	196.0155	23.94564	206.8579	199.0303	39.66212
Mean Pore System Total Volume	73476.95	52168.97	64250.68	34987.85	31835.91	22554.44	47287.03	35946.46	32084.62
Med.Maj.Por.D	91.80073	89.71967	4.916869	91.32858	89.27042	5.723148	91.31844	90.61023	4.286601
Med.Orient.Phi	191.0923	190.7201	28.27197	188.9688	192.3822	37.30396	192.5736	190.654	25.91855
Med.Orient.Theta	35.67227	35.46339	6.475035	36.14122	37.46409	5.658605	35.06225	34.00327	5.503787
Med.Por.Vol	26991.61	26768.25	1057.406	27049.51	26996.3	1217.301	27041.73	26809.71	1295.849
Med.Pore.Sph	0.603038	0.604234	0.006528	0.602752	0.599093	0.006417	0.603697	0.604452	0.004229
Med.Pore.Surf	7072.387	7032.164	228.631	7091.25	7114.477	261.2735	7073.842	7041.587	252.9123
Med.Pore.Th	18.01792	18.05207	0.282429	17.91444	18.01489	0.285778	17.98979	18.00092	0.311777
Median Pore System Mean Length	77.45663	76.05039	5.624598	76.73146	74.1589	6.366114	76.53023	76.25973	4.819167
Median Pore System Mean Radius	3.266341	3.263471	0.05152	3.261294	3.258058	0.050637	3.267708	3.261214	0.07418
Median Pore System Total Length	83.51142	81.53205	4.568035	83.08809	81.62389	5.97615	82.62487	81.85128	4.209824
Median Pore System Total Volume	2964.498	2899.235	253.67	2938.278	2875.092	287.0528	2944.041	2880.688	288.3502
Min Pore System Mean Length	23.12487	23.33685	2.233748	23.31144	23.95517	2.37392	24.57464	24.73971	1.362512
Min Pore System Total Length	24.38023	24.3568	1.146507	24.87407	24.98697	0.941736	25.08895	25.46202	1.200903

Min Pore System Total Volume	617.4219	618.967	28.94808	615.4297	616.2354	20.90335	618.7285	622.1925	18.76684
Number Closed Pores	19398.86	19118.5	6435.06	23461.86	20172	8071.889	21543	19923	7632.757
Number of objects	21954.14	21493.5	6825.283	26242	22919	8643.661	24188.36	22027	8176.99
Number Open Pores	2555.286	2530.5	502.2113	2780.143	2821.5	656.4014	2645.357	2466	726.6014
Open Pore Density	16.50357	15.85	3.609438	17.17857	17.18	3.147062	16.315	15.1	4.182787
Open Porosity (%)	1.500608	1.384746	0.8349	0.964882	0.882883	0.301652	1.142188	1.063898	0.615702
Perif.CA.Surf	2.65E+08	2.67E+08	1.24E+07	2.66E+08	2.69E+08	1.05E+07	2.70E+08	2.75E+08	1.50E+07
Perif.TA.Surf	1.61E+08	1.62E+08	6.25E+06	1.62E+08	1.63E+08	6.86E+06	1.64E+08	1.66E+08	8.42E+06
Pore Density	141.1871	138.8	45.65355	161.025	146.98	42.45907	148.2729	137.375	44.92263
Pore Fractal	2.19811	2.20631	0.120745	2.211281	2.221823	0.088779	2.197372	2.173235	0.116776
Pore Segment Mean Length	103.5061	101.4978	14.4784	98.58273	92.94501	14.97442	99.3866	97.06824	12.14204
Pore Segment Mean Radius	5.915659	5.680023	1.31056	5.012047	4.857272	0.795456	5.411118	5.218703	0.962005
Pore Separation	300.7096	290.2501	49.40502	296.3338	289.3665	43.5727	306.3603	308.896	55.39753
Pore Surface:Cortical Volume	0.002923	0.002855	0.000891	0.002808	0.002841	0.000585	0.002799	0.002627	0.00082
Pore Surface:PoreVolume	0.141985	0.141258	0.025822	0.167649	0.166593	0.022517	0.154698	0.15937	0.023817
Pore Tb.N	0.000473	0.000465	0.000146	0.000514	0.000542	0.000142	0.000484	0.000465	0.000139
Pore Thickness	47.01922	41.72279	18.81741	34.4534	32.59451	9.667331	38.64613	37.82341	8.412029
Pore Volume	3.38E+09	3.43E+09	1.43E+09	2.73E+09	2.75E+09	6.52E+08	3.06E+09	2.97E+09	1.21E+09
RCS	0.002247	0.002269	0.000159	0.002194	0.00219	0.000138	0.002199	0.002185	6.41E-05
RCV	58.18628	58.62999	4.256754	58.95844	59.18785	3.045703	58.25999	58.52213	2.611991
SD Pore Separation	116.4013	113.6197	26.20997	118.8709	112.012	29.22194	121.3316	114.4687	28.14616
SD Pore Thickness	35.55706	27.41297	19.84536	24.70519	23.08394	11.03015	27.88241	28.16256	7.976469
TA	2.68E+11	2.70E+11	1.97E+10	2.72E+11	2.76E+11	1.71E+10	2.78E+11	2.83E+11	2.24E+10
TA.Surf	2.62E+08	2.64E+08	1.22E+07	2.65E+08	2.67E+08	1.40E+07	2.68E+08	2.72E+08	1.61E+07
Total Number of Pore Segments	39557	40648.5	15894.93	43042.21	40723	14861.78	40596.43	35368.5	14690.23
Total Number of Pore Systems	17632.5	16983.5	5351.771	21037.57	18991	6875.583	19384	17472.5	6385.505
Total Pore Network Length	3935536	4048173	1222309	4125308	4130599	1160391	3963347	3526643	1311963
Total Pore Network Volume	1.25E+09	1.07E+09	1.11E+09	6.59E+08	5.59E+08	3.08E+08	8.58E+08	8.05E+08	5.07E+08
Total Porosity (%)	2.157467	2.109555	0.857079	1.686125	1.715845	0.324543	1.872641	1.909647	0.682214

Aggregate Porosity by Bone and Drug Treatment Group

Group	Control					
Bone	Femur			Tibia		
Statistic	Mean	Median	SD	Mean	Median	SD
Avg.Ecc	0.645	0.641	0.0217	0.627	0.63	0.0477
Avg.Imax	1.61E+14	1.71E+14	2.39E+13	1.56E+14	1.61E+14	2.03E+13
Avg.Imin	9.36E+13	9.75E+13	1.22E+13	9.39E+13	9.98E+13	1.35E+13
Avg.Maj.Por.D	136	133	13.3	135	135	7.68
Avg.Orient.Phi	174	175	6.47	194	195	6.58
Avg.Orient.Theta	38.6	38.6	5.3	41.2	41.4	2.38
Avg.Pol.MI	2.55E+14	2.68E+14	3.56E+13	2.5E+14	2.47E+14	3.14E+13
Avg.Por.Sph	0.599	0.6	0.00592	0.597	0.597	0.00698
Avg.Por.Surf	23900	23800	3190	18000	17500	1960
Avg.Por.Th	18.3	18.3	0.34	17.9	18	0.225
Avg.Por.Vol	201000	198000	47400	112000	112000	20400
Bone Surface	4.98E+08	5.23E+08	1.33E+08	4.15E+08	3.39E+08	1.52E+08
CA	1.56E+11	1.59E+11	1.39E+10	1.56E+11	1.56E+11	1.38E+10
CA.Surf	3.46E+08	3.44E+08	1.57E+07	3.51E+08	3.53E+08	1.69E+07
Closed Pore Density	116	121	28.8	134	124	54.4
Closed Porosity (%)	0.58	0.643	0.118	0.754	0.649	0.303

Connectivity	5480	5100	3730	4920	4120	3260
Connectivity Density	32.9	30	23.6	32.9	30	22.9
Cortical Fractal	2.43	2.46	0.0645	2.4	2.39	0.0937
Cortical Surface	3.26E+08	3.25E+08	1.45E+07	3.27E+08	3.29E+08	1.56E+07
Cortical Volume	1.56E+11	1.59E+11	1.39E+10	1.56E+11	1.56E+11	1.37E+10
CS.Th	1170	1210	116	1180	1160	84.9
Degree of Anistropy	0.396	0.378	0.0575	0.418	0.436	0.0373
Euler number	15300	15300	4490	18200	17000	5680
Intersection Surface	3670000	3340000	1290000	1710000	1600000	526000
Max Pore System Branching Nodes	2620	1360	2310	641	214	683
Max Pore System Mean Length	1450	1210	494	1640	1470	456
Max Pore System Mean Radius	32.6	32.4	5.65	36.5	36.2	8.29
Max Pore System Number of Nodes	4430	2160	3740	1040	362	1110
Max Pore System Number of Segments	4980	2490	4340	1190	406	1260
Max Pore System Terminal Nodes	1800	939	1440	397	148	436
Max Pore System Total Length	491000	276000	362000	123000	44400	136000
Max Pore System Total Volume	7.46E+08	4.77E+08	9.84E+08	6.92E+07	3.68E+07	6.77E+07
Mean Pore System Branching Nodes	0.791	0.709	0.358	0.491	0.541	0.143
Mean Pore System Mean Length	100	93.8	13	95.2	93.1	7.55
Mean Pore System Mean Radius	3.62	3.63	0.0763	3.55	3.56	0.0632
Mean Pore System Number of Nodes	3.35	3.25	0.521	2.84	2.91	0.231
Mean Pore System Number of Segments	2.5	2.36	0.644	1.93	2.02	0.265
Mean Pore System Terminal Nodes	2.56	2.54	0.167	2.35	2.37	0.0913
Mean Pore System Total Length	253	248	34.7	196	190	20.2
Mean Pore System Total Volume	114000	83900	70900	33100	33300	10100
Med.Maj.Por.D	94	89.7	6.16	89.6	89.5	1.7
Med.Orient.Phi	167	173	15.9	215	217	9.89
Med.Orient.Theta	33.5	33.7	8.23	37.8	38.2	3.53
Med.Por.Vol	27400	27200	1100	26600	26500	921

Med.Pore.Sph	0.603	0.605	0.00526	0.603	0.602	0.00803
Med.Pore.Surf	7130	7100	222	7020	6980	238
Med.Pore.Th	18.1	18.1	0.274	17.9	18	0.253
Median Pore System Mean Length	79.6	76.2	7.22	75.3	74.3	2.44
Median Pore System Mean Radius	3.29	3.27	0.056	3.25	3.26	0.04
Median Pore System Total Length	85.6	81.6	5.73	81.5	81.3	1.63
Median Pore System Total Volume	3090	2900	310	2840	2800	94.5
Min Pore System Mean Length	23.7	23.4	1.47	22.5	23.3	2.78
Min Pore System Total Length	24.2	24.9	1.2	24.5	23.8	1.16
Min Pore System Total Volume	617	615	32.8	617	620	27.2
Number Closed Pores	18100	20000	4620	20700	18200	8030
Number of objects	20800	23000	4870	23100	20200	8610
Number Open Pores	2720	2910	281	2390	2300	637
Open Pore Density	17.5	18.2	2.05	15.5	14.2	4.66
Open Porosity (%)	2.09	1.71	0.747	0.907	0.747	0.362
Perif.CA.Surf	2.67E+08	2.63E+08	1.31E+07	2.64E+08	2.68E+08	1.27E+07
Perif.TA.Surf	1.60E+08	1.62E+08	6.33E+06	1.62E+08	1.63E+08	6.62E+06
Pore Density	133	139	30.2	149	138	58.8
Pore Fractal	2.23	2.26	0.0937	2.17	2.16	0.145
Pore Segment Mean Length	104	98.2	17.4	103	105	12.2
Pore Segment Mean Radius	7.03	6.88	0.8	4.8	4.77	0.4
Pore Separation	301	284	48.6	300	296	54.1
Pore Surface:Cortical Volume	0.00317	0.00328	0.000765	0.00267	0.00232	0.000996
Pore Surface:PoreVolume	0.121	0.122	0.0151	0.163	0.162	0.0145
Pore Tb.N	0.000471	0.000466	0.000126	0.000475	0.000431	0.000174
Pore Thickness	59.2	51.1	19.9	34.8	33.5	4.77
Pore Volume	4.17E+09	3.72E+09	1.39E+09	2.58E+09	2.16E+09	1E+09
RCS	0.00223	0.00214	0.000194	0.00226	0.00228	0.000128
RCV	57.1	58.1	4.56	59.3	58.9	3.95
SD Pore Separation	127	116	29.4	106	110	19.6

SD Pore Thickness	46.5	38.2	23.8	24.7	23.3	3.15
TA	2.74E+11	2.71E+11	1.87E+10	2.63E+11	2.68E+11	2.05E+10
TA.Surf	2.59E+08	2.58E+08	1.26E+07	2.65E+08	2.66E+08	1.20E+07
Total Number of Pore Segments	42600	44900	15100	36500	28500	17300
Total Number of Pore Systems	16800	18000	3770	18400	15900	6810
Total Pore Network Length	4260000	4400000	1110000	3610000	2980000	1330000
Total Pore Network Volume	1.88E+09	1.57E+09	1.28E+09	6.17E+08	5.18E+08	3.06E+08
Total Porosity (%)	2.66	2.33	0.804	1.65	1.39	0.594

Group	**Fentanyl**						**Morphine**					
Bone	**Femur**			**Tibia**			**Femur**			**Tibia**		
Statistic	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Avg.Ecc	0.636	0.643	0.0373	0.611	0.606	0.0278	0.625	0.619	0.0379	0.623	0.621	0.0259
Avg.Imax	1.58E+14	1.61E+14	2.24E+13	1.65E+14	1.64E+14	2.59E+13	1.6E+14	1.53E+14	2.96E+13	1.74E+14	1.83E+14	2.27E+13
Avg.Imin	9.34E+13	9.4E+13	8.94E+12	1.02E+14	1E+14	1.29E+13	9.6E+13	9.68E+13	1.23E+13	1.06E+14	1.09E+14	1.06E+13
Avg.Maj.Por.D	131	132	14	130	126	11.2	131	133	13.6	132	130	8.82
Avg.Orient.Phi	168	167	5.67	196	198	6.85	176	177	5.91	192	191	5.4
Avg.Orient.Theta	39.7	40.4	3.64	41.2	42	3.9	38.5	37.4	2.66	41.2	41.8	3.63
Avg.Pol.MI	2.52E+14	2.55E+14	2.99E+13	2.68E+14	2.6E+14	3.86E+13	2.55E+14	2.46E+14	4.13E+13	2.8E+14	2.92E+14	3.29E+13
Avg.Por.Sph	0.599	0.595	0.00763	0.597	0.594	0.00562	0.602	0.601	0.00384	0.596	0.596	0.00308
Avg.Por.Surf	18800	19200	2180	16700	15700	2310	20300	17200	6330	18400	18000	2790

Avg.Por.Th	18.1	18.1	0.205	17.9	17.8	0.24	18.2	18.2	0.426	17.9	17.8	0.182
Avg.Por.Vol	123000	113000	33100	96400	92400	22400	150000	112000	78400	116000	103000	34400
Bone Surface	4.23E+08	4.16E+08	9.72E+07	4.90E+08	5.38E+08	1.42E+08	4.17E+08	3.95E+08	1.65E+08	4.99E+08	4.49E+08	1.33E+08
CA	1.59E+11	1.52E+11	1.19E+10	1.63E+11	1.63E+11	1.78E+10	1.57E+11	1.61E+11	1.02E+10	1.67E+11	1.68E+11	9.71E+09
CA.Surf	3.44E+08	3.47E+08	1.17E+07	3.57E+08	3.66E+08	2.02E+07	3.47E+08	3.51E+08	1.93E+07	3.64E+08	3.65E+08	1.92E+07
Closed Pore Density	125	117	28.9	163	171	42.6	114	106	36.7	149	138	42.1
Closed Porosity (%)	0.604	0.565	0.135	0.852	0.856	0.208	0.575	0.564	0.16	0.903	0.914	0.197
Connectivity	5030	4980	3110	6080	6320	2570	4260	4720	2020	6460	6510	2460
Connectivity density	31.4	30	20.4	37.1	30	12.5	27.1	30	12.5	40	40	14.1
Cortical Fractal	2.4	2.4	0.0507	2.45	2.46	0.0668	2.38	2.37	0.0906	2.44	2.4	0.062
Cortical Surface	3.24E+08	3.28E+08	1.05E+07	3.32E+08	3.41E+08	1.79E+07	3.27E+08	3.30E+08	1.80E+07	3.38E+08	3.41E+08	1.77E+07
Cortical Volume	1.59E+11	1.52E+11	1.19E+10	1.62E+11	1.63E+11	1.76E+10	1.57E+11	1.61E+11	1.02E+10	1.67E+11	1.68E+11	9.47E+09
CS.Th	1200	1190	81.3	1210	1210	103	1180	1190	36.7	1220	1230	33.5
Degree of Anistropy	0.366	0.353	0.0524	0.427	0.418	0.0445	0.403	0.421	0.056	0.416	0.409	0.0488
Euler number	17800	15700	5750	23600	26400	7180	16600	14400	5840	21100	20100	6420
Intersection Surface	2500000	2540000	1000000	1790000	1800000	684000	2710000	3060000	923000	1670000	1760000	603000
Max Pore System Branching Nodes	1190	953	1110	613	631	340	1680	665	2600	1130	994	1230
Max Pore System Mean Length	1350	1290	577	1630	1560	383	1280	1210	388	1970	1810	819
Max Pore System Mean Radius	27.8	28.7	6.5	32.6	37.6	12	36.8	37.2	9.6	34.2	30.8	10.4
Max Pore System Number of Nodes	1960	1690	1670	970	954	477	2870	1100	4580	1840	1580	2060
Max Pore System Number of Segments	2250	1830	2040	1130	1150	600	3200	1240	5020	2110	1910	2330
Max Pore System Terminal Nodes	771	733	561	369	322	159	1190	432	1990	711	459	835
Max Pore System Total Length	211000	200000	148000	107000	99300	43200	339000	119000	561000	180000	179000	186000
Max Pore System Total Volume	1.73E+08	1.16E+08	2.01E+08	5.21E+07	4.17E+07	4.19E+07	2.88E+08	9.63E+07	4.35E+08	7.23E+07	9.50E+07	4.98E+07

Mean Pore System Branching Nodes	0.615	0.536	0.259	0.495	0.525	0.0716	0.579	0.623	0.201	0.575	0.645	0.138
Mean Pore System Mean Length	97.5	94.9	14.6	92.9	89.3	7.46	98.4	98.7	10.3	91.2	90.9	5.2
Mean Pore System Mean Radius	3.57	3.58	0.0614	3.52	3.49	0.0848	3.63	3.61	0.118	3.54	3.55	0.0724
Mean Pore System Number of Nodes	3.05	2.92	0.375	2.84	2.89	0.12	2.99	3.02	0.338	2.96	3.08	0.203
Mean Pore System Number of Segments	2.17	2.02	0.469	1.93	1.99	0.135	2.1	2.15	0.378	2.07	2.21	0.246
Mean Pore System Terminal Nodes	2.43	2.38	0.117	2.35	2.35	0.0539	2.41	2.39	0.144	2.38	2.4	0.0691
Mean Pore System Total Length	210	221	20	188	178	23.4	214	191	53.3	199	203	20.8
Mean Pore System Total Volume	44700	33900	28000	25300	23600	9900	59500	45200	40000	35000	29600	16700
Med.Maj.Por.D	93.1	92.5	7.37	89.6	89.2	3.11	93.4	93.6	5.05	89.2	88.6	1.93
Med.Orient.Phi	154	152	12.5	223	225	9.14	169	172	9.63	216	213	9
Med.Orient.Theta	34.8	35.7	5.76	37.5	38.5	5.64	32.5	30.6	4.32	37.6	38.4	5.66
Med.Por.Vol	27200	27900	1410	26900	26600	1070	27200	27100	1650	26900	26700	922
Med.Pore.Sph	0.604	0.599	0.00732	0.602	0.599	0.00586	0.606	0.605	0.00334	0.602	0.599	0.00406
Med.Pore.Surf	7120	7280	307	7070	7030	228	7080	7070	320	7070	7020	190
Med.Pore.Th	18.1	18.1	0.169	17.8	17.7	0.316	18.1	18.2	0.324	17.8	17.9	0.229
Median Pore System Mean Length	78.6	76.9	8.29	74.8	73.7	3.28	79	78.7	5.59	74	73	2.16
Median Pore System Mean Radius	3.27	3.29	0.0426	3.25	3.25	0.0589	3.3	3.31	0.0864	3.24	3.22	0.0499
Median Pore System Total Length	84.6	85.8	7.8	81.6	81.5	3.37	84.5	84.2	5.11	80.8	80.3	2.13
Median Pore System Total Volume	3020	3110	338	2860	2870	223	3060	3120	344	2830	2760	171
Min Pore System Mean Length	23.7	24.5	1.87	22.9	23.4	2.88	25	25.6	1.52	24.2	24.6	1.15
Min Pore System Total Length	25.2	25.1	0.862	24.6	24.9	0.993	25.9	26.1	0.649	24.3	24.6	1.1

Min Pore System Total Volume	611	595	25	620	616	16.6	627	624	13.6	611	611	20.7
Number Closed Pores	20000	17700	6050	26900	31800	8790	18100	15800	6300	25000	22600	7650
Number of objects	22800	20400	6580	29700	34900	9540	20800	18300	6940	27600	25000	8390
Number Open Pores	2760	2500	539	2800	3000	801	2750	2510	745	2540	2420	752
Open Pore Density	17.3	16.4	2.31	17.1	18.2	4.01	17.4	15.2	4.14	15.2	14.8	4.24
Open Porosity (%)	1.09	1.08	0.308	0.837	0.758	0.254	1.33	1.04	0.805	0.957	1.09	0.304
Perif.CA.Surf	2.65E+08	2.68E+08	7.67E+06	2.68E+08	2.74E+08	1.32E+07	2.67E+08	2.70E+08	1.50E+07	2.73E+08	2.76E+08	1.56E+07
Perif.TA.Surf	1.60E+08	1.61E+08	4.42E+06	1.64E+08	1.68E+08	8.59E+06	1.61E+08	1.62E+08	7.88E+06	1.67E+08	1.69E+08	8.29E+06
Pore Density	142	135	31.1	180	186	46.1	132	121	40.1	165	153	46.2
Pore Fractal	2.19	2.16	0.0686	2.23	2.27	0.106	2.16	2.16	0.135	2.23	2.18	0.09
Pore Segment Mean Length	99.9	94.1	18.2	97.3	91.8	12.3	102	100	14.2	96.7	94.7	10
Pore Segment Mean Radius	5.45	5.28	0.892	4.58	4.55	0.369	5.91	5.67	1.02	4.91	4.83	0.604
Pore Separation	315	303	43.9	278	267	37.4	331	346	64	282	303	33.6
Pore Surface: Cortical Volume	0.00264	0.00245	0.000446	0.00297	0.00301	0.000692	0.00262	0.00245	0.000914	0.00298	0.00263	0.000737
Pore Surface: PoreVolume	0.158	0.163	0.0231	0.177	0.178	0.0191	0.145	0.153	0.0222	0.164	0.165	0.0231
Pore Tb.N	0.000457	0.000435	0.000108	0.000572	0.000614	0.000156	0.000427	0.00039	0.000139	0.000542	0.000473	0.000122
Pore Thickness	38.5	33.7	11.5	30.4	30.1	5.59	42.9	40.9	7.59	34.4	34.6	7.26
Pore Volume	2.7E+09	2.68E+09	6.12E+08	2.75E+09	2.81E+09	7.38E+08	3.04E+09	2.57E+09	1.55E+09	3.09E+09	3.46E+09	8.59E+08
RCS	0.00218	0.00219	0.000115	0.00221	0.00219	0.000166	0.00222	0.00218	7.65E-05	0.00218	0.00219	4.94E-05
RCV	58.1	58.6	2.76	59.8	59.5	3.27	56.9	56.8	2.62	59.6	59.3	1.92
SD Pore Separation	130	119	35.5	108	99.1	17.8	136	151	32.5	106	103	11.1
SD Pore Thickness	29.8	24.9	13.1	19.6	20.8	5.7	32.5	30.7	6.48	23.3	24.2	6.82
TA	2.73E+11	2.77E+11	1.39E+10	2.71E+11	2.74E+11	2.1E+10	2.77E+11	2.79E+11	2.38E+10	2.8E+11	2.87E+11	2.27E+10
TA.Surf	2.58E+08	2.61E+08	1.00E+07	2.71E+08	2.77E+08	1.52E+07	2.60E+08	2.61E+08	1.54E+07	2.76E+08	2.79E+08	1.37E+07
Total Number of Pore Segments	39700	37600	13900	46400	51900	16100	35600	31600	13200	45600	35900	15400

Total Number of Pore Systems	18400	16100	5400	23700	27700	7520	16900	14700	5570	21900	19700	6550
Total Pore Network Length	3830000	3540000	988000	4420000	4800000	1320000	3610000	3420000	1400000	4320000	3590000	1210000
Total Pore Network Volume	7.64E+08	7.09E+08	3.85E+08	5.55E+08	5.12E+08	1.77E+08	9.89E+08	8.07E+08	6.64E+08	7.27E+08	8.02E+08	2.77E+08
Total Porosity (%)	1.69	1.81	0.296	1.68	1.55	0.375	1.89	1.6	0.88	1.85	2.02	0.483

Descriptive Statistics: Femoral Regions

Femoral Porosity by Region

Statistic	Anterior			Lateral			Medial			Posterior		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Pore Surface	4.83E+10	1.10E+08	1.25E+11	3.47E+10	8.20E+07	9.20E+10	4.31E+10	1.22E+08	1.10E+11	5.14E+10	1.29E+08	1.29E+11
Closed Pore Density	96.4	102	48.8	103	102	53	132	147	65.8	87.4	91.4	43.3
Closed Porosity (%)	0.49	0.494	0.136	0.572	0.589	0.148	0.691	0.718	0.186	0.558	0.581	0.117
Connectivity	1140	1140	676	1050	883	789	1320	1280	710	1470	1330	942
Connectivity density	19.5	20	12.8	25.2	30	21.8	28.6	30	19.8	26.7	30	18.8
Cortical Fractal	2.34	2.33	0.0819	2.36	2.36	0.0724	2.43	2.42	0.0643	2.39	2.37	0.057
Cortical Surface	3.52E+10	1.03E+08	8.81E+10	3.05E+10	9.12E+07	7.64E+10	3.13E+10	9.25E+07	7.84E+10	3.76E+10	1.08E+08	9.42E+10
Cortical Volume	7.86E+14	4.51E+10	1.97E+15	5.59E+14	3.20E+10	1.40E+15	5.84E+14	3.55E+10	1.47E+15	7.71E+14	4.50E+10	1.94E+15
Degree of Anisotropy	0.391	0.374	0.0666	0.322	0.287	0.0806	0.451	0.456	0.0641	0.422	0.42	0.0629
Euler number	4700	4360	1510	3760	3290	1280	4960	4370	1640	4010	3720	1280
Intersection Surface	4.42E+08	9.33E+05	1.16E+09	4.20E+08	9.67E+05	1.10E+09	3.56E+08	1.04E+06	9.34E+08	4.28E+08	1.17E+06	1.10E+09

Avg. Pore Major diameter	976	127	2170	1250	129	2370	1250	135	2390	1110	141	2450
Number Closed Pores	4890	4750	1500	3890	3440	1300	5260	4840	1630	4390	4180	1190
Number of Pores	5840	5720	1670	4800	4150	1540	6280	5930	1820	5490	5140	1410
Number Open Pores	946	924	194	911	873	264	1020	948	224	1090	999	237
Pore surface	9.82E+06	1.89E+04	2.49E+07	9.73E+06	1.68E+04	2.10E+07	1.05E+07	2.01E+04	2.25E+07	1.04E+07	2.32E+04	2.62E+07
Pore volume	5.14E+09	1.43E+05	1.41E+10	3.60E+09	9.70E+04	8.03E+09	4.40E+09	1.35E+05	9.55E+09	4.50E+09	1.74E+05	1.16E+10
Open Pore Density	18.3	18.6	8.57	24.1	26.2	11.8	25.4	28.1	11.8	21.3	23	9.95
Open Porosity (%)	1.39	1.11	1.24	1.03	0.796	0.572	1.9	1.96	0.914	1.7	1.75	0.7
Avg. Pore Orientation phi	136	135	11.6	173	173	6.18	165	166	9.33	205	204	11.4
Avg. Pore Orientation theta	39.2	38.7	3.46	44.9	45.9	4.3	33.2	33.6	4.09	40.4	39.1	4.89
Pore Density	115	119	56.9	127	131	64.3	157	178	76.9	109	114	53
Pore Fractal	2.1	2.11	0.127	2.1	2.11	0.131	2.22	2.2	0.0903	2.15	2.13	0.0908
Pore Separation	2910	390	6480	2480	304	5530	2150	276	4770	2390	312	5260
Pore Surface: Cortical Volume	0.00198	0.00204	0.00113	0.0022	0.00236	0.00116	0.00293	0.00299	0.00146	0.00246	0.00258	0.00121
Pore Surface: PoreVolume	0.133	0.138	0.0625	0.152	0.183	0.0646	0.123	0.139	0.0581	0.118	0.129	0.0516
Pore Tb.N	0.000331	0.000333	0.00018	0.000419	0.000457	0.000225	0.000467	0.000509	0.000246	0.000408	0.000443	0.000201

Pore Thickness	552	43.2	1500	354	29.2	821	392	48	879	387	44.4	867
Pore Volume	2.64E+13	7.17E+08	7.78E+13	1.31E+13	4.92E+08	3.52E+13	1.69E+13	9.50E+08	4.39E+13	2.17E+13	9.98E+08	5.46E+13
SD Pore Separation	1360	167	3110	953	116	2130	841	109	1890	898	110	2010
SD Pore Thickness	379	30.2	1090	248	21.3	590	286	31.7	667	234	31.9	509
Sphericity	0.614	0.612	0.00677	0.602	0.602	0.00576	0.607	0.61	0.00953	0.598	0.597	0.00713
Total Porosity (%)	1.87	1.59	1.26	1.59	1.43	0.65	2.58	2.57	0.92	2.25	2.21	0.72

Femoral Porosity by Drug Treatment Group

Statistic	Control			Fentanyl			Morphine		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Pore Surface	9.38E+10	1.42E+08	1.58E+11	1.06E+08	99700000	29700000	3.92E+10	1.04E+08	9.84E+10
Closed Pore Density	85	90.9	63.7	125	117	37.2	104	106	54.9
Closed Porosity (%)	0.576	0.593	0.161	0.593	0.548	0.162	0.563	0.558	0.172
Connectivity	1390	1140	934	1270	1180	832	1080	1030	548
Connectivity density	17.9	15	15.5	32.1	30	21.1	25	30	16.4
Cortical Fractal	2.4	2.4	0.0752	2.38	2.37	0.0589	2.36	2.36	0.0885
Cortical Surface	6.62E+10	1.03E+08	1.07E+11	9.79E+07	9.85E+07	8.25E+06	3.47E+10	9.99E+07	8.66E+10
Cortical Volume	1.35E+15	4.39E+10	2.21E+15	3.97E+10	4.14E+10	6.55E+09	6.73E+14	4.08E+10	1.70E+15
Degree of Anisotropy	0.405	0.421	0.0866	0.376	0.37	0.0783	0.408	0.402	0.0835
Euler number	4060	3750	1220	4670	4170	1650	4350	3800	1560
Intersection Surface	8.37E+08	1.53E+06	1.44E+09	8.32E+05	7.39E+05	3.36E+05	3.97E+08	8.47E+05	1.00E+09
Avg. Pore Major diameter	1800	146	2690	508	128	1390	1130	130	2520
Number Closed Pores	4450	4710	1250	4930	4550	1590	4450	4220	1590
Number of Pores	5440	5790	1390	5940	5560	1790	5430	5060	1820
Number Open Pores	990	986	175	1010	935	253	977	889	280

Pore surface	1.69E+07	2.37E+04	2.79E+07	3.25E+06	1.88E+04	1.19E+07	1.02E+07	1.72E+04	2.55E+07
Pore volume	7.29E+09	2.08E+05	1.36E+10	1.35E+09	1.15E+05	5.07E+09	4.59E+09	1.15E+05	1.16E+10
Open Pore Density	18.9	23.3	13	25.8	24.1	6.31	22.1	22.3	11.2
Open Porosity (%)	2.06	2.09	1.08	1.11	1.08	0.477	1.35	1.02	0.907
Avg. Pore Orientation phi	170	172	27.6	166	164	24.4	173	173	27.8
Avg. Pore Orientation theta	39.2	39.1	6.62	40.1	39.3	6.03	39	38.3	5.1
Pore Density	104	116	76.1	151	141	42.3	126	130	65.4
Pore Fractal	2.18	2.2	0.125	2.14	2.13	0.0849	2.12	2.12	0.14
Pore Separation	4540	308	7050	307	304	55.5	2590	333	5680
Pore Surface:Cortical Volume	0.00225	0.00257	0.00162	0.00268	0.00255	0.000653	0.00226	0.00221	0.00134
Pore Surface:PoreVolume	0.0955	0.117	0.0644	0.163	0.16	0.0278	0.136	0.148	0.0606
Pore Tb.N	0.000361	0.000414	0.000264	0.000479	0.000449	0.000138	0.000379	0.00036	0.000216
Pore Thickness	829	56.4	1470	36.9	34.3	13.3	398	41	899
Pore Volume	4.09E+13	1.12E+09	7.88E+13	6.74E+08	6.75E+08	2.35E+08	1.76E+13	6.15E+08	4.51E+13
SD Pore Separation	1870	109	3040	120	116	37.8	1050	127	2340
SD Pore Thickness	604	39.3	1110	26.5	24.7	12.7	230	30.4	503
Sphericity	0.605	0.604	0.00993	0.604	0.601	0.0109	0.607	0.607	0.00631
Total Porosity (%)	2.62	2.63	1.11	1.69	1.63	0.501	1.9	1.64	0.983

Femoral Porosity by Region and Drug Treatment Group

Group	Control											
Region	Anterior			Lateral			Medial			Posterior		
Statistic	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Pore Surface	1.03E+11	1.36E+08	1.84E+11	7.12E+10	8.89E+07	1.33E+11	9.30E+10	1.49E+08	1.61E+11	1.08E+11	1.67E+08	1.85E+11
Closed Pore Density	76.4	80.1	57.7	83.6	86	67.6	109	144	80.5	71.3	78.5	53.8
Closed Porosity (%)	0.482	0.494	0.109	0.567	0.599	0.176	0.713	0.736	0.172	0.542	0.581	0.104
Connectivity	1310	1150	938	1070	883	989	1490	1380	879	1670	1540	1030
Connectivity density	14.3	10	12.7	15.7	10	14	21.4	20	19.5	20	20	17.3
Cortical Fractal	2.37	2.39	0.0691	2.37	2.39	0.0927	2.46	2.48	0.0601	2.41	2.43	0.0405
Cortical Surface	6.95E+10	1.04E+08	1.19E+11	5.98E+10	9.01E+07	1.02E+11	6.15E+10	9.16E+07	1.05E+11	7.39E+10	1.08E+08	1.26E+11
Cortical Volume	1.58E+15	4.68E+10	2.71E+15	1.13E+15	3.19E+10	1.92E+15	1.17E+15	3.55E+10	1.99E+15	1.53E+15	4.57E+10	2.62E+15
Degree of Anisotropy	0.425	0.424	0.0617	0.313	0.305	0.0914	0.451	0.434	0.0644	0.431	0.435	0.0624
Euler number	4270	4030	1340	3580	3140	1110	4620	4930	1270	3760	3550	1140
Intersection Surface	9.36E+08	1.04E+06	1.71E+09	8.98E+08	9.71E+05	1.62E+09	7.20E+08	1.64E+06	1.33E+09	7.93E+08	1.51E+06	1.40E+09
Avg. Pore Major diameter	1700	133	2710	1740	146	2740	1740	137	2750	2000	159	3160
Number Closed Pores	4640	5010	1260	3760	4570	1370	5070	4960	1170	4340	4400	1050
Number of Pores	5580	5950	1420	4650	5540	1560	6110	5990	1260	5430	5630	1180
Number Open Pores	941	937	160	888	954	188	1040	1020	120	1100	1140	179
Pore surface	1.89E+07	2.19E+04	3.28E+07	1.34E+07	1.88E+04	2.33E+07	1.58E+07	2.20E+04	2.73E+07	1.96E+07	2.91E+04	3.35E+07
Pore volume	1.09E+10	2.08E+05	2.12E+10	4.73E+09	1.13E+05	8.40E+09	5.98E+09	1.85E+05	1.07E+10	7.57E+09	2.44E+05	1.30E+10
Open Pore Density	15.6	19.6	11.1	19.9	23	14.1	22.5	29.1	15.8	17.6	23.8	12.3
Open Porosity (%)	2.25	1.68	1.62	1.17	0.75	0.784	2.58	2.62	0.756	2.23	2.19	0.312
Avg. Pore Orientation phi	135	132	12.7	173	173	3.83	167	167	10.4	206	204	13.1

Avg. Pore Orientation theta	38.1	38.7	4.12	45.2	44.5	5.71	33.4	33.6	4.98	40.1	39.1	6.19
Pore Density	92	96.8	68.7	103	108	81.1	131	169	95.6	88.9	102	65.7
Pore Fractal	2.15	2.18	0.107	2.1	2.13	0.177	2.26	2.29	0.0841	2.19	2.21	0.0606
Pore Separation	5430	390	8880	4620	318	7580	3870	243	6260	4250	304	6820
Pore Surface:Cortical Volume	0.00193	0.00212	0.00141	0.00185	0.00203	0.00147	0.00286	0.00372	0.00205	0.00235	0.00261	0.00164
Pore Surface:PoreVolume	0.0875	0.113	0.0596	0.124	0.167	0.0836	0.086	0.112	0.0606	0.0843	0.118	0.0562
Pore Tb.N	0.0003	0.000338	0.00022	0.000339	0.000382	0.000268	0.000437	0.00062	0.000333	0.000369	0.000445	0.000267
Pore Thickness	1250	58.9	2400	669	31.9	1110	734	57.9	1190	669	55.5	1050
Pore Volume	6.13E+13	1.13E+09	1.25E+14	2.55E+13	4.92E+08	4.92E+13	3.52E+13	1.11E+09	6.32E+13	4.17E+13	1.35E+09	7.12E+13
SD Pore Separation	2650	163	4430	1780	102	2900	1530	100	2540	1520	109	2450
SD Pore Thickness	895	40.8	1800	513	21.3	870	581	40.4	1000	427	38.5	663
Sphericity	0.616	0.615	0.00556	0.602	0.604	0.00625	0.606	0.603	0.00922	0.596	0.596	0.00655
Total Porosity (%)	2.71	2.13	1.61	1.73	1.35	0.919	3.27	3.13	0.758	2.76	2.83	0.378

Group	**Fentanyl**											
Region	**Anterior**			**Lateral**			**Medial**			**Posterior**		
Statistic	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Pore Surface	9.93E+07	9.64E+07	2.69E+07	8.81E+07	7.88E+07	2.79E+07	1.10E+08	9.82E+07	2.47E+07	1.26E+08	1.15E+08	3.09E+07
Closed Pore Density	115	109	28.3	124	111	32.7	162	148	38.7	100	91.4	20.7
Closed Porosity (%)	0.501	0.499	0.133	0.571	0.527	0.128	0.736	0.718	0.195	0.566	0.541	0.102
Connectivity	1060	1130	644	1160	995	942	1220	1280	565	1630	1580	1130

Connectivity density	24.3	20	14	35.7	30	30.5	34.3	30	17.2	34.3	30	22.3
Cortical Fractal	2.33	2.34	0.0625	2.37	2.35	0.0558	2.42	2.42	0.0454	2.38	2.37	0.0421
Cortical Surface	1.02E+08	1.01E+08	3.59E+06	9.08E+07	9.12E+07	5.87E+06	9.18E+07	9.25E+07	3.84E+06	1.07E+08	1.08E+08	4.64E+06
Cortical Volume	4.55E+10	4.37E+10	3.68E+09	3.32E+10	3.20E+10	3.17E+09	3.51E+10	3.42E+10	3.54E+09	4.49E+10	4.32E+10	3.30E+09
Degree of Anisotropy	0.373	0.362	0.0777	0.304	0.278	0.0645	0.428	0.392	0.0702	0.399	0.39	0.051
Euler number	5140	4410	1510	3940	3400	1400	5590	5170	1970	4020	3820	1320
Intersection Surface	7.41E+05	5.61E+05	4.08E+05	9.15E+05	9.67E+05	3.50E+05	7.52E+05	6.73E+05	3.53E+05	9.18E+05	9.59E+05	2.44E+05
Avg. Pore Major diameter	122	121	13.7	928	124	2120	844	136	1890	137	132	15.6
Number Closed Pores	5270	4750	1630	4150	3440	1400	5760	4760	1920	4530	4180	1110
Number of Pores	6200	5720	1790	5100	4150	1700	6810	5710	2160	5650	5140	1360
Number Open Pores	931	924	184	949	862	317	1050	948	253	1110	1050	253
Pore surface	1.62E+04	1.59E+04	3.23E+03	6.35E+06	1.83E+04	1.68E+07	6.61E+06	1.66E+04	1.75E+07	2.24E+04	2.24E+04	2.62E+03
Pore volume	1.05E+05	8.86E+04	3.92E+04	2.13E+09	9.77E+04	5.65E+09	3.27E+09	1.08E+05	8.64E+09	1.58E+05	1.49E+05	3.34E+04
Open Pore Density	20.4	19.2	3.07	28.4	26.9	7.92	29.8	30.4	4.83	24.7	23	4.72
Open Porosity (%)	0.861	0.789	0.344	0.933	0.796	0.361	1.26	1.23	0.633	1.39	1.54	0.386
Avg. Pore Orientation phi	135	136	11.6	169	171	7.68	162	159	8.25	197	197	10.4
Avg. Pore Orientation theta	39.4	38.1	3.17	46	46.1	1.79	32.9	34.4	4.54	42.1	44.5	4.84
Pore Density	135	131	30.6	152	134	40.1	192	178	42.9	125	114	25.3
Pore Fractal	2.09	2.09	0.0876	2.11	2.07	0.089	2.21	2.18	0.0605	2.15	2.11	0.0669
Pore Separation	356	342	67.5	301	304	39.9	265	248	39.6	307	318	36.1
Pore Surface: Cortical Volume	0.00216	0.0021	0.000472	0.00265	0.00236	0.000789	0.00312	0.00295	0.000488	0.00279	0.00258	0.000537
Pore Surface: PoreVolume	0.164	0.179	0.0295	0.178	0.185	0.0216	0.165	0.167	0.0349	0.145	0.148	0.0164
Pore Tb.N	0.000383	0.000372	0.000101	0.000518	0.000457	0.000175	0.00053	0.000509	0.000134	0.000486	0.000444	0.000103
Pore Thickness	36.6	31.3	12	29.7	26.8	6.36	40.7	32.5	22	40.4	38.9	6.17
Pore Volume	6.22E+08	6.87E+08	1.80E+08	4.96E+08	4.37E+08	1.42E+08	6.98E+08	5.99E+08	2.34E+08	8.82E+08	9.54E+08	2.25E+08
SD Pore Separation	151	136	46	112	105	24.2	99.3	80.7	32.9	118	116	31.4

SD Pore Thickness	27.4	23.6	13.1	19.2	20.2	6.12	31.8	25.1	20.2	27.8	27.7	3.92
Sphericity	0.615	0.61	0.00818	0.601	0.6	0.00566	0.605	0.599	0.0132	0.596	0.592	0.00635
Total Porosity (%)	1.36	1.48	0.352	1.5	1.43	0.423	1.98	1.92	0.566	1.95	2	0.399

Group	**Morphine**											
Region	**Anterior**			**Lateral**			**Medial**			**Posterior**		
Statistic	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Pore Surface	4.17E+10	8.72E+07	1.10E+11	3.29E+10	9.07E+07	8.69E+10	3.61E+10	1.22E+08	9.52E+10	4.60E+10	1.04E+08	1.21E+11
Closed Pore Density	98.2	100	54.1	101	102	52.6	125	136	68.6	90.6	98.6	49.1
Closed Porosity (%)	0.486	0.425	0.18	0.579	0.589	0.162	0.623	0.598	0.197	0.565	0.61	0.155
Connectivity	1030	1040	416	904	843	410	1260	1150	733	1120	1100	628
Connectivity density	20	20	11.5	24.3	30	15.1	30	30	23.1	25.7	30	16.2
Cortical Fractal	2.31	2.29	0.11	2.35	2.36	0.0751	2.41	2.4	0.0805	2.37	2.36	0.0781
Cortical Surface	3.59E+10	1.03E+08	9.48E+10	3.16E+10	9.12E+07	8.35E+10	3.24E+10	9.37E+07	8.54E+10	3.88E+10	1.09E+08	1.02E+11
Cortical Volume	7.74E+14	4.50E+10	2.05E+15	5.50E+14	3.34E+10	1.46E+15	5.87E+14	3.60E+10	1.55E+15	7.80E+14	4.41E+10	2.06E+15
Degree of Anisotropy	0.374	0.36	0.0536	0.349	0.335	0.0884	0.475	0.486	0.0577	0.436	0.437	0.0757
Euler number	4700	4170	1740	3760	2930	1460	4670	4360	1670	4270	3660	1510
Intersection Surface	3.88E+08	7.81E+05	1.03E+09	3.62E+08	9.19E+05	9.56E+08	3.48E+08	1.04E+06	9.17E+08	4.91E+08	9.08E+05	1.30E+09

Avg. Pore Major diameter	1110	117	2620	1070	129	2500	1160	125	2740	1200	139	2830
Number Closed Pores	4770	4200	1740	3770	3170	1280	4960	4620	1820	4310	3840	1530
Number of Pores	5730	4970	1960	4660	4010	1570	5940	5530	2080	5380	4830	1810
Number Open Pores	965	871	256	896	873	307	977	899	292	1070	995	300
Pore surface	1.06E+07	1.45E+04	2.79E+07	9.42E+06	1.64E+04	2.49E+07	9.16E+06	1.87E+04	2.42E+07	1.16E+07	1.74E+04	3.06E+07
Pore volume	4.54E+09	8.65E+04	1.20E+10	3.94E+09	8.86E+04	1.04E+10	3.95E+09	1.34E+05	1.04E+10	5.92E+09	1.17E+05	1.57E+10
Open Pore Density	18.7	18.5	9.9	24.2	26.2	12.6	23.8	25	12.5	21.7	22.5	11.3
Open Porosity (%)	1.07	0.6	1.06	0.971	0.924	0.555	1.88	1.99	0.888	1.48	1.05	0.941
Avg. Pore Orientation phi	138	135	12	175	175	5.58	167	173	9.53	211	212	6.13
Avg. Pore Orientation theta	40.1	39.8	3.2	43.5	45.6	4.69	33.3	33	3.21	39.1	38.8	3.51
Pore Density	117	119	63.7	126	131	64.7	148	163	80.4	112	122	60.1
Pore Fractal	2.06	2.05	0.172	2.08	2.11	0.131	2.2	2.18	0.117	2.12	2.09	0.126
Pore Separation	2940	411	6790	2500	320	5790	2320	280	5380	2600	322	6040
Pore Surface: Cortical Volume	0.00186	0.00156	0.00141	0.00211	0.00246	0.00112	0.00282	0.003	0.00161	0.00225	0.0023	0.00131
Pore Surface: Pore Volume	0.147	0.173	0.0694	0.155	0.185	0.0688	0.117	0.139	0.0519	0.126	0.149	0.0566
Pore Tb.N	0.000311	0.000293	0.000211	0.0004	0.000482	0.000216	0.000435	0.000371	0.000256	0.000369	0.000364	0.000206
Pore Thickness	375	34.6	897	363	29.2	883	401	48	933	451	43.4	1080
Pore Volume	1.79E+13	5.25E+08	4.72E+13	1.37E+13	5.67E+08	3.63E+13	1.56E+13	9.22E+08	4.12E+13	2.34E+13	7.35E+08	6.20E+13

SD Pore Separation	1280	186	2980	968	124	2260	897	114	2070	1060	114	2500
SD Pore Thickness	215	27.2	499	212	20.9	507	246	35.1	551	248	34.7	574
Sphericity	0.611	0.611	0.0062	0.604	0.604	0.00574	0.61	0.61	0.00517	0.604	0.604	0.00597
Total Porosity (%)	1.55	1.12	1.17	1.54	1.61	0.605	2.49	2.57	0.981	2.03	1.67	0.974

Descriptive Statistics: Tibial Regions

Tibial Porosity by Region

Statistic	Anterior			Lateral			Medial			Posterior		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Pore Surface	1.39E+08	1.33E+08	4.97E+07	9.29E+07	9.73E+07	3.11E+07	1.10E+08	1.01E+08	3.63E+07	1.27E+08	1.17E+08	3.95E+07
Closed Pore Density	138	124	55.6	170	170	55	158	152	39.3	127	116	41
Closed Porosity (%)	0.74	0.764	0.264	0.768	0.839	0.276	0.855	0.825	0.228	0.871	0.834	0.25
Connectivity	2030	2000	1050	1180	1120	625	1300	1210	597	1340	1110	707
Connectivity density	41	40	21.4	32.9	30	17.9	37.1	30	17.4	30.5	30	17.5
Cortical Fractal	2.39	2.38	0.0813	2.4	2.41	0.0807	2.42	2.42	0.0646	2.4	2.4	0.0696
Cortical Surface	1.11E+08	1.12E+08	6.79E+06	9.68E+07	9.79E+07	4.39E+06	9.14E+07	9.26E+07	4.07E+06	1.06E+08	1.06E+08	7.40E+06
Cortical Volume	4.87E+10	4.92E+10	4.93E+09	3.49E+10	3.52E+10	2.27E+09	3.51E+10	3.61E+10	3.72E+09	4.28E+10	4.22E+10	4.81E+09
Degree of Anisotropy	0.455	0.438	0.0502	0.392	0.388	0.0427	0.43	0.435	0.049	0.46	0.468	0.0619
Euler number	5910	5730	2420	5670	6220	1870	5220	4850	1230	5090	4590	1640

Intersection Surface	6.17E+05	6.25E+05	3.40E+05	3.94E+05	3.66E+05	1.41E+05	7.29E+05	6.93E+05	2.97E+05	7.81E+05	7.64E+05	2.99E+05
Avg. Pore Major diameter	131	129	12.1	121	118	6.25	126	123	9.37	140	140	13.2
Number Closed Pores	6830	5900	2930	5970	5970	2080	5540	5040	1410	5460	4780	1960
Number of Pores	7940	6920	3300	6850	7220	2360	6520	6040	1680	6430	5670	2200
Number Open Pores	1110	1030	391	884	876	306	976	912	293	964	926	258
Pore surface	1.80E+04	1.74E+04	3.35E+03	1.36E+04	1.34E+04	1.36E+03	1.67E+04	1.64E+04	2.91E+03	2.03E+04	1.92E+04	4.23E+03
Pore volume	1.13E+05	1.02E+05	3.90E+04	6.91E+04	6.63E+04	1.31E+04	9.70E+04	9.56E+04	2.55E+04	1.42E+05	1.24E+05	5.51E+04
Open Pore Density	22.7	22	7.53	25.2	24.4	8.48	27.9	26.2	8.72	22.5	21	5.77
Open Porosity (%)	0.988	0.96	0.441	0.562	0.578	0.161	0.949	0.859	0.464	1.14	1.21	0.474
Avg. Pore Orientation phi	196	197	6.32	192	193	8	184	185	7.84	190	190	8.55
Avg. Pore Orientation theta	43.4	43.8	3.93	45.2	45.3	3.33	39.1	38.8	3.65	36.6	36.6	3
Pore Density	161	146	62.9	195	195	62.8	186	177	47.3	149	139	46.2
Pore Fractal	2.16	2.15	0.132	2.14	2.17	0.142	2.2	2.2	0.106	2.17	2.17	0.114
Pore Separation	303	306	51	268	264	51.3	264	268	33.8	286	287	42.4
Pore Surface: Cortical Volume	0.00282	0.00256	0.00094	0.00265	0.00268	0.000831	0.00311	0.00296	0.000924	0.00295	0.00278	0.000825

Pore Surface: Pore Volume	0.167	0.17	0.0222	0.2	0.203	0.0146	0.177	0.177	0.0189	0.152	0.151	0.0275
Pore Tb.N	0.000534	0.000485	0.000165	0.000542	0.000554	0.000177	0.000594	0.000534	0.000184	0.000526	0.000509	0.000143
Pore Thickness	32.2	31.6	6.06	24.8	24.1	3.8	30.4	30.5	5.76	38.6	37.3	9.64
Pore Volume	8.44E+08	8.03E+08	3.27E+08	4.65E+08	4.94E+08	1.47E+08	6.37E+08	6.14E+08	2.45E+08	8.62E+08	8.12E+08	3.19E+08
SD Pore Separation	117	115	19.7	84.6	81.9	19.1	92	92.9	13.9	106	102	21.2
SD Pore Thickness	19.9	21.1	4.56	14	14.2	4.73	20.3	19.9	6.22	25.5	26.6	7.24
Sphericity	0.597	0.597	0.00464	0.605	0.603	0.00741	0.603	0.604	0.00591	0.605	0.605	0.00532
Total Porosity (%)	1.72	1.67	0.627	1.33	1.4	0.398	1.79	1.72	0.606	2	1.99	0.65

Tibial Porosity by Drug Treatment Group

Statistic	Control			Fentanyl			Morphine		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Pore Surface	1.04E+08	9.57E+07	4.42E+07	1.23E+08	1.19E+08	4.19E+07	1.25E+08	1.11E+08	4.01E+07
Closed Pore Density	134	121	56.1	162	160	46.6	150	147	45.5
Closed Porosity (%)	0.729	0.666	0.301	0.831	0.836	0.225	0.865	0.862	0.226
Connectivity	1240	1010	855	1530	1440	838	1630	1390	750
Connectivity density	31.1	20	22.8	36.1	40	16.4	38.9	40	15.9
Cortical Fractal	2.38	2.37	0.0878	2.42	2.43	0.0663	2.41	2.4	0.0607
Cortical Surface	9.91E+07	9.75E+07	9.10E+06	1.01E+08	9.90E+07	1.01E+07	1.03E+08	1.01E+08	9.19E+06
Cortical Volume	3.89E+10	3.79E+10	6.60E+09	4.06E+10	3.91E+10	7.63E+09	4.17E+10	3.89E+10	6.86E+09
Degree of Anisotropy	0.437	0.444	0.0616	0.432	0.427	0.0537	0.433	0.43	0.0585

Euler number	4750	4510	1630	6140	6540	1970	5510	5450	1700
Intersection Surface	6.09E+05	5.67E+05	3.15E+05	6.26E+05	6.03E+05	3.36E+05	6.56E+05	6.32E+05	2.95E+05
Avg. Pore Major diameter	132	130	12.7	127	125	12.3	129	124	13
Number Closed Pores	5090	4540	2070	6610	6960	2340	6150	5780	1940
Number of Pores	5990	5280	2370	7670	8100	2660	7140	6600	2200
Number Open Pores	906	827	327	1050	1070	344	992	932	283
Pore surface	1.74E+04	1.65E+04	3.81E+03	1.62E+04	1.51E+04	3.38E+03	1.79E+04	1.74E+04	4.45E+03
Pore volume	1.08E+05	9.26E+04	4.39E+04	9.36E+04	7.95E+04	3.59E+04	1.13E+05	1.06E+05	5.21E+04
Open Pore Density	23.8	20.8	9.45	25.9	25.1	7.23	24.1	24.1	6.87
Open Porosity (%)	0.914	0.745	0.515	0.837	0.703	0.394	0.978	0.906	0.446
Avg. Pore Orientation phi	190	190	9.33	193	196	9	189	188	7.62
Avg. Pore Orientation theta	41.2	41.5	4.39	41	40.8	5.21	41.1	40.5	5.06
Pore Density	157	139	64.7	188	184	53	174	168	51.7
Pore Fractal	2.12	2.11	0.148	2.18	2.22	0.116	2.19	2.17	0.0941
Pore Separation	294	289	56.8	272	263	41.1	275	270	40.1
Pore Surface:Cortical Volume	0.00268	0.00246	0.00106	0.00297	0.00312	0.000743	0.00299	0.00266	0.000804
Pore Surface:PoreVolume	0.17	0.177	0.0269	0.182	0.189	0.0257	0.169	0.168	0.0279
Pore Tb.N	0.000497	0.00046	0.00019	0.00059	0.000635	0.000163	0.00056	0.000513	0.000136
Pore Thickness	32.7	30.7	8.47	29	27	7.28	32.8	32.1	8.5
Pore Volume	6.45E+08	5.61E+08	3.32E+08	6.88E+08	6.31E+08	2.76E+08	7.72E+08	6.40E+08	3.21E+08
SD Pore Separation	99.9	100	23.1	102	97.1	22.4	98	93.3	21.9
SD Pore Thickness	21.3	21.1	6.73	17.4	16.5	6.43	21.1	22.2	7.34
Sphericity	0.603	0.603	0.00765	0.603	0.601	0.00706	0.602	0.602	0.00494
Total Porosity (%)	1.64	1.42	0.725	1.66	1.62	0.49	1.83	1.7	0.624

Tibial Porosity by Region and Drug Treatment Group

Group	Control

Region	**Anterior**			**Lateral**			**Medial**			**Posterior**		
Statistic	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Pore Surface	1.20E+08	1.03E+08	5.88E+07	7.74E+07	7.16E+07	2.96E+07	9.92E+07	7.76E+07	4.30E+07	1.19E+08	1.17E+08	3.48E+07
Closed Pore Density	125	101	71.3	144	126	57.1	155	146	52.8	110	96.7	40.5
Closed Porosity (%)	0.656	0.54	0.332	0.63	0.575	0.285	0.805	0.67	0.335	0.824	0.795	0.261
Connectivity	1630	1170	1180	1020	734	837	1150	1210	645	1160	960	725
Connectivity density	34.3	20	27.6	28.6	20	24.1	34.3	30	23	27.1	20	20.6
Cortical Fractal	2.36	2.33	0.107	2.36	2.36	0.0887	2.4	2.37	0.0891	2.39	2.39	0.0763
Cortical Surface	1.09E+08	1.10E+08	6.98E+06	9.52E+07	9.44E+07	4.22E+06	8.99E+07	8.83E+07	3.75E+06	1.03E+08	1.04E+08	7.25E+06
Cortical Volume	4.63E+10	4.69E+10	4.14E+09	3.44E+10	3.45E+10	2.32E+09	3.34E+10	3.12E+10	4.57E+09	4.14E+10	4.13E+10	4.62E+09
Degree of Anisotropy	0.451	0.457	0.0432	0.38	0.388	0.0424	0.432	0.444	0.0482	0.485	0.497	0.066
Euler number	5180	4410	2400	4670	4560	1580	4910	4810	1350	4250	4470	1130
Intersection Surface	5.24E+05	5.00E+05	1.64E+05	3.30E+05	3.43E+05	5.86E+04	7.49E+05	6.93E+05	2.80E+05	8.31E+05	7.09E+05	3.98E+05
Avg. Pore Major diameter	137	138	11.4	123	123	7.73	123	123	5.13	145	146	9.84
Number Closed Pores	5780	4540	3120	4930	4550	2000	5100	4560	1530	4540	4290	1510
Number of Pores	6810	5450	3510	5690	5270	2230	6060	5360	1890	5410	5140	1740
Number Open Pores	1040	906	402	756	703	248	958	804	384	876	850	244
Pore surface	1.77E+04	1.81E+04	1.60E+03	1.37E+04	1.38E+04	1.56E+03	1.60E+04	1.47E+04	2.98E+03	2.21E+04	2.26E+04	2.75E+03
Pore volume	1.05E+05	1.10E+05	1.66E+04	7.03E+04	6.77E+04	1.19E+04	9.29E+04	7.93E+04	2.78E+04	1.66E+05	1.63E+05	4.08E+04
Open Pore Density	22.5	20.2	9.55	22	21.4	7.26	29.2	26.4	12.9	21.4	19.9	6.85
Open Porosity (%)	0.892	0.777	0.49	0.519	0.511	0.167	0.931	0.772	0.578	1.31	1.31	0.469
Avg. Pore Orientation phi	197	197	8.95	193	195	8.14	182	186	8.22	189	190	6.47
Avg. Pore Orientation theta	44	44.1	2.45	45.4	46	2.13	38.9	38.8	3.07	36.5	36.6	2.31
Pore Density	148	122	80.6	166	146	63.8	184	171	65.1	132	119	47
Pore Fractal	2.11	2.07	0.175	2.07	2.08	0.157	2.16	2.13	0.145	2.15	2.17	0.127

Pore Separation	318	331	68.5	291	282	61.1	272	280	50.6	294	296	48.1
Pore Surface: Cortical Volume	0.00259	0.00213	0.00132	0.00225	0.00213	0.000842	0.00298	0.00255	0.00124	0.00288	0.00299	0.000856
Pore Surface: PoreVolume	0.171	0.168	0.0143	0.196	0.203	0.0106	0.177	0.178	0.0216	0.137	0.139	0.018
Pore Tb.N	0.000488	0.000425	0.000216	0.000448	0.000422	0.000158	0.000561	0.000495	0.000249	0.000492	0.000499	0.000145
Pore Thickness	30.8	31.2	4.48	25.5	25	1.76	31.2	30.3	7.04	43.4	41	6.98
Pore Volume	7.17E+08	6.39E+08	3.74E+08	3.94E+08	3.98E+08	1.44E+08	5.83E+08	4.37E+08	3.06E+08	8.87E+08	8.90E+08	2.98E+08
SD Pore Separation	115	120	23	89.7	87.1	23.8	91.2	98.8	14.3	104	106	24.7
SD Pore Thickness	19.3	21.1	4.38	15.5	15.4	3.38	21.3	21.7	7.12	28.9	27	3.48
Sphericity	0.598	0.598	0.00564	0.607	0.609	0.00837	0.603	0.604	0.00913	0.604	0.605	0.00488
Total Porosity (%)	1.54	1.32	0.789	1.15	1.19	0.409	1.73	1.44	0.792	2.13	2.13	0.605

Group	**Fentanyl**											
Region	**Anterior**			**Lateral**			**Medial**			**Posterior**		
Statistic	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Pore Surface	1.57E+08	1.82E+08	4.49E+07	1.00E+08	1.10E+08	3.89E+07	1.14E+08	1.19E+08	2.67E+07	1.19E+08	1.16E+08	3.95E+07
Closed Pore Density	158	160	48.5	183	193	60.6	166	160	33.5	139	157	38
Closed Porosity (%)	0.796	0.835	0.227	0.836	0.889	0.329	0.885	0.825	0.164	0.807	0.769	0.186
Connectivity	2370	2330	1030	1230	1430	611	1280	1190	395	1240	1430	681
Connectivity density	47.1	40	19.8	34.3	40	16.2	35.7	40	9.76	27.1	30	15
Cortical Fractal	2.42	2.45	0.0636	2.41	2.46	0.0958	2.43	2.44	0.0357	2.39	2.4	0.0665
Cortical Surface	1.11E+08	1.14E+08	7.79E+06	9.66E+07	9.74E+07	4.63E+06	9.12E+07	9.24E+07	4.87E+06	1.07E+08	1.09E+08	8.56E+06
Cortical Volume	4.94E+10	5.02E+10	6.32E+09	3.46E+10	3.61E+10	2.81E+09	3.57E+10	3.68E+10	3.77E+09	4.27E+10	4.22E+10	5.82E+09
Degree of Anisotropy	0.461	0.438	0.0514	0.402	0.403	0.049	0.431	0.417	0.0478	0.436	0.456	0.0601

Euler number	6910	7390	2600	6190	6520	2240	5660	5810	1210	5800	6560	1760
Intersection Surface	8.02E+05	6.83E+05	5.12E+05	3.96E+05	4.43E+05	1.81E+05	6.94E+05	6.43E+05	2.63E+05	6.13E+05	6.20E+05	2.02E+05
Avg. Pore Major diameter	127	129	11.1	121	118	6.6	127	124	13.1	134	132	15.3
Number Closed Pores	8000	9320	3000	6450	7200	2450	5930	5990	1360	6070	7040	2130
Number of Pores	9280	10800	3430	7420	8110	2800	6940	6830	1560	7040	8110	2370
Number Open Pores	1270	1390	437	966	1190	382	1010	997	230	972	1070	258
Pore surface	1.78E+04	1.60E+04	4.35E+03	1.34E+04	1.36E+04	9.22E+02	1.66E+04	1.47E+04	3.46E+03	1.72E+04	1.65E+04	2.50E+03
Pore volume	1.13E+05	9.37E+04	5.21E+04	6.52E+04	6.63E+04	6.50E+03	9.13E+04	7.67E+04	2.72E+04	1.04E+05	1.08E+05	2.74E+04
Open Pore Density	25.3	23.7	6.9	27.6	32.1	10.5	28.1	26.2	5.68	22.5	24.2	4.64
Open Porosity (%)	1.13	0.973	0.516	0.546	0.578	0.154	0.842	0.678	0.32	0.832	0.731	0.329
Avg. Pore Orientation phi	198	200	4.47	194	193	4.62	188	186	9.76	192	198	13
Avg. Pore Orientation theta	43.5	43.9	5.29	44.7	45.8	3.63	39	37.4	4.18	37	37	4.11
Pore Density	184	183	55.2	211	230	70	194	183	38.2	162	178	42
Pore Fractal	2.21	2.25	0.0975	2.16	2.23	0.17	2.22	2.24	0.0637	2.15	2.16	0.116
Pore Separation	283	265	44.5	259	230	58.2	261	260	14.3	285	271	36.8
Pore Surface: Cortical Volume	0.00314	0.00316	0.000684	0.00285	0.00322	0.000994	0.00315	0.00347	0.000567	0.00275	0.00275	0.000748
Pore Surface: PoreVolume	0.168	0.174	0.028	0.206	0.208	0.0102	0.185	0.192	0.0171	0.17	0.176	0.0261
Pore Tb.N	0.000607	0.000645	0.000146	0.000609	0.000709	0.000218	0.000628	0.00069	0.000129	0.000515	0.000525	0.00016
Pore Thickness	32.2	28.8	7.66	22.9	22.2	2.01	27.5	27.4	3.86	33.3	29.8	9.06

Pore Volume	9.49E+08	9.39E+08	3.06E+08	4.82E+08	5.44E+08	1.78E+08	6.20E+08	6.14E+08	1.69E+08	7.01E+08	6.60E+08	2.34E+08
SD Pore Separation	114	102	25	84.4	76	20.8	98.8	94.6	15.7	109	100	18.6
SD Pore Thickness	19.3	17.3	4.56	11.2	10.1	2.61	17.1	19.7	4.61	21.9	20.5	8
Sphericity	0.597	0.595	0.00466	0.604	0.599	0.00896	0.603	0.604	0.00419	0.607	0.606	0.00664
Total Porosity (%)	1.92	1.8	0.582	1.38	1.47	0.453	1.72	1.72	0.372	1.63	1.56	0.471

Group	**Morphine**											
Region	**Anterior**			**Lateral**			**Medial**			**Posterior**		
Statistic	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
Pore Surface	1.38E+08	1.33E+08	4.40E+07	1.01E+08	9.73E+07	2.01E+07	1.17E+08	9.48E+07	4.01E+07	1.43E+08	1.54E+08	4.45E+07
Closed Pore Density	132	122	46.4	183	170	43.9	154	152	33.5	130	116	44.9
Closed Porosity (%)	0.769	0.866	0.238	0.838	0.839	0.178	0.873	0.859	0.174	0.981	1.05	0.29
Connectivity	2100	2000	930	1310	1270	420	1480	1340	742	1620	1310	732
Connectivity density	41.4	40	16.8	35.7	40	14	41.4	30	18.6	37.1	30	17
Cortical Fractal	2.38	2.35	0.0638	2.42	2.41	0.0425	2.43	2.42	0.0645	2.42	2.4	0.072
Cortical Surface	1.12E+08	1.13E+08	6.04E+06	9.86E+07	1.00E+08	4.32E+06	9.30E+07	9.35E+07	3.43E+06	1.08E+08	1.07E+08	6.04E+06
Cortical Volume	5.05E+10	5.11E+10	3.60E+09	3.57E+10	3.52E+10	1.63E+09	3.63E+10	3.61E+10	2.32E+09	4.42E+10	4.46E+10	4.19E+09

Degree of Anisotropy	0.453	0.438	0.0619	0.393	0.382	0.0401	0.427	0.434	0.0583	0.459	0.447	0.0582
Euler number	5630	5730	2270	6150	6220	1550	5080	4750	1170	5200	4590	1790
Intersection Surface	5.24E+05	4.16E+05	1.89E+05	4.56E+05	4.24E+05	1.45E+05	7.43E+05	6.99E+05	3.79E+05	9.01E+05	9.14E+05	2.16E+05
Avg. Pore Major diameter	130	127	13.3	118	118	3.7	127	120	9.35	141	140	13.3
Number Closed Pores	6690	6000	2620	6520	6490	1570	5600	4960	1430	5780	4730	2120
Number of Pores	7720	6920	2940	7450	7470	1840	6560	5870	1710	6820	5670	2390
Number Open Pores	1030	922	335	929	980	273	965	912	292	1040	941	281
Pore surface	1.85E+04	1.74E+04	3.93E+03	1.38E+04	1.30E+04	1.66E+03	1.77E+04	1.74E+04	2.35E+03	2.17E+04	2.01E+04	5.35E+03
Pore volume	1.19E+05	1.11E+05	4.43E+04	7.17E+04	6.56E+04	1.90E+04	1.07E+05	9.80E+04	2.20E+04	1.56E+05	1.19E+05	7.19E+04
Open Pore Density	20.3	18.8	5.94	26	25.7	7.55	26.5	26	7.21	23.7	23	6.29
Open Porosity (%)	0.944	0.96	0.325	0.622	0.654	0.168	1.07	0.962	0.498	1.27	1.26	0.503
Avg. Pore Orientation phi	194	193	5	190	193	10.8	183	183	4.63	189	187	4.87
Avg. Pore Orientation theta	42.9	42.2	4.1	45.4	44.7	4.34	39.5	39.9	4.17	36.4	35.9	2.76
Pore Density	152	141	52.2	209	195	51.3	180	177	40.5	154	139	50.8
Pore Fractal	2.15	2.1	0.104	2.18	2.17	0.0716	2.21	2.19	0.0996	2.2	2.2	0.107
Pore Separation	309	330	35.1	253	264	26.2	259	268	30.7	278	296	46.5
Pore Surface: Cortical Volume	0.00271	0.00256	0.000743	0.00283	0.00268	0.000577	0.00321	0.00296	0.000977	0.00322	0.00316	0.000914
Pore Surface: PoreVolume	0.163	0.158	0.0249	0.196	0.199	0.0204	0.168	0.167	0.0162	0.15	0.16	0.0296
Pore Tb.N	0.000507	0.000481	0.000117	0.000569	0.000565	0.000125	0.000592	0.000534	0.000178	0.000571	0.000549	0.000134

Pore Thickness	33.5	33.8	6.29	26	24.9	5.88	32.6	32.6	5.47	39.2	35.6	10.9
Pore Volume	8.67E+08	8.03E+08	3.00E+08	5.18E+08	5.78E+08	1.02E+08	7.07E+08	6.21E+08	2.62E+08	9.98E+08	1.09E+09	3.78E+08
SD Pore Separation	122	118	10	79.6	75.7	12.7	86.1	89.9	9.89	105	102	22.8
SD Pore Thickness	21	22.3	5.23	15.4	15	6.48	22.4	23.5	6.2	25.7	24.6	8.39
Sphericity	0.597	0.597	0.00422	0.604	0.604	0.00493	0.603	0.604	0.00389	0.603	0.603	0.00395
Total Porosity (%)	1.71	1.67	0.515	1.45	1.67	0.307	1.94	1.74	0.651	2.24	2.27	0.76

Appendix XXI: Micro-CT Linear Mixed Model for Aggregate Porosity

Aggregate Porosity: LMM Fixed Effects and Random Effects

Cortical Fractal	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.156	-0.324	0.308	0	1		0.994	
Bone1	-0.173	0.156	-0.499	0.148	-1.1	0.277		0.278	
Group1	-0.018	0.221	-0.475	0.402	-0.0812	0.936		0.933	
Group2	0.0871	0.221	-0.362	0.535	0.394	0.696		0.691	
Bone1:Group1	0.348	0.221	-0.0767	0.815	1.57	0.125		0.118	
Bone1:Group2	-0.126	0.221	-0.533	0.311	-0.57	0.572		0.571	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.854	0	1		R^2M/R^2C	0.0874 / 0.0874
Residual	1.03	1.01	0.639	1.19	100	NA		AIC/BIC	139 / 153
Number Closed Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.158	-0.32	0.305	0	1		1	
Bone1	-0.368	0.134	-0.662	-0.0967	-2.75	0.0133	*	0.0144	*
Group1	-0.279	0.224	-0.7	0.197	-1.25	0.229		0.238	
Group2	0.269	0.224	-0.206	0.684	1.2	0.245		0.251	
Bone1:Group1	0.192	0.189	-0.198	0.597	1.01	0.324		0.334	
Bone1:Group2	-0.0925	0.189	-0.437	0.329	-0.489	0.631		0.625	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.149	0.386	0	0.849	16.5	0.48		R^2M/R^2C	0.188 / 0.322
Residual	0.752	0.867	0.54	1.07	83.5	NA		AIC/BIC	134 / 148
Number Open Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.179	-0.358	0.365	0	1		0.961	
Bone1	0.127	0.139	-0.148	0.397	0.918	0.371		0.361	
Group1	-0.168	0.253	-0.63	0.354	-0.661	0.517		0.536	
Group2	0.191	0.253	-0.272	0.695	0.755	0.46		0.479	
Bone1:Group1	0.132	0.196	-0.238	0.543	0.676	0.508		0.525	
Bone1:Group2	-0.166	0.196	-0.552	0.207	-0.85	0.407		0.415	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.271	0.52	0	0.988	25.1	0.279		R^2M/R^2C	0.0483 / 0.288
Residual	0.806	0.898	0.592	1.1	74.9			AIC/BIC	140 / 154

Closed Pore Density	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.149	-0.29	0.271	0	1		0.972	
Bone1	-0.364	0.149	-0.646	-0.0561	-2.45	0.0193	*	0.0196	*
Group1	-0.213	0.21	-0.593	0.207	-1.01	0.318		0.339	
Group2	0.25	0.21	-0.161	0.668	1.19	0.242		0.282	
Bone1:Group1	0.146	0.21	-0.305	0.577	0.694	0.492		0.476	
Bone1:Group2	-0.0887	0.21	-0.512	0.331	-0.422	0.676		0.658	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.786	0	1		R^2M/R^2C	0.166 / 0.166
Residual	0.929	0.964	0.634	1.14	100			AIC/BIC	136 / 150
Open Pore Density	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.176	-0.322	0.364	0	1		0.984	
Bone1	0.203	0.14	-0.103	0.483	1.45	0.164		0.161	
Group1	-0.045	0.249	-0.52	0.426	-0.181	0.858		0.867	
Group2	0.142	0.249	-0.369	0.651	0.573	0.574		0.573	
Bone1:Group1	0.0723	0.198	-0.303	0.483	0.365	0.719		0.712	
Bone1:Group2	-0.174	0.198	-0.547	0.241	-0.876	0.393		0.397	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.237	0.486	0	1.03	22.3	0.338		R^2M/R^2C	0.0609 / 0.27
Residual	0.824	0.908	0.588	1.1	77.7			AIC/BIC	140 / 153
Closed Porosity (%)	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.139	-0.288	0.29	0	1		0.992	
Bone1	-0.548	0.127	-0.768	-0.309	-4.3	0.000432	***	0.000175	***
Group1	-0.195	0.196	-0.571	0.179	-0.996	0.333		0.355	
Group2	0.0737	0.196	-0.318	0.465	0.376	0.712		0.745	
Bone1:Group1	0.166	0.18	-0.193	0.52	0.923	0.368		0.35	
Bone1:Group2	0.00464	0.18	-0.346	0.347	0.0258	0.98		0.995	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0633	0.252	0	0.734	8.51	0.718		R^2M/R^2C	0.318 / 0.376
Residual	0.681	0.825	0.53	1.01	91.5			AIC/BIC	128 / 142
Open Porosity (%)	**Lambda = -0.25**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.146	-0.266	0.289	0	1		0.994	

Bone1	0.463	0.11	0.23	0.678	4.21	0.000526	***	0.000175	***
Group1	0.373	0.206	-0.00582	0.77	1.81	0.0871	.	0.101	
Group2	-0.271	0.206	-0.686	0.154	-1.31	0.206		0.204	
Bone1:Group1	0.413	0.156	0.118	0.729	2.65	0.0162	*	0.0116	*
Bone1:Group2	-0.176	0.156	-0.494	0.108	-1.13	0.273		0.273	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.193	0.439	0	0.817	27.5	0.234		R^2M/R^2C	0.354 / 0.532
Residual	0.508	0.713	0.47	0.891	72.5			AIC/BIC	122 / 136
Total Porosity (%)	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.142	-0.266	0.299	0	1		1	
Bone1	0.263	0.138	0.000684	0.579	1.91	0.0725	.	0.0582	.
Group1	0.375	0.201	-0.0527	0.776	1.86	0.0788	.	0.0947	.
Group2	-0.326	0.201	-0.682	0.0549	-1.62	0.122		0.132	
Bone1:Group1	0.486	0.195	0.103	0.842	2.5	0.0225	*	0.0158	*
Bone1:Group2	-0.256	0.195	-0.618	0.123	-1.32	0.205		0.196	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0262	0.162	0	0.736	3.18	0.892		R^2M/R^2C	0.252 / 0.276
Residual	0.797	0.893	0.58	1.05	96.8			AIC/BIC	131 / 145
Cortical Volume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.203	-0.41	0.411	0	1		0.983	
Bone1	-0.166	0.0878	-0.331	0.0221	-1.89	0.0745	.	0.0737	.
Group1	-0.276	0.287	-0.855	0.289	-0.961	0.349		0.358	
Group2	0.0892	0.287	-0.495	0.722	0.31	0.76		0.786	
Bone1:Group1	0.188	0.124	-0.064	0.43	1.51	0.148		0.16	
Bone1:Group2	0.0217	0.124	-0.197	0.259	0.175	0.863		0.875	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.706	0.84	0.523	1.27	68.6	0.000721	***	R^2M/R^2C	0.0855 / 0.713
Residual	0.324	0.569	0.359	0.699	31.4			AIC/BIC	128 / 142
Pore Volume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.151	-0.291	0.293	0	1		0.993	
Bone1	0.215	0.14	-0.0651	0.492	1.54	0.141		0.122	
Group1	0.28	0.213	-0.146	0.719	1.31	0.206		0.227	
Group2	-0.287	0.213	-0.724	0.164	-1.35	0.195		0.219	
Bone1:Group1	0.478	0.198	0.0587	0.844	2.42	0.0265	*	0.013	*

Bone1:Group2	-0.239	0.198	-0.618	0.129	-1.21	0.242		0.216	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0675	0.26	0	0.767	7.59	0.747		R^2M/R^2C	0.198 / 0.259
Residual	0.822	0.906	0.594	1.08	92.4			AIC/BIC	134 / 148
Total Porosity	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.142	-0.276	0.287	0	1		0.988	
Bone1	0.263	0.138	-0.0174	0.523	1.91	0.0728	.	0.0537	.
Group1	0.375	0.201	-0.027	0.789	1.86	0.0789	.	0.107	
Group2	-0.326	0.201	-0.781	0.0817	-1.62	0.122		0.149	
Bone1:Group1	0.486	0.195	0.101	0.883	2.5	0.0225	*	0.0109	*
Bone1:Group2	-0.256	0.195	-0.63	0.105	-1.31	0.205		0.182	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0262	0.162	0	0.768	3.18	0.892		R^2M/R^2C	0.252 / 0.275
Residual	0.797	0.893	0.565	1.06	96.8			AIC/BIC	131 / 145
Cortical Surface	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.186	-0.4	0.34	0	1		0.994	
Bone1	-0.214	0.119	-0.446	0.00228	-1.79	0.09	.	0.093	.
Group1	-0.183	0.263	-0.712	0.351	-0.693	0.497		0.511	
Group2	-0.0489	0.263	-0.55	0.517	-0.186	0.855		0.847	
Bone1:Group1	0.193	0.169	-0.179	0.515	1.14	0.268		0.294	
Bone1:Group2	-0.0423	0.169	-0.342	0.338	-0.251	0.805		0.825	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.429	0.655	0.283	1.12	41.8	0.0629	.	R^2M/R^2C	0.0874 / 0.469
Residual	0.597	0.773	0.5	0.957	58.2			AIC/BIC	136 / 150
Pore Surface	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.165	-0.327	0.348	0	1		1	
Bone1	-0.0801	0.15	-0.415	0.224	-0.536	0.599		0.579	
Group1	-0.00255	0.234	-0.447	0.446	-0.0109	0.991		0.984	
Group2	-0.00515	0.234	-0.478	0.402	-0.022	0.983		0.98	
Bone1:Group1	0.387	0.212	0.0225	0.822	1.83	0.0836	.	0.067	.
Bone1:Group2	-0.167	0.212	-0.618	0.246	-0.79	0.44		0.431	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.104	0.322	0	0.882	9.94	0.672		R^2M/R^2C	0.0745 / 0.167
Residual	0.939	0.969	0.609	1.15	90.1			AIC/BIC	140 / 154

Intersection Surface	Lambda = None								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.131	-0.256	0.265	0	1		0.985	
Bone1	0.563	0.116	0.314	0.791	4.84	0.000131	***	0.000351	***
Group1	0.316	0.185	-0.0527	0.683	1.71	0.104		0.113	
Group2	-0.176	0.185	-0.566	0.16	-0.956	0.352		0.366	
Bone1:Group1	0.33	0.165	0.0377	0.659	2.01	0.06	.	0.0547	.
Bone1:Group2	-0.239	0.165	-0.554	0.0605	-1.45	0.163		0.156	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0734	0.271	0	0.699	11.4	0.627		R^2M/R^2C	0.404 / 0.472
Residual	0.569	0.754	0.463	0.915	88.6			AIC/BIC	122 / 136
Pore Surface: PoreVolume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.131	-0.257	0.252	0	1		0.997	
Bone1	-0.508	0.105	-0.721	-0.309	-4.83	0.000134	***	0.000175	***
Group1	-0.496	0.185	-0.884	-0.117	-2.68	0.0153	*	0.0172	*
Group2	0.499	0.185	0.124	0.846	2.69	0.0148	*	0.0204	*
Bone1:Group1	-0.298	0.149	-0.605	0.006	-2	0.0607	.	0.0561	.
Bone1:Group2	0.151	0.149	-0.145	0.469	1.01	0.325		0.321	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.128	0.358	0	0.767	21.6	0.354		R^2M/R^2C	0.447 / 0.566
Residual	0.465	0.682	0.435	0.831	78.4			AIC/BIC	119 / 133
Pore Surface: Cortical Volume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.158	-0.283	0.321	0	1		0.976	
Bone1	-0.0428	0.158	-0.35	0.254	-0.271	0.788		0.775	
Group1	0.104	0.224	-0.343	0.465	0.467	0.643		0.667	
Group2	-0.0466	0.224	-0.475	0.418	-0.208	0.836		0.852	
Bone1:Group1	0.372	0.224	-0.0977	0.825	1.66	0.105		0.0902	.
Bone1:Group2	-0.174	0.224	-0.639	0.26	-0.777	0.442		0.432	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.821	0	1		R^2M/R^2C	0.0694 / 0.0694
Residual	1.05	1.02	0.647	1.22	100			AIC/BIC	140 / 154
Pore Thickness	**Lambda = -1**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.

Intercept	0	0.121	-0.213	0.251	0	1		0.978	
Bone1	0.543	0.116	0.329	0.78	4.69	0.000183	***	0.000175	***
Group1	0.474	0.172	0.161	0.822	2.76	0.0128	*	0.0175	*
Group2	-0.487	0.172	-0.819	-0.15	-2.84	0.0109	*	0.0175	*
Bone1:Group1	0.201	0.164	-0.0891	0.511	1.23	0.236		0.219	
Bone1:Group2	-0.104	0.164	-0.447	0.215	-0.632	0.535		0.524	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.027	0.164	0	0.633	4.57	0.846		R^2M/R^2C	0.449 / 0.474
Residual	0.564	0.751	0.493	0.895	95.4			AIC/BIC	120 / 134
Pore Separation	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.152	-0.327	0.266	0	1		0.98	
Bone1	0.299	0.152	0.00573	0.588	1.97	0.0571	.	0.0509	.
Group1	-0.00874	0.215	-0.45	0.439	-0.0405	0.968		0.975	
Group2	-0.0987	0.215	-0.523	0.337	-0.458	0.65		0.668	
Bone1:Group1	-0.287	0.215	-0.735	0.108	-1.33	0.191		0.176	
Bone1:Group2	0.0789	0.215	-0.333	0.519	0.366	0.716		0.708	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.787	0	1		R^2M/R^2C	0.129 / 0.129
Residual	0.975	0.987	0.607	1.15	100			AIC/BIC	137 / 151
Pore Tb.N	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.154	-0.311	0.287	0	1		0.976	
Bone1	-0.277	0.154	-0.565	-0.0123	-1.81	0.0793	.	0.0793	.
Group1	-0.124	0.217	-0.56	0.348	-0.57	0.572		0.588	
Group2	0.17	0.217	-0.308	0.611	0.783	0.439		0.473	
Bone1:Group1	0.264	0.217	-0.131	0.675	1.22	0.232		0.22	
Bone1:Group2	-0.131	0.217	-0.564	0.291	-0.603	0.55		0.532	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.769	0	1		R^2M/R^2C	0.116 / 0.116
Residual	0.99	0.995	0.635	1.16	100			AIC/BIC	138 / 152
Degree of Anisotropy	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.158	-0.308	0.353	0	1		0.986	
Bone1	-0.314	0.144	-0.653	-0.03	-2.17	0.0433	*	0.0344	*
Group1	0.0555	0.223	-0.418	0.487	0.249	0.806		0.826	
Group2	-0.152	0.223	-0.525	0.294	-0.68	0.505		0.541	
Bone1:Group1	0.101	0.204	-0.322	0.542	0.494	0.627		0.622	

Bone1:Group2	-0.29	0.204	-0.694	0.11	-1.42	0.173		0.153	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0834	0.289	0	0.831	8.7	0.712		R^2M/R^2C	0.141 / 0.216
Residual	0.876	0.936	0.561	1.12	91.3			AIC/BIC	137 / 151
Pore Fractal	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.158	-0.314	0.302	0	1		0.979	
Bone1	-0.0984	0.158	-0.401	0.187	-0.624	0.537		0.522	
Group1	-0.0387	0.223	-0.453	0.382	-0.173	0.863		0.876	
Group2	0.0842	0.223	-0.344	0.517	0.378	0.708		0.716	
Bone1:Group1	0.362	0.223	-0.0449	0.793	1.62	0.113		0.101	
Bone1:Group2	-0.117	0.223	-0.56	0.371	-0.525	0.603		0.59	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.823	0	1		R^2M/R^2C	0.0739 / 0.0739
Residual	1.04	1.02	0.67	1.22	100			AIC/BIC	140 / 154
Number of Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.161	-0.304	0.325	0	1		0.994	
Bone1	-0.334	0.135	-0.607	-0.0645	-2.47	0.0237	*	0.0196	*
Group1	-0.274	0.228	-0.771	0.211	-1.2	0.245		0.261	
Group2	0.267	0.228	-0.181	0.715	1.17	0.258		0.271	
Bone1:Group1	0.19	0.191	-0.181	0.592	0.995	0.333		0.324	
Bone1:Group2	-0.0998	0.191	-0.467	0.287	-0.522	0.608		0.599	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.162	0.403	0	0.88	17.4	0.456		R^2M/R^2C	0.164 / 0.31
Residual	0.768	0.876	0.548	1.11	82.6			AIC/BIC	135 / 149
Pore Density	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.152	-0.332	0.285	0	1		0.995	
Bone1	-0.326	0.15	-0.636	-0.0175	-2.17	0.0439	*	0.0323	*
Group1	-0.204	0.215	-0.645	0.18	-0.946	0.357		0.383	
Group2	0.247	0.215	-0.187	0.687	1.14	0.267		0.32	
Bone1:Group1	0.143	0.213	-0.306	0.566	0.673	0.51		0.489	
Bone1:Group2	-0.0975	0.213	-0.481	0.299	-0.459	0.652		0.651	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0124	0.111	0	0.78	1.29	0.956		R^2M/R^2C	0.139 / 0.15
Residual	0.95	0.974	0.658	1.18	98.7			AIC/BIC	137 / 151
Euler number	**Lambda = None**								

Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.16	-0.333	0.308	0	1		0.991	
Bone1	-0.352	0.132	-0.603	-0.0993	-2.67	0.0158	*	0.0119	*
Group1	-0.32	0.227	-0.793	0.0853	-1.41	0.174		0.173	
Group2	0.309	0.227	-0.132	0.75	1.36	0.19		0.193	
Bone1:Group1	0.124	0.187	-0.251	0.497	0.666	0.514		0.5	
Bone1:Group2	-0.114	0.187	-0.504	0.271	-0.608	0.551		0.526	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.173	0.416	0	0.926	19.1	0.414		R^2M/R^2C	0.184 / 0.34
Residual	0.733	0.856	0.553	1.06	80.9			AIC/BIC	134 / 148
Connectivity	**Lambda = 0.375**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.158	-0.331	0.31	0	1		0.996	
Bone1	-0.183	0.158	-0.522	0.142	-1.16	0.253		0.233	
Group1	-0.105	0.223	-0.62	0.316	-0.468	0.642		0.66	
Group2	0.073	0.223	-0.39	0.462	0.327	0.746		0.753	
Bone1:Group1	0.255	0.223	-0.203	0.652	1.14	0.261		0.232	
Bone1:Group2	-0.035	0.223	-0.418	0.388	-0.156	0.877		0.863	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.806	0	1		R^2M/R^2C	0.0706 / 0.0706
Residual	1.05	1.02	0.625	1.25	100			AIC/BIC	140 / 154
Connectivity density	**Lambda = 0.425**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.159	-0.321	0.363	0	1		0.992	
Bone1	-0.209	0.159	-0.53	0.0902	-1.32	0.195		0.175	
Group1	-0.104	0.224	-0.534	0.308	-0.464	0.645		0.662	
Group2	0.0573	0.224	-0.356	0.54	0.256	0.8		0.806	
Bone1:Group1	0.192	0.224	-0.237	0.627	0.857	0.397		0.398	
Bone1:Group2	-0.0204	0.224	-0.417	0.445	-0.0912	0.928		0.932	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.786	0	1		R^2M/R^2C	0.0649 / 0.0649
Residual	1.06	1.03	0.656	1.24	100			AIC/BIC	141 / 155
SD Pore Thickness	**Lambda = -0.375**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.124	-0.253	0.203	0	1		0.978	
Bone1	0.549	0.124	0.299	0.803	4.44	0.000081	***	0.000175	***
Group1	0.431	0.175	0.0797	0.774	2.46	0.0186	*	0.0291	*

Group2	-0.424	0.175	-0.77	-0.104	-2.42	0.0205	*	0.0305	*
Bone1:Group1	0.0714	0.175	-0.273	0.38	0.408	0.686		0.68	
Bone1:Group2	-0.0102	0.175	-0.339	0.335	-0.0581	0.954		0.961	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.626	0	1		R^2M/R^2C	0.405 / 0.405
Residual	0.642	0.801	0.526	0.942	100			AIC/BIC	126 / 140
SD Pore Separation	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.146	-0.277	0.264	0	1		0.996	
Bone1	0.441	0.146	0.154	0.731	3.02	0.00468	**	0.00456	**
Group1	-0.0904	0.207	-0.518	0.343	-0.437	0.664		0.689	
Group2	0.000108	0.207	-0.405	0.438	0.000523	1		0.994	
Bone1:Group1	-0.0694	0.207	-0.475	0.365	-0.336	0.739		0.736	
Bone1:Group2	-0.0456	0.207	-0.463	0.362	-0.221	0.827		0.827	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.739	0	1		R^2M/R^2C	0.191 / 0.191
Residual	0.898	0.948	0.61	1.11	100			AIC/BIC	135 / 148
Avg.Por.Vol	**Lambda = -1.075**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.146	-0.272	0.263	0	1		0.96	
Bone1	0.468	0.1	0.279	0.661	4.66	0.000193	***	0.000175	***
Group1	0.49	0.206	0.0767	0.905	2.38	0.0285	*	0.0333	*
Group2	-0.459	0.206	-0.895	-0.0589	-2.23	0.039	*	0.0344	*
Bone1:Group1	0.277	0.142	-0.0114	0.549	1.95	0.0665	.	0.0691	.
Bone1:Group2	-0.0528	0.142	-0.331	0.266	-0.372	0.714		0.734	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.234	0.484	0.18	0.861	35.6	0.118		R^2M/R^2C	0.392 / 0.608
Residual	0.423	0.65	0.421	0.799	64.4			AIC/BIC	122 / 135
Avg.Por.Surf	**Lambda = -2.1**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.158	-0.313	0.306	0	1		0.995	
Bone1	0.394	0.103	0.187	0.58	3.81	0.00129	**	0.000702	***
Group1	0.457	0.224	-0.00106	0.855	2.04	0.0558	.	0.0635	.
Group2	-0.38	0.224	-0.767	0.11	-1.7	0.107		0.112	
Bone1:Group1	0.29	0.146	-0.0111	0.568	1.98	0.0631	.	0.0618	.
Bone1:Group2	0.0131	0.146	-0.268	0.288	0.0898	0.929		0.93	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit

Sample	0.3	0.548	0.18	0.943	40	0.0763	.	R^2M/R^2C	0.313 / 0.588
Residual	0.45	0.671	0.434	0.828	60			AIC/BIC	125 / 139
Avg. Pore Orientation Theta	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.156	-0.312	0.287	0	1		0.995	
Bone1	-0.309	0.156	-0.632	-0.012	-1.98	0.0549	.	0.0516	.
Group1	-0.0444	0.22	-0.489	0.357	-0.202	0.841		0.837	
Group2	0.0974	0.22	-0.335	0.515	0.443	0.661		0.663	
Bone1:Group1	-0.0398	0.22	-0.478	0.365	-0.181	0.858		0.866	
Bone1:Group2	0.0998	0.22	-0.345	0.516	0.454	0.653		0.68	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.801	0	1		R^2M/R^2C	0.0958 / 0.0958
Residual	1.02	1.01	0.647	1.2	100			AIC/BIC	139 / 153
Avg. Pore Orientation Phi	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.0758	-0.143	0.156	0	1		0.977	
Bone1	-0.852	0.0758	-1.01	-0.694	-11.2	0	***	0.000175	***
Group1	0.0364	0.107	-0.16	0.226	0.34	0.736		0.764	
Group2	-0.0989	0.107	-0.316	0.125	-0.922	0.363		0.403	
Bone1:Group1	0.0731	0.107	-0.142	0.266	0.682	0.5		0.504	
Bone1:Group2	-0.27	0.107	-0.498	-0.0598	-2.51	0.0166	*	0.0144	*
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.412	0	1		R^2M/R^2C	0.765 / 0.765
Residual	0.242	0.491	0.317	0.578	100			AIC/BIC	87.2 / 101
Avg.Por.Th	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.143	-0.269	0.311	0	1		0.992	
Bone1	0.447	0.143	0.182	0.758	3.14	0.00341	**	0.00316	**
Group1	0.196	0.202	-0.203	0.606	0.973	0.337		0.374	
Group2	-0.213	0.202	-0.593	0.197	-1.06	0.298		0.341	
Bone1:Group1	0.0566	0.202	-0.314	0.48	0.281	0.781		0.777	
Bone1:Group2	-0.176	0.202	-0.571	0.207	-0.875	0.388		0.375	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.728	0	1		R^2M/R^2C	0.227 / 0.227
Residual	0.854	0.924	0.596	1.15	100			AIC/BIC	133 / 147

Avg. Pore Major Diameter	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.161	-0.308	0.305	0	1		0.965	
Bone1	0.0234	0.161	-0.328	0.348	0.145	0.886		0.89	
Group1	0.265	0.228	-0.183	0.728	1.16	0.253		0.265	
Group2	-0.184	0.228	-0.587	0.292	-0.806	0.425		0.459	
Bone1:Group1	-0.0144	0.228	-0.511	0.417	-0.0631	0.95		0.94	
Bone1:Group2	0.0455	0.228	-0.435	0.508	0.2	0.843		0.833	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.847	0	1		R^2M/R^2C	0.0348 / 0.0348
Residual	1.09	1.05	0.645	1.23	100			AIC/BIC	142 / 156
Sphericity	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.157	-0.289	0.335	0	1		0.995	
Bone1	0.244	0.157	-0.0536	0.554	1.56	0.128		0.112	
Group1	-0.0525	0.222	-0.481	0.425	-0.237	0.814		0.845	
Group2	-0.0589	0.222	-0.508	0.363	-0.265	0.792		0.792	
Bone1:Group1	-0.101	0.222	-0.54	0.377	-0.455	0.652		0.646	
Bone1:Group2	-0.117	0.222	-0.546	0.344	-0.527	0.602		0.567	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.784	0	1		R^2M/R^2C	0.0815 / 0.0815
Residual	1.03	1.02	0.646	1.2	100			AIC/BIC	140 / 154
TA	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.195	-0.391	0.384	0	1		0.994	
Bone1	0.0793	0.111	-0.142	0.338	0.714	0.484		0.484	
Group1	-0.235	0.275	-0.796	0.325	-0.855	0.404		0.395	
Group2	-0.0372	0.275	-0.641	0.481	-0.135	0.894		0.888	
Bone1:Group1	0.195	0.157	-0.116	0.509	1.24	0.231		0.248	
Bone1:Group2	-0.0252	0.157	-0.313	0.289	-0.161	0.874		0.89	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.536	0.732	0.389	1.18	50.9	0.0202	*	R^2M/R^2C	0.0657 / 0.541
Residual	0.518	0.72	0.457	0.879	49.1			AIC/BIC	135 / 149
CA	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.203	-0.414	0.408	0	1		0.991	

Bone1	-0.169	0.0882	-0.329	0.00436	-1.92	0.0714	.	0.0744	.
Group1	-0.274	0.287	-0.82	0.321	-0.953	0.353		0.349	
Group2	0.09	0.287	-0.513	0.669	0.313	0.758		0.746	
Bone1:Group1	0.186	0.125	-0.0515	0.418	1.49	0.154		0.154	
Bone1:Group2	0.0202	0.125	-0.228	0.279	0.162	0.873		0.86	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.703	0.839	0.488	1.26	68.3	0.000778	***	R^2M/R^2C	0.0849 / 0.71
Residual	0.327	0.572	0.365	0.709	31.7			AIC/BIC	128 / 142
RCV	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.184	-0.35	0.439	0	1		0.994	
Bone1	-0.336	0.116	-0.593	-0.11	-2.91	0.00931	**	0.0109	*
Group1	-0.0851	0.26	-0.582	0.429	-0.327	0.747		0.769	
Group2	0.148	0.26	-0.374	0.629	0.569	0.576		0.596	
Bone1:Group1	0.00198	0.163	-0.326	0.324	0.0121	0.99		0.989	
Bone1:Group2	0.0694	0.163	-0.271	0.373	0.425	0.676		0.666	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.43	0.655	0.234	1.07	43.4	0.0527	.	R^2M/R^2C	0.117 / 0.5
Residual	0.561	0.749	0.473	0.947	56.6			AIC/BIC	134 / 148
TA.Surf	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.179	-0.373	0.387	0	1		0.991	
Bone1	-0.401	0.102	-0.615	-0.196	-3.91	0.00101	**	0.00175	**
Group1	-0.191	0.254	-0.713	0.296	-0.753	0.461		0.454	
Group2	-0.0206	0.254	-0.538	0.474	-0.0813	0.936		0.929	
Bone1:Group1	0.192	0.145	-0.0993	0.47	1.32	0.202		0.212	
Bone1:Group2	-0.0446	0.145	-0.317	0.236	-0.308	0.762		0.766	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.456	0.676	0.406	1.1	50.9	0.0201	*	R^2M/R^2C	0.192 / 0.603
Residual	0.44	0.663	0.43	0.814	49.1			AIC/BIC	129 / 143
Perif.TA.Surf	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.179	-0.325	0.301	0	1		0.985	
Bone1	-0.261	0.125	-0.532	0.00629	-2.08	0.0516	.	0.0582	.
Group1	-0.199	0.253	-0.654	0.269	-0.788	0.441		0.449	
Group2	-0.0237	0.253	-0.514	0.435	-0.0938	0.926		0.927	
Bone1:Group1	0.182	0.177	-0.2	0.506	1.03	0.317		0.317	
Bone1:Group2	-0.00772	0.177	-0.361	0.368	-0.0436	0.966		0.979	

Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.342	0.585	0.155	1.06	34.2	0.135		R^2M/R^2C	0.109 / 0.414
Residual	0.658	0.811	0.52	1.01	65.8			AIC/BIC	136 / 150
CA.Surf	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.18	-0.388	0.375	0	1		0.988	
Bone1	-0.325	0.117	-0.557	-0.0797	-2.78	0.0124	*	0.013	*
Group1	-0.168	0.254	-0.632	0.343	-0.663	0.516		0.528	
Group2	-0.0504	0.254	-0.549	0.435	-0.199	0.845		0.813	
Bone1:Group1	0.183	0.165	-0.11	0.511	1.11	0.284		0.3	
Bone1:Group2	-0.0449	0.165	-0.336	0.314	-0.271	0.789		0.781	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.389	0.624	0.298	1.06	40.4	0.0734	.	R^2M/R^2C	0.137 / 0.486
Residual	0.575	0.758	0.489	0.941	59.6			AIC/BIC	134 / 148
Perif.CA.Surf	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.185	-0.361	0.356	0	1		0.985	
Bone1	-0.0898	0.132	-0.355	0.164	-0.682	0.504		0.501	
Group1	-0.139	0.261	-0.65	0.371	-0.534	0.6		0.589	
Group2	-0.0842	0.261	-0.584	0.434	-0.323	0.751		0.739	
Bone1:Group1	0.175	0.186	-0.186	0.588	0.94	0.36		0.371	
Bone1:Group2	-0.0356	0.186	-0.409	0.303	-0.191	0.85		0.839	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.352	0.594	0.0841	1.05	32.6	0.155		R^2M/R^2C	0.0459 / 0.357
Residual	0.727	0.853	0.528	1.06	67.4			AIC/BIC	139 / 153
RCS	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.206	-0.463	0.393	0	1		0.992	
Bone1	-0.0524	0.0942	-0.241	0.145	-0.557	0.585		0.586	
Group1	0.267	0.291	-0.309	0.877	0.917	0.371		0.398	
Group2	-0.153	0.291	-0.733	0.437	-0.526	0.605		0.611	
Bone1:Group1	-0.0865	0.133	-0.346	0.167	-0.65	0.524		0.544	
Bone1:Group2	-0.0955	0.133	-0.353	0.17	-0.717	0.483		0.481	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.702	0.838	0.543	1.29	65.3	0.00156	**	R^2M/R^2C	0.05 / 0.67
Residual	0.373	0.611	0.373	0.78	34.7			AIC/BIC	131 / 145
Avg.Pol.MI	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.

Intercept	0	0.195	-0.378	0.331	0	1		0.986	
Bone1	-0.171	0.105	-0.363	0.0353	-1.63	0.121		0.127	
Group1	-0.217	0.276	-0.762	0.297	-0.784	0.443		0.441	
Group2	-0.00206	0.276	-0.593	0.491	-0.00747	0.994		0.992	
Bone1:Group1	0.235	0.148	-0.0767	0.518	1.58	0.131		0.143	
Bone1:Group2	-0.0544	0.148	-0.335	0.252	-0.367	0.718		0.729	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.572	0.756	0.392	1.18	55.4	0.0102	*	R^2M/R^2C	0.0827 / 0.591
Residual	0.461	0.679	0.431	0.845	44.6			AIC/BIC	133 / 147
Avg.Imax	**Lambda = 1.275**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.193	-0.38	0.395	0	1		0.991	
Bone1	-0.115	0.116	-0.318	0.108	-0.986	0.337		0.335	
Group1	-0.165	0.273	-0.69	0.372	-0.606	0.552		0.556	
Group2	-0.0263	0.273	-0.58	0.476	-0.0963	0.924		0.921	
Bone1:Group1	0.22	0.164	-0.0752	0.526	1.34	0.198		0.214	
Bone1:Group2	-0.0307	0.164	-0.36	0.291	-0.187	0.854		0.846	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.499	0.706	0.282	1.16	46.8	0.0348	*	R^2M/R^2C	0.0571 / 0.499
Residual	0.566	0.753	0.475	0.938	53.2			AIC/BIC	136 / 150
Avg.Imin	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.189	-0.362	0.354	0	1		0.973	
Bone1	-0.263	0.099	-0.466	-0.0848	-2.65	0.0162	*	0.0165	*
Group1	-0.309	0.267	-0.861	0.184	-1.16	0.262		0.265	
Group2	0.0348	0.267	-0.491	0.587	0.13	0.898		0.888	
Bone1:Group1	0.248	0.14	-0.0249	0.509	1.77	0.0935	.	0.1	
Bone1:Group2	-0.112	0.14	-0.376	0.158	-0.797	0.436		0.42	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.544	0.737	0.421	1.2	56.9	0.00795	**	R^2M/R^2C	0.144 / 0.631
Residual	0.412	0.642	0.41	0.793	43.1			AIC/BIC	130 / 144
Avg.Ecc	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.156	-0.287	0.324	0	1		0.985	
Bone1	0.218	0.156	-0.0936	0.529	1.39	0.172		0.167	
Group1	0.243	0.221	-0.139	0.694	1.1	0.279		0.292	
Group2	-0.125	0.221	-0.589	0.311	-0.567	0.575		0.603	
Bone1:Group1	0.0416	0.221	-0.4	0.471	0.188	0.852		0.849	

Bone1:Group2	0.159	0.221	-0.313	0.572	0.723	0.475		0.478	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.831	0	1		R^2M/R^2C	0.0905 / 0.0905
Residual	1.02	1.01	0.625	1.18	100			AIC/BIC	139 / 153
CS.Th	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.207	-0.426	0.399	0	1		0.998	
Bone1	-0.133	0.0906	-0.298	0.0596	-1.47	0.158		0.171	
Group1	-0.225	0.292	-0.802	0.295	-0.768	0.452		0.447	
Group2	0.16	0.292	-0.364	0.788	0.549	0.59		0.606	
Bone1:Group1	0.112	0.128	-0.154	0.357	0.874	0.394		0.395	
Bone1:Group2	0.0533	0.128	-0.209	0.315	0.416	0.682		0.69	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.726	0.852	0.564	1.27	67.8	0.000868	***	R^2M/R^2C	0.0533 / 0.695
Residual	0.344	0.587	0.383	0.723	32.2			AIC/BIC	130 / 144
Total Number of Pore Systems	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.162	-0.355	0.304	0	1		0.983	
Bone1	-0.318	0.136	-0.572	-0.0414	-2.33	0.0314	*	0.0246	*
Group1	-0.275	0.229	-0.707	0.237	-1.2	0.245		0.251	
Group2	0.27	0.229	-0.24	0.758	1.18	0.254		0.272	
Bone1:Group1	0.191	0.193	-0.176	0.594	0.993	0.334		0.326	
Bone1:Group2	-0.111	0.193	-0.468	0.277	-0.575	0.573		0.558	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.163	0.403	0	0.889	17.3	0.461		R^2M/R^2C	0.155 / 0.301
Residual	0.779	0.883	0.541	1.08	82.7			AIC/BIC	136 / 150
Total Number of Pore Segments	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.164	-0.344	0.301	0	1		0.984	
Bone1	-0.117	0.151	-0.417	0.191	-0.77	0.451		0.437	
Group1	-0.102	0.232	-0.493	0.32	-0.437	0.667		0.687	
Group2	0.133	0.232	-0.303	0.615	0.573	0.574		0.6	
Bone1:Group1	0.324	0.214	-0.116	0.712	1.51	0.147		0.135	
Bone1:Group2	-0.107	0.214	-0.49	0.302	-0.5	0.623		0.594	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit

Sample	0.0858	0.293	0	0.878	8.19	0.728		R^2M/R^2C	0.0707 / 0.147
Residual	0.962	0.981	0.637	1.2	91.8			AIC/BIC	140 / 154
Pore Segment Mean Length	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.161	-0.319	0.334	0	1		0.998	
Bone1	0.113	0.161	-0.207	0.432	0.699	0.489		0.494	
Group1	0.219	0.228	-0.215	0.648	0.962	0.342		0.387	
Group2	-0.139	0.228	-0.61	0.344	-0.609	0.546		0.594	
Bone1:Group1	-0.0676	0.228	-0.559	0.405	-0.297	0.769		0.754	
Bone1:Group2	-0.0188	0.228	-0.454	0.437	-0.0824	0.935		0.952	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.833	0	1		R^2M/R^2C	0.0374 / 0.0374
Residual	1.09	1.04	0.664	1.25	100			AIC/BIC	142 / 155
Pore Segment Mean Radius	**Lambda = -2.225**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.123	-0.283	0.253	0	1		0.998	
Bone1	0.659	0.0845	0.499	0.837	7.8	3E-07	***	0.000175	***
Group1	0.372	0.174	0.0266	0.715	2.13	0.0471	*	0.0481	*
Group2	-0.4	0.174	-0.718	-0.0268	-2.29	0.0342	*	0.0404	*
Bone1:Group1	0.282	0.119	0.019	0.495	2.36	0.0299	*	0.0298	*
Bone1:Group2	-0.136	0.119	-0.377	0.0975	-1.14	0.27		0.276	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.17	0.412	0.106	0.702	36.1	0.113		R^2M/R^2C	0.556 / 0.716
Residual	0.3	0.548	0.34	0.688	63.9			AIC/BIC	108 / 122
Total Pore Network Volume	**Lambda = -0.325**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.148	-0.299	0.29	0	1		0.996	
Bone1	0.42	0.112	0.181	0.636	3.73	0.00152	**	0.000702	***
Group1	0.351	0.209	-0.047	0.776	1.68	0.111		0.11	
Group2	-0.318	0.209	-0.686	0.111	-1.52	0.145		0.153	
Bone1:Group1	0.453	0.159	0.118	0.776	2.85	0.0107	*	0.0112	*
Bone1:Group2	-0.184	0.159	-0.482	0.156	-1.15	0.263		0.259	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.194	0.441	0	0.844	26.8	0.247		R^2M/R^2C	0.334 / 0.512
Residual	0.531	0.729	0.484	0.889	73.2			AIC/BIC	126 / 140

Total Pore Network Length	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.167	-0.298	0.349	0	1		0.995	
Bone1	-0.0902	0.149	-0.379	0.203	-0.606	0.552		0.572	
Group1	-0.0602	0.236	-0.532	0.376	-0.255	0.801		0.815	
Group2	0.0972	0.236	-0.38	0.564	0.413	0.685		0.701	
Bone1:Group1	0.359	0.211	-0.0468	0.761	1.7	0.105		0.102	
Bone1:Group2	-0.154	0.211	-0.639	0.274	-0.733	0.473		0.437	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.117	0.342	0	0.883	11.2	0.635		R^2M/R^2C	0.0707 / 0.174
Residual	0.931	0.965	0.585	1.17	88.8			AIC/BIC	140 / 154
Mean Pore System Number of Segments	**Lambda = -1.525**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.156	-0.3	0.345	0	1		0.996	
Bone1	0.286	0.142	-0.0132	0.563	2.01	0.0592	.	0.0554	.
Group1	0.148	0.221	-0.269	0.552	0.67	0.512		0.546	
Group2	-0.13	0.221	-0.579	0.337	-0.587	0.564		0.593	
Bone1:Group1	0.371	0.201	-0.0459	0.792	1.85	0.0809	.	0.0789	.
Bone1:Group2	-0.0551	0.201	-0.424	0.36	-0.275	0.787		0.772	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0903	0.3	0	0.856	9.66	0.681		R^2M/R^2C	0.161 / 0.242
Residual	0.845	0.919	0.566	1.1	90.3			AIC/BIC	134 / 148
Mean Pore System Mean Length	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.156	-0.296	0.285	0	1		0.995	
Bone1	0.276	0.156	0.0172	0.588	1.76	0.0862	.	0.0877	.
Group1	0.177	0.221	-0.307	0.572	0.801	0.428		0.444	
Group2	-0.0698	0.221	-0.506	0.416	-0.316	0.754		0.751	
Bone1:Group1	-0.0309	0.221	-0.493	0.387	-0.14	0.89		0.888	
Bone1:Group2	-0.0506	0.221	-0.463	0.353	-0.229	0.82		0.828	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.802	0	1		R^2M/R^2C	0.0869 / 0.0869
Residual	1.03	1.01	0.662	1.19	100			AIC/BIC	139 / 153

Mean Pore System Mean Radius	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.151	-0.297	0.296	0	1		0.989	
Bone1	0.421	0.136	0.139	0.68	3.09	0.00635	**	0.00526	**
Group1	0.166	0.214	-0.232	0.591	0.776	0.448		0.465	
Group2	-0.308	0.214	-0.746	0.116	-1.44	0.167		0.18	
Bone1:Group1	0.0163	0.193	-0.354	0.403	0.0847	0.933		0.921	
Bone1:Group2	-0.0942	0.193	-0.47	0.283	-0.488	0.631		0.62	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0904	0.301	0	0.834	10.4	0.659		R^2M/R^2C	0.213 / 0.295
Residual	0.78	0.883	0.558	1.08	89.6			AIC/BIC	133 / 147
Mean Pore System Total Volume	**Lambda = -0.45**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.13	-0.241	0.262	0	1		0.993	
Bone1	0.548	0.102	0.371	0.775	5.36	4.32E-05	***	0.000175	***
Group1	0.48	0.184	0.117	0.825	2.61	0.0177	*	0.0172	*
Group2	-0.468	0.184	-0.862	-0.109	-2.55	0.0202	*	0.0277	*
Bone1:Group1	0.256	0.145	0.000674	0.555	1.77	0.094	.	0.087	.
Bone1:Group2	-0.0885	0.145	-0.411	0.212	-0.612	0.548		0.532	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.135	0.368	0	0.726	23.5	0.311		R^2M/R^2C	0.463 / 0.589
Residual	0.439	0.663	0.419	0.825	76.5			AIC/BIC	122 / 136
Mean Pore System Total Length	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.15	-0.318	0.268	0	1		0.986	
Bone1	0.435	0.113	0.211	0.638	3.86	0.00115	**	0.000702	***
Group1	0.397	0.213	-0.026	0.822	1.87	0.0784	.	0.0818	.
Group2	-0.307	0.213	-0.684	0.0835	-1.44	0.166		0.168	
Bone1:Group1	0.352	0.16	0.049	0.679	2.21	0.0407	*	0.0435	*
Bone1:Group2	-0.125	0.16	-0.434	0.17	-0.781	0.445		0.432	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.208	0.456	0	0.87	28	0.226		R^2M/R^2C	0.319 / 0.51
Residual	0.535	0.731	0.481	0.915	72			AIC/BIC	126 / 140
Mean Pore System	**Lambda = -2.975**								

Number of Nodes									
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.156	-0.293	0.347	0	1		0.991	
Bone1	0.319	0.136	0.027	0.569	2.35	0.0307	*	0.0225	*
Group1	0.171	0.221	-0.238	0.575	0.774	0.449		0.462	
Group2	-0.134	0.221	-0.528	0.307	-0.606	0.552		0.551	
Bone1:Group1	0.384	0.192	0.0213	0.747	2	0.0611	.	0.0575	.
Bone1:Group2	-0.0537	0.192	-0.449	0.338	-0.279	0.783		0.789	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.125	0.353	0	0.904	13.9	0.554		R^2M/R^2C	0.188 / 0.301
Residual	0.776	0.881	0.56	1.05	86.1			AIC/BIC	133 / 147
Mean Pore System Terminal Nodes	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.143	-0.292	0.262	0	1		0.986	
Bone1	0.425	0.127	0.185	0.683	3.35	0.00358	**	0.00386	**
Group1	0.303	0.203	-0.0654	0.665	1.49	0.152		0.169	
Group2	-0.18	0.203	-0.596	0.177	-0.889	0.386		0.405	
Bone1:Group1	0.405	0.179	0.037	0.759	2.26	0.0366	*	0.0298	*
Bone1:Group2	-0.0939	0.179	-0.443	0.281	-0.524	0.607		0.575	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0944	0.307	0	0.8	12.3	0.602		R^2M/R^2C	0.296 / 0.383
Residual	0.675	0.822	0.538	0.993	87.7			AIC/BIC	129 / 143
Mean Pore System Branching Nodes	**Lambda = -0.35**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.157	-0.276	0.312	0	1		0.994	
Bone1	0.256	0.145	-0.0172	0.522	1.77	0.0944	.	0.0825	.
Group1	0.134	0.222	-0.279	0.558	0.605	0.553		0.57	
Group2	-0.126	0.222	-0.53	0.352	-0.566	0.578		0.579	
Bone1:Group1	0.369	0.205	-0.0198	0.744	1.8	0.0889	.	0.0705	.
Bone1:Group2	-0.0594	0.205	-0.466	0.346	-0.289	0.776		0.773	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0744	0.273	0	0.801	7.76	0.741		R^2M/R^2C	0.142 / 0.209
Residual	0.883	0.94	0.582	1.15	92.2			AIC/BIC	135 / 149

Median Pore System Mean Length	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.15	-0.261	0.336	0	1		0.998	
Bone1	0.392	0.15	0.0854	0.696	2.61	0.0133	*	0.0105	*
Group1	0.0998	0.213	-0.295	0.524	0.469	0.642		0.665	
Group2	-0.0317	0.213	-0.48	0.417	-0.149	0.883		0.899	
Bone1:Group1	-0.00935	0.213	-0.403	0.423	-0.0439	0.965		0.975	
Bone1:Group2	-0.0486	0.213	-0.521	0.407	-0.228	0.821		0.816	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.749	0	1		R^2M/R^2C	0.148 / 0.148
Residual	0.951	0.975	0.638	1.16	100			AIC/BIC	137 / 150
Median Pore System Mean Radius	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.156	-0.303	0.294	0	1		0.994	
Bone1	0.347	0.148	0.0698	0.62	2.34	0.0313	*	0.0256	*
Group1	0.021	0.221	-0.431	0.448	0.095	0.925		0.941	
Group2	-0.0655	0.221	-0.535	0.414	-0.296	0.771		0.797	
Bone1:Group1	0.0108	0.21	-0.432	0.414	0.0515	0.959		0.967	
Bone1:Group2	-0.162	0.21	-0.56	0.229	-0.772	0.45		0.432	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0513	0.226	0	0.833	5.25	0.824		R^2M/R^2C	0.127 / 0.173
Residual	0.926	0.962	0.626	1.17	94.7			AIC/BIC	137 / 151
Median Pore System Total Volume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.152	-0.307	0.31	0	1		0.994	
Bone1	0.393	0.149	0.0784	0.691	2.64	0.0166	*	0.013	*
Group1	0.0576	0.215	-0.368	0.512	0.267	0.792		0.798	
Group2	-0.0395	0.215	-0.461	0.412	-0.183	0.857		0.858	
Bone1:Group1	0.0556	0.21	-0.356	0.465	0.264	0.795		0.789	
Bone1:Group2	-0.1	0.21	-0.546	0.294	-0.475	0.64		0.647	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0223	0.149	0	0.789	2.35	0.921		R^2M/R^2C	0.148 / 0.168
Residual	0.929	0.964	0.594	1.14	97.7			AIC/BIC	137 / 150
Median Pore System Total Length	**Lambda = None**								

Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.152	-0.334	0.281	0	1		0.985	
Bone1	0.367	0.152	0.096	0.66	2.41	0.0211	*	0.0151	*
Group1	0.0897	0.215	-0.337	0.51	0.417	0.679		0.718	
Group2	0.00273	0.215	-0.416	0.402	0.0127	0.99		0.981	
Bone1:Group1	0.052	0.215	-0.35	0.504	0.242	0.81		0.8	
Bone1:Group2	-0.0601	0.215	-0.502	0.354	-0.279	0.782		0.768	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.782	0	1		R^2M/R^2C	0.13 / 0.13
Residual	0.973	0.986	0.624	1.17	100			AIC/BIC	137 / 151
Max Pore System Number of Segments	**Lambda = 0.025**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.145	-0.323	0.293	0	1		0.994	
Bone1	0.352	0.145	0.043	0.643	2.43	0.0201	*	0.0204	*
Group1	0.117	0.205	-0.262	0.471	0.573	0.57		0.621	
Group2	-0.0304	0.205	-0.404	0.378	-0.149	0.883		0.899	
Bone1:Group1	0.412	0.205	-0.0169	0.815	2.01	0.0517	.	0.0512	.
Bone1:Group2	-0.114	0.205	-0.552	0.296	-0.555	0.583		0.575	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.748	0	1		R^2M/R^2C	0.205 / 0.205
Residual	0.88	0.938	0.595	1.11	100			AIC/BIC	136 / 150
Max Pore System Mean Length	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.15	-0.272	0.322	0	1		0.984	
Bone1	-0.348	0.15	-0.621	-0.0707	-2.33	0.0258	*	0.0239	*
Group1	-0.00947	0.212	-0.462	0.376	-0.0447	0.965		0.982	
Group2	-0.112	0.212	-0.534	0.307	-0.53	0.599		0.608	
Bone1:Group1	0.179	0.212	-0.247	0.599	0.845	0.404		0.384	
Bone1:Group2	0.0916	0.212	-0.301	0.496	0.432	0.668		0.655	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.764	0	1		R^2M/R^2C	0.155 / 0.155
Residual	0.942	0.971	0.613	1.18	100			AIC/BIC	136 / 150
Max Pore System Mean Radius	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.155	-0.319	0.32	0	1		0.994	

Bone1	-0.113	0.155	-0.398	0.201	-0.732	0.469		0.454	
Group1	0.127	0.219	-0.355	0.559	0.581	0.565		0.594	
Group2	-0.361	0.219	-0.758	0.0908	-1.65	0.108		0.142	
Bone1:Group1	-0.108	0.219	-0.516	0.309	-0.494	0.624		0.6	
Bone1:Group2	-0.155	0.219	-0.565	0.277	-0.707	0.484		0.455	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.786	0	1		R^2M/R^2C	0.105 / 0.105
Residual	1.01	1	0.64	1.21	100			AIC/BIC	139 / 152
Max Pore System Total Volume	**Lambda = -0.15**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.143	-0.293	0.298	0	1		0.991	
Bone1	0.515	0.118	0.294	0.753	4.37	0.000369	***	0.000175	***
Group1	0.287	0.203	-0.0812	0.709	1.41	0.175		0.184	
Group2	-0.182	0.203	-0.547	0.224	-0.898	0.381		0.405	
Bone1:Group1	0.315	0.167	-0.0145	0.643	1.89	0.0748	.	0.0674	.
Bone1:Group2	-0.157	0.167	-0.52	0.157	-0.942	0.359		0.348	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.14	0.374	0	0.775	19.3	0.407		R^2M/R^2C	0.336 / 0.464
Residual	0.583	0.763	0.478	0.947	80.7			AIC/BIC	132 / 146
Max Pore System Total Length	**Lambda = -0.025**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.142	-0.288	0.271	0	1		0.994	
Bone1	0.372	0.142	0.112	0.676	2.62	0.0129	*	0.0109	*
Group1	0.179	0.201	-0.246	0.581	0.889	0.38		0.409	
Group2	-0.0387	0.201	-0.504	0.346	-0.192	0.848		0.834	
Bone1:Group1	0.415	0.201	0.00426	0.855	2.06	0.0463	*	0.0453	*
Bone1:Group2	-0.123	0.201	-0.57	0.241	-0.612	0.545		0.535	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.718	0	1		R^2M/R^2C	0.23 / 0.23
Residual	0.85	0.922	0.6	1.08	100			AIC/BIC	135 / 149
Max Pore System Number of Nodes	**Lambda = 0**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.144	-0.289	0.284	0	1		0.999	
Bone1	0.36	0.144	0.0511	0.632	2.51	0.0169	*	0.0228	*

Group1	0.119	0.203	-0.263	0.494	0.585	0.562		0.575	
Group2	-0.0227	0.203	-0.42	0.364	-0.112	0.912		0.911	
Bone1:Group1	0.419	0.203	0.0184	0.789	2.06	0.0468	*	0.0428	*
Bone1:Group2	-0.113	0.203	-0.488	0.293	-0.557	0.581		0.6	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.723	0	1		R^2M/R^2C	0.215 / 0.215
Residual	0.869	0.932	0.614	1.11	100			AIC/BIC	136 / 150
Max Pore System Terminal Nodes	**Lambda = -0.025**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.142	-0.294	0.314	0	1		0.996	
Bone1	0.38	0.142	0.0804	0.638	2.69	0.0109	*	0.0109	*
Group1	0.129	0.2	-0.28	0.505	0.646	0.522		0.532	
Group2	-0.0162	0.2	-0.382	0.381	-0.0811	0.936		0.945	
Bone1:Group1	0.43	0.2	0.0457	0.82	2.15	0.0385	*	0.0393	*
Bone1:Group2	-0.11	0.2	-0.519	0.265	-0.551	0.585		0.592	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.729	0	1		R^2M/R^2C	0.236 / 0.236
Residual	0.842	0.918	0.563	1.09	100			AIC/BIC	135 / 149
Max Pore System Branching Nodes	**Lambda = 0.025**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.145	-0.28	0.315	0	1		0.993	
Bone1	0.345	0.145	0.0853	0.621	2.37	0.023	*	0.0228	*
Group1	0.112	0.206	-0.292	0.495	0.545	0.589		0.629	
Group2	-0.0284	0.206	-0.503	0.397	-0.138	0.891		0.891	
Bone1:Group1	0.41	0.206	-0.0598	0.808	2	0.0536	.	0.0523	.
Bone1:Group2	-0.117	0.206	-0.53	0.266	-0.568	0.573		0.563	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.739	0	1		R^2M/R^2C	0.199 / 0.199
Residual	0.888	0.942	0.576	1.13	100			AIC/BIC	136 / 150
Min Pore System Mean Length	**Lambda = 6.075**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.151	-0.31	0.316	0	1		0.996	
Bone1	0.221	0.151	-0.0696	0.517	1.47	0.151		0.146	
Group1	-0.289	0.213	-0.735	0.106	-1.36	0.184		0.219	

Group2	-0.157	0.213	-0.587	0.267	-0.736	0.467		0.489	
Bone1:Group1	-0.00074	0.213	-0.403	0.432	-0.00344	0.997		0.989	
Bone1:Group2	-0.0943	0.213	-0.571	0.378	-0.442	0.661		0.651	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0	0	0	0.759	0	1		R^2M/R^2C	0.144 / 0.144
Residual	0.955	0.977	0.598	1.16	100			AIC/BIC	137 / 151
Min Pore System Total Volume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.163	-0.319	0.31	0	1		0.983	
Bone1	0.0515	0.157	-0.307	0.369	0.329	0.746		0.761	
Group1	0.01	0.23	-0.433	0.454	0.0437	0.966		0.977	
Group2	-0.0775	0.23	-0.549	0.415	-0.337	0.74		0.766	
Bone1:Group1	-0.0532	0.222	-0.444	0.402	-0.24	0.813		0.808	
Bone1:Group2	-0.248	0.222	-0.656	0.146	-1.12	0.279		0.241	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0403	0.201	0	0.837	3.76	0.873		R^2M/R^2C	0.0523 / 0.088
Residual	1.03	1.02	0.635	1.24	96.2			AIC/BIC	141 / 155
Min Pore System Total Length	**Lambda = 7.175**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
Intercept	0	0.14	-0.291	0.295	0	1		0.998	
Bone1	0.295	0.134	0.039	0.554	2.21	0.0405	*	0.0309	*
Group1	-0.358	0.198	-0.754	0.0808	-1.81	0.0876	.	0.105	
Group2	0.0451	0.198	-0.365	0.399	0.227	0.823		0.823	
Bone1:Group1	-0.436	0.189	-0.79	-0.0668	-2.3	0.0333	*	0.0253	*
Bone1:Group2	-0.0347	0.189	-0.401	0.386	-0.184	0.856		0.839	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0366	0.191	0	0.743	4.64	0.844		R^2M/R^2C	0.281 / 0.315
Residual	0.751	0.867	0.557	1.03	95.4			AIC/BIC	131 / 145

Aggregate Porosity: Post-Hoc Tests for Significant Fixed Effects

Number Closed Pores	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Bone		Femur < Tibia	0.0133	*	0.848	Large	37%

Closed Pore Density	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Bone		Femur < Tibia	0.0247	*	0.756	Medium	33%
Closed Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.000432	***	1.33	Large	30%
Open Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.000526	***	1.3	Large	65%
Bone | Group	Femur	Control > Fentanyl	0.000852	***	2.16	Large	39%
Bone | Group	Femur	Control > Morphine	0.00845	**	1.66	Large	
Bone | Group	Femur	Fentanyl < Morphine	0.399		0.507	Medium	
Bone | Group	Tibia	Control > Fentanyl	0.8		0.152	Small	
Bone | Group	Tibia	Control < Morphine	0.857		0.108	Small	
Bone | Group	Tibia	Fentanyl < Morphine	0.666		0.259	Medium	
Group | Bone	Control	Femur > Tibia	0.000309	***	2.57	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.351		0.552	Medium	
Group | Bone	Morphine	Femur > Tibia	0.182		0.801	Large	
Pore Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone | Group	Femur	Control > Fentanyl	0.00519	**	1.62	Large	39%
Bone | Group	Femur	Control > Morphine	0.0243	*	1.28	Large	
Bone | Group	Femur	Fentanyl < Morphine	0.536		0.34	Medium	
Bone | Group	Tibia	Control < Fentanyl	0.932		0.0469	Small	
Bone | Group	Tibia	Control < Morphine	0.549		0.328	Medium	
Bone | Group	Tibia	Fentanyl < Morphine	0.608		0.281	Medium	
Group | Bone	Control	Femur > Tibia	0.00566	**	1.68	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.978		0.0147	Small	
Group | Bone	Morphine	Femur > Tibia	0.893		0.0731	Small	
Pore Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone | Group	Femur	Control > Fentanyl	0.0153	*	1.42	Large	43%
Bone | Group	Femur	Control > Morphine	0.0574	.	1.09	Large	
Bone | Group	Femur	Fentanyl < Morphine	0.563		0.325	Medium	
Bone | Group	Tibia	Control < Fentanyl	0.767		0.166	Small	
Bone | Group	Tibia	Control < Morphine	0.384		0.49	Medium	
Bone | Group	Tibia	Fentanyl < Morphine	0.563		0.325	Medium	
Group | Bone	Control	Femur > Tibia	0.0104	*	1.53	Large	
Group | Bone	Fentanyl	Femur < Tibia	0.922		0.0529	Small	
Group | Bone	Morphine	Femur < Tibia	0.923		0.0526	Small	
Total Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone | Group	Femur	Control > Fentanyl	0.0052	**	1.62	Large	48%
Bone | Group	Femur	Control > Morphine	0.0243	*	1.28	Large	

Bone | Group	Femur	Fentanyl < Morphine	0.536		0.34	Medium	
Bone | Group	Tibia	Control < Fentanyl	0.932		0.0469	Small	
Bone | Group	Tibia	Control < Morphine	0.549		0.329	Medium	
Bone | Group	Tibia	Fentanyl < Morphine	0.607		0.282	Medium	
Group | Bone	Control	Femur > Tibia	0.00568	**	1.68	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.979		0.0142	Small	
Group | Bone	Morphine	Femur > Tibia	0.894		0.0721	Small	
Intersection Surface	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.000131	***	1.49	Large	44%
Pore Surface: PoreVolume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.000134	***	1.49	Large	71%
Group		Control < Fentanyl	0.0161	*	1.46	Large	95%
Group		Control < Morphine	0.298		0.723	Medium	
Group		Fentanyl > Morphine	0.286		0.737	Medium	
Pore Thickness	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.000183	***	1.45	Large	60%
Group		Control > Fentanyl	0.0122	*	1.28	Large	92%
Group		Control > Morphine	0.291		0.615	Medium	
Group		Fentanyl < Morpine	0.24		0.665	Medium	
Degree of Anisotropy	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.0433	*	0.671	Medium	31%
Number of Pores	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.0237	*	0.763	Medium	34%
Pore Density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.0439	*	0.669	Medium	35%
Group		Control < Fentanyl	0.465		0.462	Medium	46%
Euler number	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.0158	*	0.823	Large	34%
SD Pore Thickness	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.000314	***	1.37	Large	50%
Group		Control > Fentanyl	0.0289	*	1.07	Large	89%
Group		Control > Morphine	0.339		0.547	Medium	
Group		Fentanyl < Morpine	0.374		0.52	Medium	
SD Pore Separation	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.00742	**	0.931	Large	20%
Avg.Por.Vol	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power

Bone		Femur > Tibia	0.000193	***	1.44	Large	68%
Group		Control > Fentanyl	0.0403	*	1.46	Large	87%
Group		Control > Morphine	0.331		0.803	Large	
Group		Fentanyl < Morpine	0.47		0.656	Medium	
Avg.Por.Surf	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.00129	**	1.18	Large	61%
Avg. Pore Orientation Phi	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0	***	3.47	Large	53%
Avg.Por.Th	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.00572	**	0.968	Large	37%
RCV	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.00931	**	0.899	Large	25%
TA.Surf	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.00101	**	1.21	Large	35%
CA.Surf	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.0124	*	0.857	Large	23%
Avg.Imin	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.0162	*	0.819	Large	45%
Total Number of Pore Systems	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.031425	*	0.720102	Medium	35%
Pore Segment Mean Radius	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	3.50E-07	***	2.407654	Large	73%
Group		Control > Fentanyl	0.04981	*	1.409326	Large	100%
Group		Control > Morphine	0.504106		0.627802	Medium	
Group		Fentanyl < Morpine	0.353775		0.781523	Medium	
Bone | Group	Femur	Control > Fentanyl	0.000238	***	2.672798	Large	29%
Bone | Group	Femur	Control > Morphine	0.006577	**	1.884795	Large	
Bone | Group	Femur	Fentanyl < Morphine	0.234608		0.788003	Medium	
Bone | Group	Tibia	Control > Fentanyl	0.576536		0.367231	Medium	
Bone | Group	Tibia	Control < Morphine	0.775328		0.187364	Small	
Bone | Group	Tibia	Fentanyl < Morphine	0.400398		0.554595	Medium	
Group | Bone	Control	Femur > Tibia	3.55E-06	***	3.764053	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.020201	*	1.458486	Large	
Group | Bone	Morphine	Femur > Tibia	0.008469	**	1.691894	Large	
Total Pore Network Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.001524	**	1.151886	Large	68%

Bone | Group	Femur	Control > Fentanyl	0.00217	**	2.051248	Large	43%
Bone | Group	Femur	Control > Morphine	0.012273	*	1.637725	Large	
Bone | Group	Femur	Fentanyl < Morphine	0.509775		0.413522	Medium	
Bone | Group	Tibia	Control > Fentanyl	0.855749		0.113718	Small	
Bone | Group	Tibia	Control < Morphine	0.747517		0.201475	Medium	
Bone | Group	Tibia	Fentanyl < Morphine	0.614905		0.315193	Medium	
Group | Bone	Control	Femur > Tibia	0.001516	**	2.319733	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.546003		0.382203	Medium	
Group | Bone	Morphine	Femur > Tibia	0.449148		0.480533	Medium	
Mean Pore System Mean Radius	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.006352	**	0.952796	Large	29%
Mean Pore System Total Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	4.32E-05	***	1.653104	Large	67%
Group		Control > Fentanyl	0.020983	*	1.430039	Large	97%
Group		Control > Morphine	0.295187		0.741263	Medium	
Group		Fentanyl < Morpine	0.345254		0.688776	Medium	
Mean Pore System Total Length	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.001147	**	1.191205	Large	51%
Bone | Group	Femur	Control > Fentanyl	0.015064	*	1.614312	Large	39%
Bone | Group	Femur	Control > Morphine	0.026937	*	1.457699	Large	
Bone | Group	Femur	Fentanyl < Morphine	0.805153		0.156612	Small	
Bone | Group	Tibia	Control > Fentanyl	0.624741		0.310963	Medium	
Bone | Group	Tibia	Control < Morphine	0.84209		0.126459	Small	
Bone | Group	Tibia	Fentanyl < Morphine	0.492179		0.437422	Medium	
Group | Bone	Control	Femur > Tibia	0.000787	***	2.153708	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.129047		0.850359	Large	
Group | Bone	Morphine	Femur > Tibia	0.300721		0.569549	Medium	
Mean Pore System Number of Nodes	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.030667	*	0.723806	Medium	44%
Mean Pore System Termil Nodes	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.003576	**	1.033408	Large	47%
Bone | Group	Femur	Control > Fentanyl	0.043425	*	1.195219	Large	41%
Bone | Group	Femur	Control > Morphine	0.020066	*	1.389401	Large	
Bone | Group	Femur	Fentanyl > Morphine	0.735649		0.194183	Small	
Bone | Group	Tibia	Control < Fentanyl	0.973906		0.018798	Small	
Bone | Group	Tibia	Control < Morphine	0.540509		0.352672	Medium	
Bone | Group	Tibia	Fentanyl < Morphine	0.562197		0.333874	Medium	
Group | Bone	Control	Femur > Tibia	0.001381	**	2.018771	Large	

Group | Bone	Fentanyl	Femur > Tibia	0.149527		0.804755	Large	
Group | Bone	Morphine	Femur > Tibia	0.611004		0.276698	Medium	
Median Pore System Mean Length	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.017895	*	0.804045	Large	26%
Median Pore System Mean Radius	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.031273	*	0.720836	Medium	23%
Median Pore System Total Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.01655	*	0.815484	Large	24%
Median Pore System Total Length	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.026798	*	0.744142	Medium	21%
Max Pore System Number of Segments	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.025713	*	0.75034	Medium	30%
Max Pore System Mean Length	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur < Tibia	0.031941	*	0.717627	Medium	25%
Max Pore System Total Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.000369	***	1.348645	Large	46%
Max Pore System Total Length	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.017456	*	0.807687	Large	47%
Bone | Group	Femur	Control > Fentanyl	0.084335	.	0.985105	Large	52%
Bone | Group	Femur	Control > Morphine	0.341606		0.534829	Medium	
Bone | Group	Femur	Fentanyl < Morphine	0.422481		0.450276	Medium	
Bone | Group	Tibia	Control > Fentanyl	0.92094		0.055466	Small	
Bone | Group	Tibia	Control < Morphine	0.716695		0.202972	Medium	
Bone | Group	Tibia	Fentanyl < Morphine	0.644239		0.258438	Medium	
Group | Bone	Control	Femur > Tibia	0.031323	*	1.295815	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.517715		0.366176	Medium	
Group | Bone	Morphine	Femur > Tibia	0.327975		0.558014	Medium	
Max Pore System Number of Nodes	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.02206	*	0.773177	Medium	44%
Bone | Group	Femur	Control > Fentanyl	0.095758	.	0.945244	Large	38%
Bone | Group	Femur	Control > Morphine	0.286483		0.597842	Medium	
Bone | Group	Femur	Fentanyl < Morphine	0.533518		0.347402	Medium	

Bone | Group	Tibia	Control > Fentanyl	0.962482		0.026174	Small	
Bone | Group	Tibia	Control < Morphine	0.582185		0.306796	Medium	
Bone | Group	Tibia	Fentanyl < Morphine	0.550567		0.33297	Medium	
Group | Bone	Control	Femur > Tibia	0.030085	*	1.301206	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.498041		0.382136	Medium	
Group | Bone	Morphine	Femur > Tibia	0.482171		0.396568	Medium	
Max Pore System Termil Nodes	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.015082	*	0.829026	Large	40%
Bone | Group	Femur	Control > Fentanyl	0.087546	.	0.980888	Large	42%
Bone | Group	Femur	Control > Morphine	0.304631		0.581629	Medium	
Bone | Group	Femur	Fentanyl < Morphine	0.479299		0.399259	Medium	
Bone | Group	Tibia	Control > Fentanyl	0.961649		0.027043	Small	
Bone | Group	Tibia	Control < Morphine	0.596565		0.298289	Medium	
Bone | Group	Tibia	Fentanyl < Morphine	0.563857		0.325332	Medium	
Group | Bone	Control	Femur > Tibia	0.027807	*	1.33666	Large	
Group | Bone	Fentanyl	Femur > Tibia	0.501806		0.382815	Medium	
Group | Bone	Morphine	Femur > Tibia	0.424168		0.456742	Medium	
Max Pore System Branching Nodes	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.028917	*	0.732685	Medium	35%
Min Pore System Total Length	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Bone		Femur > Tibia	0.040481	*	0.681317	Medium	68%
Bone | Group	Femur	Control < Fentanyl	0.089226	.	0.977197	Large	49%
Bone | Group	Femur	Control < Morphine	0.003851	**	1.729126	Large	
Bone | Group	Femur	Fentanyl < Morphine	0.187328		0.751929	Medium	
Bone | Group	Tibia	Control < Fentanyl	0.937998		0.043817	Small	
Bone | Group	Tibia	Control > Morphine	0.640017		0.263838	Medium	
Bone | Group	Tibia	Fentanyl > Morphine	0.585725		0.307655	Medium	
Group | Bone	Control	Femur < Tibia	0.531914		0.34029	Medium	
Group | Bone	Fentanyl	Femur > Tibia	0.281251		0.59309	Medium	
Group | Bone	Morphine	Femur > Tibia	0.006241	**	1.652674	Large	

Aggregate Porosity: All Directional Trends

Cortical Fractal Trends		
Bone		Tibia > Femur
Group		Fentanyl > Control > Morphine
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur

Group | Bone	Morphine	Tibia > Femur
Number Closed Pores Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Control > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Number Open Pores Trends		
Bone		Femur > Tibia
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Morphine > Control
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Femur > Tibia
Closed Pore Density Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Control > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Open Pore Density Trends		
Bone		Femur > Tibia
Group		Fentanyl > Control > Morphine
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Closed Porosity (%) Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Fentanyl > Control > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur

Open Porosity (%) Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Total Porosity (%) Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Cortical Volume Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Fentanyl > Morphine > Control
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Pore Volume Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Cortical Surface Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Pore Surface Trends		

Bone		Tibia > Femur
Group		Morphine > Control > Fentanyl
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Intersection Surface Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Pore Surface: PoreVolume Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Morphine > Control
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Pore Surface: Cortical Volume Trends		
Bone		Tibia > Femur
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Pore Thickness Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Control > Morphine > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Pore Separation Trends		
Bone		Femur > Tibia

Group		Morphine > Control > Fentanyl
Region | Group	Femur	Morphine > Fentanyl > Control
Region | Group	Tibia	Control > Morphine > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Pore Tb.N Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Degree of Anisotropy Trends		
Bone		Tibia > Femur
Group		Morphine > Control > Fentanyl
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Pore Fractal Trends		
Bone		Tibia > Femur
Group		Fentanyl > Control > Morphine
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Number of Pores Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Morphine > Control
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Pore Density Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control

Region | Group	Femur	Fentanyl > Control > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Euler number Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Morphine > Control
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Connectivity Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Connectivity density Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
SD Pore Thickness Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Control > Morphine > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
SD Pore Separation Trends		
Bone		Femur > Tibia
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Fentanyl > Control

Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Avg.Por.Vol Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Avg.Por.Surf Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Avg.Orient.Theta Trends		
Bone		Tibia > Femur
Group		Fentanyl > Control > Morphine
Region | Group	Femur	Fentanyl > Control > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Avg.Orient.Phi Trends		
Bone		Tibia > Femur
Group		Morphine > Control > Fentanyl
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Avg.Por.Th Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Control > Fentanyl > Morphine

Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Avg. Pore Major Diameter Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Control > Morphine > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Tibia > Femur
Sphericity Trends		
Bone		Femur > Tibia
Group		Morphine > Control > Fentanyl
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
TA Trends		
Bone		Femur > Tibia
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Tibia > Femur
CA Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Fentanyl > Morphine > Control
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
RCV Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Control > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur

Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
TA.Surf Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Perif.TA.Surf Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
CA.Surf Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Perif.CA.Surf Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
RCS Trends		
Bone		Tibia > Femur
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Control > Fentanyl > Morphine
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur

Group | Bone	Morphine	Femur > Tibia
Avg.Pol.MI Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Avg.Imax Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Avg.Imin Trends		
Bone		Tibia > Femur
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Avg.Ecc Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Control > Morphine > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
CS.Th Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Morphine > Control
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur

Total Number of Pore Systems Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Fentanyl > Morphine > Control
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Total Number of Pore Segments Trends		
Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Pore Segment Mean Length Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Control > Fentanyl > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Pore Segment Mean Radius Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Total Pore Network Volume Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Total Pore Network Length Trends		

Bone		Tibia > Femur
Group		Fentanyl > Morphine > Control
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Fentanyl > Morphine > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Mean Pore System Number of Segments Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Mean Pore System Mean Length Trends		
Bone		Femur > Tibia
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Control > Fentanyl > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Mean Pore System Mean Radius Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Control > Morphine > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Mean Pore System Total Volume Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Mean Pore System Total Length Trends		
Bone		Femur > Tibia

Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Mean Pore System Number of Nodes Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Mean Pore System Terminal Nodes Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Mean Pore System Branching Nodes Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Median Pore System Mean Length Trends		
Bone		Femur > Tibia
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Control > Fentanyl > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Median Pore System Mean Radius Trends		
Bone		Femur > Tibia
Group		Morphine > Control > Fentanyl

Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Median Pore System Total Volume Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Median Pore System Total Length Trends		
Bone		Femur > Tibia
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Max Pore System Number of Segments Trends		
Bone		Femur > Tibia
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Max Pore System Mean Length Trends		
Bone		Tibia > Femur
Group		Morphine > Control > Fentanyl
Region | Group	Femur	Control > Fentanyl > Morphine
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Tibia > Femur
Max Pore System Mean Radius Trends		
Bone		Tibia > Femur
Group		Morphine > Control > Fentanyl
Region | Group	Femur	Morphine > Control > Fentanyl

Region | Group	Tibia	Control > Morphine > Fentanyl
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Femur > Tibia
Max Pore System Total Volume Trends		
Bone		Femur > Tibia
Group		Control > Morphine > Fentanyl
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Max Pore System Total Length Trends		
Bone		Femur > Tibia
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Max Pore System Number of Nodes Trends		
Bone		Femur > Tibia
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Max Pore System Terminal Nodes Trends		
Bone		Femur > Tibia
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Max Pore System Branching Nodes Trends		
Bone		Femur > Tibia
Group		Control > Fentanyl > Morphine
Region | Group	Femur	Control > Morphine > Fentanyl
Region | Group	Tibia	Morphine > Control > Fentanyl

Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Min Pore System Mean Length Trends		
Bone		Femur > Tibia
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Morphine > Fentanyl > Control
Group | Bone	Control	Femur > Tibia
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia
Min Pore System Total Volume Trends		
Bone		Femur > Tibia
Group		Morphine > Control > Fentanyl
Region | Group	Femur	Morphine > Control > Fentanyl
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Tibia > Femur
Group | Bone	Morphine	Femur > Tibia
Min Pore System Total Length Trends		
Bone		Femur > Tibia
Group		Morphine > Fentanyl > Control
Region | Group	Femur	Morphine > Fentanyl > Control
Region | Group	Tibia	Fentanyl > Control > Morphine
Group | Bone	Control	Tibia > Femur
Group | Bone	Fentanyl	Femur > Tibia
Group | Bone	Morphine	Femur > Tibia

Appendix XXII: Micro-CT Linear Mixed Model for Femoral Regions

Femoral Regions: LMM Fixed Effects and Random Effects

Cortical Fractal	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.189	-0.387	0.343	0	1		0.976	
Region1	-0.544	0.073	-0.703	-0.4	-7.45	0	***	0.000175	***
Region2	-0.206	0.073	-0.35	-0.0559	-2.83	0.00658	**	0.00596	**
Region3	0.649	0.073	0.502	0.797	8.89	0	***	0.000175	***
Group1	0.277	0.267	-0.325	0.828	1.04	0.313		0.313	
Group2	-0.0295	0.267	-0.554	0.5	-0.111	0.913		0.904	
Region1:Group1	0.086	0.103	-0.105	0.288	0.833	0.409		0.392	
Region2:Group1	-0.251	0.103	-0.45	-0.0647	-2.43	0.0183	*	0.0165	*
Region3:Group1	0.102	0.103	-0.095	0.311	0.989	0.327		0.335	
Region1:Group2	-0.0311	0.103	-0.242	0.17	-0.301	0.765		0.728	
Region2:Group2	0.119	0.103	-0.0841	0.327	1.15	0.256		0.243	
Region3:Group2	-0.0466	0.103	-0.235	0.15	-0.452	0.653		0.661	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.712	0.844	0.599	1.23	82.7	1.95E-17	***	R^2M/R^2C	0.227 / 0.866
Residual	0.149	0.386	0.307	0.448	17.3			AIC/BIC	190 / 224
Number Closed Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.206	-0.361	0.371	0	1		0.978	
Region1	0.191	0.064	0.0669	0.311	2.98	0.00428	**	0.00386	**
Region2	-0.485	0.064	-0.606	-0.348	-7.57	0	***	0.000175	***
Region3	0.44	0.064	0.318	0.571	6.88	0	***	0.000175	***
Group1	-0.107	0.292	-0.644	0.456	-0.367	0.718		0.715	
Group2	0.214	0.292	-0.395	0.699	0.734	0.473		0.499	
Region1:Group1	-0.0632	0.0905	-0.249	0.0987	-0.699	0.488		0.487	
Region2:Group1	0.017	0.0905	-0.149	0.197	0.188	0.851		0.836	
Region3:Group1	-0.0219	0.0905	-0.188	0.162	-0.242	0.81		0.793	
Region1:Group2	0.0393	0.0905	-0.146	0.218	0.434	0.666		0.666	
Region2:Group2	-0.0391	0.0905	-0.232	0.125	-0.432	0.667		0.676	
Region3:Group2	0.12	0.0905	-0.0579	0.298	1.32	0.191		0.178	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.864	0.93	0.658	1.35	88.3	6.71E-22	***	R^2M/R^2C	0.133 / 0.899
Residual	0.115	0.339	0.27	0.395	11.7			AIC/BIC	179 / 213
Number Open Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.

(Intercept)	0	0.209	-0.434	0.433	0	1		0.989	
Region1	-0.198	0.0755	-0.337	-0.0465	-2.62	0.0114	*	0.0123	*
Region2	-0.344	0.0755	-0.48	-0.209	-4.55	3.11E-05	***	0.000175	***
Region3	0.117	0.0755	-0.0382	0.276	1.55	0.127		0.137	
Group1	-0.0102	0.296	-0.601	0.568	-0.0345	0.973		0.972	
Group2	0.0767	0.296	-0.512	0.708	0.259	0.798		0.803	
Region1:Group1	-0.0104	0.107	-0.211	0.206	-0.0974	0.923		0.942	
Region2:Group1	-0.0857	0.107	-0.281	0.11	-0.802	0.426		0.42	
Region3:Group1	0.0757	0.107	-0.134	0.29	0.708	0.482		0.465	
Region1:Group2	-0.137	0.107	-0.349	0.0645	-1.28	0.205		0.205	
Region2:Group2	0.0844	0.107	-0.117	0.273	0.79	0.433		0.452	
Region3:Group2	0.041	0.107	-0.152	0.282	0.384	0.703		0.707	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.879	0.938	0.648	1.4	84.6	8.69E-19	***	R^2M/R^2C	0.0868 / 0.86
Residual	0.16	0.4	0.311	0.463	15.4			AIC/BIC	197 / 231
Closed Pore Density	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.205	-0.403	0.417	0	1		0.988	
Region1	-0.15	0.0506	-0.253	-0.048	-2.96	0.00457	**	0.00526	**
Region2	-0.031	0.0506	-0.13	0.066	-0.613	0.542		0.528	
Region3	0.494	0.0506	0.398	0.586	9.77	0	***	0.000175	***
Group1	-0.357	0.289	-0.933	0.204	-1.23	0.233		0.259	
Group2	0.375	0.289	-0.201	0.902	1.3	0.212		0.232	
Region1:Group1	-0.00731	0.0715	-0.149	0.136	-0.102	0.919		0.911	
Region2:Group1	0.00537	0.0715	-0.137	0.154	0.0751	0.94		0.926	
Region3:Group1	-0.0624	0.0715	-0.194	0.0932	-0.872	0.387		0.393	
Region1:Group2	-0.0417	0.0715	-0.183	0.0894	-0.583	0.563		0.561	
Region2:Group2	0.00396	0.0715	-0.127	0.139	0.0553	0.956		0.949	
Region3:Group2	0.177	0.0715	0.0259	0.328	2.47	0.0167	*	0.0144	*
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.862	0.928	0.676	1.33	92.3	8.92E-27	***	R^2M/R^2C	0.169 / 0.936
Residual	0.0716	0.268	0.21	0.312	7.67			AIC/BIC	153 / 187
Open Pore Density	**Lambda = 1.675**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.2	-0.412	0.37	0	1		0.989	
Region1	-0.483	0.0757	-0.628	-0.334	-6.39	0	***	0.000175	***
Region2	0.236	0.0757	0.0888	0.391	3.11	0.00295	**	0.00421	**
Region3	0.381	0.0757	0.217	0.534	5.04	5.6E-06	***	0.000175	***
Group1	-0.247	0.283	-0.821	0.262	-0.875	0.393		0.413	

Group2	0.258	0.283	-0.272	0.836	0.912	0.374		0.394	
Region1:Group1	0.09	0.107	-0.0954	0.305	0.841	0.404		0.407	
Region2:Group1	-0.125	0.107	-0.336	0.0753	-1.17	0.247		0.239	
Region3:Group1	0.0785	0.107	-0.138	0.275	0.733	0.467		0.468	
Region1:Group2	-0.169	0.107	-0.385	0.0204	-1.58	0.119		0.105	
Region2:Group2	0.104	0.107	-0.125	0.312	0.972	0.335		0.316	
Region3:Group2	0.0912	0.107	-0.123	0.332	0.852	0.398		0.396	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.799	0.894	0.638	1.27	83.3	7.62E-18	***	R^2M/R^2C	0.149 / 0.858
Residual	0.16	0.4	0.316	0.459	16.7			AIC/BIC	158 / 192
Closed Porosity (%)	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.181	-0.349	0.399	0	1		0.977	
Region1	-0.537	0.0998	-0.737	-0.344	-5.37	1.7E-06	***	0.000175	***
Region2	-0.0338	0.0998	-0.226	0.166	-0.338	0.736		0.739	
Region3	0.692	0.0998	0.499	0.884	6.93	0	***	0.000175	***
Group1	-0.01	0.256	-0.476	0.586	-0.0391	0.969		0.971	
Group2	0.0975	0.256	-0.413	0.552	0.381	0.708		0.735	
Region1:Group1	-0.0379	0.141	-0.339	0.276	-0.268	0.79		0.788	
Region2:Group1	-0.0232	0.141	-0.313	0.294	-0.165	0.87		0.866	
Region3:Group1	0.146	0.141	-0.143	0.408	1.03	0.306		0.307	
Region1:Group2	-0.029	0.141	-0.335	0.227	-0.205	0.838		0.844	
Region2:Group2	-0.105	0.141	-0.333	0.215	-0.745	0.459		0.474	
Region3:Group2	0.182	0.141	-0.108	0.449	1.29	0.204		0.193	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.617	0.786	0.512	1.15	68.9	5.95E-11	***	R^2M/R^2C	0.199 / 0.751
Residual	0.279	0.528	0.42	0.612	31.1			AIC/BIC	222 / 256
Open Porosity (%)	**Lambda = 0.125**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.149	-0.289	0.313	0	1		0.973	
Region1	-0.287	0.102	-0.477	-0.0733	-2.81	0.00691	**	0.00772	**
Region2	-0.522	0.102	-0.721	-0.311	-5.1	4.4E-06	***	0.000175	***
Region3	0.478	0.102	0.257	0.679	4.68	1.96E-05	***	0.000175	***
Group1	0.579	0.211	0.192	0.998	2.75	0.0132	*	0.0144	*
Group2	-0.353	0.211	-0.763	0.0425	-1.67	0.112		0.11	
Region1:Group1	0.38	0.145	0.0923	0.652	2.63	0.0111	*	0.0119	*
Region2:Group1	-0.428	0.145	-0.716	-0.146	-2.96	0.00456	**	0.00842	**
Region3:Group1	0.0477	0.145	-0.269	0.307	0.33	0.743		0.753	
Region1:Group2	-0.104	0.145	-0.404	0.193	-0.717	0.476		0.474	

Region2:Group2	0.296	0.145	0.0236	0.548	2.05	0.0456	*	0.0495	*
Region3:Group2	-0.29	0.145	-0.54	-0.00811	-2.01	0.0496	*	0.053	.
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.393	0.627	0.41	0.93	57.4	1.21E-07	***	R^2M/R^2C	0.372 / 0.732
Residual	0.292	0.541	0.428	0.631	42.6			AIC/BIC	229 / 263
Total Porosity (%)	**Lambda = 0.15**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.157	-0.288	0.318	0	1		0.993	
Region1	-0.329	0.0972	-0.511	-0.136	-3.38	0.00133	**	0.000702	***
Region2	-0.498	0.0972	-0.681	-0.306	-5.13	4.1E-06	***	0.000175	***
Region3	0.557	0.0972	0.364	0.741	5.73	5E-07	***	0.000175	***
Group1	0.537	0.222	0.0282	0.977	2.42	0.0262	*	0.033	*
Group2	-0.312	0.222	-0.759	0.108	-1.41	0.176		0.177	
Region1:Group1	0.353	0.137	0.0993	0.611	2.57	0.013	*	0.0112	*
Region2:Group1	-0.429	0.137	-0.708	-0.16	-3.12	0.00291	**	0.00351	**
Region3:Group1	0.0714	0.137	-0.193	0.327	0.519	0.606		0.606	
Region1:Group2	-0.119	0.137	-0.4	0.124	-0.865	0.391		0.386	
Region2:Group2	0.263	0.137	0.00255	0.536	1.91	0.0613	.	0.0618	.
Region3:Group2	-0.208	0.137	-0.429	0.0327	-1.51	0.137		0.146	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.449	0.67	0.455	0.986	62.9	4.27E-09	***	R^2M/R^2C	0.348 / 0.758
Residual	0.265	0.514	0.413	0.588	37.1			AIC/BIC	224 / 258
Cortical Volume	**Lambda = -2.475**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.173	-0.331	0.317	0	1		0.993	
Region1	0.569	0.0764	0.417	0.73	7.45	0	***	0.000175	***
Region2	-0.775	0.0764	-0.945	-0.627	-10.1	0	***	0.000175	***
Region3	-0.313	0.0764	-0.45	-0.161	-4.09	0.000145	***	0.000175	***
Group1	0.138	0.245	-0.311	0.595	0.564	0.58		0.592	
Group2	-0.171	0.245	-0.665	0.313	-0.698	0.494		0.51	
Region1:Group1	-0.0152	0.108	-0.245	0.202	-0.14	0.889		0.9	
Region2:Group1	-0.0466	0.108	-0.27	0.166	-0.431	0.668		0.665	
Region3:Group1	0.0399	0.108	-0.169	0.247	0.369	0.713		0.713	
Region1:Group2	0.0627	0.108	-0.138	0.29	0.58	0.564		0.565	
Region2:Group2	0.000676	0.108	-0.2	0.226	0.00625	0.995		0.996	
Region3:Group2	-0.145	0.108	-0.364	0.0732	-1.34	0.186		0.18	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.587	0.766	0.554	1.11	78.2	7.21E-15	***	R^2M/R^2C	0.317 / 0.851
Residual	0.164	0.404	0.32	0.467	21.8			AIC/BIC	112 / 146

Pore Volume	**Lambda = -0.7**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.186	-0.388	0.362	0	1		0.995	
Region1	-0.0886	0.0791	-0.246	0.0615	-1.12	0.268		0.266	
Region2	-0.517	0.0791	-0.679	-0.384	-6.53	0	***	0.000175	***
Region3	0.268	0.0791	0.118	0.426	3.39	0.00132	**	0.000702	***
Group1	0.48	0.263	0.0096	0.999	1.82	0.0851	.	0.0933	.
Group2	-0.298	0.263	-0.853	0.179	-1.13	0.273		0.301	
Region1:Group1	0.217	0.112	-0.00953	0.434	1.94	0.0572	.	0.0646	.
Region2:Group1	-0.219	0.112	-0.458	0.00602	-1.95	0.0559	.	0.0586	.
Region3:Group1	0.0149	0.112	-0.23	0.225	0.133	0.894		0.878	
Region1:Group2	-0.0147	0.112	-0.233	0.201	-0.132	0.896		0.888	
Region2:Group2	0.119	0.112	-0.107	0.363	1.07	0.291		0.286	
Region3:Group2	-0.17	0.112	-0.411	0.0405	-1.52	0.135		0.139	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.685	0.828	0.604	1.15	79.6	1.28E-15	***	R^2M/R^2C	0.228 / 0.843
Residual	0.175	0.419	0.336	0.488	20.4			AIC/BIC	204 / 238
Cortical Surface	**Lambda = -5.525**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.17	-0.361	0.364	0	1		0.991	
Region1	0.394	0.0797	0.244	0.541	4.94	7.9E-06	***	0.000175	***
Region2	-0.624	0.0797	-0.792	-0.475	-7.83	0	***	0.000175	***
Region3	-0.48	0.0797	-0.628	-0.312	-6.02	2E-07	***	0.000175	***
Group1	0.198	0.24	-0.332	0.673	0.826	0.419		0.431	
Group2	-0.213	0.24	-0.654	0.271	-0.888	0.386		0.393	
Region1:Group1	0.0204	0.113	-0.187	0.272	0.181	0.857		0.841	
Region2:Group1	0.0423	0.113	-0.177	0.251	0.375	0.709		0.708	
Region3:Group1	-0.0097	0.113	-0.229	0.22	-0.086	0.932		0.909	
Region1:Group2	0.0934	0.113	-0.13	0.33	0.828	0.411		0.397	
Region2:Group2	-0.136	0.113	-0.359	0.0495	-1.21	0.232		0.239	
Region3:Group2	-0.0578	0.113	-0.273	0.165	-0.513	0.61		0.59	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.561	0.749	0.536	1.08	75.9	9.35E-14	***	R^2M/R^2C	0.327 / 0.838
Residual	0.178	0.422	0.335	0.497	24.1			AIC/BIC	49.6 / 83.6
Pore Surface	**Lambda = -0.875**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.204	-0.417	0.394	0	1		0.981	
Region1	-0.0802	0.0689	-0.216	0.058	-1.16	0.249		0.233	
Region2	-0.421	0.0689	-0.541	-0.291	-6.11	1E-07	***	0.000175	***
Region3	0.22	0.0689	0.0782	0.362	3.19	0.00237	**	0.0014	**

Group1	0.37	0.288	-0.201	0.913	1.29	0.215		0.232	
Group2	-0.209	0.288	-0.786	0.329	-0.727	0.476		0.488	
Region1:Group1	0.155	0.0974	-0.0308	0.332	1.59	0.117		0.111	
Region2:Group1	-0.191	0.0974	-0.39	-0.00122	-1.96	0.0554	.	0.0502	.
Region3:Group1	0.0138	0.0974	-0.15	0.229	0.141	0.888		0.887	
Region1:Group2	-0.0284	0.0974	-0.217	0.156	-0.292	0.771		0.774	
Region2:Group2	0.0933	0.0974	-0.105	0.298	0.958	0.342		0.35	
Region3:Group2	-0.098	0.0974	-0.309	0.0875	-1.01	0.319		0.319	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.837	0.915	0.639	1.32	86.3	4.12E-20	***	R^2M/R^2C	0.14 / 0.882
Residual	0.133	0.364	0.286	0.419	13.7			AIC/BIC	124 / 158
Intersection Surface	**Lambda = -1**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.18	-0.37	0.374	0	1		0.993	
Region1	-0.196	0.08	-0.365	-0.0245	-2.45	0.0177	*	0.0158	*
Region2	-0.0195	0.08	-0.172	0.147	-0.243	0.809		0.807	
Region3	0.0259	0.08	-0.136	0.169	0.324	0.747		0.744	
Group1	0.636	0.255	0.184	1.11	2.49	0.0226	*	0.0274	*
Group2	-0.551	0.255	-1.07	-0.101	-2.16	0.0443	*	0.046	*
Region1:Group1	0.192	0.113	-0.018	0.412	1.69	0.0961	.	0.0947	.
Region2:Group1	-0.245	0.113	-0.433	-0.0319	-2.17	0.0345	*	0.0295	*
Region3:Group1	0.102	0.113	-0.105	0.324	0.901	0.371		0.348	
Region1:Group2	-0.22	0.113	-0.446	0.0306	-1.94	0.0575	.	0.0593	.
Region2:Group2	0.257	0.113	0.0325	0.461	2.27	0.0273	*	0.0309	*
Region3:Group2	-0.233	0.113	-0.439	-0.0164	-2.06	0.0445	*	0.0432	*
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.639	0.799	0.519	1.17	78.1	8.38E-15	***	R^2M/R^2C	0.262 / 0.838
Residual	0.179	0.423	0.335	0.494	21.9			AIC/BIC	86.1 / 120
Pore Surface: PoreVolume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.192	-0.39	0.397	0	1		0.981	
Region1	0.0231	0.0528	-0.0794	0.126	0.437	0.664		0.651	
Region2	0.349	0.0528	0.241	0.444	6.6	0	***	0.000175	***
Region3	-0.148	0.0528	-0.256	-0.0379	-2.81	0.00694	**	0.00842	**
Group1	-0.603	0.271	-1.14	-0.107	-2.22	0.0394	*	0.034	*
Group2	0.526	0.271	-0.084	1.03	1.94	0.0684	.	0.0695	.
Region1:Group1	-0.157	0.0747	-0.295	-0.00311	-2.11	0.0399	*	0.0393	*
Region2:Group1	0.134	0.0747	-0.0229	0.27	1.79	0.0792	.	0.0842	.
Region3:Group1	-0.0113	0.0747	-0.141	0.14	-0.151	0.881		0.891	

Region1:Group2	0.000198	0.0747	-0.16	0.16	0.00265	0.998		0.986	
Region2:Group2	-0.0953	0.0747	-0.25	0.0529	-1.28	0.208		0.21	
Region3:Group2	0.176	0.0747	0.0182	0.321	2.35	0.0223	*	0.0246	*
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.753	0.868	0.638	1.29	90.6	1.98E-24	***	R^2M/R^2C	0.251 / 0.93
Residual	0.0781	0.28	0.223	0.325	9.4			AIC/BIC	155 / 189
Pore Surface: Cortical Volume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.212	-0.382	0.393	0	1		0.981	
Region1	-0.322	0.0628	-0.444	-0.198	-5.13	0.000004	***	0.000175	***
Region2	-0.151	0.0628	-0.294	-0.0176	-2.41	0.0196	*	0.0204	*
Region3	0.421	0.0628	0.303	0.542	6.7	0	***	0.000175	***
Group1	-0.116	0.3	-0.714	0.498	-0.386	0.704		0.702	
Group2	0.223	0.3	-0.407	0.784	0.744	0.467		0.484	
Region1:Group1	0.0748	0.0888	-0.109	0.26	0.842	0.403		0.402	
Region2:Group1	-0.162	0.0888	-0.328	0.012	-1.82	0.074	.	0.0747	.
Region3:Group1	0.0608	0.0888	-0.105	0.221	0.685	0.496		0.519	
Region1:Group2	-0.0831	0.0888	-0.229	0.0937	-0.935	0.354		0.35	
Region2:Group2	0.128	0.0888	-0.0497	0.303	1.44	0.157		0.144	
Region3:Group2	-0.0778	0.0888	-0.245	0.107	-0.876	0.385		0.402	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.917	0.958	0.687	1.38	89.3	6.89E-23	***	R^2M/R^2C	0.0958 / 0.903
Residual	0.11	0.332	0.257	0.388	10.7			AIC/BIC	178 / 212
Pore Thickness	**Lambda = -1.625**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.155	-0.324	0.286	0	1		0.993	
Region1	0.000935	0.0857	-0.169	0.182	0.0109	0.991		0.989	
Region2	-0.662	0.0857	-0.831	-0.486	-7.72	0	***	0.000175	***
Region3	0.301	0.0857	0.135	0.477	3.52	0.000896	***	0.00105	**
Group1	0.617	0.219	0.156	1.06	2.82	0.0113	*	0.0144	*
Group2	-0.572	0.219	-1.04	-0.188	-2.61	0.0176	*	0.02	*
Region1:Group1	0.228	0.121	-0.00446	0.474	1.88	0.0651	.	0.0667	.
Region2:Group1	-0.0632	0.121	-0.309	0.187	-0.521	0.605		0.605	
Region3:Group1	-0.0662	0.121	-0.317	0.182	-0.546	0.587		0.587	
Region1:Group2	0.00632	0.121	-0.233	0.257	0.0521	0.959		0.941	
Region2:Group2	0.0509	0.121	-0.191	0.268	0.42	0.676		0.699	
Region3:Group2	-0.216	0.121	-0.489	0.0383	-1.78	0.081	.	0.0825	.
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.451	0.672	0.448	0.985	68.7	6.80E-11	***	R^2M/R^2C	0.395 / 0.811

Residual	0.206	0.454	0.359	0.526	31.3			AIC/BIC	200 / 234
Pore Separation	**Lambda = -2.175**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.206	-0.387	0.363	0	1		0.994	
Region1	0.42	0.0688	0.292	0.558	6.11	1E-07	***	0.000175	***
Region2	0.0393	0.0688	-0.0999	0.172	0.571	0.57		0.573	
Region3	-0.521	0.0688	-0.656	-0.388	-7.57	0	***	0.000175	***
Group1	0.0781	0.292	-0.487	0.661	0.268	0.792		0.792	
Group2	-0.18	0.292	-0.783	0.335	-0.618	0.544		0.539	
Region1:Group1	0.0379	0.0973	-0.162	0.225	0.39	0.698		0.698	
Region2:Group1	0.0821	0.0973	-0.101	0.283	0.844	0.403		0.426	
Region3:Group1	-0.138	0.0973	-0.318	0.0516	-1.42	0.162		0.161	
Region1:Group2	0.0674	0.0973	-0.11	0.234	0.693	0.491		0.491	
Region2:Group2	-0.0344	0.0973	-0.233	0.154	-0.353	0.725		0.746	
Region3:Group2	-0.0653	0.0973	-0.257	0.128	-0.671	0.505		0.493	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.86	0.927	0.661	1.32	86.7	2.10E-20	***	R^2M/R^2C	0.123 / 0.883
Residual	0.132	0.364	0.295	0.425	13.3			AIC/BIC	88.4 / 122
Pore Tb.N	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.211	-0.424	0.428	0	1		0.999	
Region1	-0.347	0.0645	-0.487	-0.218	-5.38	1.6E-06	***	0.000175	***
Region2	0.0579	0.0645	-0.0855	0.182	0.898	0.373		0.378	
Region3	0.283	0.0645	0.156	0.412	4.38	5.44E-05	***	0.000175	***
Group1	-0.209	0.298	-0.825	0.341	-0.7	0.493		0.514	
Group2	0.336	0.298	-0.265	0.897	1.13	0.274		0.298	
Region1:Group1	0.0654	0.0913	-0.114	0.245	0.717	0.477		0.483	
Region2:Group1	-0.163	0.0913	-0.344	0.00695	-1.78	0.08	.	0.0754	.
Region3:Group1	0.0694	0.0913	-0.116	0.253	0.761	0.45		0.444	
Region1:Group2	-0.0979	0.0913	-0.274	0.108	-1.07	0.288		0.308	
Region2:Group2	0.122	0.0913	-0.0449	0.292	1.34	0.187		0.192	
Region3:Group2	-0.0488	0.0913	-0.228	0.136	-0.535	0.595		0.601	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.903	0.95	0.662	1.35	88.6	3.59E-22	***	R^2M/R^2C	0.102 / 0.897
Residual	0.117	0.341	0.273	0.397	11.4			AIC/BIC	180 / 214
Degree of Anisotropy	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.162	-0.353	0.335	0	1		0.999	
Region1	-0.0695	0.0824	-0.214	0.0901	-0.843	0.403		0.382	

Region2	-0.897	0.0824	-1.07	-0.735	-10.9	0	***	0.000175	***
Region3	0.661	0.0824	0.502	0.846	8.01	0	***	0.000175	***
Group1	0.103	0.229	-0.325	0.651	0.448	0.66		0.667	
Group2	-0.247	0.229	-0.713	0.174	-1.08	0.296		0.309	
Region1:Group1	0.31	0.117	0.076	0.533	2.66	0.0104	*	0.0119	*
Region2:Group1	-0.207	0.117	-0.422	0.014	-1.77	0.0819	.	0.0884	.
Region3:Group1	-0.109	0.117	-0.351	0.108	-0.933	0.355		0.371	
Region1:Group2	0.0347	0.117	-0.186	0.273	0.297	0.767		0.768	
Region2:Group2	0.0296	0.117	-0.189	0.268	0.254	0.8		0.807	
Region3:Group2	-0.0329	0.117	-0.258	0.199	-0.282	0.779		0.777	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.505	0.71	0.479	1.04	72.6	2.41E-12	***	R^2M/R^2C	0.364 / 0.826
Residual	0.19	0.436	0.344	0.51	27.4			AIC/BIC	197 / 231
Pore Fractal	**Lambda = 4.475**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.191	-0.356	0.375	0	1		0.987	
Region1	-0.341	0.0754	-0.5	-0.191	-4.52	3.42E-05	***	0.000175	***
Region2	-0.377	0.0754	-0.527	-0.222	-5	6.4E-06	***	0.000175	***
Region3	0.67	0.0754	0.518	0.815	8.88	0	***	0.000175	***
Group1	0.286	0.27	-0.195	0.833	1.06	0.303		0.33	
Group2	-0.0734	0.27	-0.595	0.462	-0.272	0.788		0.815	
Region1:Group1	0.0922	0.107	-0.103	0.303	0.864	0.391		0.387	
Region2:Group1	-0.234	0.107	-0.465	0.00137	-2.2	0.0322	*	0.0347	*
Region3:Group1	0.094	0.107	-0.147	0.284	0.881	0.382		0.394	
Region1:Group2	-0.0489	0.107	-0.272	0.161	-0.459	0.648		0.625	
Region2:Group2	0.138	0.107	-0.0778	0.343	1.3	0.201		0.201	
Region3:Group2	-0.0904	0.107	-0.271	0.165	-0.847	0.401		0.404	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.724	0.851	0.629	1.24	82	5.45E-17	***	R^2M/R^2C	0.209 / 0.857
Residual	0.159	0.399	0.329	0.46	18			AIC/BIC	199 / 234
Number of Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.21	-0.506	0.394	0	1		0.997	
Region1	0.141	0.0598	0.016	0.257	2.35	0.0224	*	0.0253	*
Region2	-0.477	0.0598	-0.585	-0.359	-7.97	0	***	0.000175	***
Region3	0.405	0.0598	0.294	0.521	6.78	0	***	0.000175	***
Group1	-0.096	0.297	-0.687	0.425	-0.323	0.751		0.761	
Group2	0.2	0.297	-0.391	0.831	0.672	0.51		0.511	
Region1:Group1	-0.0573	0.0846	-0.211	0.121	-0.678	0.501		0.473	
Region2:Group1	0.0029	0.0846	-0.158	0.157	0.0343	0.973		0.945	

Region3:Group1	-0.00862	0.0846	-0.183	0.153	-0.102	0.919		0.916	
Region1:Group2	0.0153	0.0846	-0.161	0.17	0.181	0.857		0.828	
Region2:Group2	-0.0226	0.0846	-0.197	0.135	-0.267	0.79		0.773	
Region3:Group2	0.112	0.0846	-0.0288	0.286	1.32	0.192		0.187	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.903	0.95	0.677	1.4	90	9.63E-24	***	R^2M/R^2C	0.114 / 0.912
Residual	0.1	0.316	0.248	0.364	9.98			AIC/BIC	172 / 206
Pore Density	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.207	-0.426	0.383	0	1		0.988	
Region1	-0.188	0.0475	-0.278	-0.0928	-3.96	0.000222	***	0.000351	***
Region2	0.00231	0.0475	-0.103	0.0945	0.0485	0.961		0.972	
Region3	0.465	0.0475	0.366	0.564	9.79	0	***	0.000175	***
Group1	-0.354	0.293	-0.908	0.218	-1.21	0.242		0.274	
Group2	0.371	0.293	-0.214	0.926	1.27	0.221		0.243	
Region1:Group1	0.00536	0.0672	-0.129	0.144	0.0797	0.937		0.962	
Region2:Group1	-0.00926	0.0672	-0.142	0.127	-0.138	0.891		0.899	
Region3:Group1	-0.0449	0.0672	-0.169	0.0782	-0.668	0.507		0.528	
Region1:Group2	-0.0568	0.0672	-0.199	0.0652	-0.844	0.402		0.417	
Region2:Group2	0.014	0.0672	-0.119	0.157	0.208	0.836		0.842	
Region3:Group2	0.163	0.0672	0.049	0.29	2.42	0.019	*	0.0193	*
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.884	0.94	0.69	1.35	93.3	2.20E-28	***	R^2M/R^2C	0.158 / 0.944
Residual	0.0633	0.252	0.198	0.289	6.68			AIC/BIC	147 / 181
Euler number	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.205	-0.424	0.417	0	1		1	
Region1	0.231	0.0674	0.0935	0.367	3.42	0.00119	**	0.000702	***
Region2	-0.403	0.0674	-0.54	-0.271	-5.98	2E-07	***	0.000175	***
Region3	0.403	0.0674	0.269	0.541	5.98	2E-07	***	0.000175	***
Group1	-0.202	0.29	-0.804	0.346	-0.699	0.494		0.512	
Group2	0.208	0.29	-0.353	0.791	0.72	0.481		0.494	
Region1:Group1	-0.0872	0.0953	-0.256	0.0837	-0.915	0.364		0.366	
Region2:Group1	0.0826	0.0953	-0.0907	0.27	0.867	0.39		0.383	
Region3:Group1	-0.0273	0.0953	-0.193	0.16	-0.286	0.776		0.785	
Region1:Group2	0.0823	0.0953	-0.119	0.262	0.864	0.392		0.407	
Region2:Group2	-0.0887	0.0953	-0.274	0.0976	-0.93	0.356		0.361	
Region3:Group2	0.213	0.0953	0.0195	0.388	2.23	0.0298	*	0.0316	*
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit

Sample	0.849	0.921	0.673	1.26	87	1.11E-20	***	R^2M/R^2C	0.136 / 0.887
Residual	0.127	0.357	0.283	0.425	13			AIC/BIC	184 / 218
Connectivity	**Lambda = 0.25**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.216	-0.38	0.423	0	1		0.991	
Region1	-0.111	0.0652	-0.226	0.0121	-1.7	0.0947	.	0.0895	.
Region2	-0.299	0.0652	-0.421	-0.169	-4.58	2.78E-05	***	0.000175	***
Region3	0.145	0.0652	0.00796	0.269	2.23	0.0301	*	0.0358	*
Group1	0.118	0.305	-0.517	0.73	0.386	0.704		0.705	
Group2	0.0325	0.305	-0.54	0.657	0.107	0.916		0.941	
Region1:Group1	0.0317	0.0922	-0.143	0.189	0.344	0.732		0.728	
Region2:Group1	-0.181	0.0922	-0.354	-0.0017	-1.96	0.0554	.	0.053	.
Region3:Group1	0.0425	0.0922	-0.129	0.224	0.461	0.646		0.641	
Region1:Group2	-0.123	0.0922	-0.324	0.0537	-1.34	0.187		0.186	
Region2:Group2	0.125	0.0922	-0.0532	0.315	1.35	0.182		0.176	
Region3:Group2	-0.123	0.0922	-0.292	0.0463	-1.34	0.187		0.193	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.946	0.973	0.716	1.37	88.8	1.94E-22	***	R^2M/R^2C	0.0669 / 0.896
Residual	0.119	0.345	0.275	0.4	11.2			AIC/BIC	201 / 235
Connectivity density	**Lambda = 0.725**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.206	-0.412	0.441	0	1		0.996	
Region1	-0.259	0.0676	-0.396	-0.129	-3.84	0.000328	***	0.000702	***
Region2	-0.00238	0.0676	-0.14	0.133	-0.0353	0.972		0.953	
Region3	0.174	0.0676	0.0461	0.313	2.57	0.0129	*	0.0144	*
Group1	-0.415	0.291	-1.03	0.133	-1.43	0.171		0.187	
Group2	0.402	0.291	-0.194	0.986	1.38	0.185		0.191	
Region1:Group1	0.0785	0.0956	-0.103	0.257	0.821	0.415		0.395	
Region2:Group1	-0.105	0.0956	-0.29	0.08	-1.1	0.278		0.26	
Region3:Group1	0.00146	0.0956	-0.191	0.195	0.0153	0.988		0.983	
Region1:Group2	-0.103	0.0956	-0.287	0.078	-1.08	0.287		0.274	
Region2:Group2	0.129	0.0956	-0.0694	0.307	1.35	0.183		0.18	
Region3:Group2	-0.0467	0.0956	-0.248	0.138	-0.489	0.627		0.628	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.859	0.927	0.616	1.36	87	9.63E-21	***	R^2M/R^2C	0.127 / 0.887
Residual	0.128	0.358	0.286	0.413	13			AIC/BIC	206 / 240
SD Pore Thickness	**Lambda = -1.075**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.161	-0.318	0.323	0	1		0.993	

Region1	0.0673	0.0923	-0.122	0.277	0.729	0.469		0.481	
Region2	-0.648	0.0923	-0.825	-0.466	-7.02	0	***	0.000175	***
Region3	0.318	0.0923	0.142	0.486	3.45	0.00111	**	0.00211	**
Group1	0.546	0.227	0.0701	1.03	2.4	0.0272	*	0.0302	*
Group2	-0.575	0.227	-1.03	-0.105	-2.53	0.0209	*	0.0253	*
Region1:Group1	0.128	0.131	-0.101	0.406	0.978	0.332		0.341	
Region2:Group1	0.0749	0.131	-0.195	0.305	0.574	0.569		0.57	
Region3:Group1	-0.112	0.131	-0.358	0.154	-0.857	0.395		0.403	
Region1:Group2	0.0301	0.131	-0.209	0.279	0.231	0.819		0.79	
Region2:Group2	-0.156	0.131	-0.407	0.112	-1.19	0.238		0.255	
Region3:Group2	-0.0141	0.131	-0.276	0.251	-0.108	0.914		0.891	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.482	0.694	0.495	1.02	66.9	2.70E-10	***	R^2M/R^2C	0.342 / 0.782
Residual	0.239	0.489	0.4	0.559	33.1			AIC/BIC	206 / 240
SD Pore Separation	**Lambda = -1.375**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.2	-0.396	0.391	0	1		1	
Region1	0.451	0.0852	0.29	0.625	5.29	2.3E-06	***	0.000175	***
Region2	-0.0635	0.0852	-0.23	0.103	-0.745	0.46		0.437	
Region3	-0.399	0.0852	-0.564	-0.226	-4.68	1.95E-05	***	0.000175	***
Group1	0.113	0.283	-0.495	0.705	0.4	0.694		0.723	
Group2	-0.222	0.283	-0.781	0.357	-0.785	0.443		0.451	
Region1:Group1	0.06	0.121	-0.197	0.314	0.498	0.621		0.631	
Region2:Group1	0.0533	0.121	-0.186	0.303	0.442	0.66		0.656	
Region3:Group1	-0.202	0.121	-0.436	0.045	-1.67	0.1		0.102	
Region1:Group2	0.077	0.121	-0.146	0.343	0.639	0.526		0.512	
Region2:Group2	0.0418	0.121	-0.177	0.29	0.347	0.73		0.766	
Region3:Group2	-0.145	0.121	-0.399	0.0842	-1.2	0.234		0.225	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.79	0.889	0.617	1.29	79.5	1.45E-15	***	R^2M/R^2C	0.122 / 0.82
Residual	0.203	0.451	0.366	0.525	20.5			AIC/BIC	149 / 183
Pore volume	**Lambda = -1.075**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.166	-0.353	0.31	0	1		0.999	
Region1	-0.272	0.0991	-0.487	-0.0952	-2.74	0.00825	**	0.00912	**
Region2	-0.297	0.0991	-0.486	-0.104	-3	0.00413	**	0.00596	**
Region3	0.143	0.0991	-0.0499	0.357	1.44	0.156		0.173	
Group1	0.59	0.235	0.089	1.05	2.51	0.0218	*	0.0239	*
Group2	-0.373	0.235	-0.882	0.116	-1.59	0.13		0.14	

Region1:Group1	0.37	0.14	0.0898	0.641	2.64	0.0108	*	0.0102	*
Region2:Group1	-0.272	0.14	-0.566	0.0167	-1.94	0.0577	.	0.0519	.
Region3:Group1	0.0115	0.14	-0.266	0.271	0.082	0.935		0.947	
Region1:Group2	-0.0985	0.14	-0.402	0.204	-0.703	0.485		0.485	
Region2:Group2	0.239	0.14	-0.0615	0.503	1.7	0.0943	.	0.0891	.
Region3:Group2	-0.291	0.14	-0.567	0.00195	-2.08	0.0424	*	0.0474	*
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.511	0.715	0.517	1.08	65	1.04E-09	***	R^2M/R^2C	0.289 / 0.751
Residual	0.275	0.524	0.415	0.615	35			AIC/BIC	208 / 242
Pore surface	**Lambda = -1.725**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.172	-0.355	0.336	0	1		0.993	
Region1	-0.384	0.1	-0.585	-0.197	-3.84	0.000325	***	0.000175	***
Region2	-0.111	0.1	-0.297	0.11	-1.11	0.271		0.276	
Region3	0.0595	0.1	-0.137	0.246	0.595	0.554		0.576	
Group1	0.543	0.243	0.0725	1.06	2.23	0.0385	*	0.0375	*
Group2	-0.283	0.243	-0.765	0.2	-1.17	0.259		0.275	
Region1:Group1	0.38	0.141	0.123	0.654	2.69	0.00952	**	0.00877	**
Region2:Group1	-0.282	0.141	-0.582	-0.00711	-1.99	0.0513	.	0.0568	.
Region3:Group1	0.00438	0.141	-0.271	0.291	0.0309	0.975		0.976	
Region1:Group2	-0.138	0.141	-0.418	0.157	-0.978	0.333		0.32	
Region2:Group2	0.219	0.141	-0.0495	0.513	1.55	0.127		0.127	
Region3:Group2	-0.221	0.141	-0.512	0.0711	-1.56	0.124		0.135	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.551	0.742	0.493	1.09	66.3	4.15E-10	***	R^2M/R^2C	0.251 / 0.748
Residual	0.28	0.529	0.424	0.612	33.7			AIC/BIC	167 / 201
Avg. Pore Orientation theta	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.138	-0.248	0.28	0	1		0.999	
Region1	-0.0396	0.0809	-0.202	0.13	-0.489	0.627		0.606	
Region2	0.925	0.0809	0.768	1.08	11.4	0	***	0.000175	***
Region3	-1.06	0.0809	-1.21	-0.894	-13.1	0	***	0.000175	***
Group1	-0.038	0.196	-0.382	0.353	-0.194	0.848		0.839	
Group2	0.113	0.196	-0.261	0.488	0.576	0.571		0.578	
Region1:Group1	-0.152	0.114	-0.388	0.0638	-1.33	0.189		0.189	
Region2:Group1	0.0948	0.114	-0.12	0.311	0.829	0.411		0.408	
Region3:Group1	0.0701	0.114	-0.159	0.29	0.612	0.543		0.541	
Region1:Group2	-0.0794	0.114	-0.3	0.172	-0.694	0.491		0.5	
Region2:Group2	0.0721	0.114	-0.152	0.292	0.63	0.531		0.538	

Region3:Group2	-0.165	0.114	-0.383	0.046	-1.44	0.156		0.163	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.356	0.596	0.406	0.865	66	5.18E-10	***	R^2M/R^2C	0.497 / 0.829
Residual	0.183	0.428	0.343	0.494	34			AIC/BIC	190 / 224
Avg. Pore Orientation phi	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.0448	-0.0822	0.0855	0	1		0.985	
Region1	-1.27	0.0662	-1.41	-1.14	-19.2	0	***	0.000175	***
Region2	0.11	0.0662	-0.0206	0.231	1.67	0.101		0.0905	.
Region3	-0.164	0.0662	-0.293	-0.0307	-2.47	0.0166	*	0.0151	*
Group1	0.0269	0.0633	-0.0986	0.154	0.425	0.676		0.662	
Group2	-0.151	0.0633	-0.279	-0.0321	-2.38	0.0286	*	0.0298	*
Region1:Group1	-0.0604	0.0936	-0.247	0.138	-0.645	0.522		0.519	
Region2:Group1	-0.00035	0.0936	-0.2	0.193	-0.00373	0.997		0.997	
Region3:Group1	0.0414	0.0936	-0.135	0.25	0.442	0.66		0.677	
Region1:Group2	0.103	0.0936	-0.0936	0.292	1.1	0.277		0.274	
Region2:Group2	0.0219	0.0936	-0.16	0.207	0.234	0.816		0.823	
Region3:Group2	0.0122	0.0936	-0.157	0.195	0.13	0.897		0.894	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.0114	0.107	0	0.249	8.5	0.4		R^2M/R^2C	0.868 / 0.879
Residual	0.123	0.35	0.284	0.403	91.5			AIC/BIC	127 / 161
Avg. Pore Major diameter	**Lambda = -3.7**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.197	-0.404	0.38	0	1		0.999	
Region1	-0.38	0.082	-0.55	-0.209	-4.63	2.32E-05	***	0.000175	***
Region2	0.0963	0.082	-0.0633	0.248	1.17	0.246		0.236	
Region3	-0.0395	0.082	-0.191	0.131	-0.482	0.632		0.638	
Group1	0.418	0.279	-0.134	0.996	1.5	0.151		0.167	
Group2	-0.196	0.279	-0.74	0.344	-0.703	0.491		0.527	
Region1:Group1	0.188	0.116	-0.0284	0.41	1.62	0.112		0.109	
Region2:Group1	-0.14	0.116	-0.357	0.0923	-1.21	0.232		0.247	
Region3:Group1	0.0132	0.116	-0.228	0.233	0.114	0.909		0.914	
Region1:Group2	-0.168	0.116	-0.416	0.0701	-1.45	0.153		0.154	
Region2:Group2	0.0518	0.116	-0.167	0.271	0.447	0.657		0.649	
Region3:Group2	0.159	0.116	-0.0566	0.387	1.37	0.177		0.16	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.77	0.878	0.665	1.25	80.4	5.02E-16	***	R^2M/R^2C	0.149 / 0.833
Residual	0.188	0.434	0.34	0.511	19.6			AIC/BIC	168 / 202

Sphericity	Lambda = None								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.136	-0.297	0.272	0	1		0.994	
Region1	0.894	0.107	0.67	1.1	8.36	0	***	0.000175	***
Region2	-0.306	0.107	-0.504	-0.0789	-2.86	0.00598	**	0.00632	**
Region3	0.172	0.107	-0.0452	0.379	1.61	0.113		0.111	
Group1	-0.0627	0.192	-0.43	0.292	-0.327	0.748		0.74	
Group2	-0.134	0.192	-0.483	0.239	-0.696	0.495		0.495	
Region1:Group1	0.277	0.151	0.00358	0.562	1.83	0.0726	.	0.0674	.
Region2:Group1	-0.0196	0.151	-0.305	0.279	-0.129	0.898		0.896	
Region3:Group1	-0.0377	0.151	-0.3	0.241	-0.249	0.804		0.81	
Region1:Group2	0.248	0.151	-0.0435	0.527	1.64	0.107		0.103	
Region2:Group2	0.0064	0.151	-0.293	0.358	0.0423	0.966		0.978	
Region3:Group2	-0.0899	0.151	-0.387	0.225	-0.594	0.555		0.564	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.307	0.554	0.355	0.834	49	7.52E-06	***	R^2M/R^2C	0.421 / 0.704
Residual	0.32	0.566	0.455	0.664	51			AIC/BIC	219 / 253

Femoral Regions: Post-Hoc Tests for Significant Fixed Effects

Cortical Fractal	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior < Lateral	0.0319	*	0.874	Large	35%
Region		Anterior < Medial	0	***	3.09	Large	
Region		Anterior < Posterior	8.5E-06	***	1.67	Large	
Region		Lateral < Medial	0	***	2.21	Large	
Region		Lateral < Posterior	0.059	.	0.797	Medium	
Region		Medial > Posterior	0.000152	***	1.42	Large	
Region | Group	Anterior	Control > Fentanyl	0.402		1.1	Large	44%
Region | Group	Anterior	Control > Morphine	0.193		1.72	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.63		0.626	Medium	
Region | Group	Lateral	Control < Fentanyl	0.899		0.164	Small	
Region | Group	Lateral	Control > Morphine	0.779		0.364	Medium	
Region | Group	Lateral	Fentanyl > Morphine	0.685		0.528	Medium	
Region | Group	Medial	Control > Fentanyl	0.368		1.18	Large	
Region | Group	Medial	Control > Morphine	0.182		1.77	Large	
Region | Group	Medial	Fentanyl > Morphine	0.652		0.587	Medium	
Region | Group	Posterior	Control > Fentanyl	0.416		1.06	Large	
Region | Group	Posterior	Control > Morphine	0.231		1.58	Large	
Region | Group	Posterior	Fentanyl > Morphine	0.691		0.516	Medium	
Group | Region	Control	Anterior < Lateral	0.998		0.00127	Small	

Group | Region	Control	Anterior < Medial	3E-07	***	3.13	Large	
Group | Region	Control	Anterior < Posterior	0.0039	**	1.61	Large	
Group | Region	Control	Lateral < Medial	3E-07	***	3.13	Large	
Group | Region	Control	Lateral < Posterior	0.00392	**	1.61	Large	
Group | Region	Control	Medial > Posterior	0.00634	**	1.52	Large	
Group | Region	Fentanyl	Anterior < Lateral	0.0219	*	1.26	Large	
Group | Region	Fentanyl	Anterior < Medial	5E-07	***	3.05	Large	
Group | Region	Fentanyl	Anterior < Posterior	0.00327	**	1.65	Large	
Group | Region	Fentanyl	Lateral < Medial	0.00151	**	1.79	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.476		0.383	Medium	
Group | Region	Fentanyl	Medial > Posterior	0.0112	*	1.4	Large	
Group | Region	Morphine	Anterior < Lateral	0.0139	*	1.36	Large	
Group | Region	Morphine	Anterior < Medial	4E-07	***	3.09	Large	
Group | Region	Morphine	Anterior < Posterior	0.0018	**	1.76	Large	
Group | Region	Morphine	Lateral < Medial	0.0021	**	1.73	Large	
Group | Region	Morphine	Lateral < Posterior	0.462		0.396	Medium	
Group | Region	Morphine	Medial > Posterior	0.0158	*	1.33	Large	
Number Closed Pores	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	2E-07	***	1.99	Large	25%
Region		Anterior < Medial	0.0924	.	0.736	Medium	
Region		Anterior > Posterior	0.0112	*	0.995	Large	
Region		Lateral < Medial	0	***	2.73	Large	
Region		Lateral < Posterior	0.0108	*	0.999	Large	
Region		Medial > Posterior	4.2E-06	***	1.73	Large	
Number Open Pores	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.641		0.365	Medium	27%
Region		Anterior < Medial	0.0629	.	0.788	Medium	
Region		Anterior < Posterior	3.23E-05	***	1.56	Large	
Region		Lateral < Medial	0.00249	**	1.15	Large	
Region		Lateral < Posterior	4E-07	***	1.92	Large	
Region		Medial < Posterior	0.0733	.	0.768	Medium	
Closed Pore Density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.483		0.443	Medium	48%
Region		Anterior < Medial	0	***	2.41	Large	
Region		Anterior > Posterior	0.207		0.612	Medium	
Region		Lateral < Medial	3E-07	***	1.96	Large	
Region		Lateral > Posterior	0.00639	**	1.06	Large	
Region		Medial > Posterior	0	***	3.02	Large	
Region | Group	Anterior	Control < Fentanyl	0.191		2.61	Large	46%

Region | Group	Anterior	Control < Morphine	0.452		1.48	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.565		1.13	Large	
Region | Group	Lateral	Control < Fentanyl	0.172		2.73	Large	
Region | Group	Lateral	Control < Morphine	0.536		1.21	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.441		1.52	Large	
Region | Group	Medial	Control < Fentanyl	0.0744	.	3.63	Large	
Region | Group	Medial	Control < Morphine	0.583		1.08	Large	
Region | Group	Medial	Fentanyl > Morphine	0.2		2.55	Large	
Region | Group	Posterior	Control < Fentanyl	0.318		1.98	Large	
Region | Group	Posterior	Control < Morphine	0.505		1.31	Large	
Region | Group	Posterior	Fentanyl > Morphine	0.733		0.669	Medium	
Group | Region	Control	Anterior < Lateral	0.363		0.491	Medium	
Group | Region	Control	Anterior < Medial	0.000133	***	2.2	Large	
Group | Region	Control	Anterior > Posterior	0.522		0.345	Medium	
Group | Region	Control	Lateral < Medial	0.00232	**	1.71	Large	
Group | Region	Control	Lateral > Posterior	0.124		0.835	Large	
Group | Region	Control	Medial > Posterior	1.49E-05	***	2.54	Large	
Group | Region	Fentanyl	Anterior < Lateral	0.256		0.614	Medium	
Group | Region	Fentanyl	Anterior < Medial	2E-07	***	3.22	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.0734	.	0.976	Large	
Group | Region	Fentanyl	Lateral < Medial	9.8E-06	***	2.61	Large	
Group | Region	Fentanyl	Lateral > Posterior	0.00439	**	1.59	Large	
Group | Region	Fentanyl	Medial > Posterior	0	***	4.2	Large	
Group | Region	Morphine	Anterior < Lateral	0.675		0.225	Medium	
Group | Region	Morphine	Anterior < Medial	0.00144	**	1.8	Large	
Group | Region	Morphine	Anterior > Posterior	0.338		0.517	Medium	
Group | Region	Morphine	Lateral < Medial	0.00485	**	1.57	Large	
Group | Region	Morphine	Lateral > Posterior	0.171		0.742	Medium	
Group | Region	Morphine	Medial > Posterior	0.000066	***	2.31	Large	
Open Pore Density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.000002	***	1.8	Large	31%
Region		Anterior < Medial	0	***	2.16	Large	
Region		Anterior < Posterior	0.0321	*	0.873	Large	
Region		Lateral < Medial	0.644		0.363	Medium	
Region		Lateral > Posterior	0.0213	*	0.922	Large	
Region		Medial > Posterior	0.000637	***	1.29	Large	
Closed Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.0165	*	0.952	Large	32%
Region		Anterior < Medial	0	***	2.32	Large	
Region		Anterior < Posterior	0.0639	.	0.786	Medium	

Region		Lateral < Medial	0.000248	***	1.37	Large	
Region		Lateral > Posterior	0.95		0.166	Small	
Region		Medial > Posterior	3.92E-05	***	1.54	Large	
Open Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.502		0.434	Medium	71%
Region		Anterior < Medial	0.000156	***	1.42	Large	
Region		Anterior < Posterior	0.00278	**	1.14	Large	
Region		Lateral < Medial	0.000001	***	1.85	Large	
Region		Lateral < Posterior	2.58E-05	***	1.58	Large	
Region		Medial > Posterior	0.812		0.274	Medium	
Group		Control > Fentanyl	0.0499	*	1.72	Large	99%
Group		Control > Morphine	0.0968	.	1.49	Large	
Group		Fentanyl < Morpine	0.937		0.233	Medium	
Region | Group	Anterior	Control > Fentanyl	0.0026	**	2.72	Large	40%
Region | Group	Anterior	Control > Morphine	0.00966	**	2.31	Large	
Region | Group	Anterior	Fentanyl < Morphine	0.624		0.419	Medium	
Region | Group	Lateral	Control > Fentanyl	0.579		0.475	Medium	
Region | Group	Lateral	Control > Morphine	0.64		0.4	Medium	
Region | Group	Lateral	Fentanyl < Morphine	0.93		0.0752	Small	
Region | Group	Medial	Control > Fentanyl	0.00384	**	2.6	Large	
Region | Group	Medial	Control > Morphine	0.113		1.37	Large	
Region | Group	Medial	Fentanyl < Morphine	0.156		1.23	Large	
Region | Group	Posterior	Control > Fentanyl	0.0572	.	1.66	Large	
Region | Group	Posterior	Control > Morphine	0.0883	.	1.48	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.834		0.179	Small	
Group | Region	Control	Anterior > Lateral	0.000852	***	2.11	Large	
Group | Region	Control	Anterior < Medial	0.277		0.655	Medium	
Group | Region	Control	Anterior > Posterior	0.971		0.0221	Small	
Group | Region	Control	Lateral < Medial	2.34E-05	***	2.76	Large	
Group | Region	Control	Lateral < Posterior	0.000955	***	2.09	Large	
Group | Region	Control	Medial > Posterior	0.261		0.677	Medium	
Group | Region	Fentanyl	Anterior < Lateral	0.814		0.141	Small	
Group | Region	Fentanyl	Anterior < Medial	0.199		0.777	Medium	
Group | Region	Fentanyl	Anterior < Posterior	0.0869	.	1.04	Large	
Group | Region	Fentanyl	Lateral < Medial	0.292		0.635	Medium	
Group | Region	Fentanyl	Lateral < Posterior	0.138		0.899	Large	
Group | Region	Fentanyl	Medial < Posterior	0.66		0.264	Medium	
Group | Region	Morphine	Anterior > Lateral	0.736		0.202	Medium	
Group | Region	Morphine	Anterior < Medial	0.0103	*	1.59	Large	
Group | Region	Morphine	Anterior < Posterior	0.185		0.801	Large	
Group | Region	Morphine	Lateral < Medial	0.00411	**	1.79	Large	

Group | Region	Morphine	Lateral < Posterior	0.0985	.	1	Large	
Group | Region	Morphine	Medial > Posterior	0.194		0.785	Medium	
Total Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.996		0.0705	Small	70%
Region		Anterior < Medial	5E-07	***	1.91	Large	
Region		Anterior < Posterior	0.000567	***	1.3	Large	
Region		Lateral < Medial	2E-07	***	1.98	Large	
Region		Lateral < Posterior	0.000266	***	1.37	Large	
Region		Medial > Posterior	0.205		0.614	Medium	
Group		Control > Fentanyl	0.169		1.4	Large	100%
Group		Control > Morphine	0.135		1.49	Large	
Group		Fentanyl > Morphine	0.991		0.0969	Small	
Region | Group	Anterior	Control > Fentanyl	1		0	Small	44%
Region | Group	Anterior	Control > Morphine	1		0	Small	
Region | Group	Anterior	Fentanyl < Morphine	1		0	Small	
Region | Group	Lateral	Control > Fentanyl	1		0	Small	
Region | Group	Lateral	Control > Morphine	1		0	Small	
Region | Group	Lateral	Fentanyl < Morphine	1		0	Small	
Region | Group	Medial	Control > Fentanyl	0.0167	*	2.51	Large	
Region | Group	Medial	Control > Morphine	0.0167	*	2.51	Large	
Region | Group	Medial	Fentanyl < Morphine	1		0	Small	
Region | Group	Posterior	Control > Fentanyl	1		0	Small	
Region | Group	Posterior	Control > Morphine	1		0	Small	
Region | Group	Posterior	Fentanyl < Morphine	1		0	Small	
Group | Region	Control	Anterior > Lateral	1		0	Small	
Group | Region	Control	Anterior < Medial	0.0176	*	2.51	Large	
Group | Region	Control	Anterior < Posterior	1		0	Small	
Group | Region	Control	Lateral < Medial	0.0176	*	2.51	Large	
Group | Region	Control	Lateral < Posterior	1		0	Small	
Group | Region	Control	Medial > Posterior	0.0176	*	2.51	Large	
Group | Region	Fentanyl	Anterior < Lateral	1		0	Small	
Group | Region	Fentanyl	Anterior < Medial	1		0	Small	
Group | Region	Fentanyl	Anterior < Posterior	1		0	Small	
Group | Region	Fentanyl	Lateral < Medial	1		0	Small	
Group | Region	Fentanyl	Lateral < Posterior	1		0	Small	
Group | Region	Fentanyl	Medial < Posterior	1		0	Small	
Group | Region	Morphine	Anterior < Lateral	1		0	Small	
Group | Region	Morphine	Anterior < Medial	1		0	Small	
Group | Region	Morphine	Anterior < Posterior	1		0	Small	
Group | Region	Morphine	Lateral < Medial	1		0	Small	
Group | Region	Morphine	Lateral < Posterior	1		0	Small	

Group | Region	Morphine	Medial > Posterior	1		0	Small	
Cortical Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	3.33	Large	18%
Region		Anterior > Medial	0	***	2.18	Large	
Region		Anterior > Posterior	0.977		0.126	Small	
Region		Lateral < Medial	0.0027	**	1.14	Large	
Region		Lateral < Posterior	0	***	3.2	Large	
Region		Medial < Posterior	1E-07	***	2.05	Large	
Pore Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.00871	**	1.02	Large	43%
Region		Anterior < Medial	0.0382	*	0.852	Large	
Region		Anterior < Posterior	0.00914	**	1.02	Large	
Region		Lateral < Medial	8E-07	***	1.87	Large	
Region		Lateral < Posterior	1E-07	***	2.04	Large	
Region		Medial < Posterior	0.95		0.165	Small	
Cortical Surface	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	2.41	Large	29%
Region		Anterior > Medial	1E-07	***	2.07	Large	
Region		Anterior < Posterior	0.0836	.	0.75	Medium	
Region		Lateral < Medial	0.686		0.342	Medium	
Region		Lateral < Posterior	0	***	3.16	Large	
Region		Medial < Posterior	0	***	2.82	Large	
Pore Surface	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.0192	*	0.934	Large	49%
Region		Anterior < Medial	0.0481	*	0.823	Large	
Region		Anterior < Posterior	0.0115	*	0.992	Large	
Region		Lateral < Medial	3.1E-06	***	1.76	Large	
Region		Lateral < Posterior	4E-07	***	1.93	Large	
Region		Medial < Posterior	0.947		0.169	Small	
Intersection Surface	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.536		0.417	Medium	75%
Region		Anterior < Medial	0.335		0.524	Medium	
Region		Anterior < Posterior	0.0237	*	0.91	Large	
Region		Lateral < Medial	0.985		0.107	Small	
Region		Lateral < Posterior	0.388		0.493	Medium	
Region		Medial < Posterior	0.598		0.386	Medium	
Group		Control > Fentanyl	0.0381	*	2.81	Large	72%
Group		Control > Morphine	0.259		1.7	Large	

Group		Fentanyl < Morpine	0.552		1.1	Large	
Region | Group	Anterior	Control > Fentanyl	0.119		2.09	Large	47%
Region | Group	Anterior	Control > Morphine	0.351		1.22	Large	
Region | Group	Anterior	Fentanyl < Morphine	0.507		0.866	Large	
Region | Group	Lateral	Control > Fentanyl	0.134		2	Large	
Region | Group	Lateral	Control > Morphine	0.361		1.2	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.536		0.807	Large	
Region | Group	Medial	Control > Fentanyl	0.224		1.61	Large	
Region | Group	Medial	Control > Morphine	0.523		0.833	Large	
Region | Group	Medial	Fentanyl < Morphine	0.552		0.775	Medium	
Region | Group	Posterior	Control > Fentanyl	0.183		1.77	Large	
Region | Group	Posterior	Control > Morphine	0.604		0.675	Medium	
Region | Group	Posterior	Fentanyl < Morphine	0.403		1.09	Large	
Group | Region	Control	Anterior > Lateral	0.635		0.0849	Small	
Group | Region	Control	Anterior > Medial	0.00888	**	0.482	Medium	
Group | Region	Control	Anterior > Posterior	0.0769	.	0.32	Medium	
Group | Region	Control	Lateral > Medial	0.0294	*	0.397	Medium	
Group | Region	Control	Lateral > Posterior	0.191		0.235	Medium	
Group | Region	Control	Medial < Posterior	0.366		0.162	Small	
Group | Region	Fentanyl	Anterior < Lateral	0.998		0.000389	Small	
Group | Region	Fentanyl	Anterior < Medial	1		2.44E-05	Small	
Group | Region	Fentanyl	Anterior < Posterior	0.998		0.000394	Small	
Group | Region	Fentanyl	Lateral > Medial	0.998		0.000365	Small	
Group | Region	Fentanyl	Lateral < Posterior	1		4.7E-06	Small	
Group | Region	Fentanyl	Medial < Posterior	0.998		0.00037	Small	
Group | Region	Morphine	Anterior > Lateral	0.743		0.0585	Small	
Group | Region	Morphine	Anterior > Medial	0.611		0.0908	Small	
Group | Region	Morphine	Anterior < Posterior	0.203		0.229	Medium	
Group | Region	Morphine	Lateral > Medial	0.856		0.0323	Small	
Group | Region	Morphine	Lateral < Posterior	0.112		0.287	Medium	
Group | Region	Morphine	Medial < Posterior	0.0776	.	0.319	Medium	
Pore Surface: PoreVolume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.00221	**	1.16	Large	67%
Region		Anterior > Medial	0.206		0.613	Medium	
Region		Anterior > Posterior	0.03	*	0.882	Large	
Region		Lateral > Medial	2.4E-06	***	1.78	Large	
Region		Lateral > Posterior	1E-07	***	2.05	Large	
Region		Medial > Posterior	0.82		0.269	Medium	
Group		Control < Fentanyl	0.0671	.	4.04	Large	100%
Group		Control < Morphine	0.34		2.43	Large	
Group		Fentanyl > Morphine	0.613		1.61	Large	

Region | Group	Anterior	Control < Fentanyl	0.0154	*	4.6	Large	49%
Region | Group	Anterior	Control < Morphine	0.0543	.	3.56	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.555		1.05	Large	
Region | Group	Lateral	Control < Fentanyl	0.0792	.	3.22	Large	
Region | Group	Lateral	Control < Morphine	0.31		1.81	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.43		1.4	Large	
Region | Group	Medial	Control < Fentanyl	0.0135	*	4.71	Large	
Region | Group	Medial	Control < Morphine	0.293		1.88	Large	
Region | Group	Medial	Fentanyl > Morphine	0.12		2.82	Large	
Region | Group	Posterior	Control < Fentanyl	0.0502	.	3.62	Large	
Region | Group	Posterior	Control < Morphine	0.172		2.47	Large	
Region | Group	Posterior	Fentanyl > Morphine	0.515		1.15	Large	
Group | Region	Control	Anterior < Lateral	0.000128	***	2.21	Large	
Group | Region	Control	Anterior > Medial	0.866		0.0906	Small	
Group | Region	Control	Anterior > Posterior	0.718		0.194	Small	
Group | Region	Control	Lateral > Medial	7.31E-05	***	2.3	Large	
Group | Region	Control	Lateral > Posterior	0.000038	***	2.4	Large	
Group | Region	Control	Medial > Posterior	0.848		0.103	Small	
Group | Region	Fentanyl	Anterior < Lateral	0.13		0.823	Large	
Group | Region	Fentanyl	Anterior < Medial	0.978		0.015	Small	
Group | Region	Fentanyl	Anterior > Posterior	0.0328	*	1.17	Large	
Group | Region	Fentanyl	Lateral > Medial	0.137		0.808	Large	
Group | Region	Fentanyl	Lateral > Posterior	0.000461	***	1.99	Large	
Group | Region	Fentanyl	Medial > Posterior	0.0307	*	1.19	Large	
Group | Region	Morphine	Anterior < Lateral	0.388		0.465	Medium	
Group | Region	Morphine	Anterior > Medial	0.00172	**	1.76	Large	
Group | Region	Morphine	Anterior > Posterior	0.0201	*	1.28	Large	
Group | Region	Morphine	Lateral > Medial	0.000111	***	2.23	Large	
Group | Region	Morphine	Lateral > Posterior	0.0019	**	1.75	Large	
Group | Region	Morphine	Medial < Posterior	0.37		0.483	Medium	
Pore Surface: Cortical Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.349		0.516	Medium	26%
Region		Anterior < Medial	0	***	2.24	Large	
Region		Anterior < Posterior	0.00317	**	1.13	Large	
Region		Lateral < Medial	4.7E-06	***	1.72	Large	
Region		Lateral < Posterior	0.206		0.613	Medium	
Region		Medial > Posterior	0.00385	**	1.11	Large	
Pore Thickness	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	9.38E-05	***	1.46	Large	74%
Region		Anterior < Medial	0.152		0.662	Medium	

Region		Anterior < Posterior	0.0617	.	0.791	Medium	
Region		Lateral < Medial	0	***	2.12	Large	
Region		Lateral < Posterior	0	***	2.25	Large	
Region		Medial < Posterior	0.976		0.128	Small	
Group		Control > Fentanyl	0.015	*	2.62	Large	100%
Group		Control > Morphine	0.215		1.46	Large	
Group		Fentanyl < Morpine	0.367		1.16	Large	
Pore Separation	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.007	**	1.05	Large	29%
Region		Anterior > Medial	0	***	2.58	Large	
Region		Anterior > Posterior	0.0123	*	0.985	Large	
Region		Lateral > Medial	3.92E-05	***	1.54	Large	
Region		Lateral < Posterior	0.997		0.0612	Small	
Region		Medial < Posterior	1.94E-05	***	1.6	Large	
Pore Tb.N	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.00177	**	1.19	Large	30%
Region		Anterior < Medial	1.1E-06	***	1.85	Large	
Region		Anterior < Posterior	0.00768	**	1.04	Large	
Region		Lateral < Medial	0.155		0.659	Medium	
Region		Lateral > Posterior	0.961		0.151	Small	
Region		Medial > Posterior	0.0535	.	0.809	Large	
Degree of Anisotropy	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	6E-07	***	1.9	Large	53%
Region		Anterior < Medial	8.2E-06	***	1.67	Large	
Region		Anterior < Posterior	0.0361	*	0.859	Large	
Region		Lateral < Medial	0	***	3.57	Large	
Region		Lateral < Posterior	0	***	2.75	Large	
Region		Medial > Posterior	0.0513	.	0.815	Large	
Region | Group	Anterior	Control > Fentanyl	0.172		1.43	Large	39%
Region | Group	Anterior	Control > Morphine	0.18		1.4	Large	
Region | Group	Anterior	Fentanyl < Morphine	0.978		0.0283	Small	
Region | Group	Lateral	Control > Fentanyl	0.801		0.26	Medium	
Region | Group	Lateral	Control < Morphine	0.348		0.975	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.237		1.23	Large	
Region | Group	Medial	Control > Fentanyl	0.544		0.628	Medium	
Region | Group	Medial	Control < Morphine	0.518		0.669	Medium	
Region | Group	Medial	Fentanyl < Morphine	0.215		1.3	Large	
Region | Group	Posterior	Control > Fentanyl	0.393		0.887	Large	
Region | Group	Posterior	Control < Morphine	0.892		0.14	Small	

Region | Group	Posterior	Fentanyl < Morphine	0.323		1.03	Large	
Group | Region	Control	Anterior > Lateral	4E-07	***	3.08	Large	
Group | Region	Control	Anterior < Medial	0.187		0.715	Medium	
Group | Region	Control	Anterior < Posterior	0.762		0.163	Small	
Group | Region	Control	Lateral < Medial	0	***	3.79	Large	
Group | Region	Control	Lateral < Posterior	1E-07	***	3.24	Large	
Group | Region	Control	Medial > Posterior	0.307		0.552	Medium	
Group | Region	Fentanyl	Anterior > Lateral	0.000763	***	1.91	Large	
Group | Region	Fentanyl	Anterior < Medial	0.00632	**	1.52	Large	
Group | Region	Fentanyl	Anterior < Posterior	0.191		0.708	Medium	
Group | Region	Fentanyl	Lateral < Medial	0	***	3.43	Large	
Group | Region	Fentanyl	Lateral < Posterior	9.4E-06	***	2.62	Large	
Group | Region	Fentanyl	Medial > Posterior	0.135		0.811	Large	
Group | Region	Morphine	Anterior > Lateral	0.195		0.701	Medium	
Group | Region	Morphine	Anterior < Medial	0.000003	***	2.79	Large	
Group | Region	Morphine	Anterior < Posterior	0.00235	**	1.71	Large	
Group | Region	Morphine	Lateral < Medial	0	***	3.49	Large	
Group | Region	Morphine	Lateral < Posterior	3.61E-05	***	2.41	Large	
Group | Region	Morphine	Medial > Posterior	0.0481	*	1.08	Large	
Pore Fractal	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.991		0.0907	Small	35%
Region		Anterior < Medial	0	***	2.53	Large	
Region		Anterior < Posterior	0.0134	*	0.975	Large	
Region		Lateral < Medial	0	***	2.62	Large	
Region		Lateral < Posterior	0.00581	**	1.07	Large	
Region		Medial > Posterior	3.19E-05	***	1.56	Large	
Region | Group	Anterior	Control > Fentanyl	0.356		1.19	Large	50%
Region | Group	Anterior	Control > Morphine	0.15		1.88	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.591		0.687	Medium	
Region | Group	Lateral	Control < Fentanyl	0.783		0.351	Medium	
Region | Group	Lateral	Control > Morphine	0.851		0.239	Medium	
Region | Group	Lateral	Fentanyl > Morphine	0.644		0.59	Medium	
Region | Group	Medial	Control > Fentanyl	0.396		1.09	Large	
Region | Group	Medial	Control > Morphine	0.297		1.35	Large	
Region | Group	Medial	Fentanyl > Morphine	0.841		0.256	Medium	
Region | Group	Posterior	Control > Fentanyl	0.459		0.948	Large	
Region | Group	Posterior	Control > Morphine	0.224		1.57	Large	
Region | Group	Posterior	Fentanyl > Morphine	0.625		0.625	Medium	
Group | Region	Control	Anterior > Lateral	0.0451	*	1.16	Large	
Group | Region	Control	Anterior < Medial	0.000199	***	2.26	Large	
Group | Region	Control	Anterior < Posterior	0.118		0.898	Large	

Group | Region	Control	Lateral < Medial	1E-07	***	3.42	Large	
Group | Region	Control	Lateral < Posterior	0.000612	***	2.06	Large	
Group | Region	Control	Medial > Posterior	0.0196	*	1.36	Large	
Group | Region	Fentanyl	Anterior < Lateral	0.506		0.379	Medium	
Group | Region	Fentanyl	Anterior < Medial	0.000113	***	2.35	Large	
Group | Region	Fentanyl	Anterior < Posterior	0.0493	*	1.14	Large	
Group | Region	Fentanyl	Lateral < Medial	0.000954	***	1.98	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.185		0.758	Medium	
Group | Region	Fentanyl	Medial > Posterior	0.0357	*	1.22	Large	
Group | Region	Morphine	Anterior < Lateral	0.403		0.476	Medium	
Group | Region	Morphine	Anterior < Medial	8.2E-06	***	2.79	Large	
Group | Region	Morphine	Anterior < Posterior	0.0384	*	1.2	Large	
Group | Region	Morphine	Lateral < Medial	0.000146	***	2.31	Large	
Group | Region	Morphine	Lateral < Posterior	0.206		0.723	Medium	
Group | Region	Morphine	Medial > Posterior	0.00694	**	1.59	Large	
Number of Pores	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	3E-07	***	1.95	Large	23%
Region		Anterior < Medial	0.0432	*	0.837	Large	
Region		Anterior > Posterior	0.151		0.663	Medium	
Region		Lateral < Medial	0	***	2.79	Large	
Region		Lateral < Posterior	0.000614	***	1.29	Large	
Region		Medial > Posterior	6.12E-05	***	1.5	Large	
Pore Density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.0792	.	0.757	Medium	42%
Region		Anterior < Medial	0	***	2.6	Large	
Region		Anterior > Posterior	0.644		0.363	Medium	
Region		Lateral < Medial	1.1E-06	***	1.84	Large	
Region		Lateral > Posterior	0.00343	**	1.12	Large	
Region		Medial > Posterior	0	***	2.96	Large	
Region | Group	Anterior	Control < Fentanyl	0.217		2.64	Large	39%
Region | Group	Anterior	Control < Morphine	0.471		1.52	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.595		1.12	Large	
Region | Group	Lateral	Control < Fentanyl	0.166		2.98	Large	
Region | Group	Lateral	Control < Morphine	0.52		1.36	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.442		1.62	Large	
Region | Group	Medial	Control < Fentanyl	0.0881	.	3.71	Large	
Region | Group	Medial	Control < Morphine	0.618		1.05	Large	
Region | Group	Medial	Fentanyl > Morphine	0.213		2.66	Large	
Region | Group	Posterior	Control < Fentanyl	0.297		2.21	Large	
Region | Group	Posterior	Control < Morphine	0.499		1.43	Large	

Region | Group	Posterior	Fentanyl > Morphine	0.707		0.788	Medium	
Group | Region	Control	Anterior < Lateral	0.196		0.699	Medium	
Group | Region	Control	Anterior < Medial	3.83E-05	***	2.4	Large	
Group | Region	Control	Anterior > Posterior	0.723		0.19	Small	
Group | Region	Control	Lateral < Medial	0.00245	**	1.7	Large	
Group | Region	Control	Lateral > Posterior	0.102		0.889	Large	
Group | Region	Control	Medial > Posterior	1.12E-05	***	2.59	Large	
Group | Region	Fentanyl	Anterior < Lateral	0.0573	.	1.04	Large	
Group | Region	Fentanyl	Anterior < Medial	0	***	3.47	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.256		0.614	Medium	
Group | Region	Fentanyl	Lateral < Medial	3.09E-05	***	2.43	Large	
Group | Region	Fentanyl	Lateral > Posterior	0.00316	**	1.65	Large	
Group | Region	Fentanyl	Medial > Posterior	0	***	4.08	Large	
Group | Region	Morphine	Anterior < Lateral	0.322		0.534	Medium	
Group | Region	Morphine	Anterior < Medial	0.000686	***	1.93	Large	
Group | Region	Morphine	Anterior > Posterior	0.596		0.285	Medium	
Group | Region	Morphine	Lateral < Medial	0.0119	*	1.39	Large	
Group | Region	Morphine	Lateral > Posterior	0.131		0.819	Large	
Group | Region	Morphine	Medial > Posterior	0.000124	***	2.21	Large	
Euler number	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	2.4E-06	***	1.78	Large	47%
Region		Anterior < Medial	0.405		0.484	Medium	
Region		Anterior > Posterior	0.000582	***	1.29	Large	
Region		Lateral < Medial	0	***	2.26	Large	
Region		Lateral < Posterior	0.406		0.484	Medium	
Region		Medial > Posterior	2.4E-06	***	1.78	Large	
Region | Group	Anterior	Control < Fentanyl	0.284		1.63	Large	35%
Region | Group	Anterior	Control < Morphine	0.591		0.808	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.586		0.819	Large	
Region | Group	Lateral	Control < Fentanyl	0.655		0.672	Medium	
Region | Group	Lateral	Control < Morphine	0.823		0.335	Medium	
Region | Group	Lateral	Fentanyl > Morphine	0.823		0.336	Medium	
Region | Group	Medial	Control < Fentanyl	0.231		1.82	Large	
Region | Group	Medial	Control < Morphine	0.943		0.107	Small	
Region | Group	Medial	Fentanyl > Morphine	0.258		1.72	Large	
Region | Group	Posterior	Control < Fentanyl	0.747		0.484	Medium	
Region | Group	Posterior	Control < Morphine	0.528		0.95	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.756		0.466	Medium	
Group | Region	Control	Anterior > Lateral	0.0182	*	1.3	Large	
Group | Region	Control	Anterior < Medial	0.228		0.652	Medium	
Group | Region	Control	Anterior > Posterior	0.0781	.	0.96	Large	

Group | Region	Control	Lateral < Medial	0.000585	***	1.95	Large	
Group | Region	Control	Lateral < Posterior	0.526		0.341	Medium	
Group | Region	Control	Medial > Posterior	0.00391	**	1.61	Large	
Group | Region	Fentanyl	Anterior > Lateral	9.33E-05	***	2.26	Large	
Group | Region	Fentanyl	Anterior < Medial	0.118		0.849	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.00024	***	2.1	Large	
Group | Region	Fentanyl	Lateral < Medial	3E-07	***	3.11	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.775		0.154	Small	
Group | Region	Fentanyl	Medial > Posterior	0.000001	***	2.95	Large	
Group | Region	Morphine	Anterior > Lateral	0.00162	**	1.77	Large	
Group | Region	Morphine	Anterior > Medial	0.926		0.0497	Small	
Group | Region	Morphine	Anterior > Posterior	0.132		0.818	Large	
Group | Region	Morphine	Lateral < Medial	0.00213	**	1.72	Large	
Group | Region	Morphine	Lateral < Posterior	0.0794	.	0.956	Large	
Group | Region	Morphine	Medial > Posterior	0.156		0.769	Medium	
Connectivity	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.302		0.544	Medium	43%
Region		Anterior < Medial	0.088	.	0.743	Medium	
Region		Anterior < Posterior	0.00472	**	1.09	Large	
Region		Lateral < Medial	0.000628	***	1.29	Large	
Region		Lateral < Posterior	1.35E-05	***	1.63	Large	
Region		Medial < Posterior	0.681		0.345	Medium	
Connectivity density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.104		0.719	Medium	29%
Region		Anterior < Medial	0.00137	**	1.21	Large	
Region		Anterior < Posterior	0.0139	*	0.971	Large	
Region		Lateral < Medial	0.389		0.493	Medium	
Region		Lateral < Posterior	0.846		0.252	Medium	
Region		Medial > Posterior	0.863		0.241	Medium	
SD Pore Thickness	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	9.02E-05	***	1.46	Large	56%
Region		Anterior < Medial	0.353		0.513	Medium	
Region		Anterior < Posterior	0.569		0.4	Medium	
Region		Lateral < Medial	2E-07	***	1.98	Large	
Region		Lateral < Posterior	9E-07	***	1.87	Large	
Region		Medial > Posterior	0.983		0.113	Small	
Group		Control > Fentanyl	0.0274	*	2.3	Large	98%
Group		Control > Morphine	0.406		1.06	Large	
Group		Fentanyl < Morpine	0.299		1.24	Large	

SD Pore Separation	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.00282	**	1.14	Large	47%
Region		Anterior > Medial	7E-07	***	1.88	Large	
Region		Anterior > Posterior	0.0137	*	0.973	Large	
Region		Lateral > Medial	0.087	.	0.744	Medium	
Region		Lateral < Posterior	0.948		0.167	Small	
Region		Medial < Posterior	0.0234	*	0.911	Large	
Avg.Por.Vol	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.999		0.0478	Small	77%
Region		Anterior < Medial	0.0619	.	0.79	Medium	
Region		Anterior < Posterior	0.000392	***	1.33	Large	
Region		Lateral < Medial	0.0427	*	0.838	Large	
Region		Lateral < Posterior	0.000234	***	1.38	Large	
Region		Medial < Posterior	0.308		0.541	Medium	
Group		Control > Fentanyl	0.0719	.	1.84	Large	95%
Group		Control > Morphine	0.145		1.54	Large	
Group		Fentanyl < Morpine	0.923		0.296	Medium	
Region | Group	Anterior	Control > Fentanyl	0.0833	.	1.91	Large	35%
Region | Group	Anterior	Control > Morphine	0.303		1.11	Large	
Region | Group	Anterior	Fentanyl < Morphine	0.458		0.796	Medium	
Region | Group	Lateral	Control > Fentanyl	0.67		0.454	Medium	
Region | Group	Lateral	Control > Morphine	0.897		0.138	Small	
Region | Group	Lateral	Fentanyl < Morphine	0.767		0.316	Medium	
Region | Group	Medial	Control > Fentanyl	0.656		0.475	Medium	
Region | Group	Medial	Control > Morphine	0.739		0.356	Medium	
Region | Group	Medial	Fentanyl < Morphine	0.911		0.119	Small	
Region | Group	Posterior	Control > Fentanyl	0.22		1.33	Large	
Region | Group	Posterior	Control > Morphine	0.786		0.289	Medium	
Region | Group	Posterior	Fentanyl < Morphine	0.335		1.04	Large	
Group | Region	Control	Anterior > Lateral	0.02	*	1.08	Large	
Group | Region	Control	Anterior > Medial	0.0614	.	0.858	Large	
Group | Region	Control	Anterior > Posterior	0.203		0.579	Medium	
Group | Region	Control	Lateral < Medial	0.628		0.219	Medium	
Group | Region	Control	Lateral < Posterior	0.272		0.498	Medium	
Group | Region	Control	Medial < Posterior	0.537		0.279	Medium	
Group | Region	Fentanyl	Anterior < Lateral	0.409		0.374	Medium	
Group | Region	Fentanyl	Anterior < Medial	0.208		0.572	Medium	
Group | Region	Fentanyl	Anterior < Posterior	1		9.3E-06	Small	
Group | Region	Fentanyl	Lateral < Medial	0.66		0.198	Small	
Group | Region	Fentanyl	Lateral > Posterior	0.409		0.374	Medium	

Group | Region	Fentanyl	Medial > Posterior	0.208		0.572	Medium	
Group | Region	Morphine	Anterior > Lateral	0.815		0.105	Small	
Group | Region	Morphine	Anterior > Medial	0.817		0.104	Small	
Group | Region	Morphine	Anterior < Posterior	0.592		0.242	Medium	
Group | Region	Morphine	Lateral < Medial	0.998		0.00108	Small	
Group | Region	Morphine	Lateral < Posterior	0.443		0.347	Medium	
Group | Region	Morphine	Medial < Posterior	0.444		0.346	Medium	
Avg.Por.Surf	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.349		0.516	Medium	57%
Region		Anterior < Medial	0.0427	*	0.838	Large	
Region		Anterior < Posterior	3.48E-05	***	1.55	Large	
Region		Lateral < Medial	0.724		0.323	Medium	
Region		Lateral < Posterior	0.00787	**	1.03	Large	
Region		Medial < Posterior	0.11		0.711	Medium	
Group		Control > Fentanyl	0.151		1.56	Large	91%
Group		Control > Morphine	0.166		1.52	Large	
Group		Fentanyl < Morpine	0.998		0.0449	Small	
Region | Group	Anterior	Control > Fentanyl	0.159		1.53	Large	47%
Region | Group	Anterior	Control > Morphine	0.526		0.676	Medium	
Region | Group	Anterior	Fentanyl < Morphine	0.423		0.856	Large	
Region | Group	Lateral	Control > Fentanyl	0.59		0.573	Medium	
Region | Group	Lateral	Control > Morphine	0.76		0.324	Medium	
Region | Group	Lateral	Fentanyl < Morphine	0.814		0.249	Medium	
Region | Group	Medial	Control > Fentanyl	0.486		0.743	Medium	
Region | Group	Medial	Control > Morphine	0.614		0.536	Medium	
Region | Group	Medial	Fentanyl < Morphine	0.845		0.207	Medium	
Region | Group	Posterior	Control > Fentanyl	0.145		1.59	Large	
Region | Group	Posterior	Control > Morphine	0.543		0.648	Medium	
Region | Group	Posterior	Fentanyl < Morphine	0.38		0.938	Large	
Group | Region	Control	Anterior > Lateral	0.146		0.444	Medium	
Group | Region	Control	Anterior > Medial	0.404		0.253	Medium	
Group | Region	Control	Anterior < Posterior	0.853		0.056	Small	
Group | Region	Control	Lateral < Medial	0.527		0.191	Small	
Group | Region	Control	Lateral < Posterior	0.102		0.5	Medium	
Group | Region	Control	Medial < Posterior	0.309		0.309	Medium	
Group | Region	Fentanyl	Anterior < Lateral	0.093	.	0.514	Medium	
Group | Region	Fentanyl	Anterior < Medial	0.0807	.	0.535	Medium	
Group | Region	Fentanyl	Anterior < Posterior	0.999		0.000501	Small	
Group | Region	Fentanyl	Lateral < Medial	0.944		0.021	Small	
Group | Region	Fentanyl	Lateral > Posterior	0.0933	.	0.514	Medium	
Group | Region	Fentanyl	Medial > Posterior	0.081	.	0.535	Medium	

Group | Region	Morphine	Anterior > Lateral	0.76		0.0924	Small	
Group | Region	Morphine	Anterior > Medial	0.707		0.114	Small	
Group | Region	Morphine	Anterior < Posterior	0.783		0.0834	Small	
Group | Region	Morphine	Lateral > Medial	0.944		0.0212	Small	
Group | Region	Morphine	Lateral < Posterior	0.561		0.176	Small	
Group | Region	Morphine	Medial < Posterior	0.515		0.197	Small	
Avg.Orient.Theta	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0	***	2.25	Large	36%
Region		Anterior > Medial	0	***	2.38	Large	
Region		Anterior < Posterior	0.386		0.494	Medium	
Region		Lateral > Medial	0	***	4.63	Large	
Region		Lateral > Posterior	3.1E-06	***	1.76	Large	
Region		Medial < Posterior	0	***	2.87	Large	
Avg.Orient.Phi	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0	***	3.95	Large	50%
Region		Anterior < Medial	0	***	3.17	Large	
Region		Anterior < Posterior	0	***	7.42	Large	
Region		Lateral > Medial	0.0657	.	0.782	Medium	
Region		Lateral < Posterior	0	***	3.47	Large	
Region		Medial < Posterior	0	***	4.26	Large	
Group		Control > Fentanyl	0.264		0.507	Medium	100%
Group		Control < Morphine	0.658		0.276	Medium	
Group		Fentanyl < Morpine	0.0553	.	0.783	Medium	
Avg.Maj.Por.D	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.00428	**	1.1	Large	29%
Region		Anterior < Medial	0.0647	.	0.785	Medium	
Region		Anterior < Posterior	1.54E-05	***	1.62	Large	
Region		Lateral > Medial	0.742		0.313	Medium	
Region		Lateral < Posterior	0.337		0.523	Medium	
Region		Medial < Posterior	0.0435	*	0.836	Large	
Sphericity	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	2.12	Large	54%
Region		Anterior > Medial	0.000713	***	1.27	Large	
Region		Anterior > Posterior	0	***	2.92	Large	
Region		Lateral < Medial	0.0402	*	0.846	Large	
Region		Lateral > Posterior	0.0564	.	0.803	Large	
Region		Medial > Posterior	1.11E-05	***	1.65	Large	

Femoral Regions: All Directional Trends

Cortical Fractal Trends		
Region		Medial > Posterior > Lateral > Anterior
Group		Control > Fentanyl > Morphine
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Control > Fentanyl > Morphine
Group | Region	Control	Medial > Posterior > Lateral > Anterior
Group | Region	Fentanyl	Medial > Posterior > Lateral > Anterior
Group | Region	Morphine	Medial > Posterior > Lateral > Anterior
Number Closed Pores Trends		
Region		Medial > Anterior > Posterior > Lateral
Group		Fentanyl > Control > Morphine
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Control > Morphine
Group | Region	Control	Medial > Anterior > Posterior > Lateral
Group | Region	Fentanyl	Medial > Anterior > Posterior > Lateral
Group | Region	Morphine	Medial > Anterior > Posterior > Lateral
Number Open Pores Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Fentanyl > Control > Morphine
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Control > Morphine
Group | Region	Control	Posterior > Medial > Anterior > Lateral
Group | Region	Fentanyl	Posterior > Medial > Lateral > Anterior
Group | Region	Morphine	Posterior > Medial > Anterior > Lateral
Closed Pore Density Trends		
Region		Medial > Lateral > Anterior > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control

Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Medial > Lateral > Anterior > Posterior
Group | Region	Fentanyl	Medial > Lateral > Anterior > Posterior
Group | Region	Morphine	Medial > Lateral > Anterior > Posterior
Open Pore Density Trends		
Region		Medial > Lateral > Posterior > Anterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Medial > Lateral > Posterior > Anterior
Group | Region	Fentanyl	Medial > Lateral > Posterior > Anterior
Group | Region	Morphine	Lateral > Medial > Posterior > Anterior
Closed Porosity (%) Trends		
Region		Medial > Lateral > Posterior > Anterior
Group		Fentanyl > Control > Morphine
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Medial > Lateral > Posterior > Anterior
Group | Region	Fentanyl	Medial > Lateral > Posterior > Anterior
Group | Region	Morphine	Medial > Lateral > Posterior > Anterior
Open Porosity (%) Trends		
Region		Medial > Posterior > Anterior > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Medial > Anterior > Posterior > Lateral
Group | Region	Fentanyl	Posterior > Medial > Lateral > Anterior
Group | Region	Morphine	Medial > Posterior > Anterior > Lateral
Total Porosity (%) Trends		
Region		Medial > Posterior > Anterior > Lateral
Group		Control > Fentanyl > Morphine
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl

Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Medial > Posterior > Anterior > Lateral
Group | Region	Fentanyl	Posterior > Medial > Lateral > Anterior
Group | Region	Morphine	Medial > Posterior > Lateral > Anterior
Cortical Volume Trends		
Region		Anterior > Posterior > Medial > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Anterior > Posterior > Medial > Lateral
Group | Region	Fentanyl	Anterior > Posterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Pore Volume Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Anterior > Posterior > Medial > Lateral
Group | Region	Fentanyl	Posterior > Medial > Anterior > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Cortical Surface Trends		
Region		Posterior > Anterior > Medial > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Anterior > Medial > Lateral
Group | Region	Fentanyl	Posterior > Anterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Pore Surface Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl

Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Anterior > Medial > Lateral
Group | Region	Fentanyl	Posterior > Medial > Anterior > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Intersection Surface Trends		
Region		Posterior > Medial > Lateral > Anterior
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Anterior > Lateral > Posterior > Medial
Group | Region	Fentanyl	Posterior > Lateral > Medial > Anterior
Group | Region	Morphine	Posterior > Anterior > Lateral > Medial
Pore Surface:PoreVolume Trends		
Region		Lateral > Anterior > Medial > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Lateral > Anterior > Medial > Posterior
Group | Region	Fentanyl	Lateral > Medial > Anterior > Posterior
Group | Region	Morphine	Lateral > Anterior > Posterior > Medial
Pore Surface:Cortical Volume Trends		
Region		Medial > Posterior > Lateral > Anterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Control > Morphine
Group | Region	Control	Medial > Posterior > Anterior > Lateral
Group | Region	Fentanyl	Medial > Posterior > Lateral > Anterior
Group | Region	Morphine	Medial > Posterior > Lateral > Anterior
Pore Thickness Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl

Group | Region	Control	Anterior > Medial > Lateral > Posterior
Group | Region	Fentanyl	Medial > Posterior > Anterior > Lateral
Group | Region	Morphine	Posterior > Medial > Anterior > Lateral
Pore Separation Trends		
Region		Anterior > Posterior > Lateral > Medial
Group		Morphine > Control > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Anterior > Lateral > Posterior > Medial
Group | Region	Fentanyl	Anterior > Posterior > Lateral > Medial
Group | Region	Morphine	Anterior > Posterior > Lateral > Medial
Pore Tb.N Trends		
Region		Medial > Lateral > Posterior > Anterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Medial > Posterior > Lateral > Anterior
Group | Region	Fentanyl	Medial > Lateral > Posterior > Anterior
Group | Region	Morphine	Medial > Lateral > Posterior > Anterior
Degree of Anisotropy Trends		
Region		Medial > Posterior > Anterior > Lateral
Group		Morphine > Control > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Medial > Posterior > Anterior > Lateral
Group | Region	Fentanyl	Medial > Posterior > Anterior > Lateral
Group | Region	Morphine	Medial > Posterior > Anterior > Lateral
Pore Fractal Trends		
Region		Medial > Posterior > Anterior > Lateral
Group		Control > Fentanyl > Morphine
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Control > Fentanyl > Morphine
Group | Region	Control	Medial > Posterior > Anterior > Lateral

Group | Region	Fentanyl	Medial > Posterior > Lateral > Anterior
Group | Region	Morphine	Medial > Posterior > Lateral > Anterior
Number of Pores Trends		
Region		Medial > Anterior > Posterior > Lateral
Group		Fentanyl > Control > Morphine
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Control > Morphine
Group | Region	Control	Medial > Anterior > Posterior > Lateral
Group | Region	Fentanyl	Medial > Anterior > Posterior > Lateral
Group | Region	Morphine	Medial > Anterior > Posterior > Lateral
Pore Density Trends		
Region		Medial > Lateral > Anterior > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Medial > Lateral > Anterior > Posterior
Group | Region	Fentanyl	Medial > Lateral > Anterior > Posterior
Group | Region	Morphine	Medial > Lateral > Anterior > Posterior
Euler number Trends		
Region		Medial > Anterior > Posterior > Lateral
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Medial > Anterior > Posterior > Lateral
Group | Region	Fentanyl	Medial > Anterior > Posterior > Lateral
Group | Region	Morphine	Anterior > Medial > Posterior > Lateral
Connectivity Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Control > Fentanyl > Morphine
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Fentanyl > Morphine
Group | Region	Control	Posterior > Medial > Anterior > Lateral
Group | Region	Fentanyl	Posterior > Medial > Lateral > Anterior

Group | Region	Morphine	Medial > Posterior > Anterior > Lateral
Connectivity density Trends		
Region		Medial > Posterior > Lateral > Anterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Medial > Posterior > Lateral > Anterior
Group | Region	Fentanyl	Lateral > Medial > Posterior > Anterior
Group | Region	Morphine	Medial > Posterior > Lateral > Anterior
SD Pore Thickness Trends		
Region		Medial > Posterior > Anterior > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Anterior > Medial > Lateral > Posterior
Group | Region	Fentanyl	Medial > Posterior > Anterior > Lateral
Group | Region	Morphine	Posterior > Medial > Anterior > Lateral
SD Pore Separation Trends		
Region		Anterior > Posterior > Lateral > Medial
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Anterior > Lateral > Medial > Posterior
Group | Region	Fentanyl	Anterior > Posterior > Lateral > Medial
Group | Region	Morphine	Anterior > Posterior > Lateral > Medial
Avg. Por. Vol. Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Anterior > Posterior > Medial > Lateral
Group | Region	Fentanyl	Medial > Lateral > Posterior > Anterior
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral

Avg. Por. Surf Trends		
Region		Posterior > Medial > Lateral > Anterior
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Anterior > Medial > Lateral
Group | Region	Fentanyl	Medial > Lateral > Posterior > Anterior
Group | Region	Morphine	Posterior > Anterior > Lateral > Medial
Avg. Orientation theta Trends		
Region		Lateral > Posterior > Anterior > Medial
Group		Fentanyl > Control > Morphine
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Fentanyl > Control > Morphine
Group | Region	Control	Lateral > Posterior > Anterior > Medial
Group | Region	Fentanyl	Lateral > Posterior > Anterior > Medial
Group | Region	Morphine	Lateral > Anterior > Posterior > Medial
Avg. Orientation phi Trends		
Region		Posterior > Lateral > Medial > Anterior
Group		Morphine > Control > Fentanyl
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Posterior > Lateral > Medial > Anterior
Group | Region	Fentanyl	Posterior > Lateral > Medial > Anterior
Group | Region	Morphine	Posterior > Lateral > Medial > Anterior
Avg. Major Pore diameter Trends		
Region		Posterior > Lateral > Medial > Anterior
Group		Control > Fentanyl > Morphine
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Medial > Lateral > Anterior
Group | Region	Fentanyl	Lateral > Medial > Posterior > Anterior
Group | Region	Morphine	Posterior > Medial > Anterior > Lateral
Sphericity Trends		

Region		Anterior > Medial > Lateral > Posterior
Group		Morphine > Control > Fentanyl
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Anterior > Medial > Lateral > Posterior
Group | Region	Fentanyl	Anterior > Medial > Lateral > Posterior
Group | Region	Morphine	Anterior > Medial > Lateral > Posterior

Appendix XXIII: Micro-CT Linear Mixed Model for Tibial Regions

Tibial Regions: LMM Fixed Effects and Random Effects

Cortical Fractal	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.203	-0.366	0.386	0	1		0.993	
Region1	-0.163	0.0887	-0.32	0.0184	-1.84	0.0711	.	0.0712	.
Region2	-0.0466	0.0887	-0.201	0.124	-0.525	0.602		0.581	
Region3	0.237	0.0887	0.0593	0.402	2.67	0.0099	**	0.00772	**
Group1	-0.336	0.287	-0.885	0.245	-1.17	0.257		0.254	
Group2	0.188	0.287	-0.388	0.8	0.655	0.521		0.545	
Region1:Group1	-0.0467	0.125	-0.325	0.197	-0.372	0.711		0.704	
Region2:Group1	-0.208	0.125	-0.464	0.0717	-1.66	0.102		0.102	
Region3:Group1	0.102	0.125	-0.152	0.363	0.81	0.421		0.418	
Region1:Group2	0.283	0.125	0.027	0.53	2.26	0.0282	*	0.0309	*
Region2:Group2	0.0324	0.125	-0.207	0.286	0.258	0.798		0.818	
Region3:Group2	-0.0482	0.125	-0.308	0.187	-0.384	0.702		0.704	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.808	0.899	0.636	1.33	78.6	4.66E-15	***	R^2M/R^2C	0.0946 / 0.806
Residual	0.22	0.469	0.371	0.542	21.4			AIC/BIC	213 / 247
Number Closed Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.2	-0.394	0.36	0	1		0.999	
Region1	0.399	0.0778	0.247	0.552	5.13	0.000004	***	0.000175	***
Region2	0.00882	0.0778	-0.123	0.163	0.113	0.91		0.918	
Region3	-0.185	0.0778	-0.319	-0.0421	-2.38	0.0207	*	0.0204	*
Group1	-0.394	0.282	-0.94	0.125	-1.39	0.18		0.187	
Group2	0.303	0.282	-0.183	0.901	1.07	0.298		0.317	
Region1:Group1	-0.0835	0.11	-0.299	0.121	-0.759	0.451		0.451	
Region2:Group1	-0.0801	0.11	-0.319	0.133	-0.728	0.47		0.479	
Region3:Group1	0.192	0.11	-0.0484	0.426	1.75	0.086	.	0.094	.
Region1:Group2	0.234	0.11	0.0156	0.45	2.13	0.0377	*	0.0386	*
Region2:Group2	-0.0825	0.11	-0.27	0.125	-0.75	0.457		0.455	
Region3:Group2	-0.126	0.11	-0.345	0.0966	-1.14	0.258		0.273	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.795	0.892	0.621	1.29	82.4	2.79E-17	***	R^2M/R^2C	0.145 / 0.85
Residual	0.169	0.412	0.328	0.474	17.6			AIC/BIC	199 / 233
Number Open Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.

(Intercept)	0	0.202	-0.403	0.412	0	1		0.998	
Region1	0.401	0.0834	0.247	0.552	4.81	1.25E-05	***	0.000175	***
Region2	-0.314	0.0834	-0.467	-0.158	-3.76	0.000418	***	0.000175	***
Region3	-0.0248	0.0834	-0.192	0.144	-0.298	0.767		0.786	
Group1	-0.243	0.285	-0.806	0.337	-0.85	0.406		0.396	
Group2	0.219	0.285	-0.348	0.75	0.766	0.454		0.46	
Region1:Group1	0.000371	0.118	-0.213	0.243	0.00315	0.998		0.994	
Region2:Group1	-0.155	0.118	-0.423	0.0687	-1.31	0.195		0.199	
Region3:Group1	0.185	0.118	-0.0415	0.433	1.57	0.122		0.124	
Region1:Group2	0.283	0.118	0.0359	0.515	2.4	0.0197	*	0.0175	*
Region2:Group2	0.0371	0.118	-0.213	0.271	0.314	0.755		0.761	
Region3:Group2	-0.127	0.118	-0.356	0.106	-1.07	0.288		0.286	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.806	0.898	0.636	1.3	80.6	3.85E-16	***	R^2M/R^2C	0.116 / 0.828
Residual	0.195	0.441	0.349	0.512	19.4			AIC/BIC	207 / 241
Closed Pore Density	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.197	-0.441	0.395	0	1		0.998	
Region1	-0.197	0.0771	-0.337	-0.0532	-2.55	0.0135	*	0.0133	*
Region2	0.429	0.0771	0.289	0.585	5.56	9E-07	***	0.000175	***
Region3	0.198	0.0771	0.0586	0.348	2.56	0.0131	*	0.0147	*
Group1	-0.291	0.278	-0.873	0.281	-1.05	0.309		0.313	
Group2	0.266	0.278	-0.277	0.8	0.955	0.352		0.358	
Region1:Group1	0.0292	0.109	-0.169	0.245	0.268	0.79		0.789	
Region2:Group1	-0.23	0.109	-0.45	-0.0307	-2.11	0.0395	*	0.0474	*
Region3:Group1	0.231	0.109	0.00349	0.442	2.12	0.0388	*	0.0389	*
Region1:Group2	0.132	0.109	-0.0705	0.327	1.21	0.232		0.22	
Region2:Group2	0.00379	0.109	-0.218	0.22	0.0347	0.972		0.972	
Region3:Group2	-0.115	0.109	-0.321	0.105	-1.05	0.297		0.285	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.773	0.879	0.629	1.28	82.3	3.55E-17	***	R^2M/R^2C	0.165 / 0.852
Residual	0.167	0.408	0.322	0.473	17.7			AIC/BIC	197 / 231
Open Pore Density	**Lambda = -0.225**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.2	-0.387	0.402	0	1		0.999	
Region1	-0.259	0.0903	-0.443	-0.0912	-2.87	0.00581	**	0.00456	**
Region2	0.0458	0.0903	-0.125	0.247	0.507	0.614		0.626	
Region3	0.422	0.0903	0.24	0.611	4.68	0.00002	***	0.000175	***

Group1	-0.161	0.283	-0.826	0.431	-0.568	0.577		0.572	
Group2	0.188	0.283	-0.393	0.71	0.665	0.515		0.512	
Region1:Group1	0.0826	0.128	-0.171	0.339	0.647	0.52		0.509	
Region2:Group1	-0.215	0.128	-0.446	0.039	-1.68	0.0988	.	0.0923	.
Region3:Group1	0.165	0.128	-0.0983	0.402	1.29	0.203		0.212	
Region1:Group2	0.213	0.128	-0.0217	0.456	1.67	0.101		0.0926	.
Region2:Group2	0.0132	0.128	-0.203	0.283	0.104	0.918		0.925	
Region3:Group2	-0.0694	0.128	-0.312	0.179	-0.543	0.589		0.587	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.783	0.885	0.623	1.27	77.4	1.81E-14	***	R^2M/R^2C	0.108 / 0.798
Residual	0.229	0.478	0.379	0.559	22.6			AIC/BIC	218 / 252
Closed Porosity (%)	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.198	-0.437	0.397	0	1		0.991	
Region1	-0.265	0.0942	-0.438	-0.0921	-2.81	0.00688	**	0.00421	**
Region2	-0.157	0.0942	-0.349	0.0149	-1.67	0.1		0.105	
Region3	0.18	0.0942	-0.00342	0.345	1.91	0.062	.	0.0726	.
Group1	-0.309	0.28	-0.86	0.173	-1.11	0.283		0.289	
Group2	0.087	0.28	-0.564	0.608	0.311	0.759		0.764	
Region1:Group1	-0.0186	0.133	-0.274	0.274	-0.14	0.889		0.889	
Region2:Group1	-0.227	0.133	-0.502	0.0208	-1.7	0.094	.	0.0951	.
Region3:Group1	0.117	0.133	-0.124	0.383	0.88	0.383		0.388	
Region1:Group2	0.128	0.133	-0.14	0.394	0.96	0.341		0.338	
Region2:Group2	0.176	0.133	-0.0671	0.445	1.32	0.191		0.18	
Region3:Group2	0.0312	0.133	-0.25	0.318	0.234	0.816		0.819	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.759	0.871	0.594	1.27	75.3	1.69E-13	***	R^2M/R^2C	0.111 / 0.781
Residual	0.248	0.498	0.403	0.573	24.7			AIC/BIC	219 / 253
Open Porosity (%)	**Lambda = -0.125**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.155	-0.297	0.32	0	1		0.988	
Region1	0.223	0.11	0.0122	0.442	2.02	0.0485	*	0.054	.
Region2	-0.853	0.11	-1.08	-0.626	-7.73	0	***	0.000175	***
Region3	0.114	0.11	-0.121	0.334	1.03	0.305		0.322	
Group1	-0.0699	0.219	-0.48	0.378	-0.32	0.753		0.755	
Group2	-0.112	0.219	-0.526	0.299	-0.514	0.613		0.641	
Region1:Group1	-0.241	0.156	-0.567	0.0643	-1.55	0.128		0.131	
Region2:Group1	-0.118	0.156	-0.418	0.187	-0.755	0.454		0.44	
Region3:Group1	-0.0414	0.156	-0.368	0.24	-0.266	0.792		0.784	

Region1:Group2	0.444	0.156	0.142	0.756	2.84	0.00629	**	0.00491	**
Region2:Group2	0.0465	0.156	-0.25	0.319	0.298	0.767		0.773	
Region3:Group2	-0.0387	0.156	-0.354	0.275	-0.248	0.805		0.809	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.417	0.646	0.421	0.969	55	4.23E-07	***	R^2M/R^2C	0.311 / 0.69
Residual	0.341	0.584	0.461	0.682	45			AIC/BIC	241 / 275
Total Porosity (%)	**Lambda = 0.275**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.176	-0.311	0.355	0	1		0.993	
Region1	0.022	0.103	-0.183	0.235	0.214	0.831		0.831	
Region2	-0.634	0.103	-0.845	-0.418	-6.17	1E-07	***	0.000175	***
Region3	0.155	0.103	-0.0376	0.361	1.5	0.138		0.149	
Group1	-0.181	0.249	-0.736	0.346	-0.727	0.477		0.467	
Group2	-0.0326	0.249	-0.586	0.461	-0.131	0.897		0.92	
Region1:Group1	-0.191	0.145	-0.471	0.11	-1.31	0.195		0.195	
Region2:Group1	-0.194	0.145	-0.507	0.104	-1.33	0.189		0.203	
Region3:Group1	0.00688	0.145	-0.249	0.282	0.0473	0.962		0.968	
Region1:Group2	0.388	0.145	0.0925	0.692	2.67	0.0101	*	0.00947	**
Region2:Group2	0.113	0.145	-0.187	0.398	0.775	0.442		0.445	
Region3:Group2	-0.0187	0.145	-0.32	0.274	-0.129	0.898		0.902	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.575	0.758	0.511	1.11	66	5.20E-10	***	R^2M/R^2C	0.219 / 0.734
Residual	0.296	0.544	0.428	0.64	34			AIC/BIC	234 / 268
Cortical Volume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.108	-0.228	0.217	0	1		0.977	
Region1	1.19	0.0646	1.06	1.32	18.3	0	***	0.000175	***
Region2	-0.777	0.0646	-0.907	-0.653	-12	0	***	0.000175	***
Region3	-0.748	0.0646	-0.866	-0.61	-11.6	0	***	0.000175	***
Group1	-0.212	0.153	-0.52	0.0532	-1.38	0.183		0.187	
Group2	0.0309	0.153	-0.245	0.361	0.202	0.843		0.855	
Region1:Group1	-0.129	0.0914	-0.3	0.0563	-1.41	0.164		0.158	
Region2:Group1	0.145	0.0914	-0.0464	0.33	1.59	0.118		0.113	
Region3:Group1	-0.0378	0.0914	-0.205	0.156	-0.414	0.681		0.674	
Region1:Group2	0.0619	0.0914	-0.107	0.249	0.678	0.501		0.495	
Region2:Group2	-0.0818	0.0914	-0.261	0.108	-0.895	0.375		0.364	
Region3:Group2	0.0581	0.0914	-0.109	0.259	0.636	0.528		0.519	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit

Sample	0.217	0.466	0.296	0.681	65	1.06E-09	***	R^2M/R^2C	0.68 / 0.888
Residual	0.117	0.342	0.267	0.397	35			AIC/BIC	157 / 191
Pore Volume	**Lambda = 0.15**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.16	-0.303	0.322	0	1		0.995	
Region1	0.456	0.0941	0.278	0.628	4.85	0.000011	***	0.000175	***
Region2	-0.811	0.0941	-0.995	-0.618	-8.61	0	***	0.000175	***
Region3	-0.164	0.0941	-0.357	0.00954	-1.74	0.0874	.	0.0916	.
Group1	-0.239	0.226	-0.657	0.168	-1.06	0.304		0.324	
Group2	-0.00938	0.226	-0.509	0.4	-0.0416	0.967		0.967	
Region1:Group1	-0.197	0.133	-0.447	0.109	-1.48	0.144		0.149	
Region2:Group1	-0.104	0.133	-0.356	0.158	-0.785	0.436		0.438	
Region3:Group1	-0.0253	0.133	-0.28	0.217	-0.19	0.85		0.87	
Region1:Group2	0.341	0.133	0.0726	0.596	2.56	0.0132	*	0.014	*
Region2:Group2	0.0531	0.133	-0.195	0.309	0.399	0.692		0.686	
Region3:Group2	0.017	0.133	-0.246	0.305	0.127	0.899		0.92	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.474	0.688	0.484	1.01	65.6	6.79E-10	***	R^2M/R^2C	0.341 / 0.774
Residual	0.248	0.498	0.398	0.575	34.4			AIC/BIC	226 / 260
Cortical Surface	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.122	-0.251	0.237	0	1		0.968	
Region1	1	0.0577	0.897	1.12	17.4	0	***	0.000175	***
Region2	-0.46	0.0577	-0.582	-0.348	-7.98	0	***	0.000175	***
Region3	-1.03	0.0577	-1.14	-0.925	-17.8	0	***	0.000175	***
Group1	-0.214	0.173	-0.572	0.118	-1.24	0.23		0.231	
Group2	0.0136	0.173	-0.315	0.379	0.0787	0.938		0.945	
Region1:Group1	0.0176	0.0815	-0.132	0.202	0.216	0.83		0.819	
Region2:Group1	0.0494	0.0815	-0.115	0.201	0.606	0.547		0.567	
Region3:Group1	0.0622	0.0815	-0.113	0.225	0.763	0.449		0.451	
Region1:Group2	-0.00255	0.0815	-0.162	0.159	-0.0312	0.975		0.967	
Region2:Group2	-0.0333	0.0815	-0.192	0.121	-0.409	0.685		0.673	
Region3:Group2	-0.0311	0.0815	-0.192	0.152	-0.381	0.705		0.692	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.29	0.538	0.387	0.796	75.7	1.16E-13	***	R^2M/R^2C	0.635 / 0.911
Residual	0.0931	0.305	0.245	0.355	24.3			AIC/BIC	149 / 183
Pore Surface	**Lambda = 0.425**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.

(Intercept)	0	0.186	-0.386	0.352	0	1		0.982	
Region1	0.468	0.0838	0.303	0.632	5.59	8E-07	***	0.000175	***
Region2	-0.576	0.0838	-0.739	-0.406	-6.87	0	***	0.000175	***
Region3	-0.142	0.0838	-0.325	0.0414	-1.69	0.0968	.	0.0965	.
Group1	-0.335	0.263	-0.871	0.189	-1.27	0.219		0.238	
Group2	0.128	0.263	-0.392	0.655	0.487	0.632		0.653	
Region1:Group1	-0.111	0.119	-0.357	0.129	-0.936	0.353		0.37	
Region2:Group1	-0.0906	0.119	-0.313	0.154	-0.765	0.448		0.433	
Region3:Group1	0.0356	0.119	-0.205	0.254	0.3	0.765		0.775	
Region1:Group2	0.288	0.119	0.0715	0.508	2.43	0.0186	*	0.0175	*
Region2:Group2	0.0269	0.119	-0.206	0.281	0.227	0.821		0.795	
Region3:Group2	-0.00613	0.119	-0.246	0.245	-0.0518	0.959		0.948	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.677	0.823	0.59	1.2	77.5	1.64E-14	***	R^2M/R^2C	0.217 / 0.824
Residual	0.197	0.443	0.349	0.514	22.5			AIC/BIC	213 / 247
Intersection Surface	**Lambda = 0.1**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.143	-0.277	0.28	0	1		0.998	
Region1	-0.0311	0.115	-0.28	0.19	-0.27	0.788		0.791	
Region2	-0.885	0.115	-1.11	-0.655	-7.69	0	***	0.000175	***
Region3	0.387	0.115	0.157	0.612	3.36	0.00143	**	0.0014	**
Group1	-0.0745	0.202	-0.522	0.294	-0.37	0.716		0.719	
Group2	-0.0477	0.202	-0.446	0.368	-0.237	0.816		0.813	
Region1:Group1	-0.158	0.163	-0.448	0.122	-0.972	0.335		0.342	
Region2:Group1	-0.185	0.163	-0.486	0.121	-1.14	0.26		0.255	
Region3:Group1	0.156	0.163	-0.176	0.468	0.956	0.343		0.345	
Region1:Group2	0.527	0.163	0.235	0.816	3.24	0.00207	**	0.000351	***
Region2:Group2	-0.0332	0.163	-0.339	0.255	-0.204	0.839		0.844	
Region3:Group2	-0.0367	0.163	-0.333	0.28	-0.226	0.822		0.814	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.334	0.578	0.355	0.865	47.4	1.49E-05	***	R^2M/R^2C	0.355 / 0.661
Residual	0.371	0.609	0.486	0.696	52.6			AIC/BIC	247 / 281
Pore Surface: PoreVolume	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.13	-0.237	0.273	0	1		0.984	
Region1	-0.25	0.103	-0.461	-0.0274	-2.41	0.0193	*	0.0242	*
Region2	0.945	0.103	0.736	1.14	9.13	0	***	0.000175	***
Region3	0.107	0.103	-0.119	0.314	1.03	0.307		0.308	

Group1	-0.139	0.184	-0.534	0.217	-0.753	0.461		0.485	
Group2	0.306	0.184	-0.0533	0.676	1.66	0.114		0.12	
Region1:Group1	0.268	0.146	-0.0426	0.559	1.83	0.0727	.	0.0768	.
Region2:Group1	0.0176	0.146	-0.313	0.268	0.121	0.904		0.914	
Region3:Group1	0.138	0.146	-0.149	0.383	0.945	0.349		0.357	
Region1:Group2	-0.279	0.146	-0.565	0.00801	-1.91	0.0618	.	0.0656	.
Region2:Group2	-0.0649	0.146	-0.357	0.237	-0.443	0.659		0.65	
Region3:Group2	0.0055	0.146	-0.309	0.265	0.0376	0.97		0.952	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.281	0.53	0.33	0.791	48.4	9.69E-06	***	R^2M/R^2C	0.461 / 0.722
Residual	0.3	0.547	0.439	0.641	51.6			AIC/BIC	214 / 248
Pore Surface: Cortical Volume	**Lambda = 0.525**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.203	-0.374	0.416	0	1		0.998	
Region1	-0.0786	0.0886	-0.262	0.0912	-0.887	0.379		0.39	
Region2	-0.274	0.0886	-0.45	-0.0774	-3.09	0.00315	**	0.00456	**
Region3	0.263	0.0886	0.0832	0.429	2.97	0.0044	**	0.00386	**
Group1	-0.269	0.286	-0.844	0.301	-0.938	0.361		0.391	
Group2	0.121	0.286	-0.455	0.666	0.423	0.677		0.717	
Region1:Group1	-0.0496	0.125	-0.275	0.185	-0.396	0.694		0.691	
Region2:Group1	-0.215	0.125	-0.454	0.0372	-1.71	0.0921	.	0.0884	.
Region3:Group1	0.0773	0.125	-0.184	0.304	0.617	0.54		0.551	
Region1:Group2	0.281	0.125	0.0225	0.531	2.24	0.0289	*	0.0267	*
Region2:Group2	0.0975	0.125	-0.181	0.359	0.778	0.44		0.445	
Region3:Group2	-0.0394	0.125	-0.283	0.19	-0.314	0.755		0.754	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.807	0.898	0.613	1.3	78.6	4.67E-15	***	R^2M/R^2C	0.0963 / 0.806
Residual	0.22	0.469	0.373	0.546	21.4			AIC/BIC	217 / 251
Pore Thickness	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.134	-0.294	0.278	0	1		0.98	
Region1	0.0803	0.109	-0.13	0.294	0.737	0.464		0.459	
Region2	-0.818	0.109	-1.03	-0.589	-7.5	0	***	0.000175	***
Region3	-0.13	0.109	-0.353	0.0865	-1.19	0.239		0.226	
Group1	0.147	0.189	-0.236	0.561	0.778	0.447		0.47	
Group2	-0.308	0.189	-0.701	0.068	-1.63	0.121		0.132	
Region1:Group1	-0.309	0.154	-0.642	0.0297	-2.01	0.0496	*	0.0533	.
Region2:Group1	-0.066	0.154	-0.328	0.233	-0.428	0.67		0.678	
Region3:Group1	-0.0581	0.154	-0.356	0.225	-0.377	0.707		0.704	

Region1:Group2	0.311	0.154	7.13E-05	0.619	2.02	0.0486	*	0.0502	.
Region2:Group2	0.0783	0.154	-0.23	0.369	0.508	0.613		0.619	
Region3:Group2	-0.0476	0.154	-0.355	0.244	-0.309	0.759		0.749	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.292	0.54	0.34	0.837	46.8	1.93E-05	***	R^2M/R^2C	0.423 / 0.693
Residual	0.332	0.577	0.443	0.668	53.2			AIC/BIC	221 / 255
Pore Separation	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.191	-0.374	0.418	0	1		0.974	
Region1	0.488	0.0939	0.302	0.669	5.2	3.1E-06	***	0.000175	***
Region2	-0.268	0.0939	-0.428	-0.098	-2.85	0.00616	**	0.00632	**
Region3	-0.341	0.0939	-0.517	-0.156	-3.63	0.000621	***	0.0014	**
Group1	0.286	0.27	-0.211	0.817	1.06	0.304		0.313	
Group2	-0.171	0.27	-0.659	0.349	-0.635	0.533		0.533	
Region1:Group1	0.0208	0.133	-0.23	0.28	0.157	0.876		0.871	
Region2:Group1	0.207	0.133	-0.0591	0.483	1.56	0.125		0.132	
Region3:Group1	-0.121	0.133	-0.392	0.165	-0.909	0.367		0.36	
Region1:Group2	-0.258	0.133	-0.514	0.026	-1.94	0.0573	.	0.0544	.
Region2:Group2	-0.00654	0.133	-0.281	0.242	-0.0492	0.961		0.948	
Region3:Group2	0.107	0.133	-0.145	0.357	0.807	0.423		0.419	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.702	0.838	0.596	1.24	74	6.57E-13	***	R^2M/R^2C	0.157 / 0.781
Residual	0.247	0.497	0.385	0.583	26			AIC/BIC	217 / 251
Pore Tb.N	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.203	-0.356	0.405	0	1		0.995	
Region1	-0.0898	0.0875	-0.266	0.0952	-1.03	0.31		0.295	
Region2	-0.0416	0.0875	-0.212	0.125	-0.475	0.637		0.648	
Region3	0.269	0.0875	0.0971	0.452	3.07	0.00332	**	0.00421	**
Group1	-0.308	0.287	-0.873	0.263	-1.07	0.298		0.305	
Group2	0.243	0.287	-0.325	0.809	0.848	0.408		0.43	
Region1:Group1	0.036	0.124	-0.23	0.304	0.29	0.773		0.772	
Region2:Group1	-0.255	0.124	-0.505	0.00619	-2.06	0.0445	*	0.04	*
Region3:Group1	0.113	0.124	-0.145	0.358	0.909	0.367		0.363	
Region1:Group2	0.192	0.124	-0.0757	0.437	1.55	0.126		0.133	
Region2:Group2	0.156	0.124	-0.0709	0.406	1.26	0.213		0.207	
Region3:Group2	-0.0372	0.124	-0.249	0.222	-0.3	0.765		0.774	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit

Sample	0.812	0.901	0.635	1.29	79.1	2.47E-15	***	R^2M/R^2C	0.0962 / 0.811
Residual	0.215	0.463	0.367	0.54	20.9			AIC/BIC	212 / 246
Degree of Anisotropy	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.167	-0.349	0.324	0	1		0.998	
Region1	0.362	0.111	0.152	0.58	3.25	0.002	**	0.0014	**
Region2	-0.74	0.111	-0.966	-0.542	-6.65	0	***	0.000175	***
Region3	-0.0753	0.111	-0.285	0.132	-0.676	0.502		0.509	
Group1	0.0516	0.237	-0.373	0.562	0.218	0.83		0.838	
Group2	-0.0316	0.237	-0.526	0.402	-0.134	0.895		0.889	
Region1:Group1	-0.117	0.157	-0.476	0.197	-0.74	0.463		0.45	
Region2:Group1	-0.247	0.157	-0.565	0.0403	-1.57	0.123		0.127	
Region3:Group1	-0.0228	0.157	-0.337	0.3	-0.145	0.886		0.916	
Region1:Group2	0.131	0.157	-0.21	0.442	0.83	0.41		0.406	
Region2:Group2	0.204	0.157	-0.0818	0.536	1.3	0.2		0.191	
Region3:Group2	0.0565	0.157	-0.243	0.351	0.359	0.721		0.738	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.501	0.708	0.482	1.04	59.1	4.52E-08	***	R^2M/R^2C	0.237 / 0.688
Residual	0.347	0.589	0.475	0.678	40.9			AIC/BIC	231 / 265
Pore Fractal	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.201	-0.416	0.365	0	1		0.986	
Region1	-0.0597	0.0864	-0.227	0.102	-0.691	0.492		0.486	
Region2	-0.215	0.0864	-0.383	-0.0399	-2.49	0.0157	*	0.0147	*
Region3	0.26	0.0864	0.111	0.438	3.01	0.00401	**	0.00386	**
Group1	-0.358	0.285	-0.955	0.141	-1.26	0.225		0.239	
Group2	0.167	0.285	-0.433	0.704	0.586	0.565		0.598	
Region1:Group1	-0.0578	0.122	-0.287	0.178	-0.473	0.638		0.651	
Region2:Group1	-0.193	0.122	-0.455	0.0608	-1.58	0.12		0.121	
Region3:Group1	0.0617	0.122	-0.17	0.283	0.505	0.615		0.614	
Region1:Group2	0.293	0.122	0.0425	0.534	2.4	0.0199	*	0.0204	*
Region2:Group2	0.0105	0.122	-0.223	0.231	0.0861	0.932		0.918	
Region3:Group2	-0.00953	0.122	-0.234	0.234	-0.078	0.938		0.92	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.8	0.894	0.627	1.26	79.3	1.98E-15	***	R^2M/R^2C	0.11 / 0.816
Residual	0.209	0.457	0.359	0.528	20.7			AIC/BIC	210 / 244
Number of Pores	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.201	-0.42	0.386	0	1		0.981	

Region1	0.404	0.077	0.265	0.552	5.25	2.7E-06	***	0.000175	***
Region2	-0.0327	0.077	-0.182	0.123	-0.425	0.673		0.67	
Region3	-0.167	0.077	-0.323	-0.0242	-2.16	0.0348	*	0.0389	*
Group1	-0.378	0.284	-0.925	0.195	-1.33	0.2		0.215	
Group2	0.295	0.284	-0.247	0.848	1.04	0.313		0.33	
Region1:Group1	-0.0735	0.109	-0.274	0.125	-0.676	0.502		0.505	
Region2:Group1	-0.0906	0.109	-0.291	0.118	-0.832	0.409		0.391	
Region3:Group1	0.193	0.109	-0.0465	0.403	1.78	0.0811	.	0.0768	.
Region1:Group2	0.243	0.109	0.0325	0.461	2.23	0.0297	*	0.0263	*
Region2:Group2	-0.0679	0.109	-0.293	0.13	-0.624	0.535		0.528	
Region3:Group2	-0.127	0.109	-0.337	0.086	-1.17	0.248		0.241	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.807	0.899	0.663	1.33	83	1.25E-17	***	R^2M/R^2C	0.138 / 0.853
Residual	0.166	0.407	0.329	0.465	17			AIC/BIC	198 / 232
Pore Density	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.199	-0.374	0.441	0	1		0.999	
Region1	-0.206	0.0773	-0.355	-0.0457	-2.66	0.0102	*	0.0137	*
Region2	0.387	0.0773	0.227	0.531	5.01	6.3E-06	***	0.000175	***
Region3	0.231	0.0773	0.09	0.397	2.99	0.00415	**	0.00246	**
Group1	-0.27	0.281	-0.836	0.287	-0.96	0.35		0.36	
Group2	0.256	0.281	-0.28	0.822	0.911	0.374		0.389	
Region1:Group1	0.0363	0.109	-0.172	0.245	0.332	0.741		0.74	
Region2:Group1	-0.243	0.109	-0.43	-0.0303	-2.22	0.0306	*	0.0344	*
Region3:Group1	0.238	0.109	0.014	0.451	2.18	0.0337	*	0.0351	*
Region1:Group2	0.138	0.109	-0.0751	0.336	1.27	0.211		0.209	
Region2:Group2	0.023	0.109	-0.209	0.256	0.21	0.834		0.812	
Region3:Group2	-0.12	0.109	-0.325	0.0998	-1.1	0.276		0.264	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.786	0.886	0.619	1.26	82.4	2.73E-17	***	R^2M/R^2C	0.154 / 0.851
Residual	0.167	0.409	0.319	0.478	17.6			AIC/BIC	198 / 232
Euler number	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.2	-0.442	0.381	0	1		0.976	
Region1	0.237	0.0819	0.0904	0.399	2.9	0.00542	**	0.00491	**
Region2	0.109	0.0819	-0.0402	0.271	1.33	0.19		0.186	
Region3	-0.138	0.0819	-0.29	0.00545	-1.68	0.0986	.	0.0888	.
Group1	-0.389	0.283	-0.962	0.183	-1.38	0.186		0.204	
Group2	0.365	0.283	-0.194	0.902	1.29	0.213		0.214	

Region1:Group1	-0.00403	0.116	-0.214	0.207	-0.0348	0.972		0.984	
Region2:Group1	-0.154	0.116	-0.387	0.0747	-1.33	0.189		0.188	
Region3:Group1	0.223	0.116	-0.00935	0.467	1.92	0.0596	.	0.0674	.
Region1:Group2	0.18	0.116	-0.0279	0.384	1.55	0.126		0.134	
Region2:Group2	-0.0811	0.116	-0.333	0.138	-0.7	0.487		0.488	
Region3:Group2	-0.124	0.116	-0.339	0.104	-1.07	0.291		0.315	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.793	0.891	0.637	1.29	80.9	2.56E-16	***	R^2M/R^2C	0.132 / 0.834
Residual	0.188	0.433	0.344	0.511	19.1			AIC/BIC	204 / 238
Connectivity	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.185	-0.398	0.368	0	1		0.992	
Region1	0.69	0.0897	0.508	0.887	7.69	0	***	0.000175	***
Region2	-0.342	0.0897	-0.524	-0.167	-3.81	0.000358	***	0.000351	***
Region3	-0.196	0.0897	-0.377	-0.018	-2.18	0.0334	*	0.0351	*
Group1	-0.273	0.262	-0.767	0.257	-1.04	0.311		0.327	
Group2	0.0756	0.262	-0.435	0.576	0.289	0.776		0.81	
Region1:Group1	-0.213	0.127	-0.447	0.0578	-1.68	0.0984	.	0.0993	.
Region2:Group1	0.0708	0.127	-0.176	0.313	0.558	0.579		0.583	
Region3:Group1	0.0866	0.127	-0.181	0.347	0.683	0.498		0.491	
Region1:Group2	0.333	0.127	0.0671	0.581	2.62	0.0113	*	0.0112	*
Region2:Group2	-0.0241	0.127	-0.282	0.244	-0.19	0.85		0.849	
Region3:Group2	-0.108	0.127	-0.346	0.138	-0.851	0.398		0.418	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.665	0.815	0.586	1.2	74.7	3.32E-13	***	R^2M/R^2C	0.204 / 0.798
Residual	0.225	0.475	0.375	0.544	25.3			AIC/BIC	211 / 245
Connectivity density	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.208	-0.419	0.398	0	1		0.987	
Region1	0.299	0.0808	0.147	0.469	3.7	0.000506	***	0.000175	***
Region2	-0.134	0.0808	-0.287	0.0226	-1.65	0.104		0.102	
Region3	0.0954	0.0808	-0.0734	0.248	1.18	0.243		0.249	
Group1	-0.229	0.294	-0.779	0.4	-0.78	0.446		0.456	
Group2	0.0382	0.294	-0.59	0.655	0.13	0.898		0.894	
Region1:Group1	-0.127	0.114	-0.341	0.0985	-1.11	0.27		0.269	
Region2:Group1	0	0.114	-0.238	0.228	0	1		0.988	
Region3:Group1	0.0763	0.114	-0.145	0.298	0.668	0.507		0.505	
Region1:Group2	0.293	0.114	0.0709	0.508	2.56	0.0133	*	0.0133	*
Region2:Group2	0.0382	0.114	-0.193	0.278	0.334	0.74		0.728	

Region3:Group2	-0.114	0.114	-0.343	0.114	-1	0.321		0.312	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.859	0.927	0.639	1.35	82.5	2.64E-17	***	R^2M/R^2C	0.0847 / 0.839
Residual	0.183	0.427	0.332	0.494	17.5			AIC/BIC	204 / 238
SD Pore Thickness	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.14	-0.267	0.269	0	1		0.995	
Region1	-0.00816	0.108	-0.207	0.194	-0.0757	0.94		0.941	
Region2	-0.839	0.108	-1.03	-0.617	-7.78	0	***	0.000175	***
Region3	0.0492	0.108	-0.169	0.255	0.456	0.65		0.667	
Group1	0.191	0.197	-0.207	0.578	0.966	0.347		0.349	
Group2	-0.366	0.197	-0.777	0.0438	-1.85	0.0801	.	0.0881	.
Region1:Group1	-0.264	0.153	-0.56	0.0585	-1.73	0.0887	.	0.0958	.
Region2:Group1	0.0186	0.153	-0.264	0.316	0.122	0.903		0.929	
Region3:Group1	-0.0446	0.153	-0.334	0.262	-0.292	0.771		0.778	
Region1:Group2	0.283	0.153	-0.00408	0.596	1.85	0.0694	.	0.0789	.
Region2:Group2	-0.0435	0.153	-0.367	0.245	-0.285	0.777		0.798	
Region3:Group2	-0.0907	0.153	-0.386	0.196	-0.594	0.555		0.554	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.328	0.572	0.347	0.864	50.1	4.44E-06	***	R^2M/R^2C	0.399 / 0.7
Residual	0.326	0.571	0.44	0.664	49.9			AIC/BIC	221 / 255
SD Pore Separation	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.145	-0.282	0.313	0	1		0.987	
Region1	0.776	0.12	0.543	1.05	6.46	0	***	0.000175	***
Region2	-0.687	0.12	-0.955	-0.465	-5.72	5E-07	***	0.000175	***
Region3	-0.352	0.12	-0.578	-0.132	-2.93	0.00497	**	0.00491	**
Group1	0.00366	0.206	-0.375	0.402	0.0178	0.986		0.994	
Group2	0.0792	0.206	-0.337	0.451	0.385	0.705		0.73	
Region1:Group1	-0.0868	0.17	-0.401	0.235	-0.511	0.612		0.618	
Region2:Group1	0.227	0.17	-0.108	0.548	1.34	0.187		0.188	
Region3:Group1	-0.0415	0.17	-0.356	0.3	-0.244	0.808		0.789	
Region1:Group2	-0.201	0.17	-0.557	0.143	-1.18	0.241		0.255	
Region2:Group2	-0.0852	0.17	-0.431	0.216	-0.502	0.618		0.629	
Region3:Group2	0.226	0.17	-0.171	0.585	1.33	0.188		0.193	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.343	0.586	0.373	0.908	45.9	2.71E-05	***	R^2M/R^2C	0.32 / 0.632
Residual	0.404	0.636	0.504	0.735	54.1			AIC/BIC	234 / 268

Avg. Pore volume	**Lambda = -0.875**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.118	-0.249	0.219	0	1		0.987	
Region1	0.317	0.103	0.143	0.512	3.07	0.00331	**	0.00351	**
Region2	-1.08	0.103	-1.29	-0.884	-10.4	0	***	0.000175	***
Region3	-0.0354	0.103	-0.243	0.171	-0.343	0.733		0.709	
Group1	0.0988	0.167	-0.224	0.457	0.591	0.562		0.558	
Group2	-0.268	0.167	-0.604	0.0714	-1.6	0.126		0.138	
Region1:Group1	-0.114	0.146	-0.41	0.167	-0.779	0.439		0.458	
Region2:Group1	-0.0215	0.146	-0.294	0.26	-0.147	0.883		0.901	
Region3:Group1	-0.234	0.146	-0.539	0.0363	-1.6	0.115		0.122	
Region1:Group2	0.176	0.146	-0.122	0.468	1.2	0.234		0.231	
Region2:Group2	0.105	0.146	-0.197	0.397	0.722	0.474		0.473	
Region3:Group2	0.066	0.146	-0.232	0.331	0.452	0.653		0.669	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.219	0.467	0.274	0.723	42.3	0.000111	***	R^2M/R^2C	0.516 / 0.721
Residual	0.298	0.546	0.433	0.646	57.7			AIC/BIC	227 / 261
Avg. Pore surface	**Lambda = -1.225**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.118	-0.254	0.225	0	1		0.988	
Region1	0.325	0.106	0.112	0.518	3.07	0.00337	**	0.00421	**
Region2	-1.07	0.106	-1.28	-0.867	-10.1	0	***	0.000175	***
Region3	-0.0281	0.106	-0.25	0.191	-0.265	0.792		0.788	
Group1	0.0607	0.167	-0.258	0.43	0.364	0.72		0.728	
Group2	-0.232	0.167	-0.589	0.119	-1.39	0.182		0.192	
Region1:Group1	-0.0312	0.15	-0.319	0.254	-0.209	0.836		0.824	
Region2:Group1	-0.0423	0.15	-0.334	0.245	-0.282	0.779		0.761	
Region3:Group1	-0.289	0.15	-0.586	0.00605	-1.93	0.0588	.	0.0586	.
Region1:Group2	0.118	0.15	-0.188	0.399	0.789	0.434		0.441	
Region2:Group2	0.16	0.15	-0.0915	0.464	1.07	0.291		0.296	
Region3:Group2	0.133	0.15	-0.143	0.429	0.887	0.379		0.376	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.214	0.463	0.269	0.73	40.6	0.000208	***	R^2M/R^2C	0.506 / 0.707
Residual	0.314	0.56	0.448	0.648	59.4			AIC/BIC	222 / 256
Avg. Orientation theta	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.145	-0.28	0.275	0	1		0.991	
Region1	0.485	0.079	0.318	0.627	6.14	1E-07	***	0.000175	***

Region2	0.844	0.079	0.695	1	10.7	0	***	0.000175	***
Region3	-0.406	0.079	-0.566	-0.246	-5.14	3.9E-06	***	0.000175	***
Group1	0.0211	0.206	-0.386	0.431	0.103	0.919		0.94	
Group2	-0.0137	0.206	-0.417	0.421	-0.0667	0.948		0.962	
Region1:Group1	0.0846	0.112	-0.129	0.295	0.757	0.452		0.454	
Region2:Group1	0.0265	0.112	-0.207	0.238	0.237	0.813		0.799	
Region3:Group1	-0.0647	0.112	-0.29	0.175	-0.58	0.565		0.558	
Region1:Group2	0.0162	0.112	-0.232	0.275	0.145	0.885		0.883	
Region2:Group2	-0.0861	0.112	-0.316	0.14	-0.771	0.444		0.427	
Region3:Group2	-0.0153	0.112	-0.262	0.213	-0.137	0.892		0.9	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.4	0.632	0.442	0.905	69.6	3.26E-11	***	R^2M/R^2C	0.466 / 0.838
Residual	0.175	0.418	0.331	0.487	30.4			AIC/BIC	189 / 223
Avg. Orientation phi	**Lambda = 4.425**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.153	-0.321	0.294	0	1		0.991	
Region1	0.657	0.123	0.427	0.908	5.35	1.9E-06	***	0.000175	***
Region2	0.156	0.123	-0.0923	0.393	1.27	0.21		0.205	
Region3	-0.718	0.123	-0.933	-0.471	-5.85	3E-07	***	0.000175	***
Group1	-0.0547	0.216	-0.462	0.376	-0.253	0.803		0.818	
Group2	0.261	0.216	-0.223	0.669	1.21	0.243		0.256	
Region1:Group1	0.182	0.174	-0.153	0.53	1.05	0.3		0.294	
Region2:Group1	0.107	0.174	-0.234	0.443	0.618	0.539		0.54	
Region3:Group1	-0.161	0.174	-0.486	0.232	-0.926	0.358		0.36	
Region1:Group2	-0.101	0.174	-0.446	0.234	-0.583	0.562		0.551	
Region2:Group2	-0.11	0.174	-0.445	0.273	-0.634	0.529		0.51	
Region3:Group2	0.132	0.174	-0.242	0.466	0.761	0.45		0.428	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.387	0.622	0.379	0.925	47.8	1.24E-05	***	R^2M/R^2C	0.27 / 0.619
Residual	0.422	0.65	0.516	0.755	52.2			AIC/BIC	241 / 275
Avg. Pore thickness	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.123	-0.247	0.246	0	1		0.978	
Region1	-0.761	0.0968	-0.951	-0.537	-7.86	0	***	0.000175	***
Region2	-0.408	0.0968	-0.588	-0.221	-4.21	9.65E-05	***	0.000175	***
Region3	0.0156	0.0968	-0.176	0.215	0.161	0.873		0.864	
Group1	0.106	0.175	-0.232	0.489	0.606	0.552		0.563	
Group2	-0.0734	0.175	-0.46	0.278	-0.42	0.679		0.704	

Region1:Group1	0.136	0.137	-0.113	0.42	0.991	0.326		0.342	
Region2:Group1	0.0076	0.137	-0.279	0.279	0.0555	0.956		0.963	
Region3:Group1	-0.265	0.137	-0.566	-0.0313	-1.93	0.0585	.	0.066	.
Region1:Group2	-0.185	0.137	-0.453	0.0771	-1.35	0.181		0.195	
Region2:Group2	0.0902	0.137	-0.188	0.344	0.659	0.513		0.519	
Region3:Group2	0.244	0.137	0.00141	0.535	1.78	0.0802	.	0.0853	.
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.255	0.505	0.352	0.738	49.2	6.68E-06	***	R^2M/R^2C	0.516 / 0.754
Residual	0.262	0.512	0.406	0.592	50.8			AIC/BIC	205 / 239
Avg. Pore Major diameter	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.156	-0.328	0.29	0	1		0.983	
Region1	0.147	0.0941	-0.0166	0.318	1.57	0.123		0.121	
Region2	-0.689	0.0941	-0.862	-0.481	-7.32	0	***	0.000175	***
Region3	-0.299	0.0941	-0.488	-0.135	-3.17	0.00248	**	0.00456	**
Group1	0.219	0.22	-0.211	0.71	0.997	0.332		0.345	
Group2	-0.18	0.22	-0.62	0.284	-0.818	0.424		0.432	
Region1:Group1	0.232	0.133	-0.0509	0.497	1.74	0.0869	.	0.0895	.
Region2:Group1	-0.0326	0.133	-0.279	0.234	-0.245	0.807		0.822	
Region3:Group1	-0.387	0.133	-0.661	-0.11	-2.91	0.00525	**	0.00596	**
Region1:Group2	-0.161	0.133	-0.427	0.0925	-1.21	0.231		0.225	
Region2:Group2	0.184	0.133	-0.098	0.446	1.38	0.172		0.164	
Region3:Group2	0.264	0.133	0.0152	0.526	1.99	0.0521	.	0.0502	.
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.447	0.669	0.443	0.982	64.3	1.71E-09	***	R^2M/R^2C	0.364 / 0.773
Residual	0.248	0.498	0.404	0.59	35.7			AIC/BIC	210 / 244
Avg. Pore Sphericity	**Lambda = None**								
Fixed Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig.	pMCMC	Sig.
(Intercept)	0	0.166	-0.315	0.327	0	1		0.986	
Region1	-0.791	0.115	-0.985	-0.55	-6.9	0	***	0.000175	***
Region2	0.391	0.115	0.149	0.597	3.41	0.00124	**	0.00105	**
Region3	0.0713	0.115	-0.159	0.3	0.622	0.537		0.555	
Group1	0.0709	0.235	-0.362	0.506	0.302	0.766		0.759	
Group2	0.0289	0.235	-0.412	0.503	0.123	0.904		0.91	
Region1:Group1	-0.0118	0.162	-0.329	0.315	-0.0728	0.942		0.931	
Region2:Group1	0.249	0.162	-0.0892	0.573	1.54	0.13		0.143	
Region3:Group1	-0.0205	0.162	-0.342	0.33	-0.126	0.9		0.893	
Region1:Group2	-0.0605	0.162	-0.371	0.265	-0.373	0.71		0.72	

Region2:Group2	-0.252	0.162	-0.567	0.0494	-1.55	0.126		0.127	
Region3:Group2	-0.0317	0.162	-0.361	0.294	-0.196	0.846		0.866	
Random Factor	Estimate	Std.Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig.	Model Test	Model Fit
Sample	0.488	0.698	0.467	1.01	57	1.47E-07	***	R^2M/R^2C	0.232 / 0.67
Residual	0.368	0.607	0.486	0.699	43			AIC/BIC	234 / 268

Tibial Regions: Post-Hoc Tests for Significant Fixed Effects

Cortical Fractal	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior < Lateral	0.851		0.249	Medium	51%
Region		Anterior < Medial	0.0378	*	0.853	Large	
Region		Anterior < Posterior	0.784		0.29	Medium	
Region		Lateral < Medial	0.216		0.605	Medium	
Region		Lateral < Posterior	0.999		0.0412	Small	
Region		Medial > Posterior	0.273		0.563	Medium	
Region | Group	Anterior	Control < Fentanyl	0.128		1.82	Large	34%
Region | Group	Anterior	Control < Morphine	0.592		0.627	Medium	
Region | Group	Anterior	Fentanyl > Morphine	0.313		1.19	Large	
Region | Group	Lateral	Control < Fentanyl	0.171		1.63	Large	
Region | Group	Lateral	Control < Morphine	0.122		1.85	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.849		0.222	Medium	
Region | Group	Medial	Control < Fentanyl	0.497		0.796	Medium	
Region | Group	Medial	Control < Morphine	0.549		0.701	Medium	
Region | Group	Medial	Fentanyl > Morphine	0.935		0.0953	Small	
Region | Group	Posterior	Control < Fentanyl	0.851		0.22	Medium	
Region | Group	Posterior	Control < Morphine	0.42		0.946	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.535		0.727	Medium	
Group | Region	Control	Anterior > Lateral	0.858		0.096	Small	
Group | Region	Control	Anterior < Medial	0.0331	*	1.17	Large	
Group | Region	Control	Anterior < Posterior	0.186		0.716	Medium	
Group | Region	Control	Lateral < Medial	0.0216	*	1.27	Large	
Group | Region	Control	Lateral < Posterior	0.134		0.812	Large	
Group | Region	Control	Medial > Posterior	0.401		0.453	Medium	
Group | Region	Fentanyl	Anterior > Lateral	0.596		0.285	Medium	
Group | Region	Fentanyl	Anterior < Medial	0.783		0.148	Small	
Group | Region	Fentanyl	Anterior > Posterior	0.105		0.882	Large	
Group | Region	Fentanyl	Lateral < Medial	0.421		0.433	Medium	
Group | Region	Fentanyl	Lateral > Posterior	0.269		0.597	Medium	
Group | Region	Fentanyl	Medial > Posterior	0.0593	.	1.03	Large	
Group | Region	Morphine	Anterior < Lateral	0.0396	*	1.13	Large	

Group | Region	Morphine	Anterior < Medial	0.0239	*	1.24	Large	
Group | Region	Morphine	Anterior < Posterior	0.058	.	1.04	Large	
Group | Region	Morphine	Lateral < Medial	0.83		0.116	Small	
Group | Region	Morphine	Lateral > Posterior	0.864		0.0921	Small	
Group | Region	Morphine	Medial > Posterior	0.699		0.208	Medium	
Number Closed Pores	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.0169	*	0.949	Large	47%
Region		Anterior > Medial	0.000148	***	1.42	Large	
Region		Anterior > Posterior	5.34E-05	***	1.51	Large	
Region		Lateral > Medial	0.428		0.472	Medium	
Region		Lateral > Posterior	0.274		0.563	Medium	
Region		Medial > Posterior	0.991		0.0909	Small	
Region | Group	Anterior	Control < Fentanyl	0.0653	.	2.46	Large	42%
Region | Group	Anterior	Control < Morphine	0.435		1.01	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.266		1.45	Large	
Region | Group	Lateral	Control < Fentanyl	0.199		1.69	Large	
Region | Group	Lateral	Control < Morphine	0.179		1.77	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.951		0.08	Small	
Region | Group	Medial	Control < Fentanyl	0.478		0.919	Large	
Region | Group	Medial	Control < Morphine	0.672		0.547	Medium	
Region | Group	Medial	Fentanyl > Morphine	0.773		0.372	Medium	
Region | Group	Posterior	Control < Fentanyl	0.196		1.7	Large	
Region | Group	Posterior	Control < Morphine	0.29		1.38	Large	
Region | Group	Posterior	Fentanyl > Morphine	0.805		0.319	Medium	
Group | Region	Control	Anterior > Lateral	0.0841	.	0.941	Large	
Group | Region	Control	Anterior > Medial	0.166		0.75	Medium	
Group | Region	Control	Anterior > Posterior	0.0127	*	1.38	Large	
Group | Region	Control	Lateral < Medial	0.723		0.19	Small	
Group | Region	Control	Lateral > Posterior	0.416		0.438	Medium	
Group | Region	Control	Medial > Posterior	0.245		0.628	Medium	
Group | Region	Fentanyl	Anterior > Lateral	0.0022	**	1.72	Large	
Group | Region	Fentanyl	Anterior > Medial	7.34E-05	***	2.3	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.000187	***	2.14	Large	
Group | Region	Fentanyl	Lateral > Medial	0.285		0.577	Medium	
Group | Region	Fentanyl	Lateral > Posterior	0.429		0.426	Medium	
Group | Region	Fentanyl	Medial < Posterior	0.779		0.151	Small	
Group | Region	Morphine	Anterior > Lateral	0.728		0.187	Small	
Group | Region	Morphine	Anterior > Medial	0.0269	*	1.22	Large	
Group | Region	Morphine	Anterior > Posterior	0.0638	.	1.01	Large	

Group | Region	Morphine	Lateral > Medial	0.0595	.	1.03	Large	
Group | Region	Morphine	Lateral > Posterior	0.129		0.824	Large	
Group | Region	Morphine	Medial < Posterior	0.703		0.205	Medium	
Number Open Pores	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	1.54E-05	***	1.62	Large	51%
Region		Anterior > Medial	0.0146	*	0.965	Large	
Region		Anterior > Posterior	0.00666	**	1.05	Large	
Region		Lateral < Medial	0.16		0.654	Medium	
Region		Lateral < Posterior	0.265		0.569	Medium	
Region		Medial > Posterior	0.992		0.0858	Small	
Region | Group	Anterior	Control < Fentanyl	0.177		1.69	Large	38%
Region | Group	Anterior	Control > Morphine	0.974		0.0393	Small	
Region | Group	Anterior	Fentanyl > Morphine	0.167		1.73	Large	
Region | Group	Lateral	Control < Fentanyl	0.234		1.48	Large	
Region | Group	Lateral	Control < Morphine	0.323		1.22	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.833		0.258	Medium	
Region | Group	Medial	Control < Fentanyl	0.782		0.339	Medium	
Region | Group	Medial	Control < Morphine	0.966		0.0525	Small	
Region | Group	Medial	Fentanyl > Morphine	0.815		0.287	Medium	
Region | Group	Posterior	Control < Fentanyl	0.582		0.676	Medium	
Region | Group	Posterior	Control < Morphine	0.338		1.18	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.679		0.507	Medium	
Group | Region	Control	Anterior > Lateral	0.000526	***	1.97	Large	
Group | Region	Control	Anterior > Medial	0.311		0.547	Medium	
Group | Region	Control	Anterior > Posterior	0.0405	*	1.12	Large	
Group | Region	Control	Lateral < Medial	0.0101	*	1.42	Large	
Group | Region	Control	Lateral < Posterior	0.118		0.849	Large	
Group | Region	Control	Medial > Posterior	0.287		0.575	Medium	
Group | Region	Fentanyl	Anterior > Lateral	0.000152	***	2.18	Large	
Group | Region	Fentanyl	Anterior > Medial	0.000821	***	1.89	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.0002	***	2.13	Large	
Group | Region	Fentanyl	Lateral < Medial	0.598		0.283	Medium	
Group | Region	Fentanyl	Lateral < Posterior	0.933		0.0454	Small	
Group | Region	Fentanyl	Medial > Posterior	0.658		0.238	Medium	
Group | Region	Morphine	Anterior > Lateral	0.19		0.71	Medium	
Group | Region	Morphine	Anterior > Medial	0.398		0.455	Medium	
Group | Region	Morphine	Anterior < Posterior	0.851		0.101	Small	
Group | Region	Morphine	Lateral < Medial	0.635		0.255	Medium	
Group | Region	Morphine	Lateral < Posterior	0.135		0.811	Large	

Group | Region	Morphine	Medial < Posterior	0.303		0.556	Medium	
Closed Pore Density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	4.14E-05	***	1.53	Large	44%
Region		Anterior < Medial	0.0143	*	0.967	Large	
Region		Anterior > Posterior	0.263		0.57	Medium	
Region		Lateral > Medial	0.268		0.566	Medium	
Region		Lateral > Posterior	0	***	2.1	Large	
Region		Medial > Posterior	3.96E-05	***	1.54	Large	
Region | Group	Anterior	Control < Fentanyl	0.215		1.62	Large	47%
Region | Group	Anterior	Control < Morphine	0.809		0.311	Medium	
Region | Group	Anterior	Fentanyl > Morphine	0.314		1.31	Large	
Region | Group	Lateral	Control < Fentanyl	0.14		1.94	Large	
Region | Group	Lateral	Control < Morphine	0.149		1.9	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.973		0.0442	Small	
Region | Group	Medial	Control < Fentanyl	0.687		0.518	Medium	
Region | Group	Medial	Control > Morphine	0.954		0.0743	Small	
Region | Group	Medial	Fentanyl > Morphine	0.645		0.593	Medium	
Region | Group	Posterior	Control < Fentanyl	0.285		1.39	Large	
Region | Group	Posterior	Control < Morphine	0.45		0.974	Large	
Region | Group	Posterior	Fentanyl > Morphine	0.747		0.415	Medium	
Group | Region	Control	Anterior < Lateral	0.0987	.	0.898	Large	
Group | Region	Control	Anterior < Medial	0.00842	**	1.46	Large	
Group | Region	Control	Anterior > Posterior	0.186		0.716	Medium	
Group | Region	Control	Lateral < Medial	0.296		0.564	Medium	
Group | Region	Control	Lateral > Posterior	0.00387	**	1.61	Large	
Group | Region	Control	Medial > Posterior	0.000152	***	2.18	Large	
Group | Region	Fentanyl	Anterior < Lateral	0.0264	*	1.22	Large	
Group | Region	Fentanyl	Anterior < Medial	0.5		0.363	Medium	
Group | Region	Fentanyl	Anterior > Posterior	0.0831	.	0.944	Large	
Group | Region	Fentanyl	Lateral > Medial	0.115		0.857	Large	
Group | Region	Fentanyl	Lateral > Posterior	0.000166	***	2.16	Large	
Group | Region	Fentanyl	Medial > Posterior	0.0178	*	1.31	Large	
Group | Region	Morphine	Anterior < Lateral	2.22E-05	***	2.48	Large	
Group | Region	Morphine	Anterior < Medial	0.0489	*	1.08	Large	
Group | Region	Morphine	Anterior > Posterior	0.923		0.0517	Small	
Group | Region	Morphine	Lateral > Medial	0.0111	*	1.41	Large	
Group | Region	Morphine	Lateral > Posterior	1.59E-05	***	2.53	Large	
Group | Region	Morphine	Medial > Posterior	0.0393	*	1.13	Large	
Open Pore Density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**

Region		Anterior < Lateral	0.176		0.639	Medium	45%
Region		Anterior < Medial	0.000138	***	1.43	Large	
Region		Anterior < Posterior	0.986		0.106	Small	
Region		Lateral < Medial	0.0631	.	0.788	Medium	
Region		Lateral > Posterior	0.321		0.532	Medium	
Region		Medial > Posterior	0.00044	***	1.32	Large	
Closed Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.898		0.215	Medium	45%
Region		Anterior < Medial	0.0277	*	0.891	Large	
Region		Anterior < Posterior	0.00908	**	1.02	Large	
Region		Lateral < Medial	0.139		0.676	Medium	
Region		Lateral < Posterior	0.0563	.	0.803	Large	
Region		Medial < Posterior	0.976		0.127	Small	
Open Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	1.1E-06	***	1.84	Large	58%
Region		Anterior > Medial	0.931		0.186	Small	
Region		Anterior < Posterior	0.373		0.502	Medium	
Region		Lateral < Medial	1.02E-05	***	1.66	Large	
Region		Lateral < Posterior	0	***	2.34	Large	
Region		Medial < Posterior	0.129		0.688	Medium	
Region | Group	Anterior	Control < Fentanyl	0.278		0.897	Large	48%
Region | Group	Anterior	Control < Morphine	0.811		0.197	Small	
Region | Group	Anterior	Fentanyl > Morphine	0.396		0.7	Medium	
Region | Group	Lateral	Control < Fentanyl	0.901		0.103	Small	
Region | Group	Lateral	Control < Morphine	0.635		0.391	Medium	
Region | Group	Lateral	Fentanyl < Morphine	0.726		0.288	Medium	
Region | Group	Medial	Control > Fentanyl	0.683		0.336	Medium	
Region | Group	Medial	Control < Morphine	0.511		0.541	Medium	
Region | Group	Medial	Fentanyl < Morphine	0.289		0.877	Large	
Region | Group	Posterior	Control > Fentanyl	0.0308	*	1.82	Large	
Region | Group	Posterior	Control > Morphine	0.848		0.157	Small	
Region | Group	Posterior	Fentanyl < Morphine	0.0475	*	1.66	Large	
Group | Region	Control	Anterior > Lateral	0.0257	*	1.41	Large	
Group | Region	Control	Anterior < Medial	0.81		0.148	Small	
Group | Region	Control	Anterior < Posterior	0.0122	*	1.6	Large	
Group | Region	Control	Lateral < Medial	0.0142	*	1.56	Large	
Group | Region	Control	Lateral < Posterior	9.6E-06	***	3.01	Large	
Group | Region	Control	Medial < Posterior	0.0224	*	1.45	Large	
Group | Region	Fentanyl	Anterior > Lateral	0.000727	***	2.21	Large	

Group | Region	Fentanyl	Anterior > Medial	0.0838	.	1.08	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.0737	.	1.12	Large	
Group | Region	Fentanyl	Lateral < Medial	0.0739	.	1.12	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.0841	.	1.08	Large	
Group | Region	Fentanyl	Medial > Posterior	0.95		0.0385	Small	
Group | Region	Morphine	Anterior > Lateral	0.053	.	1.22	Large	
Group | Region	Morphine	Anterior < Medial	0.427		0.493	Medium	
Group | Region	Morphine	Anterior < Posterior	0.0486	*	1.24	Large	
Group | Region	Morphine	Lateral < Medial	0.00749	**	1.71	Large	
Group | Region	Morphine	Lateral < Posterior	0.000196	***	2.46	Large	
Group | Region	Morphine	Medial < Posterior	0.229		0.749	Medium	
Total Porosity (%)	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.00146	**	1.21	Large	54%
Region		Anterior < Medial	0.858		0.244	Medium	
Region		Anterior < Posterior	0.0576	.	0.8	Medium	
Region		Lateral < Medial	0.000107	***	1.45	Large	
Region		Lateral < Posterior	2E-07	***	2.01	Large	
Region		Medial < Posterior	0.284		0.556	Medium	
Region | Group	Anterior	Control < Fentanyl	0.236		1.11	Large	45%
Region | Group	Anterior	Control < Morphine	0.6		0.488	Medium	
Region | Group	Anterior	Fentanyl > Morphine	0.504		0.622	Medium	
Region | Group	Lateral	Control < Fentanyl	0.462		0.685	Medium	
Region | Group	Lateral	Control < Morphine	0.326		0.917	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.803		0.231	Medium	
Region | Group	Medial	Control > Fentanyl	0.979		0.0245	Small	
Region | Group	Medial	Control < Morphine	0.504		0.621	Medium	
Region | Group	Medial	Fentanyl < Morphine	0.488		0.646	Medium	
Region | Group	Posterior	Control > Fentanyl	0.12		1.47	Large	
Region | Group	Posterior	Control < Morphine	0.719		0.334	Medium	
Region | Group	Posterior	Fentanyl < Morphine	0.0584	.	1.8	Large	
Group | Region	Control	Anterior > Lateral	0.0519	.	1.17	Large	
Group | Region	Control	Anterior < Medial	0.352		0.554	Medium	
Group | Region	Control	Anterior < Posterior	0.00477	**	1.74	Large	
Group | Region	Control	Lateral < Medial	0.00499	**	1.73	Large	
Group | Region	Control	Lateral < Posterior	8.2E-06	***	2.91	Large	
Group | Region	Control	Medial < Posterior	0.0501	.	1.18	Large	
Group | Region	Fentanyl	Anterior > Lateral	0.00905	**	1.6	Large	
Group | Region	Fentanyl	Anterior > Medial	0.33		0.58	Medium	
Group | Region	Fentanyl	Anterior > Posterior	0.16		0.84	Large	

Group | Region	Fentanyl	Lateral < Medial	0.0904	.	1.02	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.205		0.757	Medium	
Group | Region	Fentanyl	Medial > Posterior	0.661		0.26	Medium	
Group | Region	Morphine	Anterior > Lateral	0.213		0.744	Medium	
Group | Region	Morphine	Anterior < Medial	0.249		0.688	Medium	
Group | Region	Morphine	Anterior < Posterior	0.00965	**	1.58	Large	
Group | Region	Morphine	Lateral < Medial	0.0186	*	1.43	Large	
Group | Region	Morphine	Lateral < Posterior	0.000232	***	2.33	Large	
Group | Region	Morphine	Medial < Posterior	0.135		0.896	Large	
Cortical Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	5.74	Large	30%
Region		Anterior > Medial	0	***	5.65	Large	
Region		Anterior > Posterior	0	***	2.47	Large	
Region		Lateral < Medial	0.992		0.0859	Small	
Region		Lateral < Posterior	0	***	3.27	Large	
Region		Medial < Posterior	0	***	3.18	Large	
Pore Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	2.54	Large	60%
Region		Anterior > Medial	0.000973	***	1.24	Large	
Region		Anterior < Posterior	0.977		0.125	Small	
Region		Lateral < Medial	0.000552	***	1.3	Large	
Region		Lateral < Posterior	0	***	2.67	Large	
Region		Medial < Posterior	0.000256	***	1.37	Large	
Region | Group	Anterior	Control < Fentanyl	0.114		1.5	Large	44%
Region | Group	Anterior	Control < Morphine	0.3		0.971	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.572		0.527	Medium	
Region | Group	Lateral	Control < Fentanyl	0.542		0.57	Medium	
Region | Group	Lateral	Control < Morphine	0.393		0.799	Medium	
Region | Group	Lateral	Fentanyl < Morphine	0.806		0.229	Medium	
Region | Group	Medial	Control < Fentanyl	0.799		0.237	Medium	
Region | Group	Medial	Control < Morphine	0.394		0.797	Medium	
Region | Group	Medial	Fentanyl < Morphine	0.548		0.56	Medium	
Region | Group	Posterior	Control > Fentanyl	0.204		1.19	Large	
Region | Group	Posterior	Control < Morphine	0.443		0.718	Medium	
Region | Group	Posterior	Fentanyl < Morphine	0.0456	*	1.91	Large	
Group | Region	Control	Anterior > Lateral	0.00148	**	2.08	Large	
Group | Region	Control	Anterior > Medial	0.171		0.863	Large	
Group | Region	Control	Anterior < Posterior	0.0838	.	1.1	Large	

Group | Region	Control	Lateral < Medial	0.0549	.	1.22	Large	
Group | Region	Control	Lateral < Posterior	4.3E-06	***	3.18	Large	
Group | Region	Control	Medial < Posterior	0.00267	**	1.96	Large	
Group | Region	Fentanyl	Anterior > Lateral	1.12E-05	***	3.01	Large	
Group | Region	Fentanyl	Anterior > Medial	0.00122	**	2.12	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.013	*	1.6	Large	
Group | Region	Fentanyl	Lateral < Medial	0.159		0.887	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.027	*	1.41	Large	
Group | Region	Fentanyl	Medial < Posterior	0.401		0.527	Medium	
Group | Region	Morphine	Anterior > Lateral	0.000637	***	2.26	Large	
Group | Region	Morphine	Anterior > Medial	0.101		1.04	Large	
Group | Region	Morphine	Anterior < Posterior	0.181		0.842	Large	
Group | Region	Morphine	Lateral < Medial	0.0552	.	1.22	Large	
Group | Region	Morphine	Lateral < Posterior	6.9E-06	***	3.1	Large	
Group | Region	Morphine	Medial < Posterior	0.00384	**	1.88	Large	
Cortical Surface	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	4.8	Large	26%
Region		Anterior > Medial	0	***	6.66	Large	
Region		Anterior > Posterior	0.000006	***	1.7	Large	
Region		Lateral > Medial	9E-07	***	1.86	Large	
Region		Lateral < Posterior	0	***	3.1	Large	
Region		Medial < Posterior	0	***	4.96	Large	
Pore Surface	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	2.35	Large	39%
Region		Anterior > Medial	0.000243	***	1.37	Large	
Region		Anterior > Posterior	0.387		0.494	Medium	
Region		Lateral < Medial	0.013	*	0.979	Large	
Region		Lateral < Posterior	9E-07	***	1.86	Large	
Region		Medial < Posterior	0.0301	*	0.881	Large	
Region | Group	Anterior	Control < Fentanyl	0.0909	.	1.97	Large	42%
Region | Group	Anterior	Control < Morphine	0.405		0.953	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.373		1.02	Large	
Region | Group	Lateral	Control < Fentanyl	0.295		1.2	Large	
Region | Group	Lateral	Control < Morphine	0.276		1.25	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.965		0.0497	Small	
Region | Group	Medial	Control < Fentanyl	0.509		0.754	Medium	
Region | Group	Medial	Control < Morphine	0.408		0.948	Large	
Region | Group	Medial	Fentanyl < Morphine	0.865		0.194	Small	

Region | Group	Posterior	Control < Fentanyl	0.984		0.0228	Small	
Region | Group	Posterior	Control < Morphine	0.263		1.29	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.271		1.27	Large	
Group | Region	Control	Anterior > Lateral	0.000306	***	2.25	Large	
Group | Region	Control	Anterior > Medial	0.065	.	1.1	Large	
Group | Region	Control	Anterior > Posterior	0.909		0.0673	Small	
Group | Region	Control	Lateral < Medial	0.0534	.	1.15	Large	
Group | Region	Control	Lateral < Posterior	0.000441	***	2.19	Large	
Group | Region	Control	Medial < Posterior	0.0826	.	1.03	Large	
Group | Region	Fentanyl	Anterior > Lateral	3.4E-06	***	3.03	Large	
Group | Region	Fentanyl	Anterior > Medial	0.000212	***	2.32	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.00107	**	2.02	Large	
Group | Region	Fentanyl	Lateral < Medial	0.232		0.705	Medium	
Group | Region	Fentanyl	Lateral < Posterior	0.0904	.	1.01	Large	
Group | Region	Fentanyl	Medial < Posterior	0.608		0.301	Medium	
Group | Region	Morphine	Anterior > Lateral	0.00149	**	1.95	Large	
Group | Region	Morphine	Anterior > Medial	0.0638	.	1.11	Large	
Group | Region	Morphine	Anterior < Posterior	0.647		0.269	Medium	
Group | Region	Morphine	Lateral < Medial	0.152		0.849	Large	
Group | Region	Morphine	Lateral < Posterior	0.000361	***	2.22	Large	
Group | Region	Morphine	Medial < Posterior	0.0223	*	1.37	Large	
Intersection Surface	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.000182	***	1.4	Large	51%
Region		Anterior < Medial	0.13		0.686	Medium	
Region		Anterior < Posterior	0.0218	*	0.919	Large	
Region		Lateral < Medial	1E-07	***	2.09	Large	
Region		Lateral < Posterior	0	***	2.32	Large	
Region		Medial < Posterior	0.874		0.233	Medium	
Region | Group	Anterior	Control < Fentanyl	0.0657	.	1.46	Large	38%
Region | Group	Anterior	Control < Morphine	1		0.000305	Small	
Region | Group	Anterior	Fentanyl > Morphine	0.0658	.	1.46	Large	
Region | Group	Lateral	Control < Fentanyl	0.657		0.347	Medium	
Region | Group	Lateral	Control < Morphine	0.399		0.66	Medium	
Region | Group	Lateral	Fentanyl < Morphine	0.688		0.313	Medium	
Region | Group	Medial	Control > Fentanyl	0.71		0.29	Medium	
Region | Group	Medial	Control > Morphine	0.963		0.0364	Small	
Region | Group	Medial	Fentanyl < Morphine	0.745		0.254	Medium	

Region | Group	Posterior	Control > Fentanyl	0.147		1.14	Large	
Region | Group	Posterior	Control < Morphine	0.637		0.369	Medium	
Region | Group	Posterior	Fentanyl < Morphine	0.0567	.	1.51	Large	
Group | Region	Control	Anterior > Lateral	0.118		1.02	Large	
Group | Region	Control	Anterior < Medial	0.0708	.	1.18	Large	
Group | Region	Control	Anterior < Posterior	0.0152	*	1.61	Large	
Group | Region	Control	Lateral < Medial	0.00116	**	2.2	Large	
Group | Region	Control	Lateral < Posterior	0.000142	***	2.63	Large	
Group | Region	Control	Medial < Posterior	0.51		0.426	Medium	
Group | Region	Fentanyl	Anterior > Lateral	0.00161	**	2.13	Large	
Group | Region	Fentanyl	Anterior > Medial	0.381		0.566	Medium	
Group | Region	Fentanyl	Anterior > Posterior	0.127		0.994	Large	
Group | Region	Fentanyl	Lateral < Medial	0.018	*	1.56	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.082	.	1.14	Large	
Group | Region	Fentanyl	Medial > Posterior	0.508		0.427	Medium	
Group | Region	Morphine	Anterior > Lateral	0.578		0.359	Medium	
Group | Region	Morphine	Anterior < Medial	0.0798	.	1.15	Large	
Group | Region	Morphine	Anterior < Posterior	0.00324	**	1.98	Large	
Group | Region	Morphine	Lateral < Medial	0.0227	*	1.5	Large	
Group | Region	Morphine	Lateral < Posterior	0.000608	***	2.34	Large	
Group | Region	Morphine	Medial < Posterior	0.201		0.831	Large	
Pore Surface: PoreVolume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0	***	2.18	Large	54%
Region		Anterior < Medial	0.163		0.651	Medium	
Region		Anterior > Posterior	0.00982	**	1.01	Large	
Region		Lateral > Medial	4.26E-05	***	1.53	Large	
Region		Lateral > Posterior	0	***	3.19	Large	
Region		Medial > Posterior	9.6E-06	***	1.66	Large	
Pore Surface: Cortical Volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.536		0.417	Medium	50%
Region		Anterior < Medial	0.0966	.	0.729	Medium	
Region		Anterior < Posterior	0.655		0.358	Medium	
Region		Lateral < Medial	0.00266	**	1.15	Large	
Region		Lateral < Posterior	0.0699	.	0.774	Medium	
Region		Medial > Posterior	0.626		0.372	Medium	
Region | Group	Anterior	Control < Fentanyl	0.265		1.32	Large	46%
Region | Group	Anterior	Control < Morphine	0.804		0.292	Medium	
Region | Group	Anterior	Fentanyl > Morphine	0.382		1.03	Large	
Region | Group	Lateral	Control < Fentanyl	0.222		1.45	Large	

Region | Group	Lateral	Control < Morphine	0.237		1.41	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.967		0.0479	Small	
Region | Group	Medial	Control < Fentanyl	0.731		0.404	Medium	
Region | Group	Medial	Control < Morphine	0.641		0.549	Medium	
Region | Group	Medial	Fentanyl < Morphine	0.902		0.145	Small	
Region | Group	Posterior	Control > Fentanyl	0.788		0.315	Medium	
Region | Group	Posterior	Control < Morphine	0.48		0.834	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.332		1.15	Large	
Group | Region	Control	Anterior > Lateral	0.141		0.83	Large	
Group | Region	Control	Anterior < Medial	0.0958	.	0.942	Large	
Group | Region	Control	Anterior < Posterior	0.224		0.684	Medium	
Group | Region	Control	Lateral < Medial	0.00238	**	1.77	Large	
Group | Region	Control	Lateral < Posterior	0.00867	**	1.51	Large	
Group | Region	Control	Medial > Posterior	0.645		0.258	Medium	
Group | Region	Fentanyl	Anterior > Lateral	0.214		0.699	Medium	
Group | Region	Fentanyl	Anterior < Medial	0.969		0.0214	Small	
Group | Region	Fentanyl	Anterior > Posterior	0.0913	.	0.955	Large	
Group | Region	Fentanyl	Lateral < Medial	0.2		0.721	Medium	
Group | Region	Fentanyl	Lateral > Posterior	0.647		0.256	Medium	
Group | Region	Fentanyl	Medial > Posterior	0.0844	.	0.977	Large	
Group | Region	Morphine	Anterior < Lateral	0.61		0.285	Medium	
Group | Region	Morphine	Anterior < Medial	0.0354	*	1.2	Large	
Group | Region	Morphine	Anterior < Posterior	0.0316	*	1.23	Large	
Group | Region	Morphine	Lateral < Medial	0.106		0.914	Large	
Group | Region	Morphine	Lateral < Posterior	0.0962	.	0.941	Large	
Group | Region	Morphine	Medial < Posterior	0.961		0.0271	Small	
Pore Thickness	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	3.16E-05	***	1.56	Large	51%
Region		Anterior > Medial	0.641		0.364	Medium	
Region		Anterior < Posterior	0.000272	***	1.36	Large	
Region		Lateral < Medial	0.00166	**	1.19	Large	
Region		Lateral < Posterior	0	***	2.92	Large	
Region		Medial < Posterior	4.3E-06	***	1.73	Large	
Region | Group	Anterior	Control < Fentanyl	0.697		0.287	Medium	44%
Region | Group	Anterior	Control < Morphine	0.45		0.558	Medium	
Region | Group	Anterior	Fentanyl < Morphine	0.713		0.272	Medium	
Region | Group	Lateral	Control > Fentanyl	0.466		0.539	Medium	
Region | Group	Lateral	Control < Morphine	0.874		0.117	Small	
Region | Group	Lateral	Fentanyl < Morphine	0.375		0.656	Medium	
Region | Group	Medial	Control > Fentanyl	0.298		0.771	Medium	

Region | Group	Medial	Control < Morphine	0.676		0.308	Medium	
Region | Group	Medial	Fentanyl < Morphine	0.148		1.08	Large	
Region | Group	Posterior	Control > Fentanyl	0.00563	**	2.13	Large	
Region | Group	Posterior	Control > Morphine	0.233		0.887	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.0959	.	1.25	Large	
Group | Region	Control	Anterior > Lateral	0.0383	*	1.14	Large	
Group | Region	Control	Anterior < Medial	0.894		0.0715	Small	
Group | Region	Control	Anterior < Posterior	7.3E-06	***	2.65	Large	
Group | Region	Control	Lateral < Medial	0.028	*	1.21	Large	
Group | Region	Control	Lateral < Posterior	0	***	3.79	Large	
Group | Region	Control	Medial < Posterior	1.16E-05	***	2.58	Large	
Group | Region	Fentanyl	Anterior > Lateral	0.000559	***	1.96	Large	
Group | Region	Fentanyl	Anterior > Medial	0.0705	.	0.986	Large	
Group | Region	Fentanyl	Anterior < Posterior	0.665		0.233	Medium	
Group | Region	Fentanyl	Lateral < Medial	0.0737	.	0.975	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.000138	***	2.19	Large	
Group | Region	Fentanyl	Medial < Posterior	0.0265	*	1.22	Large	
Group | Region	Morphine	Anterior > Lateral	0.0047	**	1.58	Large	
Group | Region	Morphine	Anterior > Medial	0.74		0.179	Small	
Group | Region	Morphine	Anterior < Posterior	0.0279	*	1.21	Large	
Group | Region	Morphine	Lateral < Medial	0.0115	*	1.4	Large	
Group | Region	Morphine	Lateral < Posterior	0.000003	***	2.78	Large	
Group | Region	Morphine	Medial < Posterior	0.0122	*	1.39	Large	
Pore Separation	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	4.76E-05	***	1.52	Large	43%
Region		Anterior > Medial	8.6E-06	***	1.67	Large	
Region		Anterior > Posterior	0.0897	.	0.74	Medium	
Region		Lateral > Medial	0.963		0.148	Small	
Region		Lateral < Posterior	0.066	.	0.782	Medium	
Region		Medial < Posterior	0.0199	*	0.93	Large	
Pore Tb.N	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.987		0.104	Small	56%
Region		Anterior < Medial	0.0696	.	0.775	Medium	
Region		Anterior > Posterior	0.987		0.103	Small	
Region		Lateral < Medial	0.144		0.671	Medium	
Region		Lateral > Posterior	0.907		0.207	Medium	
Region		Medial > Posterior	0.0309	*	0.878	Large	
Region | Group	Anterior	Control < Fentanyl	0.203		1.53	Large	44%

Region | Group	Anterior	Control < Morphine	0.843		0.234	Medium	
Region | Group	Anterior	Fentanyl > Morphine	0.279		1.29	Large	
Region | Group	Lateral	Control < Fentanyl	0.0878	.	2.08	Large	
Region | Group	Lateral	Control < Morphine	0.192		1.57	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.666		0.51	Medium	
Region | Group	Medial	Control < Fentanyl	0.465		0.867	Large	
Region | Group	Medial	Control < Morphine	0.736		0.399	Medium	
Region | Group	Medial	Fentanyl > Morphine	0.692		0.469	Medium	
Region | Group	Posterior	Control < Fentanyl	0.807		0.289	Medium	
Region | Group	Posterior	Control < Morphine	0.393		1.02	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.539		0.728	Medium	
Group | Region	Control	Anterior > Lateral	0.332		0.523	Medium	
Group | Region	Control	Anterior < Medial	0.0843	.	0.94	Large	
Group | Region	Control	Anterior < Posterior	0.928		0.0485	Small	
Group | Region	Control	Lateral < Medial	0.00836	**	1.46	Large	
Group | Region	Control	Lateral < Posterior	0.289		0.572	Medium	
Group | Region	Control	Medial > Posterior	0.101		0.891	Large	
Group | Region	Fentanyl	Anterior < Lateral	0.961		0.0263	Small	
Group | Region	Fentanyl	Anterior < Medial	0.603		0.28	Medium	
Group | Region	Fentanyl	Anterior > Posterior	0.0302	*	1.19	Large	
Group | Region	Fentanyl	Lateral < Medial	0.638		0.253	Medium	
Group | Region	Fentanyl	Lateral > Posterior	0.0269	*	1.22	Large	
Group | Region	Fentanyl	Medial > Posterior	0.00811	**	1.47	Large	
Group | Region	Morphine	Anterior < Lateral	0.136		0.809	Large	
Group | Region	Morphine	Anterior < Medial	0.0436	*	1.1	Large	
Group | Region	Morphine	Anterior < Posterior	0.126		0.831	Large	
Group | Region	Morphine	Lateral < Medial	0.583		0.295	Medium	
Group | Region	Morphine	Lateral < Posterior	0.967		0.0219	Small	
Group | Region	Morphine	Medial > Posterior	0.611		0.273	Medium	
Degree of Anisotropy	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	8E-07	***	1.87	Large	37%
Region		Anterior > Medial	0.0885	.	0.742	Medium	
Region		Anterior < Posterior	0.957		0.157	Small	
Region		Lateral < Medial	0.00315	**	1.13	Large	
Region		Lateral < Posterior	1E-07	***	2.03	Large	
Region		Medial < Posterior	0.0261	*	0.898	Large	
Pore Fractal	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.689		0.341	Medium	54%
Region		Anterior < Medial	0.119		0.699	Medium	
Region		Anterior < Posterior	0.951		0.164	Small	

Region		Lateral < Medial	0.00744	**	1.04	Large	
Region		Lateral < Posterior	0.367		0.505	Medium	
Region		Medial > Posterior	0.318		0.534	Medium	
Region | Group	Anterior	Control < Fentanyl	0.115		1.92	Large	38%
Region | Group	Anterior	Control < Morphine	0.496		0.812	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.356		1.1	Large	
Region | Group	Lateral	Control < Fentanyl	0.187		1.59	Large	
Region | Group	Lateral	Control < Morphine	0.0974	.	2.02	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.718		0.429	Medium	
Region | Group	Medial	Control < Fentanyl	0.406		0.992	Large	
Region | Group	Medial	Control < Morphine	0.426		0.951	Large	
Region | Group	Medial	Fentanyl > Morphine	0.972		0.0414	Small	
Region | Group	Posterior	Control < Fentanyl	0.939		0.0904	Small	
Region | Group	Posterior	Control < Morphine	0.396		1.02	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.438		0.925	Large	
Group | Region	Control	Anterior > Lateral	0.239		0.637	Medium	
Group | Region	Control	Anterior < Medial	0.078	.	0.96	Large	
Group | Region	Control	Anterior < Posterior	0.193		0.705	Medium	
Group | Region	Control	Lateral < Medial	0.00422	**	1.6	Large	
Group | Region	Control	Lateral < Posterior	0.0151	*	1.34	Large	
Group | Region	Control	Medial > Posterior	0.635		0.255	Medium	
Group | Region	Fentanyl	Anterior > Lateral	0.0784	.	0.959	Large	
Group | Region	Fentanyl	Anterior < Medial	0.946		0.0366	Small	
Group | Region	Fentanyl	Anterior > Posterior	0.0408	*	1.12	Large	
Group | Region	Fentanyl	Lateral < Medial	0.068	.	0.996	Large	
Group | Region	Fentanyl	Lateral > Posterior	0.764		0.162	Small	
Group | Region	Fentanyl	Medial > Posterior	0.0349	*	1.16	Large	
Group | Region	Morphine	Anterior < Lateral	0.288		0.574	Medium	
Group | Region	Morphine	Anterior < Medial	0.0445	*	1.1	Large	
Group | Region	Morphine	Anterior < Posterior	0.095	.	0.908	Large	
Group | Region	Morphine	Lateral < Medial	0.33		0.526	Medium	
Group | Region	Morphine	Lateral < Posterior	0.534		0.335	Medium	
Group | Region	Morphine	Medial > Posterior	0.722		0.191	Small	
Number of Pores	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.00548	**	1.07	Large	42%
Region		Anterior > Medial	0.000184	***	1.4	Large	
Region		Anterior > Posterior	6.54E-05	***	1.49	Large	
Region		Lateral > Medial	0.712		0.329	Medium	
Region		Lateral > Posterior	0.525		0.422	Medium	
Region		Medial > Posterior	0.99		0.0929	Small	

Region | Group	Anterior	Control < Fentanyl	0.0729	.	2.43	Large	45%
Region | Group	Anterior	Control < Morphine	0.495		0.897	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.248		1.53	Large	
Region | Group	Lateral	Control < Fentanyl	0.2		1.71	Large	
Region | Group	Lateral	Control < Morphine	0.191		1.74	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.979		0.0351	Small	
Region | Group	Medial	Control < Fentanyl	0.51		0.866	Large	
Region | Group	Medial	Control < Morphine	0.706		0.495	Medium	
Region | Group	Medial	Fentanyl > Morphine	0.777		0.372	Medium	
Region | Group	Posterior	Control < Fentanyl	0.227		1.61	Large	
Region | Group	Posterior	Control < Morphine	0.292		1.39	Large	
Region | Group	Posterior	Fentanyl > Morphine	0.871		0.213	Medium	
Group | Region	Control	Anterior > Lateral	0.042	*	1.11	Large	
Group | Region	Control	Anterior > Medial	0.169		0.745	Medium	
Group | Region	Control	Anterior > Posterior	0.0123	*	1.38	Large	
Group | Region	Control	Lateral < Medial	0.493		0.369	Medium	
Group | Region	Control	Lateral > Posterior	0.614		0.271	Medium	
Group | Region	Control	Medial > Posterior	0.236		0.64	Medium	
Group | Region	Fentanyl	Anterior > Lateral	0.00115	**	1.84	Large	
Group | Region	Fentanyl	Anterior > Medial	0.000067	***	2.31	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.000126	***	2.21	Large	
Group | Region	Fentanyl	Lateral > Medial	0.379		0.474	Medium	
Group | Region	Fentanyl	Lateral > Posterior	0.488		0.373	Medium	
Group | Region	Fentanyl	Medial < Posterior	0.85		0.101	Small	
Group | Region	Morphine	Anterior > Lateral	0.621		0.266	Medium	
Group | Region	Morphine	Anterior > Medial	0.0364	*	1.15	Large	
Group | Region	Morphine	Anterior > Posterior	0.103		0.887	Large	
Group | Region	Morphine	Lateral > Medial	0.105		0.881	Large	
Group | Region	Morphine	Lateral > Posterior	0.25		0.621	Medium	
Group | Region	Morphine	Medial < Posterior	0.629		0.26	Medium	
Pore Density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.000107	***	1.45	Large	51%
Region		Anterior < Medial	0.00562	**	1.07	Large	
Region		Anterior > Posterior	0.367		0.505	Medium	
Region		Lateral > Medial	0.61		0.38	Medium	
Region		Lateral > Posterior	3E-07	***	1.95	Large	
Region		Medial > Posterior	2.61E-05	***	1.57	Large	
Region | Group	Anterior	Control < Fentanyl	0.241		1.53	Large	42%
Region | Group	Anterior	Control < Morphine	0.891		0.177	Small	

Region | Group	Anterior	Fentanyl > Morphine	0.298		1.36	Large	
Region | Group	Lateral	Control < Fentanyl	0.143		1.93	Large	
Region | Group	Lateral	Control < Morphine	0.166		1.82	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.932		0.11	Small	
Region | Group	Medial	Control < Fentanyl	0.752		0.408	Medium	
Region | Group	Medial	Control > Morphine	0.89		0.178	Small	
Region | Group	Medial	Fentanyl > Morphine	0.65		0.586	Medium	
Region | Group	Posterior	Control < Fentanyl	0.333		1.26	Large	
Region | Group	Posterior	Control < Morphine	0.464		0.949	Large	
Region | Group	Posterior	Fentanyl > Morphine	0.809		0.312	Medium	
Group | Region	Control	Anterior < Lateral	0.157		0.767	Medium	
Group | Region	Control	Anterior < Medial	0.00505	**	1.56	Large	
Group | Region	Control	Anterior > Posterior	0.214		0.672	Medium	
Group | Region	Control	Lateral < Medial	0.142		0.796	Medium	
Group | Region	Control	Lateral > Posterior	0.00945	**	1.44	Large	
Group | Region	Control	Medial > Posterior	0.000107	***	2.23	Large	
Group | Region	Fentanyl	Anterior < Lateral	0.0334	*	1.17	Large	
Group | Region	Fentanyl	Anterior < Medial	0.418		0.436	Medium	
Group | Region	Fentanyl	Anterior > Posterior	0.083	.	0.944	Large	
Group | Region	Fentanyl	Lateral > Medial	0.177		0.73	Medium	
Group | Region	Fentanyl	Lateral > Posterior	0.000229	***	2.11	Large	
Group | Region	Fentanyl	Medial > Posterior	0.0125	*	1.38	Large	
Group | Region	Morphine	Anterior < Lateral	3.46E-05	***	2.41	Large	
Group | Region	Morphine	Anterior < Medial	0.0279	*	1.21	Large	
Group | Region	Morphine	Anterior < Posterior	0.851		0.101	Small	
Group | Region	Morphine	Lateral > Medial	0.0282	*	1.21	Large	
Group | Region	Morphine	Lateral > Posterior	6.56E-05	***	2.31	Large	
Group | Region	Morphine	Medial > Posterior	0.0431	*	1.11	Large	
Euler number	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.771		0.297	Medium	50%
Region		Anterior > Medial	0.0343	*	0.865	Large	
Region		Anterior > Posterior	0.00826	**	1.03	Large	
Region		Lateral > Medial	0.266		0.568	Medium	
Region		Lateral > Posterior	0.0954	.	0.731	Medium	
Region		Medial > Posterior	0.952		0.163	Small	
Connectivity	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	2.17	Large	40%
Region		Anterior > Medial	8E-07	***	1.87	Large	
Region		Anterior > Posterior	2.5E-06	***	1.78	Large	

Region		Lateral < Medial	0.752		0.307	Medium	
Region		Lateral < Posterior	0.572		0.399	Medium	
Region		Medial < Posterior	0.991		0.0915	Small	
Region | Group	Anterior	Control < Fentanyl	0.0873	.	1.89	Large	39%
Region | Group	Anterior	Control < Morphine	0.272		1.19	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.519		0.695	Medium	
Region | Group	Lateral	Control < Fentanyl	0.619		0.535	Medium	
Region | Group	Lateral	Control < Morphine	0.49		0.744	Medium	
Region | Group	Lateral	Fentanyl < Morphine	0.845		0.209	Medium	
Region | Group	Medial	Control < Fentanyl	0.762		0.325	Medium	
Region | Group	Medial	Control < Morphine	0.428		0.854	Large	
Region | Group	Medial	Fentanyl < Morphine	0.622		0.529	Medium	
Region | Group	Posterior	Control < Fentanyl	0.856		0.195	Small	
Region | Group	Posterior	Control < Morphine	0.277		1.18	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.362		0.984	Large	
Group | Region	Control	Anterior > Lateral	0.00473	**	1.58	Large	
Group | Region	Control	Anterior > Medial	0.0248	*	1.23	Large	
Group | Region	Control	Anterior > Posterior	0.0279	*	1.21	Large	
Group | Region	Control	Lateral < Medial	0.527		0.341	Medium	
Group | Region	Control	Lateral < Posterior	0.495		0.367	Medium	
Group | Region	Control	Medial < Posterior	0.96		0.0267	Small	
Group | Region	Fentanyl	Anterior > Lateral	1.2E-06	***	2.93	Large	
Group | Region	Fentanyl	Anterior > Medial	2.8E-06	***	2.79	Large	
Group | Region	Fentanyl	Anterior > Posterior	1.4E-06	***	2.9	Large	
Group | Region	Fentanyl	Lateral < Medial	0.808		0.131	Small	
Group | Region	Fentanyl	Lateral < Posterior	0.96		0.0271	Small	
Group | Region	Fentanyl	Medial > Posterior	0.847		0.104	Small	
Group | Region	Morphine	Anterior > Lateral	0.000392	***	2.02	Large	
Group | Region	Morphine	Anterior > Medial	0.00485	**	1.57	Large	
Group | Region	Morphine	Anterior > Posterior	0.0265	*	1.22	Large	
Group | Region	Morphine	Lateral < Medial	0.403		0.451	Medium	
Group | Region	Morphine	Lateral < Posterior	0.139		0.802	Large	
Group | Region	Morphine	Medial < Posterior	0.514		0.351	Medium	
Connectivity density	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.00961	**	1.01	Large	35%
Region		Anterior > Medial	0.42		0.476	Medium	
Region		Anterior > Posterior	0.000494	***	1.31	Large	
Region		Lateral < Medial	0.316		0.536	Medium	
Region		Lateral > Posterior	0.77		0.298	Medium	

Region		Medial > Posterior	0.0444	*	0.833	Large	
Region | Group	Anterior	Control < Fentanyl	0.22		1.61	Large	46%
Region | Group	Anterior	Control < Morphine	0.491		0.893	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.581		0.714	Medium	
Region | Group	Lateral	Control < Fentanyl	0.581		0.714	Medium	
Region | Group	Lateral	Control < Morphine	0.491		0.893	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.89		0.179	Small	
Region | Group	Medial	Control < Fentanyl	0.89		0.179	Small	
Region | Group	Medial	Control < Morphine	0.491		0.893	Large	
Region | Group	Medial	Fentanyl < Morphine	0.581		0.714	Medium	
Region | Group	Posterior	Control > Fentanyl	1		0	Small	
Region | Group	Posterior	Control < Morphine	0.337		1.25	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.337		1.25	Large	
Group | Region	Control	Anterior > Lateral	0.187		0.714	Medium	
Group | Region	Control	Anterior < Medial	1		0	Small	
Group | Region	Control	Anterior > Posterior	0.101		0.893	Large	
Group | Region	Control	Lateral < Medial	0.187		0.714	Medium	
Group | Region	Control	Lateral > Posterior	0.74		0.179	Small	
Group | Region	Control	Medial > Posterior	0.101		0.893	Large	
Group | Region	Fentanyl	Anterior > Lateral	0.00401	**	1.61	Large	
Group | Region	Fentanyl	Anterior > Medial	0.00994	**	1.43	Large	
Group | Region	Fentanyl	Anterior > Posterior	1.99E-05	***	2.5	Large	
Group | Region	Fentanyl	Lateral < Medial	0.74		0.179	Small	
Group | Region	Fentanyl	Lateral > Posterior	0.101		0.893	Large	
Group | Region	Fentanyl	Medial > Posterior	0.0501	.	1.07	Large	
Group | Region	Morphine	Anterior > Lateral	0.187		0.714	Medium	
Group | Region	Morphine	Anterior < Medial	1		0	Small	
Group | Region	Morphine	Anterior > Posterior	0.321		0.536	Medium	
Group | Region	Morphine	Lateral < Medial	0.187		0.714	Medium	
Group | Region	Morphine	Lateral < Posterior	0.74		0.179	Small	
Group | Region	Morphine	Medial > Posterior	0.321		0.536	Medium	
SD Pore Thickness	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.0001	***	1.46	Large	40%
Region		Anterior < Medial	0.988		0.101	Small	
Region		Anterior < Posterior	0.000162	***	1.41	Large	
Region		Lateral < Medial	3.22E-05	***	1.56	Large	
Region		Lateral < Posterior	0	***	2.87	Large	
Region		Medial < Posterior	0.000483	***	1.31	Large	
SD Pore Separation	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**

Region		Anterior > Lateral	0	***	2.3	Large	38%
Region		Anterior > Medial	2.5E-06	***	1.77	Large	
Region		Anterior > Posterior	0.0544	.	0.807	Large	
Region		Lateral < Medial	0.328		0.528	Medium	
Region		Lateral < Posterior	6.41E-05	***	1.5	Large	
Region		Medial < Posterior	0.0143	*	0.967	Large	
Avg. Pore volume	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	2.55	Large	49%
Region		Anterior > Medial	0.168		0.646	Medium	
Region		Anterior < Posterior	0.0313	*	0.877	Large	
Region		Lateral < Medial	5E-07	***	1.91	Large	
Region		Lateral < Posterior	0	***	3.43	Large	
Region		Medial < Posterior	4.71E-05	***	1.52	Large	
Avg. Pore surface	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0	***	2.5	Large	54%
Region		Anterior > Medial	0.186		0.63	Medium	
Region		Anterior < Posterior	0.0547	.	0.806	Large	
Region		Lateral < Medial	9E-07	***	1.87	Large	
Region		Lateral < Posterior	0	***	3.3	Large	
Region		Medial < Posterior	0.000124	***	1.44	Large	
Avg. Orientation theta	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.0362	*	0.859	Large	26%
Region		Anterior > Medial	0	***	2.13	Large	
Region		Anterior > Posterior	0	***	3.37	Large	
Region		Lateral > Medial	0	***	2.99	Large	
Region		Lateral > Posterior	0	***	4.23	Large	
Region		Medial > Posterior	0.00104	**	1.24	Large	
Avg. Orientation phi	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	0.0716	.	0.771	Medium	26%
Region		Anterior > Medial	0	***	2.12	Large	
Region		Anterior > Posterior	0.00244	**	1.15	Large	
Region		Lateral > Medial	0.000336	***	1.35	Large	
Region		Lateral > Posterior	0.602		0.384	Medium	
Region		Medial < Posterior	0.0152	*	0.961	Large	
Avg. Pore thickness	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	0.127		0.689	Medium	39%
Region		Anterior < Medial	5.09E-05	***	1.52	Large	

Region		Anterior < Posterior	0	***	3.74	Large	
Region		Lateral < Medial	0.0468	*	0.826	Large	
Region		Lateral < Posterior	0	***	3.05	Large	
Region		Medial < Posterior	0	***	2.22	Large	
Avg. Pore Major diameter	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior > Lateral	7.8E-06	***	1.68	Large	63%
Region		Anterior > Medial	0.0267	*	0.896	Large	
Region		Anterior < Posterior	0.000205	***	1.39	Large	
Region		Lateral < Medial	0.0656	.	0.783	Medium	
Region		Lateral < Posterior	0	***	3.07	Large	
Region		Medial < Posterior	0	***	2.29	Large	
Region | Group	Anterior	Control > Fentanyl	0.0847	.	1.59	Large	45%
Region | Group	Anterior	Control > Morphine	0.216		1.13	Large	
Region | Group	Anterior	Fentanyl < Morphine	0.608		0.464	Medium	
Region | Group	Lateral	Control > Fentanyl	0.685		0.367	Medium	
Region | Group	Lateral	Control > Morphine	0.403		0.759	Medium	
Region | Group	Lateral	Fentanyl > Morphine	0.664		0.392	Medium	
Region | Group	Medial	Control < Fentanyl	0.575		0.506	Medium	
Region | Group	Medial	Control < Morphine	0.577		0.505	Medium	
Region | Group	Medial	Fentanyl > Morphine	0.999		0.00157	Small	
Region | Group	Posterior	Control > Fentanyl	0.0583	.	1.76	Large	
Region | Group	Posterior	Control > Morphine	0.442		0.697	Medium	
Region | Group	Posterior	Fentanyl < Morphine	0.245		1.06	Large	
Group | Region	Control	Anterior > Lateral	0.000125	***	2.21	Large	
Group | Region	Control	Anterior > Medial	0.000193	***	2.14	Large	
Group | Region	Control	Anterior < Posterior	0.0182	*	1.3	Large	
Group | Region	Control	Lateral < Medial	0.896		0.0705	Small	
Group | Region	Control	Lateral < Posterior	0	***	3.51	Large	
Group | Region	Control	Medial < Posterior	0	***	3.44	Large	
Group | Region	Fentanyl	Anterior > Lateral	0.0709	.	0.985	Large	
Group | Region	Fentanyl	Anterior > Medial	0.938		0.0414	Small	
Group | Region	Fentanyl	Anterior < Posterior	0.038	*	1.14	Large	
Group | Region	Fentanyl	Lateral < Medial	0.0832	.	0.943	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.000214	***	2.12	Large	
Group | Region	Fentanyl	Medial < Posterior	0.0318	*	1.18	Large	
Group | Region	Morphine	Anterior > Lateral	0.00112	**	1.84	Large	
Group | Region	Morphine	Anterior > Medial	0.348		0.507	Medium	
Group | Region	Morphine	Anterior < Posterior	0.00203	**	1.73	Large	

Group | Region	Morphine	Lateral < Medial	0.0157	*	1.33	Large	
Group | Region	Morphine	Lateral < Posterior	0	***	3.57	Large	
Group | Region	Morphine	Medial < Posterior	0.000104	***	2.24	Large	
Avg. Pore Sphericity	**Interaction Contrast**	**Comparison**	**p-value**	**Sig.**	**Cohen's D**	**Effect Group**	**Power**
Region		Anterior < Lateral	3E-07	***	1.95	Large	28%
Region		Anterior < Medial	0.000147	***	1.42	Large	
Region		Anterior < Posterior	1.1E-06	***	1.85	Large	
Region		Lateral > Medial	0.33		0.527	Medium	
Region		Lateral > Posterior	0.987		0.103	Small	
Region		Medial < Posterior	0.521		0.424	Medium	

Tibial Regions: All Directional Trends

Cortical Fractal Trends		
Region		Medial > Posterior > Lateral > Anterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Medial > Posterior > Anterior > Lateral
Group | Region	Fentanyl	Medial > Anterior > Lateral > Posterior
Group | Region	Morphine	Medial > Lateral > Posterior > Anterior
Number Closed Pores Trends		
Region		Anterior > Lateral > Medial > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Anterior > Medial > Lateral > Posterior
Group | Region	Fentanyl	Anterior > Lateral > Posterior > Medial
Group | Region	Morphine	Anterior > Lateral > Posterior > Medial
Number Open Pores Trends		
Region		Anterior > Medial > Posterior > Lateral
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control

Group | Region	Control	Anterior > Medial > Posterior > Lateral
Group | Region	Fentanyl	Anterior > Medial > Posterior > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Closed Pore Density Trends		
Region		Lateral > Medial > Anterior > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Medial > Lateral > Anterior > Posterior
Group | Region	Fentanyl	Lateral > Medial > Anterior > Posterior
Group | Region	Morphine	Lateral > Medial > Anterior > Posterior
Open Pore Density Trends		
Region		Medial > Lateral > Posterior > Anterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Medial > Anterior > Lateral > Posterior
Group | Region	Fentanyl	Medial > Lateral > Anterior > Posterior
Group | Region	Morphine	Medial > Lateral > Posterior > Anterior
Closed Porosity (%) Trends		
Region		Posterior > Medial > Lateral > Anterior
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Posterior > Medial > Anterior > Lateral
Group | Region	Fentanyl	Medial > Lateral > Posterior > Anterior
Group | Region	Morphine	Posterior > Medial > Lateral > Anterior
Open Porosity (%) Trends		
Region		Posterior > Anterior > Medial > Lateral
Group		Morphine > Control > Fentanyl
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Medial > Anterior > Lateral

Group | Region	Fentanyl	Anterior > Medial > Posterior > Lateral
Group | Region	Morphine	Posterior > Medial > Anterior > Lateral
Total Porosity (%) Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Posterior > Medial > Anterior > Lateral
Group | Region	Fentanyl	Anterior > Medial > Posterior > Lateral
Group | Region	Morphine	Posterior > Medial > Anterior > Lateral
Cortical Volume Trends		
Region		Anterior > Posterior > Medial > Lateral
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Anterior > Posterior > Lateral > Medial
Group | Region	Fentanyl	Anterior > Posterior > Medial > Lateral
Group | Region	Morphine	Anterior > Posterior > Medial > Lateral
Pore Volume Trends		
Region		Posterior > Anterior > Medial > Lateral
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Posterior > Anterior > Medial > Lateral
Group | Region	Fentanyl	Anterior > Posterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Cortical Surface Trends		
Region		Anterior > Posterior > Lateral > Medial
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Anterior > Posterior > Lateral > Medial
Group | Region	Fentanyl	Anterior > Posterior > Lateral > Medial

Group | Region	Morphine	Anterior > Posterior > Lateral > Medial
Pore Surface Trends		
Region		Anterior > Posterior > Medial > Lateral
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Anterior > Posterior > Medial > Lateral
Group | Region	Fentanyl	Anterior > Posterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Intersection Surface Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Posterior > Medial > Anterior > Lateral
Group | Region	Fentanyl	Anterior > Medial > Posterior > Lateral
Group | Region	Morphine	Posterior > Medial > Anterior > Lateral
Pore Surface:PoreVolume Trends		
Region		Lateral > Medial > Anterior > Posterior
Group		Fentanyl > Control > Morphine
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Lateral > Medial > Anterior > Posterior
Group | Region	Fentanyl	Lateral > Medial > Posterior > Anterior
Group | Region	Morphine	Lateral > Medial > Anterior > Posterior
Pore Surface:Cortical Volume Trends		
Region		Medial > Posterior > Anterior > Lateral
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Medial > Posterior > Anterior > Lateral
Group | Region	Fentanyl	Medial > Anterior > Lateral > Posterior
Group | Region	Morphine	Posterior > Medial > Lateral > Anterior

Pore Thickness Trends		
Region		Posterior > Anterior > Medial > Lateral
Group		Morphine > Control > Fentanyl
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Medial > Anterior > Lateral
Group | Region	Fentanyl	Posterior > Anterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Pore Separation Trends		
Region		Anterior > Posterior > Lateral > Medial
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Fentanyl > Morphine
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Control > Fentanyl > Morphine
Group | Region	Control	Anterior > Posterior > Lateral > Medial
Group | Region	Fentanyl	Posterior > Anterior > Medial > Lateral
Group | Region	Morphine	Anterior > Posterior > Medial > Lateral
Pore Tb.N Trends		
Region		Medial > Lateral > Anterior > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Medial > Posterior > Anterior > Lateral
Group | Region	Fentanyl	Medial > Lateral > Anterior > Posterior
Group | Region	Morphine	Medial > Posterior > Lateral > Anterior
Degree of Anisotropy Trends		
Region		Posterior > Anterior > Medial > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Anterior > Medial > Lateral
Group | Region	Fentanyl	Anterior > Posterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Pore Fractal Trends		

Region		Medial > Posterior > Anterior > Lateral
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Medial > Posterior > Anterior > Lateral
Group | Region	Fentanyl	Medial > Anterior > Lateral > Posterior
Group | Region	Morphine	Medial > Posterior > Lateral > Anterior
Number of Objects Trends		
Region		Anterior > Lateral > Medial > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Anterior > Medial > Lateral > Posterior
Group | Region	Fentanyl	Anterior > Lateral > Posterior > Medial
Group | Region	Morphine	Anterior > Lateral > Posterior > Medial
Pore Density Trends		
Region		Lateral > Medial > Anterior > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Medial > Lateral > Anterior > Posterior
Group | Region	Fentanyl	Lateral > Medial > Anterior > Posterior
Group | Region	Morphine	Lateral > Medial > Posterior > Anterior
Euler number Trends		
Region		Anterior > Lateral > Medial > Posterior
Group		Fentanyl > Morphine > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Anterior > Medial > Lateral > Posterior
Group | Region	Fentanyl	Anterior > Lateral > Posterior > Medial
Group | Region	Morphine	Lateral > Anterior > Posterior > Medial
Connectivity Trends		
Region		Anterior > Posterior > Medial > Lateral

Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Group | Region	Control	Anterior > Posterior > Medial > Lateral
Group | Region	Fentanyl	Anterior > Medial > Posterior > Lateral
Group | Region	Morphine	Anterior > Posterior > Medial > Lateral
Connectivity density Trends		
Region		Anterior > Medial > Lateral > Posterior
Group		Morphine > Fentanyl > Control
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
Group | Region	Control	Medial > Anterior > Lateral > Posterior
Group | Region	Fentanyl	Anterior > Medial > Lateral > Posterior
Group | Region	Morphine	Medial > Anterior > Posterior > Lateral
SD Pore Thickness Trends		
Region		Posterior > Medial > Anterior > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Medial > Anterior > Lateral
Group | Region	Fentanyl	Posterior > Anterior > Medial > Lateral
Group | Region	Morphine	Posterior > Medial > Anterior > Lateral
SD Pore Separation Trends		
Region		Anterior > Posterior > Medial > Lateral
Group		Fentanyl > Control > Morphine
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Control > Fentanyl > Morphine
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Anterior > Posterior > Medial > Lateral
Group | Region	Fentanyl	Anterior > Posterior > Medial > Lateral
Group | Region	Morphine	Anterior > Posterior > Medial > Lateral
Avg. Pore volume Trends		
Region		Posterior > Anterior > Medial > Lateral
Group		Morphine > Control > Fentanyl

Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Anterior > Medial > Lateral
Group | Region	Fentanyl	Anterior > Posterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Avg. Pore surface Trends		
Region		Posterior > Anterior > Medial > Lateral
Group		Morphine > Control > Fentanyl
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Anterior > Medial > Lateral
Group | Region	Fentanyl	Anterior > Posterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Avg. Orientation theta Trends		
Region		Lateral > Anterior > Medial > Posterior
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Control > Morphine
Group | Region	Control	Lateral > Anterior > Medial > Posterior
Group | Region	Fentanyl	Lateral > Anterior > Medial > Posterior
Group | Region	Morphine	Lateral > Anterior > Medial > Posterior
Orientation phi Trends		
Region		Anterior > Lateral > Posterior > Medial
Group		Fentanyl > Control > Morphine
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Group | Region	Control	Anterior > Lateral > Posterior > Medial
Group | Region	Fentanyl	Anterior > Lateral > Posterior > Medial
Group | Region	Morphine	Anterior > Lateral > Posterior > Medial
Avg. Pore thickness Trends		
Region		Posterior > Medial > Lateral > Anterior
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl

Region | Group	Lateral	Control > Fentanyl > Morphine
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Medial > Lateral > Anterior
Group | Region	Fentanyl	Posterior > Medial > Lateral > Anterior
Group | Region	Morphine	Posterior > Medial > Lateral > Anterior
Avg. Pore Major diameter Trends		
Region		Posterior > Anterior > Medial > Lateral
Group		Control > Morphine > Fentanyl
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Fentanyl > Morphine
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Control > Morphine > Fentanyl
Group | Region	Control	Posterior > Anterior > Medial > Lateral
Group | Region	Fentanyl	Posterior > Anterior > Medial > Lateral
Group | Region	Morphine	Posterior > Anterior > Medial > Lateral
Sphericity Trends		
Region		Lateral > Posterior > Medial > Anterior
Group		Control > Fentanyl > Morphine
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Fentanyl > Control > Morphine
Group | Region	Control	Lateral > Posterior > Medial > Anterior
Group | Region	Fentanyl	Posterior > Lateral > Medial > Anterior
Group | Region	Morphine	Lateral > Posterior > Medial > Anterior

Appendix XXIV: Femoral Histology Descriptive Statistics

Whole-Section Morphometry by Drug Treatment Group

	Control	Control	Control	Fentanyl	Fentanyl	Fentanyl	Morphine	Morphine	Morphine
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
% Cortical Area	55.5	54.9	4.84	57.6	58.1	2.69	55	54.7	2.77
% Marrow Area	44.5	45.1	4.84	42.4	41.9	2.69	45	45.3	2.77
% Remodeling Area	18.6	18.5	2.95	17.1	17	0.565	21	20.6	1.44
a.Rm.Cr/CA (a.Rm.Cr. OPD.CA) (#/mm^2)	7.72	7.64	0.601	7.34	7.9	1.55	10.8	11.3	2.64
a.Rm.Cr/RA (a.Rm.Cr. OPD.RA) (#/mm^2)	23.6	23.9	4.93	24.7	25.6	4.86	28.1	28.7	6.57
Ac.F.C (#/mm^2/year)	120	114	43.4	101	85.4	40.4	141	135	43.7
Ac.F.I (#/mm^2/year)	119	113	36.9	103	87.5	38.8	143	143	54.1
Ac.F.I.dL (#/mm^2/year)	173	170	44.6	122	116	28.3	198	195	70.9
a.Rm.Cr	182	182	14.8	180	176	33.1	269	278	75.2
C.On Mean Area (um^2)	16300	16300	3410	11700	12600	3580	12100	11100	2110
C.On Mean Aspect Ratio	1.65	1.67	0.0805	1.64	1.64	0.123	1.59	1.6	0.0417
C.On Mean Circularity	0.674	0.677	0.0376	0.715	0.712	0.0369	0.703	0.699	0.0291
C.On Mean Roundness	0.658	0.657	0.0138	0.67	0.664	0.0339	0.672	0.666	0.0181
C.On Mean Solidity	0.919	0.918	0.0223	0.933	0.933	0.017	0.933	0.934	0.0124
C.On Mean Wall Thickness (W.Th.C) (um)	35.9	39.4	9.22	36	39	6.42	36.7	36.6	2.09
C.On/CA (C.On. OPD.CA) (#/mm^2)	3.71	3.8	0.397	3.24	3.27	0.587	4.86	5.06	1.18
C.On/RA (C.On. OPD.RA) (#/mm^2)	11.3	11.6	2.51	10.9	10.6	1.87	12.7	12.5	3.32
Combined Mean Inner Label (um)	41.6	40.1	6.34	42.4	44.7	8.36	40.5	38.6	8.42
Combined Mean Label (um)	41.6	40.3	5.16	41.3	42.3	7.81	40.5	39.5	6.37

	Control	Control	Control	Fentanyl	Fentanyl	Fentanyl	Morphine	Morphine	Morphine
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Complete Osteon Count (C.On)	87.8	86.5	11.2	79.8	76.5	13.6	122	128	32.7
Cortical Area (mm^2)	23.7	24.5	2.72	24.9	25.1	3.2	24.9	24.5	1.11
dL.On Mean Area (um^2)	17300	17700	3290	14100	15000	3470	12900	11600	3220
dL.On Mean Aspect Ratio	1.65	1.67	0.0989	1.54	1.49	0.113	1.56	1.57	0.0697
dL.On Mean Circularity	0.668	0.665	0.0428	0.715	0.718	0.0155	0.7	0.699	0.0406
dL.On Mean Inner Label (um)	61.6	63	9.3	51.9	46.7	13.2	56.5	53	10.7
dL.On Mean Roundness	0.652	0.64	0.0256	0.691	0.709	0.0409	0.681	0.677	0.0292
dL.On Mean Solidity	0.918	0.918	0.0221	0.938	0.938	0.0126	0.932	0.931	0.0165
dL.On Mean Wall Thickness (W.Th.dL) (um)	38.6	41.7	9.04	42.1	41.9	4.63	39	38.4	2.6
dL.On/CA (dL.On .OPD.CA) (#/mm^2)	2.26	2.19	0.165	1.63	1.24	0.862	2.81	2.92	0.556
dL.On/RA (dL.On. OPD.RA) (#/mm^2)	6.9	7.36	1.35	5.42	4.18	2.61	7.34	7.16	1.47
Double-Labeled Osteon Count (dL.On)	53.8	54	7.93	38.8	32.5	14.9	70	74	14.8
Es.MS/BS (%)	48.1	56.1	20.9	42	50.9	28.1	34.3	31	20.3
F.On Mean Area (um^2)	4190	4280	1570	2700	2670	745	2620	2160	1170
F.On Mean Aspect Ratio	1.82	1.81	0.182	1.8	1.82	0.204	1.83	1.79	0.197
F.On Mean Circularity	0.757	0.765	0.0259	0.755	0.743	0.0444	0.748	0.754	0.0379
F.On Mean Roundness	0.644	0.636	0.0274	0.65	0.646	0.0386	0.639	0.65	0.0422
F.On Mean Solidity	0.937	0.939	0.00757	0.925	0.922	0.0221	0.926	0.932	0.0199
F.On/CA (F.On. OPD.CA) (#/mm^2)	3.77	3.68	0.33	3.74	4.26	1.13	5.53	5.79	1.62
F.On/RA (F.On. OPD.RA) (#/mm^2)	11.5	11.6	2.55	12.5	14	3.55	14.4	14.5	3.68
Forming Osteon Count (F.On)	88.8	88	4.65	91	96.5	23.6	138	142	45.2

	Control	Control	Control	Fentanyl	Fentanyl	Fentanyl	Morphine	Morphine	Morphine
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Imax (mm^4)	90.1	86.8	20.4	94.1	96.8	23.1	102	98	9.98
Imin (mm^4)	156	150	40	161	168	28.2	169	158	33.3
Marrow Area (mm^2)	19.2	17.9	4.08	18.3	18.5	1.85	20.5	20.1	2.9
Mean Pore Area (um^2)	795	787	54.8	619	643	57.5	581	488	230
Mean Pore Aspect Ratio	2.42	2.36	0.191	2.49	2.37	0.291	2.34	2.26	0.272
Mean Pore Circularity	0.635	0.631	0.0328	0.616	0.628	0.0417	0.664	0.678	0.0688
Mean Pore Max Feret Diameter (um)	42.5	43	2.62	40.3	40.4	1.63	38.4	36.7	6.49
Mean Pore Min Feret Diameter (um)	20.3	21.2	1.83	19	19	0.735	18.3	17.1	2.91
Mean Pore Perimeter (um)	108	110	6.86	103	103	3.77	96.9	92.3	16.4
Mean Pore Roundness	0.54	0.546	0.023	0.532	0.549	0.0412	0.552	0.558	0.0389
Mean Pore Solidity	0.851	0.848	0.0183	0.839	0.846	0.0176	0.864	0.869	0.0368
On.MAR.C (Combined Labels) (um/day)	2.97	2.88	0.369	2.95	3.02	0.558	2.89	2.82	0.455
On.MAR.I (Inner Labels) (um/day)	2.97	2.86	0.453	3.03	3.19	0.597	2.89	2.76	0.601
On.MAR.I.dL (dL Inner Labels) (um/day)	4.4	4.5	0.665	3.71	3.34	0.942	4.03	3.79	0.767
σ_f C (days)	12.1	12.1	3.1	12.6	12.1	3.92	12.8	13	1.26
σ_f I (days)	12	12.2	2.58	12.3	11.4	3.98	13	13.1	1.94
σ_f dL (days)	8.06	8.17	1.28	9.96	9.73	2.19	9.26	9.45	1.19
Parabolic Index (Y)	0.245	0.247	0.00612	0.244	0.243	0.00379	0.247	0.247	0.00283
Percent Porosity (%)	2.02	1.81	0.726	1.45	1.38	0.297	1.81	1.54	0.669
Pore Density CA (1/mm^2)	25.4	22.8	8.57	23.6	23.6	5.43	31.5	31.6	0.836
Pore Density RA (1/mm^2)	74.2	75.1	13.7	79.2	79.7	14.7	82.6	82.5	7.35
Remodeling Area (mm^2)	8.09	7.62	2.35	7.35	7.42	0.62	9.53	9.54	0.992
Rs.N/CA (Rs.N. OPD.CA) (#/mm^2)	0.242	0.246	0.058	0.371	0.422	0.194	0.368	0.369	0.146
Rs.N/RA (Rs.N. OPD.RA) (#/mm^2)	0.722	0.735	0.133	1.26	1.48	0.658	0.978	1.03	0.41

	Control	Control	Control	Fentanyl	Fentanyl	Fentanyl	Morphine	Morphine	Morphine
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Single-Labeled Osteon Count (sL.On)	27.2	28	8.77	37.8	38	23.3	45.8	44.5	18.2
sL.On Mean Area (um^2)	9020	9000	736	8160	7890	2580	8360	8290	916
sL.On Mean Aspect Ratio	1.61	1.6	0.0646	1.81	1.8	0.282	1.63	1.63	0.0934
sL.On Mean Circularity	0.707	0.703	0.0195	0.695	0.71	0.0994	0.721	0.723	0.0217
sL.On Mean Roundness	0.677	0.679	0.0141	0.63	0.64	0.0784	0.665	0.662	0.0331
sL.On Mean Solidity	0.932	0.933	0.019	0.919	0.928	0.0487	0.941	0.944	0.00886
sL.On Mean Wall Thickness (W.Th.sL) (um)	27.6	30.2	8.37	27.5	26.8	7.35	31	32	2.81
sL.On/CA (sL.On. OPD.CA) (#/mm^2)	1.15	1.23	0.346	1.46	1.46	0.851	1.82	1.82	0.658
sL.On/RA (sL.On. OPD.RA) (#/mm^2)	3.5	3.57	1.27	5.02	5	3	4.8	4.78	1.83
T.On / Rs.N	32.3	32.1	7.38	31.9	15.3	35.5	31.3	24.6	14.3
T.On Mean Area (um^2)	10200	10000	2210	7000	7080	2270	7110	6230	2050
T.On Mean Aspect Ratio	1.73	1.71	0.103	1.72	1.77	0.129	1.72	1.68	0.123
T.On Mean Circularity	0.716	0.721	0.0239	0.737	0.728	0.0399	0.726	0.732	0.0314
T.On Mean Roundness	0.651	0.651	0.012	0.659	0.649	0.0296	0.653	0.665	0.0278
T.On Mean Solidity	0.929	0.93	0.0122	0.93	0.928	0.0182	0.929	0.934	0.0162
T.On/CA (T.On. OPD.CA) (#/mm^2)	7.48	7.36	0.576	6.98	7.54	1.59	10.4	11	2.57
T.On/RA (T.On. OPD.RA) (#/mm^2)	22.8	23.2	4.85	23.4	24.6	4.94	27.1	27.8	6.27
tL.On Mean Area (um^2)	33300	31700	13400	12900	12100	14900	30800	31500	9390
tL.On Mean Aspect Ratio	1.77	1.71	0.172	0.92	0.86	1.07	1.68	1.72	0.18
tL.On Mean Circularity	0.647	0.611	0.149	0.318	0.309	0.368	0.597	0.603	0.0322
tL.On Mean Inner Label (um)	21.7	20	6.06	9.8	8.81	11.4	24.5	24.9	7.3

	Control	Control	Control	Fentanyl	Fentanyl	Fentanyl	Morphine	Morphine	Morphine
	Mean	**Median**	**SD**	**Mean**	**Median**	**SD**	**Mean**	**Median**	**SD**
tL.On Mean Outer Label (um)	41.5	43	6.63	15.9	15.8	18.3	40.4	39	9.4
tL.On Mean Roundness	0.625	0.616	0.0337	0.292	0.278	0.337	0.62	0.606	0.0696
tL.On Mean Solidity	0.893	0.883	0.0659	0.453	0.443	0.523	0.885	0.882	0.0103
tL.On Mean Wall Thickness (W.Th.tL) (um)	37.2	40.2	21.1	24.1	21.3	28.2	53	52.8	8.42
tL.ON/CA (tL.On. OPD.CA) (#/mm^2)	0.297	0.322	0.197	0.14	0.0885	0.182	0.231	0.216	0.0676
tL.ON/RA (tL.On. OPD.RA) (#/mm^2)	0.926	0.924	0.701	0.461	0.312	0.585	0.608	0.533	0.198
Total Area (mm^2)	42.9	41.3	5.71	43.1	44.4	4.46	45.3	44.2	3.81
Total Osteon Count (T.On)	176	177	13.3	171	169	32.3	260	270	72.4
Total Pore Area (um^2)	487000	415000	214000	356000	333000	56400	452000	392000	167000
Total Pore Number	612	554	257	576	570	75	782	778	30
Triple-Labeled Osteon Count (tL.On)	6.75	8	4.03	3.25	2.5	3.95	5.75	5.5	1.71
Rs.N	5.75	5.5	1.71	9.5	11.5	5.07	9.25	9	4.03
Zpol (mm^3)	52500000	50500000	9570000	55200000	57600000	8990000	57400000	56000000	4480000

Regional Morphometry

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
a.Rm.Cr/CA (a.Rm.Cr.OPD.CA) (#/mm^2)	9.15	8.68	3.86	5.22	4.41	2.9	8.73	8.23	3.42	10.5	10.7	3.22
a.Rm.Cr/RA (a.Rm.Cr.OPD.RA) (#/mm^2)	28.1	29	9.93	21.9	21.7	9.31	25.9	24.1	9.03	24.4	25.3	5.13
Ac.F.C (#/mm^2/year)	86.9	83.6	46.1	47.3	36.9	41.1	75.7	76.3	32	95.9	97.4	48.2
Ac.F.I (#/mm^2/year)	77.5	76.3	35.1	41.3	35.3	33.8	66.4	60.1	27.8	85.8	89.3	43.4
Ac.F.I.dL (#/mm^2/year)	80.5	78.6	35.5	45.5	38.4	36.5	70.3	64.7	28.8	84.8	88	36.5
a.Rm.Cr	64	62.5	25.7	27.3	23	16.7	46.9	47	19.4	72.3	69.5	25.2
C.On Mean Area (um^2)	12200	12400	4760	9790	9930	1900	15000	14500	6520	13800	14500	3230
C.On Mean Aspect Ratio	1.64	1.65	0.137	1.56	1.49	0.204	1.67	1.6	0.198	1.59	1.58	0.133
C.On Mean Circularity	0.703	0.694	0.0606	0.749	0.737	0.0593	0.685	0.682	0.0526	0.697	0.705	0.0474
C.On Mean Roundness	0.666	0.653	0.0372	0.681	0.683	0.0635	0.652	0.665	0.0608	0.679	0.686	0.0471
C.On Mean Solidity	0.928	0.932	0.0245	0.952	0.95	0.0252	0.924	0.924	0.0223	0.927	0.934	0.0262
C.On Mean Wall Thickness (W.Th.C) (um)	33.8	34.2	6.94	34	34.7	8.33	37	38.6	10.9	37.5	40.3	6.44
C.On/CA (C.On.OPD.CA) (#/mm^2)	4.28	4.24	1.88	2.2	2.12	1.4	4.09	4.23	1.69	4.73	5.14	1.7
C.On/RA (C.On.OPD.RA) (#/mm^2)	13.2	14.7	5.21	8.92	8.44	4.77	12.1	12.2	5.16	11	12.5	3.16
Combined Mean Inner Label (um)	24.3	22.6	6.22	21.3	20.6	5.54	23.4	22.9	6.25	24.6	21.4	8.1
Combined Mean Label (um)	26.3	23.8	6.4	23.6	23	5.84	26.6	26.6	7.85	27.1	26.8	7.07
Complete Osteon Count (C.On)	30	31	12.6	11.6	10	7.87	22.2	22	10.2	32.5	32	12.3

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
Cortical Area (mm^2)	7.13	7.15	0.731	5.15	5.24	0.704	5.32	5.25	0.517	6.89	6.81	0.654
dL.On Mean Area (um^2)	14000	13400	4080	11000	10600	2080	15800	15100	5200	14900	15600	3620
dL.On Mean Aspect Ratio	1.59	1.59	0.112	1.56	1.48	0.219	1.64	1.64	0.176	1.57	1.55	0.176
dL.On Mean Circularity	0.695	0.692	0.0585	0.741	0.748	0.0634	0.682	0.679	0.0485	0.694	0.694	0.0504
dL.On Mean Inner Label (um)	25.3	23.3	6.49	23.2	21.2	5.43	24.7	23.1	6.54	24.8	23.5	5.46
dL.On Mean Roundness	0.671	0.668	0.0373	0.672	0.688	0.0784	0.656	0.659	0.0595	0.682	0.689	0.0667
dL.On Mean Solidity	0.927	0.927	0.0254	0.951	0.952	0.0259	0.926	0.927	0.0244	0.928	0.936	0.023
dL.On Mean Wall Thickness (W.Th.dL) (um)	38.5	39.3	5.05	36.2	38.6	10.1	40.1	40.1	10.1	40.6	40.8	5.36
dL.On/CA (dL.On. OPD.CA) (#/mm^2)	2.3	2.51	1.25	1.2	0.914	0.858	2.39	2.22	1.34	2.79	2.87	1.08
dL.On/RA (dL.On. OPD.RA) (#/mm^2)	6.91	7.95	3.22	4.92	4.19	3.06	6.98	6.51	3.85	6.53	6.44	2.35
Double-Labeled Osteon Count (dL.On)	16	17	8.24	6.25	4.5	4.69	12.9	13	7.48	19	19	6.8
F.On Mean Area (um^2)	3200	2070	2040	2010	1490	1740	3540	2410	3080	3380	3070	1340
F.On Mean Aspect Ratio	1.7	1.68	0.159	1.78	1.74	0.311	1.89	1.83	0.284	1.91	1.84	0.317
F.On Mean Circularity	0.774	0.768	0.0348	0.775	0.776	0.0481	0.735	0.741	0.0443	0.736	0.748	0.0606
F.On Mean Roundness	0.668	0.665	0.0376	0.664	0.652	0.0631	0.622	0.633	0.0519	0.626	0.632	0.0564
F.On Mean Solidity	0.938	0.937	0.0139	0.94	0.936	0.019	0.92	0.925	0.0279	0.923	0.93	0.0328
F.On/CA (F.On. OPD.CA) (#/mm^2)	4.57	4.24	2.22	2.83	2.32	1.45	4.32	3.73	1.96	5.29	4.76	1.81
F.On/RA (F.On.	14	13.8	5.56	12.3	11.5	5.22	12.9	12.1	5.16	12.3	11.6	2.92

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
OPD.RA) (#/mm^2)												
Forming Osteon Count (F.On)	31.8	31.5	14.8	14.7	12	8.29	22.9	19	10.4	36.7	35	14.6
Mean Pore Area (um^2)	671	735	251	510	493	172	696	643	247	690	653	203
Mean Pore Aspect Ratio	2.31	2.24	0.281	2.67	2.58	0.482	2.39	2.3	0.278	2.43	2.39	0.202
Mean Pore Circularity	0.651	0.651	0.0523	0.618	0.595	0.0744	0.644	0.65	0.0598	0.632	0.624	0.0423
Mean Pore Max Feret Diameter (um)	38.9	40.6	6.1	39.1	38.9	5.04	42.2	41.5	5.08	40.9	41.6	4.89
Mean Pore Min Feret Diameter (um)	19.2	19.5	2.75	17.6	17.8	2.42	19.9	19.3	2.46	19.3	19.8	2.57
Mean Pore Perimeter (um)	99.6	102	15.8	97.9	97.6	12	108	106	12.1	104	106	12.7
Mean Pore Roundness	0.559	0.574	0.0371	0.519	0.517	0.0641	0.548	0.553	0.0401	0.531	0.532	0.0273
Mean Pore Solidity	0.857	0.856	0.0273	0.844	0.834	0.0354	0.853	0.86	0.0305	0.849	0.846	0.0226
On.MAR.C (Combined Labels) (um/day)	1.88	1.7	0.457	1.68	1.64	0.417	1.9	1.9	0.561	1.94	1.91	0.505
On.MAR.I (Inner Labels) (um/day)	1.74	1.61	0.444	1.52	1.47	0.396	1.67	1.63	0.446	1.76	1.53	0.579
On.MAR.I. dL (dL Inner Labels) (um/day)	1.81	1.67	0.464	1.66	1.52	0.388	1.77	1.65	0.467	1.77	1.68	0.39
σ_f C (days)	18.6	17.2	4.87	21.4	20.7	7.68	19.9	19	5.01	20.7	19.6	6.6
σ_f I (days)	20.1	19.3	5.35	23.3	21.8	7.72	22.7	23	6.24	23.1	21.5	7.34
σ_f dL (days)	19.2	19.1	4.49	21.4	20.1	7.34	21.2	22.2	4.83	21.9	21.1	5.61
Percent Porosity (%)	1.85	1.83	0.916	0.912	0.799	0.511	1.86	1.77	0.692	2.24	2.01	0.888
Pore Density CA (1/mm^2)	27.6	30.8	10.3	17.4	16.6	5.82	28	24.3	9.92	32.4	31.5	6.8
Pore Density RA (1/mm^2)	83.6	86.3	19.3	74.2	71.8	9.3	81.9	79.6	15	76.3	77.4	12.3
RA/CA (%)	0.327	0.338	0.0902	0.239	0.23	0.0843	0.341	0.319	0.0992	0.426	0.419	0.0696

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
Remodeling Area (mm^2)	2.3	2.41	0.615	1.26	1.19	0.558	1.81	1.81	0.512	2.95	2.83	0.671
Rs.N/CA (Rs.N.OPD.CA) (#/mm^2)	0.3	0.271	0.287	0.197	0.085	0.335	0.325	0.36	0.277	0.453	0.375	0.293
Rs.N/RA (Rs.N.OPD.RA) (#/mm^2)	0.906	0.805	0.834	0.685	0.254	0.923	0.979	0.905	0.836	1.1	0.829	0.751
Single-Labeled Osteon Count (sL.On)	12.7	13.5	6.87	4.92	4	4.01	7.83	7.5	6.18	11.5	10	7.55
sL.On Mean Area (um^2)	7520	6560	3790	5560	5750	3640	8600	8930	3990	9660	10800	2610
sL.On Mean Aspect Ratio	1.72	1.66	0.282	1.29	1.45	0.657	1.46	1.56	0.514	1.55	1.54	0.165
sL.On Mean Circularity	0.715	0.717	0.0742	0.629	0.737	0.299	0.653	0.692	0.22	0.705	0.714	0.0868
sL.On Mean Roundness	0.654	0.65	0.0751	0.579	0.662	0.283	0.617	0.651	0.211	0.688	0.692	0.0551
sL.On Mean Solidity	0.934	0.942	0.0261	0.794	0.951	0.372	0.854	0.93	0.271	0.92	0.946	0.0711
sL.On Mean Wall Thickness (W.Th.sL) (um)	25.4	25.2	9.04	23.6	26.9	12.6	27.9	32.8	12.8	31.5	30.3	7.79
sL.On/CA (sL.On.OPD.CA) (#/mm^2)	1.77	2.07	0.924	0.909	0.852	0.694	1.43	1.45	1.09	1.64	1.35	0.998
sL.On/RA (sL.On.OPD.RA) (#/mm^2)	5.74	6.16	3.3	3.62	4.61	2.26	4.25	4.29	3.38	3.77	3.42	1.99
T.On / Rs.N	39.5	26.2	34.2	21	21	13	26.8	25.5	13.3	33.8	26.1	24
T.On Mean Area (um^2)	7680	7420	3440	5210	5440	2000	9200	8180	5530	8280	8980	2260
T.On Mean Aspect Ratio	1.67	1.64	0.114	1.7	1.71	0.21	1.77	1.76	0.176	1.76	1.75	0.164
T.On Mean Circularity	0.738	0.73	0.0444	0.761	0.756	0.0451	0.713	0.712	0.0418	0.718	0.727	0.0464
T.On Mean Roundness	0.667	0.669	0.027	0.667	0.662	0.0516	0.639	0.645	0.0357	0.651	0.656	0.04

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
T.On Mean Solidity	0.933	0.934	0.0156	0.944	0.943	0.0178	0.922	0.917	0.0197	0.925	0.932	0.0232
T.On/CA (T.On. OPD.CA) (#/mm^2)	8.85	8.34	3.88	5.02	4.32	2.7	8.41	7.99	3.23	10	10.3	3.25
T.On/RA (T.On. OPD.RA) (#/mm^2)	27.2	27.1	10.1	21.2	21	8.96	25	23.1	8.48	23.3	24.6	5.23
tL.On Mean Area (um^2)	14300	5000	19400	8010	0	10500	20700	19100	20600	19000	23600	13500
tL.On Mean Aspect Ratio	0.817	0.668	0.87	0.635	0	0.802	1.02	1.43	0.926	1.42	1.66	0.915
tL.On Mean Circularity	0.296	0.24	0.318	0.296	0	0.372	0.363	0.558	0.326	0.448	0.56	0.286
tL.On Mean Inner Label (um)	10.5	5.31	11.9	6.2	0	8.03	12.8	13.6	12.5	18.6	16.4	16.1
tL.On Mean Outer Label (um)	17.7	11	19.3	14.8	0	19.5	24	26.1	23.2	26.5	29.8	18.5
tL.On Mean Roundness	0.317	0.254	0.338	0.281	0	0.357	0.362	0.562	0.323	0.442	0.536	0.277
tL.On Mean Solidity	0.436	0.394	0.458	0.388	0	0.481	0.521	0.843	0.461	0.668	0.86	0.406
tL.On Mean Wall Thickness (W.Th.tL) (um)	24.2	19	26.4	16.7	0	23.4	32.2	46.3	29.3	30.3	37.4	22.2
tL.ON/CA (tL.On. OPD.CA) (#/mm^2)	0.204	0.07	0.271	0.0837	0	0.106	0.267	0.195	0.318	0.303	0.204	0.297
tL.ON/RA (tL.On. OPD.RA) (#/mm^2)	9.57E-09	3.13E-09	1.37E-08	6.29E-09	0	9.11E-09	1.39E-08	7.33E-09	1.96E-08	1.18E-08	8.99E-09	1.17E-08
Total Osteon Count (T.On)	61.8	62.5	25.7	26.2	22.5	15.4	45.2	45.5	18.4	69.2	68.5	25.3
Total Pore Area (um^2)	129000	115000	64500	48400	38100	31900	100000	94900	39500	154000	136000	63400
Total Pore Number	193	216	66.7	91.6	87	39.4	148	142	49.5	224	210	57.5
Triple-Labeled Osteon	1.33	0.5	1.72	0.417	0	0.515	1.5	1	1.83	2	1.5	1.86

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
Count (tL.On)												
Rs.N	2.17	2	2.08	1.08	0.5	1.98	1.75	2	1.48	3.17	2.5	2.12

Regional Morphometry by Drug Treatment Group

Control Group by Region

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
a.Rm.Cr/CA (a.Rm.Cr.OPD.CA) (#/mm^2)	8.84	8.33	1.35	5.3	5.2	1.08	6.79	6.65	1.31	9.13	9.36	1.49
a.Rm.Cr/RA (a.Rm.Cr.OPD.RA) (#/mm^2)	26.5	24.7	7.31	26.7	24.7	8.27	21.8	22	4.35	21.3	22.3	4.82
Ac.F.C (#/mm^2/year)	97	82	49.5	63.4	38.4	52.2	67.2	64.4	19.4	100	103	49
Ac.F.I (#/mm^2/year)	83.4	76.3	31	50.4	38.4	33.2	60.9	54.3	21.2	93.7	110	44.5
Ac.F.I.dL (#/mm^2/year)	86.7	78.6	17.8	56.7	38.4	41.1	65.6	60.2	18.4	89.5	95	37.4
a.Rm.Cr	61	61	1.15	25.2	24	4.27	35.2	35	7.37	60.8	61	9.95
C.On Mean Area (um^2)	16100	15500	4460	10200	9930	786	19200	18900	8530	16400	16900	1210
C.On Mean Aspect Ratio	1.66	1.66	0.187	1.52	1.49	0.108	1.66	1.66	0.141	1.68	1.7	0.107
C.On Mean Circularity	0.667	0.663	0.0468	0.736	0.728	0.0475	0.662	0.668	0.0456	0.67	0.673	0.0444
C.On Mean Roundness	0.661	0.647	0.0413	0.685	0.683	0.0277	0.648	0.646	0.0485	0.653	0.658	0.0495
C.On Mean Solidity	0.915	0.914	0.0239	0.946	0.943	0.0239	0.919	0.924	0.0213	0.913	0.917	0.0341
C.On Mean Wall Thickness (W.Th.C) (um)	35.7	38.1	7.94	28.9	32.8	11.7	39.1	42.9	16.8	35.4	36.9	7.81
C.On/CA (C.On.OPD.CA) (#/mm^2)	4.69	4.6	0.628	2.1	2.12	0.45	3.35	3.5	0.913	4.2	4.38	1.15
C.On/RA (C.On.OPD.RA) (#/mm^2)	14	14	3.65	10.8	9.21	4.29	10.7	10.7	2.97	9.82	9.88	3.19
Combined Mean	23.2	21.4	4.62	21.9	21.2	4.51	25.3	25.6	6.26	28.5	26.8	11.6

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
Inner Label (um)												
Combined Mean Label (um)	26.3	25.1	6.81	25.9	26.3	4.52	28.4	31.2	7.47	29.9	29.8	7.32
Complete Osteon Count (C.On)	32.5	31	3.7	10	10	1.63	17.5	19	5.26	27.8	28.5	6.6
Cortical Area (mm^2)	7	7.33	0.875	4.85	4.96	0.805	5.18	5.28	0.33	6.7	6.78	0.848
dL.On Mean Area (um^2)	17100	17500	3410	11900	12000	2300	18200	19000	5930	17500	17200	1880
dL.On Mean Aspect Ratio	1.62	1.65	0.138	1.56	1.49	0.174	1.64	1.65	0.183	1.68	1.66	0.131
dL.On Mean Circularity	0.667	0.664	0.0525	0.705	0.691	0.0671	0.673	0.668	0.05	0.664	0.659	0.0328
dL.On Mean Inner Label (um)	25.1	25.1	5.69	24.1	24.1	4.29	27.8	30.5	6.94	27.2	27.2	5.58
dL.On Mean Roundness	0.665	0.655	0.041	0.678	0.69	0.0368	0.655	0.652	0.0505	0.636	0.639	0.0539
dL.On Mean Solidity	0.913	0.91	0.0244	0.932	0.927	0.0341	0.924	0.927	0.0217	0.917	0.916	0.0228
dL.On Mean Wall Thickness (W.Th.dL) (um)	39.1	41.2	7.37	29.4	36.1	16.3	40	42.9	15.1	38.9	40.3	5.75
dL.On/CA (dL.On. OPD.CA) (#/mm^2)	2.76	2.71	0.349	1.03	0.914	0.401	2.52	2.76	1.13	2.5	2.57	0.652
dL.On/RA (dL.On. OPD.RA) (#/mm^2)	8.24	8.75	1.86	5.42	4.19	3.28	7.91	7.77	3.43	5.86	6.05	1.88
Double-Labeled Osteon Count (dL.On)	19.2	19	2.99	4.75	4.5	0.957	13.2	15	6.18	16.5	16.5	3.7
F.On Mean Area (um^2)	4780	5460	1920	2640	1630	2770	5270	3600	5080	4100	3570	1650
F.On Mean Aspect Ratio	1.64	1.64	0.127	1.83	1.74	0.257	2.07	1.95	0.358	1.82	1.81	0.196

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
F.On Mean Circularity	0.77	0.768	0.00521	0.784	0.788	0.0618	0.714	0.708	0.0414	0.756	0.762	0.0476
F.On Mean Roundness	0.681	0.678	0.0313	0.641	0.639	0.0346	0.596	0.609	0.0541	0.639	0.635	0.0305
F.On Mean Solidity	0.94	0.938	0.00663	0.946	0.949	0.0245	0.925	0.925	0.0201	0.938	0.938	0.0153
F.On/CA (F.On. OPD.CA) (#/mm^2)	3.89	3.76	1.16	3.03	2.8	0.841	3.2	3.26	0.774	4.67	4.55	0.406
F.On/RA (F.On. OPD.RA) (#/mm^2)	11.7	10.2	4.77	15.1	15.8	4.32	10.2	10.1	2.23	10.9	11.6	1.99
Forming Osteon Count (F.On)	26.5	27.5	4.65	14.5	13.5	3.87	16.5	16.5	3.7	31.2	31.5	4.57
Mean Pore Area (um^2)	864	870	146	618	660	195	839	784	288	792	841	157
Mean Pore Aspect Ratio	2.32	2.21	0.281	2.59	2.58	0.377	2.41	2.42	0.122	2.45	2.41	0.15
Mean Pore Circularity	0.651	0.651	0.0356	0.63	0.626	0.0501	0.629	0.629	0.0273	0.626	0.622	0.0362
Mean Pore Max Feret Diameter (um)	42	41.8	1.81	40.1	39.8	3.97	44.6	44.3	5.38	42.9	44.6	5.23
Mean Pore Min Feret Diameter (um)	20.9	21.3	2.26	18.8	20	2.62	20.8	20.6	2.07	20.3	21.5	2.75
Mean Pore Perimeter (um)	108	108	6.89	101	102	10.4	113	113	13.1	109	113	12.7
Mean Pore Roundness	0.558	0.566	0.0293	0.533	0.535	0.0524	0.538	0.541	0.0179	0.528	0.535	0.0172
Mean Pore Solidity	0.858	0.857	0.0184	0.852	0.85	0.0231	0.851	0.852	0.0155	0.845	0.838	0.0232
On.MAR.C (Combined Labels) (um/day)	1.88	1.79	0.486	1.85	1.88	0.323	2.03	2.23	0.534	2.14	2.13	0.523
On.MAR.I (Inner Labels) (um/day)	1.66	1.53	0.33	1.56	1.52	0.322	1.81	1.83	0.447	2.03	1.92	0.827
On.MAR.I.dL (dL Inner Labels) (um/day)	1.8	1.79	0.407	1.72	1.72	0.307	1.99	2.18	0.495	1.95	1.94	0.398

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
σ_f C (days)	20.1	20.5	7.06	15.8	17.5	6.36	18.7	18	5.4	17.6	15.8	7.1
σ_f I (days)	21.9	21.8	5.56	18	20.2	5.67	21.5	21.3	8.37	19.9	17.4	10.1
σ_f dL (days)	20	19.3	2.77	16.5	18.4	5.78	19.2	19.3	5.66	18.7	17.1	5.8
Percent Porosity (%)	2.24	2.26	0.778	0.891	0.799	0.369	2.14	2.05	1.09	2.54	2.22	0.931
Pore Density CA (1/mm^2)	25.8	24.6	7.84	14.5	14.7	3.03	26.4	19.4	15.6	32.4	31.2	10.2
Pore Density RA (1/mm^2)	74.2	74.8	14.7	70.4	68.4	4.49	77.4	73.9	14.7	74	72.6	19.3
RA/CA (%)	0.343	0.329	0.0539	0.205	0.217	0.0354	0.326	0.27	0.123	0.437	0.41	0.081
Remodeling Area (mm^2)	2.43	2.43	0.604	1.01	1.07	0.304	1.69	1.43	0.634	2.97	2.69	0.892
Rs.N/CA (Rs.N. OPD.CA) (#/mm^2)	0.269	0.271	0.217	0.163	0.194	0.114	0.244	0.211	0.287	0.261	0.254	0.132
Rs.N/RA (Rs.N. OPD.RA) (#/mm^2)	0.729	0.824	0.529	0.849	0.845	0.7	0.902	0.793	1.06	0.587	0.553	0.258
Single-Labeled Osteon Count (sL.On)	11	10	5.1	4.75	4	2.36	2.5	2	1.73	9	9	4.4
sL.On Mean Area (um^2)	8330	7410	2860	7390	7090	2290	11300	11200	2670	10300	11300	2600
sL.On Mean Aspect Ratio	1.74	1.71	0.246	1.5	1.52	0.0754	1.54	1.57	0.258	1.5	1.53	0.181
sL.On Mean Circularity	0.692	0.696	0.0505	0.753	0.752	0.0326	0.669	0.638	0.106	0.708	0.715	0.0837
sL.On Mean Roundness	0.646	0.631	0.0409	0.686	0.678	0.0399	0.674	0.651	0.122	0.702	0.681	0.0714
sL.On Mean Solidity	0.933	0.943	0.0243	0.957	0.954	0.0152	0.913	0.918	0.0486	0.924	0.94	0.0589
sL.On Mean Wall Thickness (W.Th.sL) (um)	25.2	27.9	8.08	28.7	31.7	10.7	27.1	25.7	14.9	29.6	28.5	9.28
sL.On/CA (sL.On.	1.58	1.56	0.69	0.959	0.925	0.359	0.5	0.379	0.383	1.33	1.35	0.594

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
OPD.CA) (#/mm^2)												
sL.On/RA (sL.On. OPD.RA) (#/mm^2)	4.78	4.69	2.43	4.67	4.88	1.38	1.7	1.06	1.54	3.04	2.86	1.37
T.On / Rs.N	33.6	29	20.1	24.5	23.5	4.04	24.6	21.5	14.8	41.8	37.8	22.7
T.On Mean Area (um^2)	11000	10800	2540	5680	5100	2350	12600	11600	7640	9840	9690	1090
T.On Mean Aspect Ratio	1.65	1.62	0.138	1.69	1.67	0.112	1.87	1.77	0.207	1.75	1.76	0.0869
T.On Mean Circularity	0.713	0.706	0.0231	0.766	0.755	0.049	0.687	0.681	0.0444	0.717	0.731	0.0415
T.On Mean Roundness	0.67	0.671	0.0198	0.663	0.657	0.0134	0.62	0.633	0.0363	0.646	0.646	0.0356
T.On Mean Solidity	0.927	0.928	0.0103	0.947	0.942	0.0205	0.922	0.917	0.0165	0.927	0.935	0.0207
T.On/CA (T.On. OPD.CA) (#/mm^2)	8.58	7.92	1.53	5.13	4.98	0.998	6.55	6.17	1.36	8.87	8.98	1.48
T.On/RA (T.On. OPD.RA) (#/mm^2)	25.7	23.9	7.83	25.8	24.2	7.67	20.9	21.2	3.56	20.8	21.9	4.83
tL.On Mean Area (um^2)	25600	19600	27900	8760	7920	10200	20500	18500	23900	21100	26800	14200
tL.On Mean Aspect Ratio	1.07	1.4	0.72	0.764	0.688	0.891	0.938	0.888	1.09	1.6	1.9	1.14
tL.On Mean Circularity	0.465	0.553	0.33	0.403	0.378	0.467	0.288	0.278	0.333	0.413	0.518	0.285
tL.On Mean Inner Label (um)	16.1	17.6	13.4	7.67	6.97	8.93	10.3	9.49	12	22.1	17.2	23.1
tL.On Mean Outer Label (um)	27.3	32.6	20.9	21.8	19.7	25.4	22	17.8	26.4	25.2	29.8	17.7
tL.On Mean Roundness	0.535	0.691	0.359	0.33	0.298	0.385	0.303	0.288	0.351	0.438	0.539	0.303
tL.On Mean Solidity	0.662	0.841	0.447	0.482	0.474	0.557	0.443	0.433	0.511	0.637	0.83	0.426
tL.On Mean Wall	37.1	41.2	27.5	14.6	6.52	21.3	27.2	22.1	32.5	21.8	23.2	18.9

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
Thickness (W.Th.tL) (um)												
tL.ON/CA (tL.On. OPD.CA) (#/mm^2)	0.343	0.336	0.327	0.114	0.0961	0.135	0.327	0.194	0.436	0.368	0.289	0.398
tL.ON/RA (tL.On. OPD.RA) (#/mm^2)	1.69E-08	1.41E-08	1.74E-08	1.1E-08	7.85E-09	1.37E-08	1.83E-08	6.35E-09	2.87E-08	1.53E-08	1.2E-08	1.7E-08
Total Osteon Count (T.On)	59	58	2	24.5	23.5	4.04	34	33.5	7.53	59	58.5	9.83
Total Pore Area (um^2)	157000	147000	62700	44600	36100	24800	112000	111000	57700	173000	136000	82800
Total Pore Number	182	178	67.7	71.8	73	24.9	137	106	80.2	221	198	92.3
Triple-Labeled Osteon Count (tL.On)	2.25	2.5	2.06	0.5	0.5	0.577	1.75	1	2.36	2.25	2	2.22
Rs.N	2	2	1.63	0.75	1	0.5	1.25	1	1.5	1.75	1.5	0.957

Fentanyl Group by Region

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
a.Rm.Cr/CA (a.Rm.Cr.OPD.CA) (#/mm^2)	8.68	9.79	4.67	2.62	2.6	1.2	8.06	9.08	3.36	8.81	8.75	2.5
a.Rm.Cr/RA (a.Rm.Cr.OPD.RA) (#/mm^2)	29	32.2	10.4	14.6	12.3	8.1	25.7	28.4	7.41	22.6	22.7	3.92
Ac.F.C (#/mm^2/year)	65.3	76.1	29.1	13.9	7.21	15.8	62.4	64.3	41.1	65.4	67.8	36.3
Ac.F.I (#/mm^2/year)	61.4	69.8	26.3	14.1	7.21	16.3	59.7	64.3	37	59.6	56.2	33.3
Ac.F.I.dL (#/mm^2/year)	61.8	70.5	26.5	15.3	7.21	18.7	60.3	64.3	37.9	63.6	64.2	31.6
a.Rm.Cr	61	72.5	29.1	14	13	8.12	43.8	52.5	19.2	61.5	59	17.1
C.On Mean Area (um^2)	11400	11500	2980	8830	9860	2760	12300	13700	4810	12300	13800	3990
C.On Mean Aspect Ratio	1.64	1.63	0.137	1.52	1.41	0.329	1.79	1.8	0.263	1.55	1.57	0.194
C.On Mean Circularity	0.729	0.721	0.0657	0.776	0.805	0.0862	0.694	0.705	0.0706	0.723	0.728	0.0587
C.On Mean Roundness	0.672	0.668	0.0436	0.702	0.722	0.105	0.62	0.622	0.0796	0.7	0.688	0.0612
C.On Mean Solidity	0.94	0.942	0.0277	0.958	0.974	0.0389	0.922	0.931	0.0315	0.936	0.946	0.0279
C.On Mean Wall Thickness (W.Th.C) (um)	36.3	36.9	6.8	37.3	37.2	7.84	32.2	32	8.84	38.4	41.1	8.41
C.On/CA (C.On.OPD.CA) (#/mm^2)	3.77	4.1	2.08	0.987	0.684	0.966	3.4	4.11	2.02	4.14	4.11	1.79
C.On/RA (C.On.OPD.RA) (#/mm^2)	12.7	13.7	5.03	4.72	4.95	3.2	10.5	12.2	5.93	10.5	10.4	3.38
Combined Mean Inner Label (um)	25.7	22.2	10.5	19.8	18.3	7.29	22.9	21.8	6.81	19.7	19	1.61
Combined Mean Label (um)	26.7	22.7	9.57	19.7	18.1	7.32	23.8	21.8	8.43	21.5	21.5	2.98
Complete Osteon Count (C.On)	26.5	30.5	12.9	5.5	3.5	5.92	19	21.5	12.2	28.8	29.5	11.6

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
Cortical Area (mm^2)	7.38	7.43	0.923	5.15	5.37	0.894	5.33	5.25	0.747	7.02	7.1	0.687
dL.On Mean Area (um^2)	12700	12700	1140	10400	9860	2810	16200	16400	5110	13800	14700	4120
dL.On Mean Aspect Ratio	1.54	1.53	0.0698	1.51	1.41	0.297	1.73	1.69	0.199	1.49	1.44	0.262
dL.On Mean Circularity	0.724	0.716	0.0547	0.781	0.805	0.0664	0.678	0.681	0.0651	0.724	0.723	0.0557
dL.On Mean Inner Label (um)	25.8	22.2	10.4	20.4	19.5	7.23	23.1	21.8	7.18	21.7	19.4	5.16
dL.On Mean Roundness	0.689	0.684	0.0203	0.685	0.722	0.121	0.615	0.63	0.0611	0.721	0.718	0.0915
dL.On Mean Solidity	0.943	0.939	0.0231	0.968	0.972	0.0179	0.923	0.927	0.0365	0.939	0.946	0.0233
dL.On Mean Wall Thickness (W.Th.dL) (um)	40.8	40.4	1.57	41.7	41.5	2.9	40.7	43.9	10.5	42.5	41.5	7.38
dL.On/CA (dL.On. OPD.CA) (#/mm^2)	1.84	1.42	1.64	0.433	0.425	0.206	1.21	1.12	0.944	2.54	1.99	1.75
dL.On/RA (dL.On. OPD.RA) (#/mm^2)	5.92	4.66	4.23	2.29	2.4	0.82	3.85	3.58	2.78	6.32	5.03	3.7
Double-Labeled Osteon Count (dL.On)	12.5	10.5	9.68	2.25	2	1.26	6.75	5.5	5.85	17.2	15	10.3
F.On Mean Area (um^2)	2010	1720	941	1150	1210	423	3150	3380	1130	3170	3240	801
F.On Mean Aspect Ratio	1.7	1.67	0.196	1.59	1.53	0.329	1.76	1.73	0.121	2.04	2.02	0.495
F.On Mean Circularity	0.781	0.772	0.0363	0.786	0.778	0.0398	0.762	0.762	0.0267	0.711	0.703	0.0904
F.On Mean Roundness	0.671	0.669	0.0338	0.712	0.694	0.0726	0.642	0.65	0.0237	0.607	0.603	0.0916
F.On Mean Solidity	0.94	0.936	0.0114	0.941	0.94	0.021	0.924	0.927	0.0241	0.907	0.905	0.0483
F.On/CA (F.On. OPD.CA) (#/mm^2)	4.46	4.94	2.53	1.6	1.65	0.449	4.39	4.42	1.86	4.05	4.2	0.867
F.On/RA (F.On.	14.8	16.1	5.52	9.76	7.97	6.22	14.4	15.1	4.44	10.5	11.1	1.5

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
OPD.RA) (#/mm^2)												
Forming Osteon Count (F.On)	31.2	36.5	15.6	8.25	8	2.99	23.2	23.5	9.74	28.2	27	5.74
Mean Pore Area (um^2)	646	651	231	372	371	74.8	604	607	178	665	653	60.2
Mean Pore Aspect Ratio	2.44	2.5	0.341	2.8	2.58	0.726	2.42	2.32	0.281	2.5	2.45	0.258
Mean Pore Circularity	0.619	0.629	0.0438	0.586	0.587	0.0874	0.632	0.65	0.054	0.615	0.605	0.0334
Mean Pore Max Feret Diameter (um)	39.9	39.9	4.93	36.9	39.6	5.63	40.5	41.2	3.17	41.3	41.6	1.76
Mean Pore Min Feret Diameter (um)	19.2	19.5	1.68	16.2	16.8	1.92	19	18.8	2.32	19.5	19.8	1.03
Mean Pore Perimeter (um)	102	102	12	93.3	98.2	12.5	104	104	6.48	105	106	4
Mean Pore Roundness	0.545	0.551	0.0529	0.507	0.517	0.0914	0.544	0.559	0.0471	0.523	0.52	0.0301
Mean Pore Solidity	0.841	0.848	0.0213	0.825	0.825	0.0294	0.845	0.856	0.0259	0.838	0.837	0.0137
On.MAR.C (Combined Labels) (um/day)	1.91	1.62	0.684	1.41	1.29	0.523	1.7	1.56	0.602	1.54	1.54	0.213
On.MAR.I (Inner Labels) (um/day)	1.84	1.59	0.747	1.42	1.31	0.521	1.64	1.56	0.486	1.41	1.36	0.115
On.MAR.I. dL (dL Inner Labels) (um/day)	1.84	1.59	0.74	1.46	1.39	0.516	1.65	1.56	0.513	1.55	1.39	0.369
σ_f C (days)	19.9	19.7	4.23	28.1	28.9	7.58	19.7	18.4	5.12	25.1	23.9	5.69
σ_f I (days)	21.3	19.7	6.75	28	28.6	7.7	20.1	19.2	4.79	27.3	28.2	5.76
σ_f dL (days)	21.2	19.7	6.46	27.4	27.3	8.37	20	19	4.86	25.3	24.3	6.66
Percent Porosity (%)	1.57	1.52	1.04	0.566	0.517	0.253	1.49	1.59	0.378	1.94	1.88	0.226
Pore Density CA (1/mm^2)	23.2	23.8	12.4	14.8	14	4.28	25.2	23.1	5.46	29.2	28.2	3.87
Pore Density RA (1/mm^2)	79.5	78.8	21.1	80	81.8	14.5	85.7	81.8	22.5	76.6	76.1	11.8

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
RA/CA (%)	0.278	0.306	0.0915	0.197	0.172	0.0957	0.301	0.319	0.0572	0.385	0.389	0.048
Remodeling Area (mm^2)	1.99	2.1	0.504	1.03	0.761	0.624	1.62	1.77	0.433	2.7	2.72	0.37
Rs.N/CA (Rs.N. OPD.CA) (#/mm^2)	0.444	0.34	0.399	0.0425	0	0.085	0.264	0.291	0.216	0.618	0.686	0.336
Rs.N/RA (Rs.N. OPD.RA) (#/mm^2)	1.5	1.37	1.09	0.127	0	0.254	0.807	0.852	0.668	1.65	1.98	0.869
Single-Labeled Osteon Count (sL.On)	13	12	8.49	3	1.5	4.24	11.8	14	8.22	10	8.5	7.96
sL.On Mean Area (um^2)	9280	7470	4550	3010	1450	4310	5260	5220	4320	9770	10800	3280
sL.On Mean Aspect Ratio	1.82	1.79	0.391	0.82	0.62	1	1.26	1.49	0.884	1.61	1.57	0.207
sL.On Mean Circularity	0.709	0.713	0.109	0.375	0.325	0.441	0.562	0.703	0.381	0.688	0.74	0.141
sL.On Mean Roundness	0.632	0.638	0.117	0.35	0.293	0.415	0.499	0.626	0.34	0.682	0.692	0.0469
sL.On Mean Solidity	0.93	0.931	0.0407	0.466	0.443	0.54	0.71	0.932	0.474	0.892	0.94	0.115
sL.On Mean Wall Thickness (W.Th.sL) (um)	29.6	26.4	12	12.8	12.8	14.8	21.8	26.1	15.5	31.5	33.6	10.1
sL.On/CA (sL.On. OPD.CA) (#/mm^2)	1.77	1.75	1.1	0.512	0.259	0.721	2.11	2.49	1.49	1.37	1.18	1.04
sL.On/RA (sL.On. OPD.RA) (#/mm^2)	6.3	5.36	3.66	2.3	2.29	2.66	6.43	7.53	4.53	3.58	3.42	2.46
T.On / Rs.N	30.8	16.6	33	13.8	13	7.72	27.8	21	18.2	23.7	10.6	28.3
T.On Mean Area (um^2)	6360	6550	1270	3800	3130	2080	6700	5650	3940	7630	8320	2390
T.On Mean Aspect Ratio	1.68	1.69	0.107	1.62	1.68	0.282	1.76	1.75	0.166	1.78	1.86	0.264
T.On Mean Circularity	0.758	0.762	0.0371	0.772	0.776	0.061	0.741	0.734	0.0295	0.718	0.715	0.072

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
T.On Mean Roundness	0.672	0.673	0.0237	0.694	0.671	0.0787	0.64	0.642	0.0349	0.655	0.636	0.0614
T.On Mean Solidity	0.94	0.941	0.0153	0.943	0.946	0.0262	0.925	0.928	0.025	0.922	0.925	0.034
T.On/CA (T.On. OPD.CA) (#/mm^2)	8.24	9.04	4.54	2.58	2.6	1.13	7.79	8.75	3.22	8.19	8.14	2.59
T.On/RA (T.On. OPD.RA) (#/mm^2)	27.5	29.8	10.3	14.5	12.1	8.15	24.9	27.1	7.01	20.9	21.4	4.28
tL.On Mean Area (um^2)	6810	0	13600	2890	0	5780	12100	0	24200	8830	7060	10600
tL.On Mean Aspect Ratio	0.47	0	0.941	0.451	0	0.902	0.551	0	1.1	0.837	0.78	0.971
tL.On Mean Circularity	0.17	0	0.34	0.132	0	0.264	0.14	0	0.28	0.337	0.314	0.391
tL.On Mean Inner Label (um)	4.28	0	8.56	4.01	0	8.02	7.8	0	15.6	8.22	7.38	9.59
tL.On Mean Outer Label (um)	7.29	0	14.6	4.27	0	8.54	10.7	0	21.3	15.6	14.3	18.2
tL.On Mean Roundness	0.137	0	0.274	0.139	0	0.278	0.137	0	0.274	0.309	0.281	0.36
tL.On Mean Solidity	0.234	0	0.469	0.216	0	0.432	0.205	0	0.41	0.469	0.456	0.542
tL.On Mean Wall Thickness (W.Th.tL) (um)	14.4	0	28.8	8.04	0	16.1	12.1	0	24.2	23	21	26.7
tL.ON/CA (tL.On. OPD.CA) (#/mm^2)	0.161	0	0.321	0.0425	0	0.085	0.0795	0	0.159	0.226	0.13	0.305
tL.ON/RA (tL.On. OPD.RA) (#/mm^2)	7.72E-09	0	1.54E-08	2.12E-09	0	4.24E-09	4.22E-09	0	8.45E-09	9.25E-09	6E-09	1.19E-08
Total Osteon Count (T.On)	57.8	67	27.9	13.8	13	7.72	42.2	50	18.3	57	55.5	16.8
Total Pore Area (um^2)	111000	103000	70900	29400	22700	16100	80700	84600	27500	135000	134000	12300
Total Pore Number	163	170	72.8	77	66	30.8	133	126	24.2	203	202	6.85

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
Triple-Labeled Osteon Count (tL.On)	1	0	2	0.25	0	0.5	0.5	0	1	1.5	1	1.91
Rs.N	3.25	2.5	2.87	0.25	0	0.5	1.5	1.5	1.29	4.5	5	2.65

Morphine Group by Region

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
a.Rm.Cr/CA (a.Rm.Cr.OPD.CA) (#/mm^2)	9.93	9.53	5.45	7.74	8.12	3.28	11.3	11	3.89	13.5	12.4	3.37
a.Rm.Cr/RA (a.Rm.Cr.OPD.RA) (#/mm^2)	28.9	31.6	13.9	24.4	24.4	8.6	30.3	29	13.3	29.3	29.5	2.89
Ac.F.C (#/mm^2/year)	98.4	110	59.6	64.5	57.1	31.4	97.4	86.5	27.1	122	142	51.3
Ac.F.I (#/mm^2/year)	87.6	103	48.3	59.4	49.8	36.1	78.5	68.7	27	104	110	48.8
Ac.F.I.dL (#/mm^2/year)	93	107	53.7	64.6	57.2	31.3	85	81.9	28.7	101	105	38.7
a.Rm.Cr	70	68	38.7	42.8	43.5	19.6	61.8	62	21.6	94.8	81.5	30.5
C.On Mean Area (um^2)	9040	9320	4420	10300	10100	1800	13400	12200	4870	12800	12200	2770
C.On Mean Aspect Ratio	1.61	1.62	0.115	1.63	1.64	0.15	1.55	1.56	0.134	1.55	1.55	0.0347
C.On Mean Circularity	0.712	0.701	0.0645	0.735	0.732	0.0418	0.699	0.686	0.0448	0.698	0.705	0.0304
C.On Mean Roundness	0.666	0.671	0.0372	0.655	0.651	0.0388	0.687	0.676	0.0428	0.683	0.687	0.0208
C.On Mean Solidity	0.93	0.928	0.0213	0.953	0.95	0.0117	0.93	0.924	0.0171	0.933	0.936	0.0119
C.On Mean Wall Thickness (W.Th.C) (um)	29.2	30.9	5.1	35.8	35.9	1.83	39.6	39.6	5.54	38.7	39.2	3.41
C.On/CA (C.On.OPD.CA) (#/mm^2)	4.38	4.78	2.77	3.5	3.66	1.34	5.51	5.63	1.22	5.85	5.96	1.87
C.On/RA (C.On.OPD.RA) (#/mm^2)	13	14.8	7.72	11.3	11.3	4.42	14.9	14.4	6.1	12.7	14	2.96
Combined Mean	23.9	23.3	2.64	22.3	22.6	5.88	21.8	20	6.98	25.6	25.5	7.15

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**	**Mean**	**Med**	**SD**
Inner Label (um)												
Combined Mean Label (um)	26	25.3	3.43	25.1	25.2	4.57	27.6	27.4	9.1	29.9	32.4	7.6
Complete Osteon Count (C.On)	31	34	19.4	19.2	21	7.63	30.2	29	8.88	41	39	15.1
Cortical Area (mm^2)	7.01	6.91	0.466	5.45	5.39	0.352	5.44	5.25	0.52	6.96	6.89	0.546
dL.On Mean Area (um^2)	12200	11900	5360	10800	10600	1110	13100	11400	4510	13500	12200	3760
dL.On Mean Aspect Ratio	1.61	1.6	0.131	1.6	1.51	0.228	1.54	1.54	0.127	1.54	1.53	0.0489
dL.On Mean Circularity	0.693	0.694	0.068	0.736	0.748	0.0433	0.695	0.685	0.0396	0.693	0.707	0.0519
dL.On Mean Inner Label (um)	25.1	23.3	3.79	25.2	25.2	4.57	23.3	22.1	6.19	25.5	24.6	5.53
dL.On Mean Roundness	0.658	0.654	0.0481	0.654	0.685	0.076	0.699	0.709	0.0439	0.689	0.689	0.00984
dL.On Mean Solidity	0.925	0.919	0.0252	0.953	0.952	0.0105	0.93	0.927	0.0185	0.929	0.935	0.0235
dL.On Mean Wall Thickness (W.Th.dL) (um)	35.5	37.1	4.12	37.6	37.8	1.06	39.7	39.2	5.77	40.5	40.6	3.01
dL.On/CA (dL.On. OPD.CA) (#/mm^2)	2.31	2.41	1.55	2.15	2.11	0.682	3.44	3.54	1.03	3.34	3.51	0.445
dL.On/RA (dL.On. OPD.RA) (#/mm^2)	6.57	8.16	3.58	7.06	6.73	2.68	9.17	9.28	3.76	7.42	7.27	1.18
Double-Labeled Osteon Count (dL.On)	16.2	17	10.8	11.8	12	3.69	18.8	20	6.02	23.2	23	3.69

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
F.On Mean Area (um^2)	2820	1850	2290	2260	1950	1260	2200	1940	995	2860	2190	1470
F.On Mean Aspect Ratio	1.75	1.71	0.172	1.91	1.9	0.325	1.83	1.79	0.287	1.87	1.87	0.222
F.On Mean Circularity	0.77	0.774	0.0546	0.756	0.735	0.0484	0.729	0.734	0.0561	0.74	0.741	0.04
F.On Mean Roundness	0.653	0.65	0.0501	0.639	0.634	0.0598	0.628	0.631	0.0701	0.631	0.636	0.0402
F.On Mean Solidity	0.933	0.938	0.0222	0.932	0.93	0.012	0.91	0.908	0.0409	0.925	0.926	0.0274
F.On/CA (F.On. OPD.CA) (#/mm^2)	5.36	4.53	2.97	3.85	3.87	1.83	5.37	4.92	2.63	7.14	6.44	2.01
F.On/RA (F.On. OPD.RA) (#/mm^2)	15.5	16.2	7.04	12	10.9	4.83	14.2	12.6	7.64	15.5	15.4	2.09
Forming Osteon Count (F.On)	37.8	32.5	21.3	21.2	20.5	10.8	29	27.5	13.6	50.5	43.5	18.4
Mean Pore Area (um^2)	501	419	259	540	572	150	643	553	257	612	535	316
Mean Pore Aspect Ratio	2.17	2.12	0.209	2.62	2.61	0.386	2.35	2.2	0.431	2.35	2.32	0.215
Mean Pore Circularity	0.682	0.691	0.0651	0.638	0.644	0.0899	0.671	0.687	0.0895	0.656	0.659	0.0539
Mean Pore Max Feret Diameter (um)	34.9	33.5	8.54	40.3	37.6	6.03	41.5	40.7	6.61	38.3	35.8	6.51
Mean Pore Min Feret Diameter (um)	17.4	16.5	3.42	17.8	18.4	2.48	19.8	18.6	3.22	18.2	17.4	3.59
Mean Pore Perimeter (um)	88.6	85.1	21.4	99.4	93.1	14.7	106	103	16.4	96.2	89.7	17.3
Mean Pore	0.572	0.581	0.0304	0.517	0.526	0.0591	0.562	0.567	0.0539	0.543	0.54	0.0354

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
Roundness												
Mean Pore Solidity	0.871	0.872	0.0368	0.856	0.857	0.0493	0.862	0.871	0.0481	0.864	0.864	0.0258
On.MAR.C (Combined Labels) (um/day)	1.86	1.81	0.245	1.8	1.8	0.326	1.97	1.96	0.65	2.14	2.32	0.543
On.MAR.I (Inner Labels) (um/day)	1.71	1.67	0.189	1.59	1.61	0.42	1.56	1.43	0.499	1.83	1.82	0.511
On.MAR.I.dL (dL Inner Labels) (um/day)	1.79	1.67	0.271	1.8	1.8	0.326	1.66	1.58	0.442	1.82	1.76	0.395
σ_f C (days)	15.7	16.1	1.72	20.4	20.3	3.75	21.3	19.7	5.67	19.2	17.2	5.93
σ_f I (days)	17.2	16.8	3.29	23.9	22.1	7.75	26.5	27.3	4.49	22.1	21.3	4.88
σ_f dL (days)	16.4	16	2.88	20.4	20.2	3.72	24.4	25.2	2.97	21.7	22.5	2.9
Percent Porosity (%)	1.73	1.58	1.04	1.28	1.15	0.645	1.97	1.94	0.356	2.23	1.92	1.32
Pore Density CA (1/mm^2)	33.9	35.7	9.42	22.9	20.7	6.04	32.4	32.6	6.94	35.5	35.2	4.85
Pore Density RA (1/mm^2)	97.2	99.3	17.8	72.2	71.8	4.32	82.7	81.9	7.84	78.4	77.4	5.16
RA/CA (%)	0.359	0.385	0.117	0.314	0.296	0.0648	0.396	0.37	0.106	0.455	0.437	0.0728
Remodeling Area (mm^2)	2.49	2.56	0.76	1.73	1.54	0.465	2.12	1.94	0.423	3.19	2.94	0.743
Rs.N/CA (Rs.N.OPD.CA) (#/mm^2)	0.187	0.152	0.224	0.386	0.182	0.557	0.466	0.36	0.337	0.48	0.361	0.313
Rs.N/RA (Rs.N.OPD.RA) (#/mm^2)	0.484	0.405	0.574	1.08	0.708	1.38	1.23	0.905	0.933	1.08	0.883	0.706
Single-Labeled Osteon Count (sL.On)	14	16.5	8.29	7	7.5	4.97	9.25	9	2.87	15.5	14.5	9.68
sL.On Mean Area (um^2)	4950	5390	3180	6270	6610	3330	9270	8770	2710	8950	9750	2520

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
sL.On Mean Aspect Ratio	1.59	1.62	0.204	1.54	1.48	0.367	1.58	1.63	0.202	1.55	1.5	0.127
sL.On Mean Circularity	0.743	0.739	0.0623	0.759	0.764	0.0526	0.728	0.716	0.0521	0.72	0.714	0.0136
sL.On Mean Roundness	0.685	0.67	0.0569	0.699	0.686	0.126	0.677	0.66	0.0723	0.68	0.685	0.0585
sL.On Mean Solidity	0.94	0.942	0.0135	0.958	0.955	0.0117	0.938	0.934	0.0124	0.945	0.946	0.00507
sL.On Mean Wall Thickness (W.Th.sL) (um)	21.4	23.9	6.83	29.2	28.9	3.88	34.9	35.6	4.9	33.3	33.3	5.1
sL.On/CA (sL.On. OPD.CA) (#/mm^2)	1.97	2.3	1.16	1.26	1.4	0.863	1.68	1.71	0.372	2.2	2.22	1.25
sL.On/RA (sL.On. OPD.RA) (#/mm^2)	6.14	6.16	4.34	3.87	4.25	2.42	4.62	4.63	1.98	4.68	5.25	2.17
T.On / Rs.N	54.2	43.2	48.5	24.8	18	20.9	28	30	9.33	36	30.8	24
T.On Mean Area (um^2)	5630	5140	3510	6160	6240	827	8330	7470	3720	7350	6720	2600
T.On Mean Aspect Ratio	1.69	1.68	0.126	1.78	1.76	0.228	1.7	1.67	0.154	1.74	1.71	0.142
T.On Mean Circularity	0.744	0.736	0.0627	0.744	0.745	0.0272	0.711	0.699	0.0407	0.719	0.716	0.0315
T.On Mean Roundness	0.659	0.654	0.04	0.645	0.65	0.0419	0.656	0.659	0.036	0.652	0.666	0.028
T.On Mean Solidity	0.931	0.933	0.0211	0.942	0.943	0.00624	0.92	0.92	0.0225	0.927	0.927	0.0191
T.On/CA (T.On. OPD.CA) (#/mm^2)	9.74	9.31	5.54	7.36	7.46	3.04	10.9	10.6	3.58	13	11.8	3.44
T.On/RA (T.On. OPD.RA) (#/mm^2)	28.4	31	14.3	23.3	22.2	8.62	29.1	28.2	12.5	28.3	28.1	3.1

	A	A	A	L	L	L	M	M	M	P	P	P
	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
tL.On Mean Area (um^2)	10500	10300	12100	12400	12000	14300	29400	26600	14200	27200	29500	11000
tL.On Mean Aspect Ratio	0.906	0.829	1.05	0.691	0.564	0.825	1.57	1.58	0.184	1.82	1.86	0.347
tL.On Mean Circularity	0.252	0.242	0.292	0.352	0.336	0.407	0.661	0.661	0.0925	0.593	0.56	0.143
tL.On Mean Inner Label (um)	11.2	10	13	6.92	4.28	9.09	20.3	18.7	8.48	25.6	27.1	9.92
tL.On Mean Outer Label (um)	18.4	18	21.3	18.3	17	21.2	39.3	39.6	16.3	38.6	38.3	15.9
tL.On Mean Roundness	0.279	0.254	0.325	0.374	0.305	0.447	0.648	0.638	0.0694	0.578	0.572	0.111
tL.On Mean Solidity	0.411	0.397	0.476	0.467	0.456	0.54	0.916	0.916	0.0415	0.899	0.882	0.0563
tL.On Mean Wall Thickness (W.Th.tL) (um)	21.3	21.2	24.5	27.6	26.3	32	57.3	55	9.15	46	52.3	15.5
tL.ON/CA (tL.On. OPD.CA) (#/mm^2)	0.108	0.0741	0.136	0.0945	0.0912	0.109	0.394	0.289	0.289	0.314	0.231	0.239
tL.ON/RA (tL.On. OPD.RA) (#/mm^2)	4.07E-09	3.13E-09	4.94E-09	5.72E-09	5.55E-09	6.62E-09	1.92E-08	1.28E-08	1.79E-08	1.08E-08	8.99E-09	6.41E-09
Total Osteon Count (T.On)	68.8	66.5	39.4	40.5	42.5	17.7	59.2	60	20.1	91.5	77	31.1
Total Pore Area (um^2)	119000	108000	68400	71300	61700	40700	108000	99800	29400	153000	140000	82200
Total Pore Number	234	246	53.4	126	108	41.8	174	172	24.3	248	236	46.5
Triple-Labeled Osteon Count (tL.On)	0.75	0.5	0.957	0.5	0.5	0.577	2.25	1.5	1.89	2.25	1.5	1.89
Rs.N	1.25	1	1.5	2.25	1	3.3	2.5	2	1.73	3.25	2.5	1.89

Appendix XXV: Histology ANOVA for Whole-Section Femur

Whole-Section Femur: ANOVA Results

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
Ps.MS/BS (%)	Kruskal-Wallis	Group	2			4.19	0.123		0.244		Large	0.518
Es.MS/BS (%)	ANOVA	Group	2	384	192	0.352	0.712		0.073	-0.121	Very Small	0.05
	Lambda = None	Residuals	9	4910	545							
Total Area (mm^2)	ANOVA	Group	2	14.7	7.34	0.329	0.728		0.068	-0.126	Very Small	0.05
	Lambda = None	Residuals	9	201	22.3							
Marrow Area (mm^2)	ANOVA	Group	2	9.93	4.97	0.523	0.61		0.104	-0.086	Very Small	0.05
	Lambda = None	Residuals	9	85.5	9.5							
Cortical Area (mm^2)	ANOVA	Group	2	3.49	1.74	0.277	0.764		0.058	-0.137	Very Small	0.05
	Lambda = None	Residuals	9	56.7	6.3							
Remodeling Area (mm^2)	ANOVA	Group	2	9.84	4.92	2.14	0.174		0.322	0.16	Large	0.603
	Lambda = None	Residuals	9	20.7	2.3							
% Cortical Area	ANOVA	Group	2	14.9	7.46	0.584	0.577		0.115	-0.074	Very Small	0.05
	Lambda = None	Residuals	9	115	12.8							
% Marrow Area	ANOVA	Group	2	14.9	7.46	0.584	0.577		0.115	-0.074	Very Small	0.05
	Lambda = None	Residuals	9	115	12.8							
% Remodeling Area	ANOVA	Group	2	31.8	15.9	4.3	0.0489	*	0.489	0.355	Large	0.975
	Lambda = None	Residuals	9	33.3	3.7							
Parabolic Index (Y)	ANOVA	Group	2	2.09E-05	1.04E-05	0.524	0.609		0.104	-0.086	Very Small	0.05
	Lambda = None	Residuals	9	0.000179	1.99E-05							
Imin (mm^4)	ANOVA	Group	2	385	193	0.165	0.85		0.035	-0.162	Very Small	0.05
	Lambda = None	Residuals	9	10500	1170							
Imax (mm^4)	ANOVA	Group	2	275	138	0.394	0.685		0.08	-0.112	Very Small	0.05

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
	Lambda = None	Residuals	9	3150	350							
Zpol (mm^3)	ANOVA	Group	2	4.91 E+13	2.46 E+13	0.383	0.692		0.078	-0.115	Very Small	0.05
	Lambda = None	Residuals	9	5.77 E+14	6.41 E+13							
Percent Porosity (%)	ANOVA	Group	2	0.0902	0.0451	1.39	0.298		0.236	0.061	Medium	0.239
	Lambda = -2.17	Residuals	9	0.292	0.0325							
Pore Density CA (1/mm^2)	ANOVA	Group	2	136	67.9	1.97	0.196		0.304	0.139	Medium	0.529
	Lambda = None	Residuals	9	311	34.5							
Pore Density RA (1/mm^2)	Kruskal-Wallis	Group	2			0.731	0.694		-0.141		Large	0.125
Total Pore Number	ANOVA	Group	2	96800	48400	2.01	0.19		0.308	0.144	Large	0.547
	Lambda = None	Residuals	9	217000	24100							
Total Pore Area (um^2)	ANOVA	Group	2	3.64 E+10	1.82 E+10	0.71	0.517		0.136	-0.051	Very Small	0.05
	Lambda = None	Residuals	9	2.31 E+11	2.56 E+10							
Mean Pore Area (um^2)	Kruskal-Wallis	Group	2			4.19	0.123		0.244		Large	0.416
Mean Pore Perimeter (um)	ANOVA	Group	2	255	128	1.16	0.356		0.205	0.026	Small	0.122
	Lambda = None	Residuals	9	991	110							
Mean Pore Circularity	ANOVA	Group	2	0.00461	0.0023	0.914	0.435		0.169	-0.014	Very Small	0.05
	Lambda = None	Residuals	9	0.0227	0.00252							
Mean Pore Max Feret Diameter (um)	ANOVA	Group	2	33	16.5	0.957	0.42		0.175	-0.007	Very Small	0.05
	Lambda = None	Residuals	9	155	17.2							
Mean Pore Min Feret Diameter (um)	ANOVA	Group	2	7.97	3.99	0.968	0.416		0.177	-0.005	Very Small	0.05
	Lambda = None	Residuals	9	37.1	4.12							
Mean Pore Aspect Ratio	ANOVA	Group	2	5.99 E-05	0.00003	0.629	0.555		0.123	-0.066	Very Small	0.05

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
	Lambda = -4.72	Residuals	9	0.000429	4.77 E-05							
Mean Pore Roundness	ANOVA	Group	2	0.000796	0.000398	0.319	0.735		0.066	-0.128	Very Small	0.05
	Lambda = None	Residuals	9	0.0112	0.00125							
Mean Pore Solidity	ANOVA	Group	2	0.00131	0.000655	0.983	0.411		0.179	-0.003	Very Small	0.05
	Lambda = None	Residuals	9	0.006	0.000667							
Total Osteon Count (T.On)	ANOVA	Group	2	20000	9980	4.63	0.0414	*	0.507	0.377	Large	0.985
	Lambda = None	Residuals	9	19400	2160							
Complete Osteon Count (C.On)	ANOVA	Group	2	3930	1960	4.27	0.0497	*	0.487	0.353	Large	0.974
	Lambda = None	Residuals	9	4140	460							
Forming Osteon Count (F.On)	ANOVA	Group	2	6320	3160	3.61	0.0705	.	0.445	0.303	Large	0.933
	Lambda = None	Residuals	9	7860	874							
Single-Labeled Osteon Count (sL.On)	ANOVA	Group	2	689	344	1.09	0.377		0.195	0.014	Small	0.0869
	Lambda = None	Residuals	9	2850	316							
Double-Labeled Osteon Count (dL.On)	ANOVA	Group	2	1950	977	5.79	0.0241	*	0.563	0.444	Large	0.997
	Lambda = None	Residuals	9	1520	169							
Triple-Labeled Osteon Count (tL.On)	ANOVA	Group	2	26	13	1.12	0.367		0.2	0.02	Small	0.104
	Lambda = None	Residuals	9	104	11.6							
dL.On Mean Inner Label (um)	ANOVA	Group	2	188	93.8	0.749	0.5		0.143	-0.044	Very Small	0.05

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
	Lambda = None	Residuals	9	1130	125							
tL.On Mean Inner Label (um)	ANOVA	Group	2	364000	182000	2.31	0.155		0.34	0.18	Large	0.669
	Lambda = 2	Residuals	9	707000	78600							
tL.On Mean Outer Label (um)	ANOVA	Group	2	23000000	11500000	4.65	0.041	*	0.508	0.378	Large	0.985
	Lambda = 2.22	Residuals	9	22300000	2480000							
Combined Mean Inner Label (um)	ANOVA	Group	2	7.56	3.78	0.0627	0.94		0.014	-0.185	Very Small	0.05
	Lambda = None	Residuals	9	542	60.3							
Combined Mean Label (um)	ANOVA	Group	2	2.63	1.31	0.0308	0.97		0.007	-0.193	Very Small	0.05
	Lambda = None	Residuals	9	385	42.7							
C.On Mean Wall Thickness (W.Th.C) (um)	ANOVA	Group	2	2.21 E+21	1.1 E+21	0.337	0.723		0.07	-0.124	Very Small	0.05
	Lambda = 6.92	Residuals	9	2.95 E+22	3.28 E+21							
sL.On Mean Wall Thickness (W.Th.sL) (um)	ANOVA	Group	2	30.7	15.3	0.349	0.715		0.072	-0.122	Very Small	0.05
	Lambda = None	Residuals	9	396	44							
dL.On Mean Wall Thickness (W.Th.dL) (um)	ANOVA	Group	2	28.6	14.3	0.39	0.688		0.08	-0.113	Very Small	0.05
	Lambda = None	Residuals	9	330	36.6							
tL.On Mean Wall Thickness (W.Th.tL) (um)	ANOVA	Group	2	1.13 E+10	5.67 E+09	1.67	0.242		0.27	0.1	Medium	0.383
	Lambda = 2.92	Residuals	9	3.06 E+10	3.4 E+09							

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
T.On Mean Area (um^2)	ANOVA	Group	2	26200000	13100000	2.76	0.116		0.38	0.227	Large	0.8
	Lambda = None	Residuals	9	42700000	4740000							
T.On Mean Circularity	ANOVA	Group	2	0.000878	0.000439	0.418	0.67		0.085	-0.107	Very Small	0.05
	Lambda = None	Residuals	9	0.00945	0.00105							
T.On Mean Aspect Ratio	ANOVA	Group	2	0.00032	0.00016	0.0113	0.989		0.003	-0.197	Very Small	0.05
	Lambda = None	Residuals	9	0.127	0.0141							
T.On Mean Roundness	ANOVA	Group	2	0.000143	7.15 E-05	0.12	0.888		0.026	-0.172	Very Small	0.05
	Lambda = None	Residuals	9	0.00537	0.000597							
T.On Mean Solidity	ANOVA	Group	2	4.26 E-06	2.13 E-06	0.00862	0.991		0.002	-0.198	Very Small	0.05
	Lambda = None	Residuals	9	0.00222	0.000247							
C.On Mean Area (um^2)	ANOVA	Group	2	53700000	26800000	2.79	0.114		0.382	0.229	Large	0.805
	Lambda = None	Residuals	9	86700000	9630000							
C.On Mean Circularity	ANOVA	Group	2	0.0036	0.0018	1.49	0.276		0.249	0.076	Medium	0.293
	Lambda = None	Residuals	9	0.0109	0.00121							
C.On Mean Aspect Ratio	ANOVA	Group	2	0.0091	0.00455	0.583	0.578		0.115	-0.075	Very Small	0.05
	Lambda = None	Residuals	9	0.0703	0.00781							
C.On Mean Roundness	ANOVA	Group	2	0.000486	0.000243	0.438	0.658		0.089	-0.103	Very Small	0.05
	Lambda = None	Residuals	9	0.00499	0.000555							
C.On Mean Solidity	ANOVA	Group	2	0.0121	0.00605	0.765	0.493		0.145	-0.041	Very Small	0.05
	Lambda = 9.93	Residuals	9	0.0712	0.00791							
F.On Mean Area (um^2)	ANOVA	Group	2	6310000	3150000	2.15	0.172		0.324	0.161	Large	0.607
	Lambda = None	Residuals	9	13200000	1460000							
F.On Mean Circularity	ANOVA	Group	2	0.000202	0.000101	0.0742	0.929		0.016	-0.182	Very Small	0.05
	Lambda = None	Residuals	9	0.0122	0.00136							
F.On Mean Aspect Ratio	ANOVA	Group	2	0.00208	0.00104	0.0275	0.973		0.006	-0.193	Very Small	0.05

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
	Lambda = None	Residuals	9	0.34	0.0378							
F.On Mean Roundness	ANOVA	Group	2	0.00023	0.000115	0.0857	0.919		0.019	-0.18	Very Small	0.05
	Lambda = None	Residuals	9	0.0121	0.00134							
F.On Mean Solidity	ANOVA	Group	2	0.000365	0.000183	0.582	0.578		0.115	-0.075	Very Small	0.05
	Lambda = None	Residuals	9	0.00282	0.000314							
sL.On Mean Area (um^2)	ANOVA	Group	2	5.33 E+08	2.67 E+08	0.26	0.777		0.055	-0.141	Very Small	0.05
	Lambda = 1.3	Residuals	9	9.23 E+09	1.03 E+09							
sL.On Mean Circularity	ANOVA	Group	2	0.00133	0.000666	0.186	0.833		0.04	-0.157	Very Small	0.05
	Lambda = None	Residuals	9	0.0322	0.00357							
sL.On Mean Aspect Ratio	ANOVA	Group	2	0.000236	0.000118	0.557	0.591		0.11	-0.08	Very Small	0.05
	Lambda = -7.45	Residuals	9	0.00191	0.000212							
sL.On Mean Roundness	ANOVA	Group	2	6.84E-05	3.42E-05	0.333	0.725		0.069	-0.125	Very Small	0.05
	Lambda = 9.9	Residuals	9	0.000924	0.000103							
sL.On Mean Solidity	ANOVA	Group	2	0.00103	0.000513	0.547	0.597		0.108	-0.082	Very Small	0.05
	Lambda = None	Residuals	9	0.00843	0.000937							
dL.On Mean Area (um^2)	ANOVA	Group	2	41500000	20800000	1.88	0.209		0.294	0.127	Medium	0.485
	Lambda = None	Residuals	9	99700000	11100000							
dL.On Mean Circularity	ANOVA	Group	2	0.000715	0.000358	1.48	0.277		0.248	0.075	Medium	0.289
	Lambda = 9.32	Residuals	9	0.00217	0.000241							
dL.On Mean Aspect Ratio	ANOVA	Group	2	0.0269	0.0135	1.47	0.279		0.247	0.073	Medium	0.282
	Lambda = None	Residuals	9	0.0823	0.00914							
dL.On Mean Roundness	ANOVA	Group	2	0.00324	0.00162	1.53	0.269		0.253	0.081	Medium	0.312
	Lambda = None	Residuals	9	0.00954	0.00106							
dL.On Mean Solidity	ANOVA	Group	2	0.000771	0.000386	1.26	0.33		0.219	0.041	Small	0.17
	Lambda = None	Residuals	9	0.00276	0.000306							

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
tL.On Mean Area (um^2)	ANOVA	Group	2	9.9 E+08	4.95 E+08	3.02	0.099	.	0.402	0.252	Large	0.855
	Lambda = None	Residuals	9	1.47 E+09	1.64 E+08							
tL.On Mean Circularity	ANOVA	Group	2	0.098	0.049	1.58	0.258		0.26	0.088	Medium	0.338
	Lambda = 2.2	Residuals	9	0.279	0.031							
tL.On Mean Aspect Ratio	ANOVA	Group	2	77.4	38.7	0.605	0.567		0.119	-0.07	Very Small	0.05
	Lambda = 4.6	Residuals	9	576	64							
tL.On Mean Roundness	ANOVA	Group	2	0.0123	0.00616	2.9	0.107		0.392	0.24	Large	0.83
	Lambda = 4.88	Residuals	9	0.0191	0.00212							
tL.On Mean Solidity	ANOVA	Group	2	0.117	0.0583	1.09	0.375		0.196	0.016	Small	0.0925
	Lambda = 7.42	Residuals	9	0.479	0.0533							
Unlabeled Resorption Space Count (Rs.N)	ANOVA	Group	2	35.2	17.6	1.18	0.352		0.207	0.029	Small	0.131
	Lambda = None	Residuals	9	134	14.9							
Active Remodeling Centers (a.Rm.Cr)	ANOVA	Group	2	20700	10300	4.44	0.0455	*	0.497	0.365	Large	0.98
	Lambda = None	Residuals	9	20900	2330							
a.Rm.Cr/CA (a.Rm.Cr. OPD.CA) (#/mm^2)	ANOVA	Group	2	28.1	14.1	4.34	0.048	*	0.491	0.357	Large	0.976
	Lambda = None	Residuals	9	29.2	3.24							
T.On/CA (T.On. OPD.CA) (#/mm^2)	ANOVA	Group	2	27.7	13.8	4.39	0.0467	*	0.494	0.361	Large	0.978
	Lambda = None	Residuals	9	28.4	3.15							
C.On/CA (C.On. OPD.CA) (#/mm^2)	ANOVA	Group	2	5.62	2.81	4.45	0.0454	*	0.497	0.365	Large	0.98
	Lambda = None	Residuals	9	5.69	0.632							

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
F.On/CA (F.On. OPD.CA) (#/mm^2)	ANOVA	Group	2	8.43	4.21	3.14	0.0922	.	0.411	0.263	Large	0.875
	Lambda = None	Residuals	9	12.1	1.34							
sL.On/CA (sL.On. OPD.CA) (#/mm^2)	ANOVA	Group	2	0.899	0.449	1.06	0.387		0.19	0.009	Very Small	0.0732
	Lambda = None	Residuals	9	3.83	0.426							
dL.On/CA (dL.On. OPD.CA) (#/mm^2)	ANOVA	Group	2	2.79	1.4	3.88	0.061	.	0.463	0.324	Large	0.953
	Lambda = None	Residuals	9	3.24	0.36							
tL.ON/CA (tL.On. OPD.CA) (#/mm^2)	ANOVA	Group	2	0.0499	0.0249	0.981	0.412		0.179	-0.003	Very Small	0.05
	Lambda = None	Residuals	9	0.229	0.0254							
Rs.N/CA (Rs.N. OPD.CA) (#/mm^2)	ANOVA	Group	2	0.0438	0.0219	1.05	0.39		0.189	0.008	Very Small	0.0705
	Lambda = None	Residuals	9	0.188	0.0209							
a.Rm.Cr/RA (a.Rm.Cr. OPD.RA) (#/mm^2)	ANOVA	Group	2	43.7	21.9	0.72	0.513		0.138	-0.049	Very Small	0.05
	Lambda = None	Residuals	9	273	30.4							
T.On/RA (T.On. OPD.RA) (#/mm^2)	ANOVA	Group	2	42.5	21.3	0.731	0.508		0.14	-0.047	Very Small	0.05
	Lambda = None	Residuals	9	262	29.1							
C.On/RA (C.On. OPD.RA) (#/mm^2)	ANOVA	Group	2	7.63	3.81	0.548	0.596		0.109	-0.081	Very Small	0.05
	Lambda = None	Residuals	9	62.6	6.95							
F.On/RA (F.On.	ANOVA	Group	2	16.5	8.25	0.758	0.496		0.144	-0.042	Very Small	0.05

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
OPD.RA) (#/mm^2)												
	Lambda = None	Residuals	9	97.9	10.9							
sL.On/RA (sL.On. OPD.RA) (#/mm^2)	ANOVA	Group	2	5.39	2.7	0.581	0.579		0.114	-0.075	Very Small	0.05
	Lambda = None	Residuals	9	41.8	4.65							
dL.On/RA (dL.On. OPD.RA) (#/mm^2)	ANOVA	Group	2	8.12	4.06	1.12	0.367		0.2	0.02	Small	0.104
	Lambda = None	Residuals	9	32.5	3.61							
tL.ON/RA (tL.On. OPD.RA) (#/mm^2)	ANOVA	Group	2	0.451	0.226	0.775	0.489		0.147	-0.039	Very Small	0.05
	Lambda = None	Residuals	9	2.62	0.291							
Rs.N/RA (Rs.N. OPD.RA) (#/mm^2)	ANOVA	Group	2	0.584	0.292	1.42	0.292		0.239	0.065	Medium	0.253
	Lambda = None	Residuals	9	1.86	0.206							
T.On / Rs.N	ANOVA	Group	2	0.00579	0.0029	0.74	0.504		0.141	-0.045	Very Small	0.05
	Lambda = -0.3	Residuals	9	0.0352	0.00391							
On.MAR.I.dL (um/day)	ANOVA	Group	2	0.957	0.478	0.749	0.5		0.143	-0.044	Very Small	0.05
	Lambda = None	Residuals	9	5.75	0.639							
On.MAR.I (um/day)	ANOVA	Group	2	0.0386	0.0193	0.0627	0.94		0.014	-0.185	Very Small	0.05
	Lambda = None	Residuals	9	2.77	0.308							
On.MAR.C (um/day)	ANOVA	Group	2	0.0134	0.0067	0.0308	0.97		0.007	-0.193	Very Small	0.05
	Lambda = None	Residuals	9	1.96	0.218							
σ_f dL (days)	ANOVA	Group	2	7.36	3.68	1.41	0.294		0.238	0.064	Medium	0.249
	Lambda = None	Residuals	9	23.5	2.61							
σ_f I (days)	ANOVA	Group	2	1.92	0.961	0.11	0.897		0.024	-0.174	Very Small	0.05

Variable	Test	Effect	df	Sumsq	Meansq	F	P-Value	Sig	η_p^2	ω_p^2	Effect Size	Pwr
	Lambda = None	Residuals	9	78.7	8.74							
σ_f C (days)	ANOVA	Group	2	1.21	0.605	0.0684	0.934		0.015	-0.184	Very Small	0.05
	Lambda = None	Residuals	9	79.6	8.85							
Ac.F.I.dL (#/mm^2/ year)	ANOVA	Group	2	12200	6120	2.35	0.151		0.343	0.183	Large	0.679
	Lambda = None	Residuals	9	23500	2610							
Ac.F.I (#/mm^2/ year)	ANOVA	Group	2	3270	1640	0.846	0.46		0.158	-0.026	Very Small	0.05
	Lambda = None	Residuals	9	17400	1930							
Ac.F.C (#/mm^2/ year)	ANOVA	Group	2	3160	1580	0.874	0.45		0.163	-0.021	Very Small	0.05
	Lambda = None	Residuals	9	16300	1810							

Whole-Section Femur: Tukey HSD Post-Hoc Comparisons for Significant Main Effects

Variable	Contrast	Estimate	ConfLow	ConfHigh	P-value Adj	Sig
% Remodeling Area	Control > Fentanyl	-1.54	-5.34	2.25	0.518	
	Control < Morphine	2.41	-1.38	6.21	0.232	
	Fentanyl < Morphine	3.96	0.16	7.75	0.0416	*
Total Osteon Count (T.On)	Control > Fentanyl	-5.75	-97.4	85.9	0.983	
	Control < Morphine	83.5	-8.16	175	0.0736	.
	Fentanyl < Morphine	89.2	-2.41	181	0.0561	.
Complete Osteon Count (C.On)	Control > Fentanyl	-8	-50.3	34.3	0.86	
	Control < Morphine	33.8	-8.6	76.1	0.12	
	Fentanyl < Morphine	41.8	-0.595	84.1	0.0532	.
Double-Labeled Osteon Count (dL.On)	Control > Fentanyl	-15	-40.6	10.6	0.282	
	Control < Morphine	16.2	-9.39	41.9	0.234	
	Fentanyl < Morphine	31.2	5.61	56.9	0.0193	*
tL.On Mean Outer Label (um)	Control > Fentanyl	-3000	-6110	108	0.0581	.
	Control > Morphine	-124	-3230	2980	0.993	
	Fentanyl < Morphine	2880	-232	5980	0.0692	.
Active Remodeling Centers (a.Rm.Cr)	Control > Fentanyl	-2	-97.2	93.2	0.998	
	Control < Morphine	87	-8.2	182	0.0727	.
	Fentanyl < Morphine	89	-6.2	184	0.0664	.
a.Rm.Cr.OPD.CA (#/mm^2)	Control > Fentanyl	-0.377	-3.93	3.18	0.953	
	Control < Morphine	3.04	-0.512	6.59	0.0933	.
	Fentanyl < Morphine	3.42	-0.135	6.97	0.059	.
T.On.OPD.CA (#/mm^2)	Control > Fentanyl	-0.505	-4.01	3	0.916	
	Control < Morphine	2.94	-0.565	6.45	0.1	
	Fentanyl < Morphine	3.44	-0.0605	6.95	0.0539	.
C.On.OPD.CA (#/mm^2)	Control > Fentanyl	-0.478	-2.05	1.09	0.683	
	Control < Morphine	1.15	-0.417	2.72	0.156	
	Fentanyl < Morphine	1.63	0.0607	3.2	0.0423	*

Whole-Section Femur: All Directional Trends

Factor	Trend
Ps.MS/BS (%)	Fentanyl > Control > Morphine
Es.MS/BS (%)	Control > Fentanyl > Morphine
Total Area (mm^2)	Morphine > Fentanyl > Control
Marrow Area (mm^2)	Morphine > Control > Fentanyl
Cortical Area (mm^2)	Fentanyl > Morphine > Control
Remodeling Area (mm^2)	Morphine > Control > Fentanyl
% Cortical Area	Fentanyl > Control > Morphine
% Marrow Area	Morphine > Control > Fentanyl
% Remodeling Area	Morphine > Control > Fentanyl
Parabolic Index (Y)	Morphine > Control > Fentanyl
Imin (mm^4)	Morphine > Fentanyl > Control
Imax (mm^4)	Morphine > Fentanyl > Control
Zpol (mm^3)	Morphine > Fentanyl > Control
Percent Porosity (%)	Control > Morphine > Fentanyl
Pore Density CA (1/mm^2)	Morphine > Control > Fentanyl
Pore Density RA (1/mm^2)	Morphine > Fentanyl > Control
Total Pore Number	Morphine > Control > Fentanyl
Total Pore Area (um^2)	Control > Morphine > Fentanyl
Mean Pore Area (um^2)	Control > Fentanyl > Morphine
Mean Pore Perimeter (um)	Control > Fentanyl > Morphine
Mean Pore Circularity	Morphine > Control > Fentanyl
Mean Pore Max Feret Diameter (um)	Control > Fentanyl > Morphine
Mean Pore Min Feret Diameter (um)	Control > Fentanyl > Morphine
Mean Pore Aspect Ratio	Fentanyl > Control > Morphine
Mean Pore Roundness	Morphine > Control > Fentanyl
Mean Pore Solidity	Morphine > Control > Fentanyl
Total Osteon Count (T.On)	Morphine > Control > Fentanyl
Complete Osteon Count (C.On)	Morphine > Control > Fentanyl
Forming Osteon Count (F.On)	Morphine > Fentanyl > Control
Single-Labeled Osteon Count (sL.On)	Morphine > Fentanyl > Control
Double-Labeled Osteon Count (dL.On)	Morphine > Control > Fentanyl
Triple-Labeled Osteon Count (tL.On)	Control > Morphine > Fentanyl
dL.On Mean Inner Label (um)	Control > Morphine > Fentanyl
tL.On Mean Inner Label (um)	Morphine > Control > Fentanyl
tL.On Mean Outer Label (um)	Control > Morphine > Fentanyl

Factor	Trend
Combined Mean Inner Label (um)	Fentanyl > Control > Morphine
Combined Mean Label (um)	Control > Fentanyl > Morphine
C.On Mean Wall Thickness (W.Th.C) (um)	Morphine > Fentanyl > Control
sL.On Mean Wall Thickness (W.Th.sL) (um)	Morphine > Control > Fentanyl
dL.On Mean Wall Thickness (W.Th.dL) (um)	Fentanyl > Morphine > Control
tL.On Mean Wall Thickness (W.Th.tL) (um)	Morphine > Control > Fentanyl
T.On Mean Area (um^2)	Control > Morphine > Fentanyl
T.On Mean Circularity	Fentanyl > Morphine > Control
T.On Mean Aspect Ratio	Control > Morphine > Fentanyl
T.On Mean Roundness	Fentanyl > Morphine > Control
T.On Mean Solidity	Fentanyl > Morphine > Control
C.On Mean Area (um^2)	Control > Morphine > Fentanyl
C.On Mean Circularity	Fentanyl > Morphine > Control
C.On Mean Aspect Ratio	Control > Fentanyl > Morphine
C.On Mean Roundness	Morphine > Fentanyl > Control
C.On Mean Solidity	Fentanyl > Morphine > Control
F.On Mean Area (um^2)	Control > Fentanyl > Morphine
F.On Mean Circularity	Control > Fentanyl > Morphine
F.On Mean Aspect Ratio	Morphine > Control > Fentanyl
F.On Mean Roundness	Fentanyl > Control > Morphine
F.On Mean Solidity	Control > Morphine > Fentanyl
sL.On Mean Area (um^2)	Control > Morphine > Fentanyl
sL.On Mean Circularity	Morphine > Control > Fentanyl
sL.On Mean Aspect Ratio	Fentanyl > Morphine > Control
sL.On Mean Roundness	Control > Morphine > Fentanyl
sL.On Mean Solidity	Morphine > Control > Fentanyl
dL.On Mean Area (um^2)	Control > Fentanyl > Morphine
dL.On Mean Circularity	Fentanyl > Morphine > Control
dL.On Mean Aspect Ratio	Control > Morphine > Fentanyl
dL.On Mean Roundness	Fentanyl > Morphine > Control
dL.On Mean Solidity	Fentanyl > Morphine > Control
tL.On Mean Area (um^2)	Control > Morphine > Fentanyl
tL.On Mean Circularity	Control > Morphine > Fentanyl
tL.On Mean Aspect Ratio	Control > Morphine > Fentanyl
tL.On Mean Roundness	Control > Morphine > Fentanyl
tL.On Mean Solidity	Control > Morphine > Fentanyl
Unlabeled Resorption Space Count (Rs.N)	Fentanyl > Morphine > Control
Active Remodeling Centers (a.Rm.Cr) (T.On + Rs.N)	Morphine > Control > Fentanyl
a.Rm.Cr/CA (a.Rm.Cr.OPD.CA) (#/mm^2)	Morphine > Control > Fentanyl
T.On/CA (T.On.OPD.CA) (#/mm^2)	Morphine > Control > Fentanyl

Factor	Trend
C.On/CA (C.On.OPD.CA) (#/mm^2)	Morphine > Control > Fentanyl
F.On/CA (F.On.OPD.CA) (#/mm^2)	Morphine > Control > Fentanyl
sL.On/CA (sL.On.OPD.CA) (#/mm^2)	Morphine > Fentanyl > Control
dL.On/CA (dL.On.OPD.CA) (#/mm^2)	Morphine > Control > Fentanyl
tL.ON/CA (tL.On.OPD.CA) (#/mm^2)	Control > Morphine > Fentanyl
Rs.N/CA (Rs.N.OPD.CA) (#/mm^2)	Fentanyl > Morphine > Control
a.Rm.Cr/RA (a.Rm.Cr.OPD.RA) (#/mm^2)	Morphine > Fentanyl > Control
T.On/RA (T.On.OPD.RA) (#/mm^2)	Morphine > Fentanyl > Control
C.On/RA (C.On.OPD.RA) (#/mm^2)	Morphine > Control > Fentanyl
F.On/RA (F.On.OPD.RA) (#/mm^2)	Morphine > Fentanyl > Control
sL.On/RA (sL.On.OPD.RA) (#/mm^2)	Fentanyl > Morphine > Control
dL.On/RA (dL.On.OPD.RA) (#/mm^2)	Morphine > Control > Fentanyl
tL.ON/RA (tL.On.OPD.RA) (#/mm^2)	Control > Morphine > Fentanyl
Rs.N/RA (Rs.N.OPD.RA) (#/mm^2)	Fentanyl > Morphine > Control
T.On / Rs.N	Control > Fentanyl > Morphine
On.MAR.I.dL (dL Inner Labels) (um/day)	Control > Morphine > Fentanyl
On.MAR.I (Inner Labels) (um/day)	Fentanyl > Control > Morphine
On.MAR.C (Combined Labels) (um/day)	Control > Fentanyl > Morphine
Osteon Formation Time (W.Th.C / On.MAR.I.dL) (days)	Fentanyl > Morphine > Control
Osteon Formation Time (W.Th.C / On.MAR.I) (days)	Morphine > Fentanyl > Control
Osteon Formation Time (W.Th.C / On.MAR.C) (days)	Morphine > Fentanyl > Control
Ac.F.I.dL ((C.On.OPD.CA/OFT.I.dL)*365) (#/mm^2/year)	Morphine > Control > Fentanyl
Ac.F.I ((C.On.OPD.CA/OFT.I)*365) (#/mm^2/year)	Morphine > Control > Fentanyl
Ac.F.C ((C.On.OPD.CA/OFT.C)*365) (#/mm^2/year)	Morphine > Control > Fentanyl

Appendix XXVI: Histology Linear Mixed Model for Regional Femur

Regional Femur: LMM Fixed Effects and Random Effects

Cortical Area (mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	6.12	0.181	5.76	6.49	33.8	0	***	0.975	
Region1	1.01	0.0878	0.824	1.18	11.5	0	***	0.000175	***
Region2	-0.973	0.0878	-1.15	-0.793	-11.1	0	***	0.000175	***
Region3	-0.806	0.0878	-0.954	-0.635	-9.18	0	***	0.000175	***
Group1	-0.191	0.256	-0.703	0.337	-0.744	0.476		0.491	
Group2	0.0968	0.256	-0.401	0.61	0.378	0.714		0.729	
Region1:Group1	0.0594	0.124	-0.187	0.312	0.479	0.636		0.647	
Region2:Group1	-0.111	0.124	-0.369	0.121	-0.896	0.378		0.354	
Region3:Group1	0.0577	0.124	-0.185	0.317	0.465	0.646		0.638	
Region1:Group2	0.152	0.124	-0.101	0.422	1.23	0.23		0.209	
Region2:Group2	-0.0999	0.124	-0.346	0.152	-0.805	0.428		0.444	
Region3:Group2	-0.0837	0.124	-0.333	0.168	-0.674	0.506		0.492	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.363	0.602	0.356	1.02	74.6	2.67E-07	***	R2M / R2C	0.636 / 0.908
Residual	0.123	0.351	0.244	0.43	25.4	NA		AIC/BIC	111 / 138
Remodeling Area (mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	2.08	0.109	1.85	2.29	19	0	***	0.996	
Region1	0.222	0.13	-0.0228	0.479	1.71	0.0991	.	0.102	
Region2	-0.823	0.13	-1.08	-0.565	-6.34	9E-07	***	0.000175	***
Region3	-0.27	0.13	-0.509	0.00136	-2.08	0.0471	*	0.0551	.
Group1	-0.0577	0.155	-0.351	0.248	-0.373	0.718		0.724	
Group2	-0.244	0.155	-0.547	0.0655	-1.58	0.15		0.152	
Region1:Group1	0.182	0.183	-0.194	0.547	0.99	0.331		0.323	
Region2:Group1	-0.189	0.183	-0.536	0.151	-1.03	0.311		0.306	
Region3:Group1	-0.0648	0.183	-0.449	0.279	-0.353	0.727		0.715	
Region1:Group2	-0.0643	0.183	-0.4	0.304	-0.351	0.729		0.729	
Region2:Group2	0.0202	0.183	-0.37	0.353	0.11	0.913		0.922	
Region3:Group2	0.0559	0.183	-0.323	0.431	0.305	0.763		0.768	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit

Sample	0.0763	0.276	0	0.54	22.1	0.141		R2M / R2C	0.572 / 0.666
Residual	0.269	0.519	0.364	0.624	77.9	NA		AIC/BIC	123 / 150
RA/CA (%)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.333	0.0131	0.308	0.36	25.4	0	***	0.995	
Region1	-0.00639	0.0202	-0.0444	0.0336	-0.316	0.754		0.747	
Region2	-0.0942	0.0202	-0.134	-0.0541	-4.67	7.37E-05	***	0.000175	***
Region3	0.00803	0.0202	-0.0292	0.0502	0.398	0.694		0.696	
Group1	-0.00522	0.0185	-0.0395	0.0296	-0.281	0.785		0.801	
Group2	-0.0427	0.0185	-0.0806	-0.0044	-2.3	0.0467	*	0.0607	.
Region1:Group1	0.022	0.0285	-0.0365	0.0789	0.769	0.448		0.431	
Region2:Group1	-0.029	0.0285	-0.0829	0.024	-1.02	0.319		0.302	
Region3:Group1	-0.00976	0.0285	-0.0695	0.0437	-0.342	0.735		0.715	
Region1:Group2	-0.006	0.0285	-0.0581	0.0513	-0.21	0.835		0.837	
Region2:Group2	0.0013	0.0285	-0.0594	0.053	0.0456	0.964		0.968	
Region3:Group2	0.00296	0.0285	-0.0561	0.0612	0.104	0.918		0.916	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00044	0.0209	0	0.0625	6.33	0.657		R2M / R2C	0.47 / 0.503
Residual	0.00651	0.0807	0.0566	0.0968	93.7	NA		AIC/BIC	-1.38182
Percent Porosity (%)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	1.71	0.165	1.37	2.05	10.4	2.6E-06	***	0.993	
Region1	0.133	0.157	-0.163	0.444	0.845	0.406		0.39	
Region2	-0.802	0.157	-1.11	-0.49	-5.1	0.000023	***	0.000175	***
Region3	0.149	0.157	-0.142	0.477	0.946	0.352		0.36	
Group1	0.236	0.233	-0.207	0.702	1.01	0.339		0.354	
Group2	-0.324	0.233	-0.778	0.141	-1.39	0.198		0.221	
Region1:Group1	0.154	0.222	-0.301	0.598	0.694	0.493		0.485	
Region2:Group1	-0.257	0.222	-0.677	0.156	-1.16	0.258		0.246	
Region3:Group1	0.0365	0.222	-0.429	0.453	0.164	0.871		0.882	
Region1:Group2	0.0483	0.222	-0.358	0.494	0.217	0.83		0.835	
Region2:Group2	-0.0213	0.222	-0.495	0.382	-0.0959	0.924		0.922	
Region3:Group2	-0.0512	0.222	-0.511	0.403	-0.23	0.819		0.816	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.228	0.477	0.176	0.845	36.5	0.0178	*	R2M / R2C	0.34 / 0.581
Residual	0.395	0.629	0.441	0.757	63.5	NA		AIC/BIC	141 / 167

Pore Density CA (1/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	26.3	1.66	22.9	29.6	15.9	1E-07	***	0.979	
Region1	1.27	1.74	-2.01	4.73	0.732	0.471		0.46	
Region2	-8.97	1.74	-12.4	-5.5	-5.15	2.04E-05	***	0.000175	***
Region3	1.67	1.74	-1.55	5.31	0.958	0.346		0.358	
Group1	-1.59	2.34	-6	3.07	-0.68	0.513		0.527	
Group2	-3.23	2.34	-7.75	1.44	-1.38	0.201		0.21	
Region1:Group1	-0.264	2.46	-5.31	4.65	-0.107	0.915		0.932	
Region2:Group1	-1.32	2.46	-5.98	3.25	-0.537	0.596		0.592	
Region3:Group1	0.0118	2.46	-5.15	4.63	0.00477	0.996		0.998	
Region1:Group2	-1.17	2.46	-5.67	3.78	-0.473	0.64		0.645	
Region2:Group2	0.668	2.46	-4.58	5.13	0.271	0.788		0.813	
Region3:Group2	0.416	2.46	-4.68	5.45	0.169	0.867		0.869	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	20.8	4.56	0.686	8.39	30	0.0499	*	R2M / R2C	0.39 / 0.573
Residual	48.5	6.97	4.89	8.39	70	NA		AIC/BIC	313 / 339
Pore Density RA (1/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	79	3.21	72.4	85.5	24.6	0	***	0.985	
Region1	4.62	2.72	-0.507	10	1.7	0.101		0.105	
Region2	-4.84	2.72	-10.2	0.567	-1.78	0.0863	.	0.0888	.
Region3	2.91	2.72	-2.11	8.6	1.07	0.293		0.299	
Group1	-5.03	4.54	-13.5	3.85	-1.11	0.297		0.33	
Group2	1.42	4.54	-7.53	10.5	0.313	0.761		0.769	
Region1:Group1	-4.41	3.84	-12.3	3.26	-1.15	0.262		0.258	
Region2:Group1	1.24	3.84	-6.03	8.38	0.323	0.749		0.746	
Region3:Group1	0.461	3.84	-7.59	7.67	0.12	0.905		0.911	
Region1:Group2	-5.58	3.84	-12.6	2.14	-1.45	0.159		0.149	
Region2:Group2	4.37	3.84	-3.82	11.3	1.14	0.266		0.27	
Region3:Group2	2.36	3.84	-5.59	10.2	0.613	0.545		0.556	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	94.1	9.7	4.27	16.7	44.3	0.00419	**	R2M / R2C	0.186 / 0.547
Residual	118	10.9	7.63	13.1	55.7	NA		AIC/BIC	349 / 375
Total Pore Number	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig

(Intercept)	164	11.2	141	187	14.7	1E-07	***	0.999	
Region1	29.1	10.6	9.05	50.2	2.73	0.0109	*	0.0119	*
Region2	-72.6	10.6	-93.4	-51.5	-6.83	2E-07	***	0.000175	***
Region3	-16.4	10.6	-36.1	5.85	-1.54	0.135		0.144	
Group1	-11.2	15.9	-41.3	20.5	-0.704	0.499		0.515	
Group2	-20.2	15.9	-51	11.4	-1.27	0.235		0.254	
Region1:Group1	0.333	15	-30.5	30.3	0.0221	0.982		0.963	
Region2:Group1	-8.67	15	-37.1	19.3	-0.576	0.569		0.56	
Region3:Group1	0.333	15	-31.2	28.6	0.0221	0.982		0.986	
Region1:Group2	-9.92	15	-37.4	20.3	-0.659	0.516		0.514	
Region2:Group2	5.58	15	-26.5	32.9	0.371	0.714		0.732	
Region3:Group2	5.08	15	-26	35.8	0.338	0.738		0.74	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	1050	32.5	12	57.5	36.8	0.017	*	R2M / R2C	0.52 / 0.697
Residual	1810	42.6	29.9	51.2	63.2	NA		AIC/BIC	445 / 471
Total Pore Area (um^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	108000	11600	84000	132000	9.34	6.3E-06	***	0.993	
Region1	21300	10400	1610	41900	2.04	0.0514	.	0.0533	.
Region2	-59400	10400	-79800	-38700	-5.7	4.7E-06	***	0.000175	***
Region3	-7740	10400	-27000	14100	-0.742	0.464		0.471	
Group1	13800	16300	-17300	46000	0.843	0.421		0.437	
Group2	-18800	16300	-50700	13800	-1.15	0.28		0.295	
Region1:Group1	14100	14800	-16200	43500	0.953	0.349		0.339	
Region2:Group1	-17600	14800	-45500	9810	-1.19	0.243		0.227	
Region3:Group1	-2120	14800	-33000	25500	-0.144	0.887		0.876	
Region1:Group2	778	14800	-26200	30400	0.0527	0.958		0.965	
Region2:Group2	-262	14800	-31700	26500	-0.0177	0.986		0.981	
Region3:Group2	-677	14800	-31200	29500	-0.0459	0.964		0.957	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	1.17E+09	34100	13700	59700	40.1	0.00949	**	R2M / R2C	0.39 / 0.635
Residual	1.74E+09	41700	29300	50200	59.9	NA		AIC/BIC	942 / 968
Mean Pore Area (um^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	641	37	565	712	17.3	0	***	0.996	
Region1	29.2	47.1	-59.6	123	0.619	0.541		0.531	

Region2	-132	47.1	-224	-38	-2.79	0.00944	**	0.0109	*
Region3	54.2	47.1	-32.9	153	1.15	0.261		0.267	
Group1	137	52.3	38	243	2.62	0.0279	*	0.0284	*
Group2	-69.5	52.3	-171	34.8	-1.33	0.216		0.232	
Region1:Group1	56.8	66.7	-79.8	190	0.852	0.402		0.39	
Region2:Group1	-29	66.7	-155	94.8	-0.435	0.667		0.664	
Region3:Group1	7.03	66.7	-133	132	0.105	0.917		0.924	
Region1:Group2	45.3	66.7	-76.5	179	0.679	0.503		0.499	
Region2:Group2	-68.3	66.7	-210	52.6	-1.02	0.315		0.315	
Region3:Group2	-22	66.7	-160	114	-0.329	0.744		0.734	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	7510	86.7	0	182	17.4	0.239		R2M / R2C	0.301 / 0.423
Residual	35600	189	132	227	82.6	NA		AIC/BIC	547 / 573
Mean Pore Perimeter (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	102	2.96	96	108	34.5	0	***	0.987	
Region1	-2.55	2.4	-7.07	2.2	-1.06	0.297		0.308	
Region2	-4.23	2.4	-8.91	0.542	-1.76	0.0892	.	0.0884	.
Region3	5.37	2.4	0.946	10.4	2.24	0.0334	*	0.0351	*
Group1	5.57	4.19	-2.27	13.8	1.33	0.216		0.236	
Group2	-0.858	4.19	-9.22	7.61	-0.205	0.842		0.868	
Region1:Group1	2.8	3.39	-4.14	9.57	0.827	0.415		0.403	
Region2:Group1	-2.42	3.39	-8.83	3.88	-0.714	0.481		0.474	
Region3:Group1	-0.369	3.39	-7.47	5.99	-0.109	0.914		0.904	
Region1:Group2	3.54	3.39	-2.66	10.3	1.04	0.306		0.322	
Region2:Group2	-3.8	3.39	-11	2.34	-1.12	0.272		0.275	
Region3:Group2	-2.36	3.39	-9.37	4.57	-0.696	0.492		0.487	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	82.3	9.07	4.16	15.5	47.2	0.00228	**	R2M / R2C	0.205 / 0.58
Residual	92	9.59	6.73	11.6	52.8	NA		AIC/BIC	340 / 367
Mean Pore Circularity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.636	0.0156	0.604	0.668	40.9	0	***	1	
Region1	0.0144	0.00736	0.000538	0.029	1.96	0.0607	.	0.06	.
Region2	-0.0184	0.00736	-0.0328	-0.0038	-2.51	0.0186	*	0.0182	*
Region3	0.00789	0.00736	-0.00571	0.0233	1.07	0.293		0.298	

Group1	-0.00207	0.022	-0.0446	0.0398	-0.0941	0.927		0.924	
Group2	-0.0233	0.022	-0.0705	0.0224	-1.06	0.318		0.333	
Region1:Group1	0.00278	0.0104	-0.0185	0.0235	0.267	0.791		0.774	
Region2:Group1	0.0143	0.0104	-0.00542	0.0336	1.37	0.182		0.185	
Region3:Group1	-0.0126	0.0104	-0.0344	0.00691	-1.21	0.236		0.24	
Region1:Group2	-0.00844	0.0104	-0.0275	0.0124	-0.811	0.424		0.42	
Region2:Group2	-0.00871	0.0104	-0.0309	0.0102	-0.837	0.41		0.397	
Region3:Group2	0.0114	0.0104	-0.0101	0.0327	1.1	0.283		0.282	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00269	0.0518	0.0284	0.0843	75.6	1.6E-07	***	R2M / R2C	0.15 / 0.793
Residual	0.00087	0.0294	0.0206	0.0355	24.4	NA		AIC/BIC	1.648515
Mean Pore Max Feret Diameter (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	40.3	1.18	37.8	42.6	34.1	0	***	0.987	
Region1	-1.33	0.982	-3.18	0.616	-1.35	0.187		0.191	
Region2	-1.17	0.982	-3.09	0.781	-1.19	0.243		0.234	
Region3	1.93	0.982	0.111	3.98	1.96	0.0604	.	0.0621	.
Group1	2.12	1.67	-1.01	5.39	1.27	0.236		0.262	
Group2	-0.6	1.67	-3.91	2.76	-0.359	0.728		0.748	
Region1:Group1	0.964	1.39	-1.88	3.73	0.693	0.494		0.485	
Region2:Group1	-1.17	1.39	-3.8	1.41	-0.844	0.406		0.395	
Region3:Group1	0.245	1.39	-2.67	2.85	0.176	0.861		0.872	
Region1:Group2	1.55	1.39	-0.987	4.34	1.12	0.274		0.285	
Region2:Group2	-1.57	1.39	-4.53	0.951	-1.13	0.269		0.273	
Region3:Group2	-1.06	1.39	-3.94	1.78	-0.764	0.452		0.452	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	12.9	3.59	1.59	6.18	45.5	0.00329	**	R2M / R2C	0.185 / 0.556
Residual	15.4	3.93	2.76	4.74	54.5	NA		AIC/BIC	276 / 302
Mean Pore Min Feret Diameter (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	19	0.569	17.8	20.1	33.4	0	***	0.984	
Region1	0.189	0.466	-0.688	1.11	0.407	0.687		0.678	
Region2	-1.41	0.466	-2.32	-0.481	-3.02	0.00544	**	0.00702	**
Region3	0.898	0.466	0.0372	1.87	1.93	0.0645	.	0.0653	.
Group1	1.2	0.805	-0.307	2.77	1.49	0.17		0.185	
Group2	-0.521	0.805	-2.12	1.1	-0.647	0.533		0.556	

Region1:Group1	0.515	0.659	-0.834	1.83	0.782	0.441		0.434	
Region2:Group1	0.0215	0.659	-1.22	1.24	0.0326	0.974		0.973	
Region3:Group1	-0.278	0.659	-1.66	0.957	-0.422	0.676		0.669	
Region1:Group2	0.569	0.659	-0.635	1.89	0.863	0.396		0.402	
Region2:Group2	-0.904	0.659	-2.31	0.29	-1.37	0.181		0.178	
Region3:Group2	-0.334	0.659	-1.7	1.01	-0.506	0.617		0.617	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	3.02	1.74	0.782	2.98	46.5	0.00266	**	R2M / R2C	0.224 / 0.585
Residual	3.47	1.86	1.31	2.25	53.5	NA		AIC/BIC	222 / 248
Mean Pore Aspect Ratio	Lambda = -2.65								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	-0.1	0.00742	-0.114	-0.0849	-13.5	3E-07	***	0.993	
Region1	-0.0153	0.00478	-0.0241	-0.0055	-3.2	0.00348	**	0.00491	**
Region2	0.0168	0.00478	0.00749	0.0262	3.53	0.00153	**	0.00105	**
Region3	-0.00428	0.00478	-0.0137	0.00594	-0.896	0.378		0.38	
Group1	0.00233	0.0105	-0.0167	0.0228	0.222	0.829		0.861	
Group2	0.00703	0.0105	-0.0142	0.0283	0.67	0.52		0.526	
Region1:Group1	0.000919	0.00676	-0.0117	0.0134	0.136	0.893		0.901	
Region2:Group1	-0.00538	0.00676	-0.0181	0.00642	-0.796	0.433		0.441	
Region3:Group1	0.0037	0.00676	-0.0101	0.016	0.547	0.589		0.61	
Region1:Group2	0.00706	0.00676	-0.00706	0.0199	1.04	0.306		0.323	
Region2:Group2	-0.00271	0.00676	-0.0144	0.011	-0.401	0.692		0.688	
Region3:Group2	-0.00336	0.00676	-0.0154	0.0109	-0.497	0.623		0.627	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00057	0.0239	0.0129	0.0397	60.6	6.29E-05	***	R2M / R2C	0.182 / 0.68
Residual	0.00037	0.0191	0.0137	0.0229	39.4	NA		AIC/BIC	73.3 / 99.5
Mean Pore Roundness	Lambda = 3.925								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.092	0.007	0.0791	0.106	13.1	4E-07	***	0.989	
Region1	0.012	0.00455	0.00357	0.0213	2.63	0.0139	*	0.013	*
Region2	-0.00976	0.00455	-0.0187	-0.00087	-2.14	0.0412	*	0.0382	*
Region3	0.00496	0.00455	-0.00402	0.0147	1.09	0.286		0.287	
Group1	-0.00171	0.0099	-0.0197	0.0176	-0.172	0.867		0.882	
Group2	-0.00467	0.0099	-0.0247	0.0153	-0.471	0.649		0.652	
Region1:Group1	0.00048	0.00644	-0.0115	0.0124	0.0746	0.941		0.951	
Region2:Group1	0.00782	0.00644	-0.00436	0.0191	1.21	0.236		0.248	

Region3:Group1	-0.00734	0.00644	-0.0205	0.00441	-1.14	0.264		0.269	
Region1:Group2	-0.00308	0.00644	-0.0165	0.00919	-0.478	0.636		0.629	
Region2:Group2	0.00141	0.00644	-0.00971	0.0145	0.219	0.828		0.843	
Region3:Group2	0.00216	0.00644	-0.00928	0.0157	0.335	0.74		0.729	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0005	0.0225	0.0121	0.0375	60.2	7.56E-05	***	R2M / R2C	0.129 / 0.655
Residual	0.00033	0.0182	0.0131	0.0219	39.8	NA		AIC/BIC	1.561028
Mean Pore Solidity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.851	0.00781	0.835	0.867	109	0	***	1	
Region1	0.00613	0.00356	-0.00057	0.0132	1.72	0.096	.	0.0982	.
Region2	-0.00629	0.00356	-0.0132	0.000786	-1.77	0.0884	.	0.0874	.
Region3	0.0018	0.00356	-0.00477	0.00923	0.506	0.617		0.621	
Group1	0.00106	0.011	-0.0203	0.022	0.0957	0.926		0.945	
Group2	-0.0135	0.011	-0.0371	0.00942	-1.22	0.253		0.269	
Region1:Group1	0.000344	0.00503	-0.00995	0.0104	0.0684	0.946		0.925	
Region2:Group1	0.00689	0.00503	-0.00262	0.0162	1.37	0.182		0.186	
Region3:Group1	-0.0025	0.00503	-0.013	0.00693	-0.497	0.623		0.611	
Region1:Group2	-0.00209	0.00503	-0.0113	0.00799	-0.416	0.68		0.679	
Region2:Group2	-0.00595	0.00503	-0.0167	0.00316	-1.18	0.247		0.249	
Region3:Group2	0.00581	0.00503	-0.00458	0.0161	1.16	0.258		0.253	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00068	0.0261	0.0143	0.0423	77.3	7.19E-08	***	R2M / R2C	0.146 / 0.804
Residual	0.0002	0.0142	0.00998	0.0171	22.7	NA		AIC/BIC	1.281216
Total Osteon Count (T.On)	Lambda = 0.525								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	7.56	0.263	7.04	8.1	28.7	0	***	0.998	
Region1	0.959	0.384	0.25	1.75	2.5	0.019	*	0.0165	*
Region2	-2.21	0.384	-2.97	-1.46	-5.76	0.000004	***	0.000175	***
Region3	-0.314	0.384	-1.07	0.509	-0.816	0.422		0.42	
Group1	-0.388	0.372	-1.11	0.31	-1.04	0.324		0.359	
Group2	-0.749	0.372	-1.48	0.009	-2.01	0.0749	.	0.0902	.
Region1:Group1	0.376	0.543	-0.638	1.38	0.693	0.494		0.502	
Region2:Group1	0.393	0.543	-0.634	1.34	0.724	0.475		0.491	

Region3:Group1	-0.516	0.543	-1.63	0.475	-0.95	0.351		0.353	
Region1:Group2	0.38	0.543	-0.755	1.42	0.699	0.49		0.515	
Region2:Group2	-0.758	0.543	-1.7	0.342	-1.4	0.174		0.177	
Region3:Group2	0.469	0.543	-0.495	1.61	0.864	0.395		0.398	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.24	0.489	0	1.27	9.2	0.521		R2M / R2C	0.538 / 0.58
Residual	2.36	1.54	1.1	1.82	90.8	NA		AIC/BIC	380 / 406
Complete Osteon Count (C.On)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	24.1	1.55	21.3	27	15.6	1E-07	***	0.998	
Region1	5.92	2.59	1.04	11	2.28	0.0304	*	0.026	*
Region2	-12.5	2.59	-17.6	-7.35	-4.83	4.86E-05	***	0.000175	***
Region3	-1.83	2.59	-6.62	3.58	-0.708	0.485		0.481	
Group1	-2.15	2.19	-6.1	1.88	-0.98	0.353		0.4	
Group2	-4.15	2.19	-8.23	0.357	-1.89	0.0908	.	0.116	
Region1:Group1	4.65	3.66	-2.86	12	1.27	0.215		0.192	
Region2:Group1	0.562	3.66	-6.36	7.37	0.154	0.879		0.875	
Region3:Group1	-2.6	3.66	-10.3	4.26	-0.711	0.483		0.461	
Region1:Group2	0.646	3.66	-6.05	8	0.176	0.861		0.86	
Region2:Group2	-1.94	3.66	-9.74	4.7	-0.529	0.601		0.582	
Region3:Group2	0.896	3.66	-6.68	8.38	0.245	0.809		0.805	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	1.92	1.39	0	7.57	1.76	0.898		R2M / R2C	0.466 / 0.475
Residual	107	10.4	7.27	12.3	98.2	NA		AIC/BIC	333 / 359
Forming Osteon Count (F.On)	Lambda = 0.25								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	2.21	0.0427	2.12	2.29	51.6	0	***	1	
Region1	0.124	0.0494	0.0326	0.225	2.51	0.0185	*	0.0193	*
Region2	-0.298	0.0494	-0.394	-0.201	-6.03	0.000002	***	0.000175	***
Region3	-0.054	0.0494	-0.151	0.0517	-1.09	0.284		0.29	
Group1	-0.0628	0.0604	-0.18	0.0595	-1.04	0.326		0.344	
Group2	-0.0953	0.0604	-0.21	0.0322	-1.58	0.149		0.153	
Region1:Group1	-0.00384	0.0698	-0.134	0.125	-0.055	0.957		0.951	
Region2:Group1	0.0963	0.0698	-0.0357	0.218	1.38	0.179		0.2	
Region3:Group1	-0.0816	0.0698	-0.224	0.0458	-1.17	0.253		0.259	
Region1:Group2	0.0655	0.0698	-0.0804	0.198	0.938	0.357		0.382	

Region2:Group2	-0.135	0.0698	-0.255	0.00661	-1.93	0.0642	.	0.0649	.
Region3:Group2	0.109	0.0698	-0.0146	0.256	1.57	0.129		0.129	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0122	0.11	0	0.218	23.8	0.115		R2M / R2C	0.534 / 0.645
Residual	0.039	0.197	0.142	0.236	76.2	NA		AIC/BIC	336 / 363
Single-Labeled Osteon Count (sL.On)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	9.23	1.28	6.57	11.8	7.19	5.15E-05	***	0.992	
Region1	3.44	1.27	1.04	5.96	2.7	0.0117	*	0.014	*
Region2	-4.31	1.27	-6.8	-1.78	-3.39	0.00215	**	0.00316	**
Region3	-1.4	1.27	-3.74	1.26	-1.1	0.282		0.289	
Group1	-2.42	1.82	-5.85	1.21	-1.33	0.216		0.243	
Group2	0.208	1.82	-3.32	3.84	0.115	0.911		0.919	
Region1:Group1	0.75	1.8	-2.93	4.34	0.417	0.68		0.667	
Region2:Group1	2.25	1.8	-1.15	5.59	1.25	0.221		0.224	
Region3:Group1	-2.92	1.8	-6.68	0.455	-1.62	0.116		0.116	
Region1:Group2	0.125	1.8	-3.16	3.73	0.0695	0.945		0.953	
Region2:Group2	-2.12	1.8	-5.95	1.13	-1.18	0.248		0.252	
Region3:Group2	3.71	1.8	-0.0101	7.38	2.06	0.0489	*	0.0488	*
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	13.3	3.65	1.17	6.54	34	0.027	*	R2M / R2C	0.298 / 0.537
Residual	25.9	5.09	3.57	6.12	66	NA		AIC/BIC	291 / 317
Double-Labeled Osteon Count (dL.On)	Lambda = 0.8								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	7.77	0.432	6.98	8.7	18	0	***	0.981	
Region1	1.2	0.749	-0.183	2.74	1.6	0.118		0.116	
Region2	-3.61	0.749	-5.08	-2.15	-4.82	2.61E-05	***	0.000175	***
Region3	-0.261	0.749	-1.74	1.34	-0.348	0.73		0.728	
Group1	0.05	0.612	-1.13	1.14	0.0817	0.935		0.965	
Group2	-2	0.612	-3.26	-0.789	-3.27	0.0024	**	0.0126	*
Region1:Group1	1.62	1.06	-0.358	3.58	1.53	0.135		0.122	
Region2:Group1	-0.741	1.06	-2.74	1.11	-0.699	0.489		0.494	
Region3:Group1	0.221	1.06	-1.94	2.15	0.208	0.836		0.85	
Region1:Group2	0.298	1.06	-1.92	2.32	0.281	0.78		0.788	
Region2:Group2	-0.285	1.06	-2.11	1.86	-0.269	0.789		0.775	
Region3:Group2	-1.13	1.06	-3.01	1.1	-1.06	0.294		0.293	

Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	2.1	0	1		R2M / R2C	0.508 / 0.508
Residual	8.98	3	2.15	3.53	100	NA		AIC/BIC	296 / 323
Triple-Labeled Osteon Count (tL.On)	Lambda = 0.55								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.852	0.136	0.582	1.13	6.27	0.000146	***	0.981	
Region1	-0.0272	0.206	-0.408	0.396	-0.132	0.896		0.877	
Region2	-0.435	0.206	-0.839	-0.0319	-2.11	0.0445	*	0.0379	*
Region3	0.0849	0.206	-0.322	0.527	0.411	0.684		0.686	
Group1	0.175	0.192	-0.191	0.533	0.911	0.386		0.418	
Group2	-0.338	0.192	-0.722	0.0422	-1.76	0.112		0.134	
Region1:Group1	0.322	0.292	-0.223	0.863	1.1	0.279		0.271	
Region2:Group1	-0.0917	0.292	-0.643	0.418	-0.314	0.756		0.756	
Region3:Group1	-0.14	0.292	-0.736	0.393	-0.479	0.636		0.635	
Region1:Group2	0.0496	0.292	-0.56	0.606	0.17	0.866		0.881	
Region2:Group2	0.172	0.292	-0.332	0.763	0.588	0.562		0.569	
Region3:Group2	-0.232	0.292	-0.751	0.382	-0.796	0.433		0.437	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0511	0.226	0	0.653	6.97	0.623		R2M / R2C	0.205 / 0.26
Residual	0.682	0.826	0.591	0.975	93	NA		AIC/BIC	200 / 226
dL.On Mean Inner Label (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	24.5	1.43	21.6	27.3	17.2	0	***	0.992	
Region1	0.8	1.11	-1.3	3.01	0.718	0.479		0.466	
Region2	-1.3	1.11	-3.48	0.91	-1.17	0.252		0.243	
Region3	0.198	1.11	-1.86	2.52	0.177	0.86		0.855	
Group1	1.54	2.02	-2.25	5.49	0.761	0.466		0.505	
Group2	-1.77	2.02	-5.84	2.36	-0.876	0.404		0.449	
Region1:Group1	-1.73	1.57	-4.96	1.41	-1.1	0.281		0.28	
Region2:Group1	-0.669	1.57	-3.65	2.26	-0.425	0.674		0.669	
Region3:Group1	1.53	1.57	-1.77	4.48	0.973	0.339		0.333	
Region1:Group2	2.23	1.57	-0.642	5.39	1.42	0.167		0.175	
Region2:Group2	-1.05	1.57	-4.41	1.8	-0.67	0.509		0.5	
Region3:Group2	0.154	1.57	-3.1	3.37	0.0978	0.923		0.92	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	19.5	4.42	2.09	7.52	49.6	0.00134	**	R2M / R2C	0.0982 / 0.545

Residual	19.8	4.45	3.12	5.37	50.4	NA		AIC/BIC	286 / 312
tL.On Mean Inner Label (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	12	2.09	7.86	16.2	5.76	0.000274	***	0.999	
Region1	-1.51	2.89	-6.96	4.22	-0.523	0.605		0.604	
Region2	-5.85	2.89	-11.5	-0.098	-2.02	0.0531	.	0.0502	.
Region3	0.771	2.89	-4.57	6.81	0.267	0.792		0.793	
Group1	2.01	2.96	-3.67	7.86	0.678	0.515		0.54	
Group2	-5.97	2.96	-11.9	0.0196	-2.02	0.0744	.	0.08	.
Region1:Group1	3.61	4.09	-4.77	11.8	0.882	0.386		0.371	
Region2:Group1	-0.54	4.09	-8.27	7.05	-0.132	0.896		0.898	
Region3:Group1	-4.5	4.09	-13.1	3.16	-1.1	0.281		0.287	
Region1:Group2	-0.284	4.09	-7.75	7.92	-0.0695	0.945		0.945	
Region2:Group2	3.78	4.09	-4.92	11.2	0.924	0.363		0.371	
Region3:Group2	0.951	4.09	-7.5	9.3	0.233	0.818		0.818	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	19.1	4.37	0	10.2	12.5	0.389		R2M / R2C	0.243 / 0.338
Residual	134	11.6	8.11	13.9	87.5	NA		AIC/BIC	344 / 370
tL.On Mean Outer Label (um)	Lambda = 1.45								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	108	16.4	75.2	142	6.57	0.000103	***	0.974	
Region1	-18.5	27	-68.3	36.8	-0.687	0.498		0.495	
Region2	-32.1	27	-85	20.6	-1.19	0.244		0.237	
Region3	23.5	27	-29.7	81.2	0.87	0.392		0.368	
Group1	18.9	23.2	-25.5	62.3	0.813	0.437		0.495	
Group2	-62.9	23.2	-109	-17.8	-2.71	0.0241	*	0.027	*
Region1:Group1	33.1	38.1	-38.2	104	0.867	0.394		0.393	
Region2:Group1	24.8	38.1	-47.2	91.4	0.651	0.52		0.527	
Region3:Group1	-27.6	38.1	-105	42	-0.723	0.476		0.466	
Region1:Group2	6.78	38.1	-72.9	79.4	0.178	0.86		0.873	
Region2:Group2	2.47	38.1	-63.4	79.7	0.0647	0.949		0.963	
Region3:Group2	-10.7	38.1	-78.4	69.5	-0.281	0.781		0.773	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	325	18	0	78.7	2.72	0.844		R2M / R2C	0.226 / 0.248
Residual	11600	108	76.7	127	97.3	NA		AIC/BIC	378 / 404
Combined Mean Inner Label (um)	Lambda = None								

Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	23.4	1.45	20.4	26.4	16.1	1E-07	***	0.992	
Region1	0.903	1.35	-1.64	3.58	0.668	0.51		0.501	
Region2	-2.05	1.35	-4.69	0.641	-1.52	0.141		0.15	
Region3	-0.0422	1.35	-2.54	2.78	-0.0312	0.975		0.987	
Group1	1.34	2.06	-2.56	5.43	0.652	0.531		0.55	
Group2	-1.36	2.06	-5.35	2.75	-0.659	0.526		0.554	
Region1:Group1	-2.41	1.91	-6.33	1.4	-1.26	0.218		0.221	
Region2:Group1	-0.782	1.91	-4.4	2.77	-0.409	0.686		0.684	
Region3:Group1	0.65	1.91	-3.36	4.24	0.34	0.736		0.74	
Region1:Group2	2.77	1.91	-0.723	6.61	1.45	0.159		0.173	
Region2:Group2	-0.165	1.91	-4.24	3.3	-0.0864	0.932		0.929	
Region3:Group2	0.912	1.91	-3.04	4.82	0.477	0.637		0.645	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	18	4.25	1.62	7.48	38.2	0.0134	*	R2M / R2C	0.115 / 0.453
Residual	29.2	5.41	3.79	6.51	61.8	NA		AIC/BIC	297 / 323
Combined Mean Label (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	25.9	1.38	23	28.6	18.7	0	***	0.998	
Region1	0.424	1.44	-2.29	3.28	0.294	0.771		0.768	
Region2	-2.33	1.44	-5.15	0.539	-1.62	0.118		0.122	
Region3	0.71	1.44	-1.95	3.73	0.492	0.626		0.633	
Group1	1.72	1.95	-1.96	5.61	0.882	0.4		0.409	
Group2	-2.98	1.95	-6.75	0.929	-1.53	0.161		0.175	
Region1:Group1	-1.75	2.04	-5.93	2.32	-0.857	0.399		0.411	
Region2:Group1	0.577	2.04	-3.28	4.37	0.283	0.779		0.775	
Region3:Group1	0.0894	2.04	-4.18	3.91	0.0438	0.965		0.973	
Region1:Group2	3.34	2.04	-0.39	7.43	1.64	0.113		0.122	
Region2:Group2	-0.875	2.04	-5.22	2.82	-0.429	0.671		0.662	
Region3:Group2	0.16	2.04	-4.06	4.33	0.0785	0.938		0.938	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	14.6	3.82	0.686	7	30.5	0.0462	*	R2M / R2C	0.157 / 0.414
Residual	33.3	5.77	4.05	6.94	69.5	NA		AIC/BIC	299 / 325
C.On Mean Wall Thickness (W.Th.C) (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig

(Intercept)	35.6	2.01	31.4	39.5	17.7	0	***	0.988	
Region1	-1.78	1.42	-4.45	1.03	-1.26	0.22		0.223	
Region2	-1.56	1.42	-4.33	1.26	-1.1	0.281		0.273	
Region3	1.4	1.42	-1.21	4.36	0.99	0.331		0.334	
Group1	-0.771	2.85	-6.1	4.66	-0.271	0.792		0.805	
Group2	0.502	2.85	-5.35	6.45	0.177	0.864		0.877	
Region1:Group1	2.74	2	-1.37	6.73	1.37	0.183		0.174	
Region2:Group1	-4.31	2	-8.1	-0.585	-2.15	0.0407	*	0.0347	*
Region3:Group1	2.9	2	-1.3	6.66	1.45	0.159		0.156	
Region1:Group2	2.05	2	-1.61	6.07	1.02	0.315		0.324	
Region2:Group2	2.8	2	-1.46	6.44	1.4	0.173		0.178	
Region3:Group2	-5.28	2	-9.42	-1.19	-2.63	0.0138	*	0.0123	*
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	40.5	6.37	3.13	10.7	55.8	0.000281	***	R2M / R2C	0.146 / 0.622
Residual	32.1	5.67	3.98	6.83	44.2	NA		AIC/BIC	305 / 331
sL.On Mean Wall Thickness (W.Th.sL) (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	27.1	2.21	22.5	31.6	12.3	6E-07	***	0.998	
Region1	-1.68	2.05	-5.53	2.37	-0.822	0.418		0.424	
Region2	-3.51	2.05	-7.51	0.559	-1.72	0.0976	.	0.0996	.
Region3	0.815	2.05	-2.96	5.09	0.399	0.693		0.699	
Group1	0.555	3.12	-5.36	6.76	0.178	0.863		0.888	
Group2	-3.18	3.12	-9.25	3.06	-1.02	0.335		0.364	
Region1:Group1	-0.79	2.89	-6.72	4.98	-0.273	0.787		0.8	
Region2:Group1	4.6	2.89	-0.867	9.98	1.59	0.123		0.123	
Region3:Group1	-1.41	2.89	-7.47	4.01	-0.488	0.629		0.619	
Region1:Group2	7.38	2.89	2.1	13.2	2.55	0.0167	*	0.0158	*
Region2:Group2	-7.6	2.89	-13.8	-2.36	-2.63	0.014	*	0.013	*
Region3:Group2	-2.95	2.89	-8.93	2.96	-1.02	0.317		0.317	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	41.7	6.46	2.48	11.4	38.4	0.013	*	R2M / R2C	0.242 / 0.533
Residual	66.9	8.18	5.74	9.85	61.6	NA		AIC/BIC	326 / 353
dL.On Mean Wall Thickness (W.Th.dL) (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	38.9	1.93	34.8	42.6	20.1	0	***	0.989	

Region1	-0.389	1.4	-3.02	2.38	-0.279	0.782		0.775	
Region2	-2.64	1.4	-5.36	0.141	-1.89	0.0698	.	0.0698	.
Region3	1.25	1.4	-1.33	4.17	0.895	0.379		0.385	
Group1	-1.99	2.73	-7.1	3.28	-0.73	0.484		0.516	
Group2	2.55	2.73	-3.04	8.23	0.935	0.374		0.403	
Region1:Group1	2.66	1.97	-1.38	6.6	1.35	0.189		0.176	
Region2:Group1	-4.79	1.97	-8.53	-1.13	-2.43	0.0221	*	0.02	*
Region3:Group1	1.88	1.97	-2.26	5.58	0.95	0.35		0.347	
Region1:Group2	-0.234	1.97	-3.84	3.73	-0.118	0.907		0.909	
Region2:Group2	2.88	1.97	-1.32	6.46	1.46	0.155		0.161	
Region3:Group2	-2.01	1.97	-6.09	2.03	-1.02	0.319		0.317	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	36.9	6.07	2.98	10.2	54.2	0.000432	***	R2M / R2C	0.144 / 0.608
Residual	31.2	5.58	3.92	6.73	45.8	NA		AIC/BIC	303 / 330
tL.On Mean Wall Thickness (W.Th.tL) (um)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	25.9	4.23	17.2	34.1	6.12	0.000176	***	0.992	
Region1	-1.62	5.52	-12	9.31	-0.294	0.771		0.766	
Region2	-9.12	5.52	-19.9	1.85	-1.65	0.11		0.114	
Region3	6.34	5.52	-3.85	17.9	1.15	0.26		0.267	
Group1	-0.695	5.98	-12.3	11.3	-0.116	0.91		0.915	
Group2	-11.5	5.98	-23.2	0.43	-1.92	0.0868	.	0.0923	.
Region1:Group1	13.6	7.8	-2.42	29.1	1.74	0.0935	.	0.0804	.
Region2:Group1	-1.48	7.8	-16.2	13	-0.19	0.851		0.848	
Region3:Group1	-4.33	7.8	-20.7	10.3	-0.555	0.583		0.571	
Region1:Group2	1.62	7.8	-12.6	17.3	0.208	0.837		0.845	
Region2:Group2	2.79	7.8	-13.8	16.9	0.358	0.723		0.741	
Region3:Group2	-8.6	7.8	-24.7	7.34	-1.1	0.28		0.278	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	92.8	9.63	0	20.8	16	0.277		R2M / R2C	0.256 / 0.375
Residual	487	22.1	15.5	26.6	84	NA		AIC/BIC	392 / 418
T.On Mean Area (um^2)	Lambda = 0.175								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	4.7	0.0693	4.56	4.84	67.8	0	***	0.985	
Region1	0.0144	0.0739	-0.122	0.166	0.195	0.847		0.863	
Region2	-0.273	0.0739	-0.418	-0.129	-3.69	0.000989	***	0.00105	**

Region3	0.134	0.0739	-0.012	0.292	1.81	0.0816	.	0.0849	.
Group1	0.223	0.098	0.0397	0.418	2.28	0.0486	*	0.0604	.
Group2	-0.165	0.098	-0.351	0.0466	-1.68	0.127		0.141	
Region1:Group1	0.15	0.105	-0.0453	0.344	1.43	0.163		0.158	
Region2:Group1	-0.147	0.105	-0.344	0.0359	-1.4	0.172		0.178	
Region3:Group1	0.0469	0.105	-0.167	0.238	0.449	0.657		0.681	
Region1:Group2	0.0741	0.105	-0.144	0.273	0.709	0.484		0.516	
Region2:Group2	-0.089	0.105	-0.27	0.123	-0.851	0.402		0.419	
Region3:Group2	-0.0789	0.105	-0.265	0.141	-0.755	0.457		0.469	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0357	0.189	0.0268	0.358	29	0.0573	.	R2M / R2C	0.365 / 0.549
Residual	0.0874	0.296	0.212	0.354	71	NA		AIC/BIC	744 / 771
T.On Mean Circularity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.732	0.00898	0.714	0.75	81.5	0	***	0.993	
Region1	0.00576	0.00968	-0.0125	0.0249	0.596	0.556		0.549	
Region2	0.0282	0.00968	0.00934	0.0475	2.92	0.007	**	0.00737	**
Region3	-0.0195	0.00968	-0.0374	0.000713	-2.02	0.0538	.	0.0604	.
Group1	-0.0115	0.0127	-0.0354	0.0137	-0.908	0.388		0.42	
Group2	0.0145	0.0127	-0.0101	0.0396	1.14	0.283		0.302	
Region1:Group1	-0.0135	0.0137	-0.0415	0.0138	-0.983	0.334		0.34	
Region2:Group1	0.0171	0.0137	-0.00878	0.0425	1.25	0.222		0.22	
Region3:Group1	-0.0145	0.0137	-0.0432	0.0112	-1.06	0.3		0.309	
Region1:Group2	0.00484	0.0137	-0.0202	0.0323	0.354	0.726		0.735	
Region2:Group2	-0.00317	0.0137	-0.0323	0.0216	-0.231	0.819		0.808	
Region3:Group2	0.0131	0.0137	-0.0152	0.0411	0.959	0.346		0.36	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00059	0.0244	0.000728	0.0454	28.2	0.0626	.	R2M / R2C	0.226 / 0.446
Residual	0.0015	0.0387	0.0272	0.0466	71.8	NA		AIC/BIC	1.738028
T.On Mean Aspect Ratio	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	1.73	0.0371	1.65	1.8	46.6	0	***	0.993	
Region1	-0.0514	0.0357	-0.119	0.0195	-1.44	0.162		0.167	
Region2	-0.0265	0.0357	-0.0964	0.0446	-0.742	0.465		0.452	
Region3	0.0475	0.0357	-0.0186	0.122	1.33	0.195		0.2	
Group1	0.0136	0.0524	-0.0856	0.118	0.259	0.801		0.821	

Group2	-0.0168	0.0524	-0.119	0.0877	-0.32	0.756		0.763	
Region1:Group1	-0.0342	0.0506	-0.138	0.0667	-0.676	0.505		0.525	
Region2:Group1	-0.0216	0.0506	-0.117	0.0723	-0.427	0.673		0.671	
Region3:Group1	0.0799	0.0506	-0.026	0.175	1.58	0.126		0.129	
Region1:Group2	0.0176	0.0506	-0.0748	0.119	0.348	0.73		0.74	
Region2:Group2	-0.0589	0.0506	-0.167	0.0328	-1.16	0.254		0.259	
Region3:Group2	-0.0012	0.0506	-0.106	0.102	-0.0236	0.981		0.978	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0114	0.107	0.0385	0.189	35.7	0.0204	*	R2M / R2C	0.116 / 0.432
Residual	0.0204	0.143	0.1	0.172	64.3	NA		AIC/BIC	34.4 / 60.6
T.On Mean Roundness	Lambda = -2.475								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	-2.88	0.0792	-3.04	-2.72	-36.4	0	***	0.995	
Region1	0.138	0.0935	-0.0343	0.33	1.48	0.151		0.163	
Region2	0.1	0.0935	-0.083	0.283	1.07	0.293		0.285	
Region3	-0.188	0.0935	-0.372	0.0125	-2.01	0.0549	.	0.0579	.
Group1	-0.0563	0.112	-0.272	0.169	-0.503	0.627		0.618	
Group2	0.077	0.112	-0.136	0.311	0.687	0.509		0.515	
Region1:Group1	0.0981	0.132	-0.149	0.343	0.742	0.465		0.478	
Region2:Group1	0.0648	0.132	-0.185	0.296	0.49	0.628		0.645	
Region3:Group1	-0.17	0.132	-0.441	0.071	-1.29	0.209		0.218	
Region1:Group2	-0.0233	0.132	-0.3	0.229	-0.176	0.862		0.845	
Region2:Group2	0.14	0.132	-0.0884	0.408	1.06	0.299		0.303	
Region3:Group2	-0.0503	0.132	-0.285	0.228	-0.38	0.707		0.704	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0403	0.201	0	0.402	22.4	0.137		R2M / R2C	0.162 / 0.349
Residual	0.14	0.374	0.269	0.448	77.6	NA		AIC/BIC	1.642157
T.On Mean Solidity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.931	0.00443	0.922	0.94	210	0	***	0.993	
Region1	0.00174	0.00413	-0.00604	0.00993	0.422	0.677		0.668	
Region2	0.0127	0.00413	0.00462	0.0209	3.07	0.00482	**	0.00526	**
Region3	-0.00894	0.00413	-0.0166	-0.0003	-2.16	0.0395	*	0.046	*
Group1	-0.00035	0.00627	-0.0122	0.0121	-0.0562	0.956		0.965	
Group2	0.00129	0.00627	-0.0109	0.0138	0.205	0.842		0.847	
Region1:Group1	-0.00525	0.00584	-0.0172	0.00641	-0.898	0.377		0.381	

Region2:Group1	0.00338	0.00584	-0.00767	0.0142	0.578	0.568		0.564	
Region3:Group1	-0.00019	0.00584	-0.0124	0.0108	-0.0324	0.974		0.971	
Region1:Group2	0.00561	0.00584	-0.00506	0.0173	0.961	0.345		0.355	
Region2:Group2	-0.00239	0.00584	-0.0148	0.00821	-0.408	0.686		0.677	
Region3:Group2	0.00147	0.00584	-0.0106	0.0134	0.252	0.803		0.803	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00017	0.0129	0.00493	0.0228	38.6	0.0138	*	R2M / R2C	0.155 / 0.476
Residual	0.00027	0.0165	0.0116	0.0199	61.4	NA		AIC/BIC	1.275239
C.On Mean Area (um^2)	Lambda = 0.35								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	26.9	0.7	25.6	28.3	38.4	0	***	1	
Region1	-0.451	0.577	-1.52	0.733	-0.781	0.442		0.448	
Region2	-2.08	0.577	-3.21	-0.947	-3.59	0.00128	**	0.00105	**
Region3	1.47	0.577	0.336	2.71	2.55	0.0166	*	0.0165	*
Group1	2.01	0.989	0.17	3.94	2.03	0.0724	.	0.0835	.
Group2	-1.1	0.989	-3.04	0.89	-1.11	0.297		0.329	
Region1:Group1	1.05	0.817	-0.477	2.56	1.28	0.21		0.203	
Region2:Group1	-1.53	0.817	-3.08	-0.109	-1.88	0.0711	.	0.0754	.
Region3:Group1	0.601	0.817	-1.07	2.09	0.736	0.468		0.484	
Region1:Group2	0.784	0.817	-0.922	2.34	0.96	0.346		0.366	
Region2:Group2	0.0883	0.817	-1.32	1.74	0.108	0.915		0.93	
Region3:Group2	-0.724	0.817	-2.17	0.993	-0.887	0.383		0.405	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	4.54	2.13	1.01	3.68	46	0.00297	**	R2M / R2C	0.334 / 0.64
Residual	5.33	2.31	1.66	2.77	54	NA		AIC/BIC	756 / 782
C.On Mean Circularity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.708	0.0101	0.688	0.728	70.4	0	***	0.997	
Region1	-0.00561	0.0126	-0.0294	0.0194	-0.445	0.66		0.659	
Region2	0.0407	0.0126	0.0161	0.0658	3.23	0.00326	**	0.00351	**
Region3	-0.0234	0.0126	-0.0467	0.00295	-1.86	0.0744	.	0.0853	.
Group1	-0.0249	0.0142	-0.0519	0.00396	-1.75	0.115		0.132	
Group2	0.0222	0.0142	-0.0054	0.0506	1.56	0.154		0.165	
Region1:Group1	-0.0113	0.0178	-0.0479	0.0243	-0.635	0.531		0.551	
Region2:Group1	0.0119	0.0178	-0.0218	0.045	0.667	0.511		0.508	
Region3:Group1	0.00175	0.0178	-0.0356	0.0352	0.098	0.923		0.931	

Region1:Group2	0.0045	0.0178	-0.0281	0.0403	0.252	0.803		0.804	
Region2:Group2	0.00483	0.0178	-0.0332	0.0372	0.271	0.789		0.815	
Region3:Group2	-0.0132	0.0178	-0.0501	0.0232	-0.741	0.465		0.463	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00058	0.024	0	0.0498	18.5	0.213		R2M / R2C	0.255 / 0.393
Residual	0.00255	0.0505	0.0354	0.0607	81.5	NA		AIC/BIC	2.371728
C.On Mean Aspect Ratio	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	1.61	0.0252	1.57	1.66	64	0	***	0.999	
Region1	0.0254	0.0437	-0.0569	0.112	0.582	0.564		0.55	
Region2	-0.0573	0.0437	-0.143	0.0297	-1.31	0.198		0.192	
Region3	0.0542	0.0437	-0.0266	0.146	1.24	0.223		0.224	
Group1	0.0172	0.0357	-0.0504	0.0835	0.481	0.633		0.68	
Group2	0.0109	0.0357	-0.0544	0.0814	0.306	0.761		0.771	
Region1:Group1	0.00589	0.0618	-0.121	0.129	0.0953	0.925		0.902	
Region2:Group1	-0.0504	0.0618	-0.167	0.0644	-0.816	0.42		0.402	
Region3:Group1	-0.0228	0.0618	-0.152	0.0931	-0.37	0.714		0.694	
Region1:Group2	-0.00619	0.0618	-0.119	0.118	-0.1	0.921		0.921	
Region2:Group2	-0.0485	0.0618	-0.18	0.0636	-0.784	0.438		0.42	
Region3:Group2	0.109	0.0618	-0.0186	0.235	1.77	0.0856	.	0.0811	.
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	0.126	0	1		R2M / R2C	0.162 / 0.162
Residual	0.0306	0.175	0.123	0.208	100	NA		AIC/BIC	38.3 / 64.5
C.On Mean Roundness	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.669	0.00783	0.655	0.685	85.5	0	***	0.996	
Region1	-0.00307	0.0136	-0.0286	0.0238	-0.226	0.822		0.81	
Region2	0.0113	0.0136	-0.0152	0.0383	0.836	0.408		0.401	
Region3	-0.0177	0.0136	-0.0427	0.0107	-1.3	0.201		0.207	
Group1	-0.00762	0.0111	-0.0286	0.013	-0.688	0.496		0.553	
Group2	0.00427	0.0111	-0.016	0.0261	0.386	0.702		0.73	
Region1:Group1	0.00213	0.0192	-0.0372	0.0404	0.111	0.912		0.889	
Region2:Group1	0.0121	0.0192	-0.0241	0.0478	0.633	0.531		0.525	
Region3:Group1	0.0036	0.0192	-0.0366	0.0396	0.188	0.852		0.859	
Region1:Group2	0.0013	0.0192	-0.0338	0.0398	0.0676	0.947		0.953	
Region2:Group2	0.0174	0.0192	-0.0235	0.0522	0.906	0.371		0.378	
Region3:Group2	-0.0359	0.0192	-0.0755	0.00333	-1.87	0.0697	.	0.0681	.

Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	0.0392	0	1		R2M / R2C	0.153 / 0.153
Residual	0.00294	0.0543	0.0381	0.0645	100	NA		AIC/BIC	2.329949
C.On Mean Solidity	Lambda = 4.425								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.74	0.0166	0.708	0.774	44.6	0	***	0.994	
Region1	-0.0163	0.0191	-0.0516	0.0228	-0.856	0.4		0.402	
Region2	0.0702	0.0191	0.0327	0.107	3.67	0.00104	**	0.000702	***
Region3	-0.0337	0.0191	-0.0714	0.00717	-1.76	0.089	.	0.0968	.
Group1	-0.0334	0.0235	-0.0787	0.0141	-1.42	0.188		0.203	
Group2	0.0233	0.0235	-0.0213	0.0728	0.992	0.347		0.371	
Region1:Group1	-0.0114	0.027	-0.0619	0.0386	-0.424	0.675		0.683	
Region2:Group1	0.00809	0.027	-0.043	0.0553	0.3	0.767		0.772	
Region3:Group1	0.0167	0.027	-0.0385	0.066	0.618	0.542		0.562	
Region1:Group2	0.0159	0.027	-0.0405	0.0674	0.589	0.561		0.586	
Region2:Group2	0.00255	0.027	-0.0441	0.0572	0.0944	0.925		0.941	
Region3:Group2	-0.0255	0.027	-0.0735	0.0313	-0.945	0.353		0.372	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00184	0.0429	0	0.0847	24	0.112		R2M / R2C	0.244 / 0.425
Residual	0.00584	0.0764	0.0549	0.0915	76	NA		AIC/BIC	1.342894
F.On Mean Area (um^2)	Lambda = -0.075								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	-0.557	0.00447	-0.566	-0.548	-125	0	***	0.995	
Region1	0.00308	0.00545	-0.00698	0.0143	0.566	0.576		0.585	
Region2	-0.0193	0.00545	-0.03	-0.00866	-3.54	0.00146	**	0.0014	**
Region3	0.00557	0.00545	-0.00517	0.0172	1.02	0.316		0.315	
Group1	0.011	0.00632	-0.00119	0.0236	1.74	0.116		0.124	
Group2	-0.00714	0.00632	-0.0192	0.00579	-1.13	0.288		0.287	
Region1:Group1	0.00945	0.00771	-0.00494	0.0237	1.23	0.231		0.228	
Region2:Group1	-0.0063	0.00771	-0.0209	0.00715	-0.818	0.421		0.436	
Region3:Group1	-0.00054	0.00771	-0.0163	0.0135	-0.0698	0.945		0.933	
Region1:Group2	-0.00726	0.00771	-0.0234	0.00742	-0.942	0.355		0.361	
Region2:Group2	-0.00877	0.00771	-0.0221	0.00683	-1.14	0.265		0.278	
Region3:Group2	0.00978	0.00771	-0.00391	0.026	1.27	0.215		0.225	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit

Sample	0.00012	0.011	0	0.0225	20	0.174		R2M / R2C	0.304 / 0.445
Residual	0.00048	0.0218	0.0157	0.0261	80	NA		AIC/BIC	712 / 739
F.On Mean Circularity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.755	0.00993	0.734	0.774	76.1	0	***	0.998	
Region1	0.0187	0.0104	-0.00082	0.0393	1.81	0.0821	.	0.0849	.
Region2	0.0203	0.0104	4.91E-05	0.0409	1.96	0.0606	.	0.0523	.
Region3	-0.0199	0.0104	-0.039	0.0018	-1.92	0.0659	.	0.0726	.
Group1	0.00112	0.014	-0.0253	0.0291	0.0798	0.938		0.935	
Group2	0.00505	0.014	-0.022	0.0331	0.359	0.728		0.745	
Region1:Group1	-0.00492	0.0147	-0.035	0.0243	-0.336	0.74		0.757	
Region2:Group1	0.00767	0.0147	-0.0201	0.0349	0.523	0.605		0.592	
Region3:Group1	-0.0223	0.0147	-0.053	0.00517	-1.52	0.139		0.137	
Region1:Group2	0.00263	0.0147	-0.0242	0.032	0.179	0.859		0.865	
Region2:Group2	0.00547	0.0147	-0.0257	0.0321	0.373	0.712		0.73	
Region3:Group2	0.0219	0.0147	-0.00838	0.0519	1.5	0.146		0.144	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00075	0.0274	0.00484	0.0503	30.4	0.0466	*	R2M / R2C	0.205 / 0.447
Residual	0.00172	0.0415	0.0291	0.0499	69.6	NA		AIC/BIC	1.873333
F.On Mean Aspect Ratio	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	1.82	0.0593	1.69	1.94	30.6	0	***	0.986	
Region1	-0.12	0.0537	-0.222	-0.0139	-2.24	0.0334	*	0.0312	*
Region2	-0.0416	0.0537	-0.147	0.0652	-0.775	0.445		0.438	
Region3	0.0686	0.0537	-0.0306	0.181	1.28	0.213		0.218	
Group1	0.0227	0.0839	-0.137	0.189	0.27	0.793		0.808	
Group2	-0.0449	0.0839	-0.209	0.123	-0.535	0.605		0.624	
Region1:Group1	-0.083	0.0759	-0.239	0.0685	-1.09	0.284		0.286	
Region2:Group1	0.0359	0.0759	-0.108	0.177	0.473	0.64		0.631	
Region3:Group1	0.162	0.0759	0.00285	0.304	2.13	0.0422	*	0.0463	*
Region1:Group2	0.053	0.0759	-0.0858	0.205	0.698	0.491		0.491	
Region2:Group2	-0.144	0.0759	-0.306	-0.00642	-1.9	0.0685	.	0.0632	.
Region3:Group2	-0.0837	0.0759	-0.241	0.0714	-1.1	0.28		0.284	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0307	0.175	0.07	0.307	40	0.00971	**	R2M / R2C	0.204 / 0.522

Residual	0.0461	0.215	0.151	0.259	60	NA		AIC/BIC	64.9 / 91.1
F.On Mean Roundness	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.645	0.0108	0.622	0.667	59.5	0	***	0.997	
Region1	0.0231	0.0109	0.00259	0.0447	2.12	0.0432	*	0.0446	*
Region2	0.0191	0.0109	-0.00218	0.0408	1.75	0.0908	.	0.0832	.
Region3	-0.0228	0.0109	-0.0429	-4.8E-05	-2.09	0.0457	*	0.0498	*
Group1	-0.0056	0.0153	-0.0346	0.025	-0.365	0.723		0.731	
Group2	0.0129	0.0153	-0.0168	0.0436	0.839	0.423		0.42	
Region1:Group1	0.0185	0.0154	-0.013	0.0492	1.2	0.24		0.234	
Region2:Group1	-0.0171	0.0154	-0.0463	0.0115	-1.11	0.276		0.266	
Region3:Group1	-0.0205	0.0154	-0.0527	0.00842	-1.33	0.195		0.199	
Region1:Group2	-0.0104	0.0154	-0.0385	0.0205	-0.673	0.507		0.505	
Region2:Group2	0.0348	0.0154	0.00205	0.0628	2.26	0.0319	*	0.0302	*
Region3:Group2	0.00692	0.0154	-0.0249	0.0384	0.45	0.657		0.658	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00094	0.0306	0.00878	0.0551	33.1	0.0312	*	R2M / R2C	0.246 / 0.495
Residual	0.0019	0.0436	0.0306	0.0524	66.9	NA		AIC/BIC	2.019455
F.On Mean Solidity	Lambda = 9.975								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.501	0.0241	0.455	0.55	20.8	0	***	0.99	
Region1	0.031	0.027	-0.0189	0.0865	1.15	0.261		0.268	
Region2	0.0478	0.027	-0.0052	0.101	1.77	0.0886	.	0.0884	.
Region3	-0.051	0.027	-0.104	0.00682	-1.89	0.0699	.	0.0775	.
Group1	0.0322	0.034	-0.0332	0.101	0.945	0.369		0.388	
Group2	-0.00628	0.034	-0.0706	0.0665	-0.185	0.858		0.851	
Region1:Group1	-0.0213	0.0382	-0.0927	0.0496	-0.556	0.583		0.594	
Region2:Group1	0.00897	0.0382	-0.0633	0.0757	0.235	0.816		0.817	
Region3:Group1	-0.0139	0.0382	-0.092	0.0559	-0.363	0.719		0.719	
Region1:Group2	0.0167	0.0382	-0.0632	0.0895	0.437	0.666		0.686	
Region2:Group2	0.0138	0.0382	-0.0523	0.0912	0.36	0.722		0.736	
Region3:Group2	0.0222	0.0382	-0.0457	0.103	0.58	0.567		0.561	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00402	0.0634	0	0.124	25.6	0.0913	.	R2M / R2C	0.153 / 0.369
Residual	0.0117	0.108	0.0777	0.129	74.4	NA		AIC/BIC	1.335927
sL.On Mean Area (um^2)	Lambda = None								

Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	7830	626	6520	9050	12.5	5E-07	***	0.993	
Region1	-314	719	-1670	1110	-0.437	0.666		0.67	
Region2	-2280	719	-3680	-846	-3.17	0.00382	**	0.00491	**
Region3	766	719	-562	2270	1.07	0.296		0.308	
Group1	1480	885	-187	3240	1.67	0.128		0.14	
Group2	-1010	885	-2740	768	-1.14	0.285		0.28	
Region1:Group1	-670	1020	-2750	1360	-0.659	0.516		0.536	
Region2:Group1	354	1020	-1570	2240	0.348	0.73		0.73	
Region3:Group1	1200	1020	-934	3110	1.18	0.249		0.261	
Region1:Group2	2760	1020	903	4800	2.71	0.0114	*	0.0133	*
Region2:Group2	-1540	1020	-3710	306	-1.51	0.142		0.139	
Region3:Group2	-2340	1020	-4440	-261	-2.3	0.0294	*	0.0291	*
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	2630000	1620	0	3110	24.1	0.11		R2M / R2C	0.347 / 0.504
Residual	8280000	2880	2020	3460	75.9	NA		AIC/BIC	745 / 771
sL.On Mean Circularity	Lambda = 4.425								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.238	0.0212	0.196	0.281	11.2	1.4E-06	***	0.978	
Region1	0.00552	0.0309	-0.0515	0.0689	0.179	0.859		0.876	
Region2	0.0131	0.0309	-0.0475	0.0735	0.423	0.675		0.662	
Region3	-0.0139	0.0309	-0.0748	0.0522	-0.45	0.656		0.659	
Group1	-0.00823	0.03	-0.0666	0.0481	-0.274	0.79		0.792	
Group2	-0.0218	0.03	-0.0807	0.0396	-0.727	0.486		0.516	
Region1:Group1	-0.0337	0.0437	-0.115	0.0472	-0.771	0.447		0.459	
Region2:Group1	0.0459	0.0437	-0.0367	0.122	1.05	0.303		0.314	
Region3:Group1	-0.0209	0.0437	-0.11	0.0589	-0.478	0.636		0.641	
Region1:Group2	0.0269	0.0437	-0.0644	0.11	0.616	0.543		0.565	
Region2:Group2	-0.0688	0.0437	-0.144	0.0197	-1.57	0.127		0.132	
Region3:Group2	0.0214	0.0437	-0.0562	0.113	0.49	0.628		0.613	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00158	0.0398	0	0.103	9.37	0.513		R2M / R2C	0.0856 / 0.171
Residual	0.0153	0.124	0.0885	0.146	90.6	NA		AIC/BIC	37.5 / 63.7
sL.On Mean Aspect Ratio	Lambda = 2.225								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	2.77	0.186	2.43	3.17	14.9	0	***	0.979	

Region1	0.672	0.323	0.0757	1.33	2.08	0.0447	*	0.0449	*
Region2	-0.484	0.323	-1.12	0.147	-1.5	0.143		0.133	
Region3	-0.119	0.323	-0.756	0.572	-0.369	0.714		0.715	
Group1	0.0242	0.264	-0.487	0.496	0.0917	0.927		0.942	
Group2	-0.0229	0.264	-0.568	0.498	-0.087	0.931		0.936	
Region1:Group1	0.0467	0.457	-0.806	0.893	0.102	0.919		0.927	
Region2:Group1	0.162	0.457	-0.701	0.959	0.355	0.725		0.729	
Region3:Group1	0.00195	0.457	-0.931	0.835	0.0042 7	0.997		0.989	
Region1:Group2	0.542	0.457	-0.412	1.41	1.19	0.243		0.255	
Region2:Group2	-0.639	0.457	-1.43	0.285	-1.4	0.17		0.175	
Region3:Group2	-0.163	0.457	-0.974	0.797	-0.358	0.723		0.716	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	0.907	0	1		R2M / R2C	0.155 / 0.155
Residual	1.67	1.29	0.928	1.52	100	NA		AIC/BIC	106 / 132
sL.On Mean Roundness	Lambda = 3.175								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.283	0.02	0.247	0.326	14.2	0	***	0.979	
Region1	-0.0124	0.0346	-0.0763	0.0586	-0.358	0.722		0.709	
Region2	-0.00644	0.0346	-0.0743	0.0612	-0.186	0.853		0.848	
Region3	-0.00906	0.0346	-0.0773	0.065	-0.262	0.795		0.791	
Group1	0.0177	0.0283	-0.0371	0.0683	0.626	0.536		0.601	
Group2	-0.0474	0.0283	-0.106	0.0084	-1.68	0.102		0.162	
Region1:Group1	-0.0357	0.049	-0.127	0.055	-0.728	0.471		0.479	
Region2:Group1	0.0109	0.049	-0.0816	0.0964	0.223	0.825		0.82	
Region3:Group1	0.019	0.049	-0.0811	0.108	0.387	0.701		0.714	
Region1:Group2	0.0296	0.049	-0.0726	0.123	0.605	0.549		0.566	
Region2:Group2	-0.0528	0.049	-0.137	0.0463	-1.08	0.288		0.293	
Region3:Group2	-0.0131	0.049	-0.1	0.0899	-0.267	0.791		0.787	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	0.0973	0	1		R2M / R2C	0.109 / 0.109
Residual	0.0192	0.138	0.0994	0.163	100	NA		AIC/BIC	36.1 / 62.3
sL.On Mean Solidity	Lambda = 9.975								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.509	0.0391	0.435	0.588	13	4E-07	***	0.986	
Region1	0.0142	0.048	-0.0743	0.113	0.297	0.769		0.784	
Region2	0.0183	0.048	-0.0756	0.112	0.382	0.705		0.692	
Region3	-0.0355	0.048	-0.13	0.0671	-0.74	0.466		0.471	

Group1	0.0182	0.0553	-0.0881	0.128	0.329	0.75		0.778	
Group2	-0.0838	0.0553	-0.19	0.0287	-1.52	0.164		0.175	
Region1:Group1	-0.0282	0.0678	-0.155	0.0974	-0.416	0.681		0.686	
Region2:Group1	0.102	0.0678	-0.0267	0.22	1.5	0.146		0.159	
Region3:Group1	-0.052	0.0678	-0.191	0.0717	-0.767	0.45		0.458	
Region1:Group2	0.0739	0.0678	-0.0678	0.203	1.09	0.286		0.304	
Region2:Group2	-0.166	0.0678	-0.283	-0.0288	-2.45	0.0211	*	0.0193	*
Region3:Group2	0.06	0.0678	-0.0604	0.203	0.885	0.384		0.393	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00914	0.0956	0	0.196	19.9	0.182		R2M / R2C	0.17 / 0.335
Residual	0.0368	0.192	0.138	0.23	80.1	NA		AIC/BIC	48.4 / 74.6
dL.On Mean Area (um^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	13900	884	12100	15700	15.8	1E-07	***	0.993	
Region1	26	645	-1190	1300	0.0404	0.968		0.965	
Region2	-2910	645	-4170	-1630	-4.51	0.000114	***	0.000175	***
Region3	1910	645	716	3260	2.96	0.00639	**	0.00632	**
Group1	2220	1250	-125	4640	1.77	0.11		0.125	
Group2	-680	1250	-3240	1910	-0.544	0.6		0.608	
Region1:Group1	879	912	-990	2700	0.964	0.344		0.329	
Region2:Group1	-1390	912	-3120	303	-1.53	0.139		0.12	
Region3:Group1	156	912	-1760	1870	0.171	0.865		0.874	
Region1:Group2	-601	912	-2270	1230	-0.659	0.516		0.513	
Region2:Group2	60	912	-1880	1710	0.0658	0.948		0.951	
Region3:Group2	1020	912	-870	2880	1.11	0.275		0.278	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	7700000	2780	1360	4680	53.6	0.000498	***	R2M / R2C	0.316 / 0.683
Residual	6660000	2580	1810	3110	46.4	NA		AIC/BIC	745 / 771
dL.On Mean Circularity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.703	0.0105	0.681	0.723	67	0	***	0.989	
Region1	-0.00822	0.0119	-0.0307	0.0154	-0.69	0.496		0.498	
Region2	0.0378	0.0119	0.0145	0.0615	3.17	0.00374	**	0.00421	**
Region3	-0.0205	0.0119	-0.0425	0.00442	-1.72	0.097	.	0.107	
Group1	-0.0256	0.0148	-0.0536	0.00385	-1.73	0.118		0.125	
Group2	0.0242	0.0148	-0.00482	0.0538	1.63	0.138		0.153	

Region1:Group1	-0.00198	0.0169	-0.0365	0.0316	-0.118	0.907		0.927	
Region2:Group1	-0.0103	0.0169	-0.0422	0.021	-0.612	0.546		0.542	
Region3:Group1	0.0168	0.0169	-0.0185	0.0484	0.998	0.327		0.328	
Region1:Group2	0.00514	0.0169	-0.0257	0.039	0.305	0.763		0.773	
Region2:Group2	0.0166	0.0169	-0.0193	0.0471	0.984	0.334		0.346	
Region3:Group2	-0.0281	0.0169	-0.063	0.00636	-1.67	0.107		0.109	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00075	0.0274	0	0.0524	24.8	0.0999	.	R2M / R2C	0.263 / 0.447
Residual	0.00227	0.0477	0.0335	0.0574	75.2	NA		AIC/BIC	2.224299
dL.On Mean Aspect Ratio	Lambda = -0.8								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	-0.696	0.0102	-0.716	-0.675	-68.2	0	***	0.959	
Region1	0.00397	0.0143	-0.0224	0.0332	0.278	0.783		0.798	
Region2	-0.0137	0.0143	-0.0417	0.0141	-0.963	0.344		0.35	
Region3	0.0168	0.0143	-0.0113	0.0474	1.18	0.249		0.248	
Group1	0.0134	0.0144	-0.0145	0.0415	0.927	0.378		0.41	
Group2	-0.0108	0.0144	-0.039	0.0183	-0.748	0.474		0.512	
Region1:Group1	-0.00555	0.0202	-0.0432	0.0318	-0.275	0.785		0.786	
Region2:Group1	-0.00971	0.0202	-0.0478	0.0255	-0.481	0.634		0.648	
Region3:Group1	-0.0115	0.0202	-0.0527	0.0253	-0.57	0.574		0.578	
Region1:Group2	-0.00465	0.0202	-0.0468	0.0338	-0.23	0.819		0.801	
Region2:Group2	-0.0107	0.0202	-0.0456	0.0301	-0.532	0.599		0.597	
Region3:Group2	0.0402	0.0202	0.00437	0.0826	1.99	0.0566	.	0.0519	.
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00043	0.0208	0	0.0493	11.7	0.417		R2M / R2C	0.148 / 0.248
Residual	0.00326	0.0571	0.0408	0.0677	88.3	NA		AIC/BIC	40 / 66.2
dL.On Mean Roundness	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.67	0.00991	0.651	0.691	67.6	0	***	1	
Region1	0.000304	0.0148	-0.0276	0.0297	0.0205	0.984		0.981	
Region2	0.0019	0.0148	-0.0271	0.0314	0.128	0.899		0.902	
Region3	-0.014	0.0148	-0.0413	0.017	-0.941	0.355		0.355	
Group1	-0.012	0.014	-0.0381	0.0148	-0.859	0.412		0.446	
Group2	0.0072	0.014	-0.0215	0.0357	0.514	0.62		0.647	
Region1:Group1	0.00642	0.021	-0.0365	0.0482	0.306	0.762		0.744	
Region2:Group1	0.0173	0.021	-0.0223	0.0563	0.827	0.415		0.409	
Region3:Group1	0.0105	0.021	-0.0334	0.0498	0.501	0.62		0.608	

Region1:Group2	0.011	0.021	-0.0273	0.0531	0.525	0.604		0.604	
Region2:Group2	0.00598	0.021	-0.0387	0.044	0.285	0.778		0.803	
Region3:Group2	-0.0487	0.021	-0.0921	-0.0059	-2.32	0.0279	*	0.026	*
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0003	0.0173	0	0.0481	7.85	0.582		R2M / R2C	0.173 / 0.238
Residual	0.00352	0.0593	0.0416	0.0712	92.1	NA		AIC/BIC	3.448598
dL.On Mean Solidity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.933	0.00502	0.923	0.943	186	0	***	0.98	
Region1	-0.00605	0.00493	-0.0153	0.00371	-1.23	0.23		0.24	
Region2	0.0181	0.00493	0.00846	0.0279	3.67	0.00105	**	0.00175	**
Region3	-0.00733	0.00493	-0.0164	0.00297	-1.49	0.148		0.158	
Group1	-0.0114	0.00709	-0.0248	0.00276	-1.61	0.142		0.171	
Group2	0.0102	0.00709	-0.0036	0.0243	1.43	0.185		0.207	
Region1:Group1	-0.00268	0.00697	-0.017	0.0112	-0.385	0.703		0.722	
Region2:Group1	-0.00758	0.00697	-0.0207	0.00537	-1.09	0.286		0.277	
Region3:Group1	0.0101	0.00697	-0.00445	0.0232	1.46	0.157		0.158	
Region1:Group2	0.0055	0.00697	-0.00723	0.0195	0.79	0.436		0.443	
Region2:Group2	0.00676	0.00697	-0.00807	0.0194	0.971	0.34		0.349	
Region3:Group2	-0.0127	0.00697	-0.0272	0.00149	-1.83	0.0784	.	0.0796	.
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0002	0.0143	0.0048	0.0256	33.9	0.0247	*	R2M / R2C	0.278 / 0.527
Residual	0.00039	0.0197	0.0138	0.0237	66.1	NA		AIC/BIC	1.322816
tL.On Mean Area (um^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	15500	2500	10900	20400	6.19	0.000161	***	0.994	
Region1	-1200	4030	-8800	6780	-0.299	0.767		0.757	
Region2	-7490	4030	-15400	528	-1.86	0.0741	.	0.0649	.
Region3	5150	4030	-2290	13600	1.28	0.212		0.21	
Group1	3480	3540	-3110	10100	0.983	0.351		0.391	
Group2	-7840	3540	-14900	-558	-2.21	0.0542	.	0.0695	.
Region1:Group1	7850	5700	-3830	19200	1.38	0.18		0.158	
Region2:Group1	-2730	5700	-13500	7850	-0.479	0.635		0.627	
Region3:Group1	-3670	5700	-15600	7020	-0.644	0.525		0.504	
Region1:Group2	354	5700	-10100	11800	0.062	0.951		0.956	
Region2:Group2	2710	5700	-9420	13000	0.476	0.638		0.648	

Region3:Group2	-694	5700	-12500	11000	-0.122	0.904		0.904	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	10300000	3210	0	11900	3.81	0.785		R2M / R2C	0.213 / 0.243
Residual	2.6E+08	16100	11300	19200	96.2	NA		AIC/BIC	863 / 889
tL.On Mean Circularity	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.351	0.0501	0.253	0.452	7	6.33E-05	***	0.999	
Region1	-0.0546	0.077	-0.2	0.098	-0.709	0.484		0.48	
Region2	-0.055	0.077	-0.205	0.0982	-0.714	0.481		0.461	
Region3	0.0124	0.077	-0.13	0.173	0.161	0.873		0.864	
Group1	0.0417	0.0708	-0.0891	0.175	0.589	0.57		0.595	
Group2	-0.156	0.0708	-0.3	-0.00941	-2.2	0.0554	.	0.0653	.
Region1:Group1	0.128	0.109	-0.0955	0.345	1.17	0.252		0.234	
Region2:Group1	0.0661	0.109	-0.14	0.268	0.606	0.549		0.54	
Region3:Group1	-0.117	0.109	-0.345	0.0875	-1.07	0.293		0.291	
Region1:Group2	0.0301	0.109	-0.169	0.249	0.276	0.784		0.787	
Region2:Group2	-0.00802	0.109	-0.24	0.189	-0.0737	0.942		0.937	
Region3:Group2	-0.0675	0.109	-0.293	0.155	-0.619	0.541		0.526	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00637	0.0798	0	0.239	6.29	0.656		R2M / R2C	0.209 / 0.258
Residual	0.0949	0.308	0.216	0.369	93.7	NA		AIC/BIC	81.3 / 107
tL.On Mean Aspect Ratio	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.974	0.15	0.673	1.27	6.49	0.000113	***	0.998	
Region1	-0.157	0.21	-0.552	0.259	-0.747	0.462		0.459	
Region2	-0.338	0.21	-0.748	0.0791	-1.61	0.119		0.12	
Region3	0.0478	0.21	-0.34	0.487	0.228	0.822		0.816	
Group1	0.121	0.212	-0.285	0.536	0.571	0.582		0.6	
Group2	-0.396	0.212	-0.823	0.0344	-1.87	0.0946	.	0.102	
Region1:Group1	0.137	0.297	-0.472	0.728	0.46	0.649		0.638	
Region2:Group1	0.00753	0.297	-0.554	0.559	0.0254	0.98		0.98	
Region3:Group1	-0.204	0.297	-0.826	0.353	-0.688	0.498		0.484	
Region1:Group2	0.0499	0.297	-0.493	0.645	0.168	0.868		0.869	
Region2:Group2	0.212	0.297	-0.42	0.75	0.714	0.482		0.498	
Region3:Group2	-0.0737	0.297	-0.688	0.533	-0.248	0.806		0.802	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit

Sample	0.0939	0.306	0	0.734	11.8	0.417		R2M / R2C	0.2 / 0.294
Residual	0.705	0.84	0.589	1.01	88.2	NA		AIC/BIC	155 / 181
tL.On Mean Roundness	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.351	0.0544	0.24	0.457	6.44	0.000119	***	0.994	
Region1	-0.0334	0.0719	-0.169	0.109	-0.465	0.646		0.645	
Region2	-0.0695	0.0719	-0.21	0.0735	-0.967	0.342		0.341	
Region3	0.0118	0.0719	-0.121	0.162	0.165	0.87		0.861	
Group1	0.0509	0.0769	-0.0992	0.205	0.661	0.525		0.545	
Group2	-0.17	0.0769	-0.321	-0.0163	-2.21	0.0543	.	0.0586	.
Region1:Group1	0.167	0.102	-0.0411	0.37	1.64	0.112		0.0972	.
Region2:Group1	-0.0017	0.102	-0.194	0.187	-0.0167	0.987		0.987	
Region3:Group1	-0.111	0.102	-0.324	0.0802	-1.09	0.287		0.295	
Region1:Group2	-0.0101	0.102	-0.196	0.194	-0.0989	0.922		0.923	
Region2:Group2	0.0278	0.102	-0.189	0.212	0.274	0.786		0.812	
Region3:Group2	-0.0554	0.102	-0.266	0.152	-0.545	0.59		0.587	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0148	0.122	0	0.267	15.2	0.3		R2M / R2C	0.222 / 0.341
Residual	0.0827	0.288	0.202	0.346	84.8	NA		AIC/BIC	79 / 105
tL.On Mean Solidity	Lambda = 6.725								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.286	0.0417	0.21	0.376	6.86	0	***	0.982	
Region1	-0.0648	0.0722	-0.198	0.0833	-0.898	0.375		0.369	
Region2	-0.0168	0.0722	-0.158	0.124	-0.232	0.818		0.807	
Region3	-0.00027	0.0722	-0.143	0.154	-0.0038	0.997		0.992	
Group1	0.0232	0.0589	-0.091	0.129	0.393	0.696		0.753	
Group2	-0.124	0.0589	-0.246	-0.00722	-2.1	0.043	*	0.086	.
Region1:Group1	0.119	0.102	-0.0716	0.308	1.17	0.251		0.234	
Region2:Group1	0.103	0.102	-0.0896	0.282	1.01	0.318		0.318	
Region3:Group1	-0.0868	0.102	-0.295	0.0995	-0.85	0.401		0.395	
Region1:Group2	0.0647	0.102	-0.149	0.259	0.634	0.53		0.547	
Region2:Group2	-0.0528	0.102	-0.229	0.154	-0.517	0.608		0.602	
Region3:Group2	-0.0963	0.102	-0.278	0.118	-0.943	0.352		0.359	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	0.203	0	1		R2M / R2C	0.225 / 0.225
Residual	0.0834	0.289	0.207	0.34	100	NA		AIC/BIC	105 / 131
Rs.N	Lambda = None								

Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	2.04	0.279	1.53	2.57	7.32	4.48E-05	***	1	
Region1	0.125	0.47	-0.76	1.06	0.266	0.792		0.784	
Region2	-0.958	0.47	-1.88	-0.0237	-2.04	0.0513	.	0.0432	*
Region3	-0.292	0.47	-1.16	0.691	-0.621	0.54		0.536	
Group1	-0.604	0.395	-1.31	0.118	-1.53	0.16		0.213	
Group2	0.333	0.395	-0.398	1.14	0.845	0.42		0.467	
Region1:Group1	0.438	0.664	-0.924	1.76	0.658	0.516		0.497	
Region2:Group1	0.271	0.664	-0.986	1.51	0.408	0.687		0.669	
Region3:Group1	0.104	0.664	-1.29	1.35	0.157	0.877		0.883	
Region1:Group2	0.75	0.664	-0.464	2.08	1.13	0.269		0.269	
Region2:Group2	-1.17	0.664	-2.58	0.038	-1.76	0.0905	.	0.0772	.
Region3:Group2	-0.583	0.664	-1.96	0.774	-0.878	0.388		0.374	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0509	0.226	0	1.37	1.42	0.918		R2M / R2C	0.272 / 0.282
Residual	3.53	1.88	1.32	2.23	98.6	NA		AIC/BIC	210 / 236
a.Rm.Cr	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	52.6	3.48	45.7	59.6	15.1	1E-07	***	1	
Region1	11.4	4.77	2.36	20.8	2.38	0.0246	*	0.0242	*
Region2	-25.3	4.77	-34.6	-15.8	-5.31	1.34E-05	***	0.000175	***
Region3	-5.73	4.77	-14.5	4.24	-1.2	0.24		0.248	
Group1	-7.08	4.92	-16.6	2.68	-1.44	0.184		0.206	
Group2	-7.58	4.92	-17.4	2.36	-1.54	0.158		0.162	
Region1:Group1	4.08	6.75	-9.74	17.5	0.605	0.55		0.541	
Region2:Group1	5	6.75	-7.76	17.5	0.741	0.465		0.462	
Region3:Group1	-4.58	6.75	-18.7	8.07	-0.679	0.503		0.489	
Region1:Group2	4.58	6.75	-7.75	18.1	0.679	0.503		0.5	
Region2:Group2	-5.75	6.75	-20.1	6.48	-0.852	0.402		0.391	
Region3:Group2	4.42	6.75	-9.54	18.2	0.655	0.518		0.528	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	54.3	7.37	0	16.9	13	0.373		R2M / R2C	0.512 / 0.576
Residual	364	19.1	13.4	23	87	NA		AIC/BIC	380 / 407
a.Rm.Cr/CA (a.Rm.Cr. OPD.CA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig

(Intercept)	8.39	0.504	7.38	9.43	16.7	0	***	0.997	
Region1	0.757	0.736	-0.63	2.22	1.03	0.313		0.303	
Region2	-3.17	0.736	-4.61	-1.71	-4.31	0.000195	***	0.000351	***
Region3	0.337	0.736	-1.02	1.88	0.458	0.65		0.654	
Group1	-0.878	0.713	-2.22	0.498	-1.23	0.249		0.288	
Group2	-1.35	0.713	-2.8	0.0857	-1.89	0.0907	.	0.0986	.
Region1:Group1	0.572	1.04	-1.56	2.65	0.55	0.587		0.571	
Region2:Group1	0.953	1.04	-1.02	2.89	0.915	0.368		0.358	
Region3:Group1	-1.06	1.04	-3.24	0.894	-1.02	0.318		0.316	
Region1:Group2	0.88	1.04	-1.02	2.97	0.845	0.405		0.408	
Region2:Group2	-1.25	1.04	-3.46	0.642	-1.2	0.242		0.24	
Region3:Group2	0.675	1.04	-1.48	2.8	0.648	0.522		0.529	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.878	0.937	0	2.47	9.19	0.521		R2M / R2C	0.428 / 0.48
Residual	8.68	2.95	2.07	3.54	90.8	NA		AIC/BIC	245 / 271
T.On/CA (T.On. OPD.CA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	8.07	0.487	7.11	9.08	16.6	0	***	0.994	
Region1	0.776	0.73	-0.6	2.22	1.06	0.297		0.292	
Region2	-3.05	0.73	-4.48	-1.6	-4.18	0.000275	***	0.000351	***
Region3	0.331	0.73	-1.02	1.86	0.454	0.654		0.656	
Group1	-0.794	0.688	-2.07	0.521	-1.15	0.279		0.318	
Group2	-1.37	0.688	-2.78	0.0265	-2	0.0771	.	0.0849	.
Region1:Group1	0.519	1.03	-1.6	2.58	0.503	0.619		0.604	
Region2:Group1	0.902	1.03	-1.05	2.82	0.874	0.39		0.38	
Region3:Group1	-1.06	1.03	-3.23	0.873	-1.03	0.312		0.312	
Region1:Group2	0.759	1.03	-1.13	2.83	0.735	0.468		0.465	
Region2:Group2	-1.07	1.03	-3.27	0.804	-1.03	0.31		0.305	
Region3:Group2	0.759	1.03	-1.38	2.87	0.735	0.469		0.477	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.711	0.843	0	2.36	7.69	0.589		R2M / R2C	0.417 / 0.461
Residual	8.53	2.92	2.05	3.51	92.3	NA		AIC/BIC	244 / 270
C.On/CA (C.On. OPD.CA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	3.82	0.227	3.4	4.26	16.8	0	***	0.998	
Region1	0.457	0.394	-0.285	1.24	1.16	0.253		0.249	

Region2	-1.63	0.394	-2.4	-0.843	-4.13	0.000204	***	0.000351	***
Region3	0.262	0.394	-0.465	1.08	0.665	0.51		0.511	
Group1	-0.24	0.321	-0.849	0.357	-0.748	0.459		0.524	
Group2	-0.749	0.321	-1.34	-0.114	-2.33	0.0255	*	0.0558	.
Region1:Group1	0.645	0.557	-0.495	1.76	1.16	0.254		0.232	
Region2:Group1	0.146	0.557	-0.907	1.18	0.262	0.795		0.784	
Region3:Group1	-0.497	0.557	-1.66	0.546	-0.893	0.378		0.366	
Region1:Group2	0.24	0.557	-0.777	1.36	0.432	0.669		0.665	
Region2:Group2	-0.461	0.557	-1.65	0.548	-0.829	0.413		0.394	
Region3:Group2	0.0607	0.557	-1.09	1.2	0.109	0.914		0.912	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	1.14	0	1		R2M / R2C	0.405 / 0.405
Residual	2.48	1.57	1.1	1.87	100	NA		AIC/BIC	197 / 223
F.On/CA (F.On. OPD.CA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	4.25	0.325	3.57	4.87	13.1	4E-07	***	0.999	
Region1	0.319	0.387	-0.41	1.09	0.824	0.417		0.406	
Region2	-1.42	0.387	-2.18	-0.655	-3.68	0.00102	**	0.0014	**
Region3	0.0697	0.387	-0.645	0.878	0.18	0.858		0.852	
Group1	-0.553	0.46	-1.43	0.355	-1.2	0.26		0.278	
Group2	-0.625	0.46	-1.53	0.294	-1.36	0.208		0.205	
Region1:Group1	-0.126	0.547	-1.25	0.965	-0.23	0.82		0.839	
Region2:Group1	0.757	0.547	-0.278	1.77	1.38	0.178		0.183	
Region3:Group1	-0.566	0.547	-1.71	0.46	-1.03	0.31		0.313	
Region1:Group2	0.519	0.547	-0.48	1.62	0.949	0.351		0.364	
Region2:Group2	-0.607	0.547	-1.77	0.385	-1.11	0.277		0.283	
Region3:Group2	0.698	0.547	-0.433	1.82	1.28	0.213		0.213	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.672	0.82	0	1.61	21.9	0.144		R2M / R2C	0.369 / 0.507
Residual	2.39	1.55	1.09	1.86	78.1	NA		AIC/BIC	202 / 228
sL.On/CA (sL.On. OPD.CA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	1.44	0.185	1.05	1.81	7.77	0.000028	***	0.985	
Region1	0.336	0.186	-0.0145	0.705	1.81	0.082	.	0.0856	.
Region2	-0.528	0.186	-0.892	-0.158	-2.84	0.00857	**	0.0109	*
Region3	-0.0069	0.186	-0.351	0.382	-0.0371	0.971		0.98	

Group1	-0.344	0.262	-0.837	0.178	-1.31	0.221		0.253	
Group2	0.0049	0.262	-0.501	0.529	0.0187	0.985		0.997	
Region1:Group1	0.152	0.263	-0.387	0.677	0.578	0.568		0.563	
Region2:Group1	0.394	0.263	-0.104	0.883	1.5	0.146		0.152	
Region3:Group1	-0.587	0.263	-1.14	-0.0927	-2.23	0.0344	*	0.033	*
Region1:Group2	-0.00819	0.263	-0.489	0.52	-0.0311	0.975		0.976	
Region2:Group2	-0.402	0.263	-0.963	0.0755	-1.53	0.139		0.136	
Region3:Group2	0.677	0.263	0.132	1.21	2.57	0.016	*	0.0161	*
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.272	0.522	0.145	0.94	32.9	0.0321	*	R2M / R2C	0.266 / 0.507
Residual	0.555	0.745	0.523	0.896	67.1	NA		AIC/BIC	152 / 179
dL.On/CA (dL.On. OPD.CA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	2.17	0.15	1.89	2.46	14.5	2E-07	***	0.997	
Region1	0.131	0.258	-0.354	0.641	0.509	0.615		0.593	
Region2	-0.968	0.258	-1.47	-0.455	-3.76	0.000837	***	0.00105	**
Region3	0.216	0.258	-0.26	0.754	0.839	0.409		0.404	
Group1	0.0294	0.212	-0.356	0.42	0.139	0.892		0.905	
Group2	-0.667	0.212	-1.05	-0.247	-3.15	0.0118	*	0.0165	*
Region1:Group1	0.428	0.364	-0.318	1.15	1.17	0.25		0.223	
Region2:Group1	-0.205	0.364	-0.894	0.471	-0.563	0.578		0.56	
Region3:Group1	0.103	0.364	-0.66	0.786	0.283	0.78		0.779	
Region1:Group2	0.205	0.364	-0.461	0.935	0.562	0.579		0.566	
Region2:Group2	-0.105	0.364	-0.88	0.555	-0.289	0.775		0.757	
Region3:Group2	-0.516	0.364	-1.27	0.228	-1.42	0.168		0.159	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00354	0.0595	0	0.742	0.333	0.981		R2M / R2C	0.42 / 0.422
Residual	1.06	1.03	0.723	1.22	99.7	NA		AIC/BIC	166 / 192
tL.ON/CA (tL.On. OPD.CA) (#/mm^2)	Lambda = 0.6								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.301	0.0488	0.204	0.401	6.15	0.000168	***	0.975	
Region1	-0.0211	0.0752	-0.16	0.133	-0.281	0.781		0.769	
Region2	-0.142	0.0752	-0.289	0.00479	-1.89	0.0696	.	0.0642	.
Region3	0.0501	0.0752	-0.098	0.211	0.667	0.51		0.488	
Group1	0.0737	0.0691	-0.0576	0.203	1.07	0.314		0.346	
Group2	-0.124	0.0691	-0.263	0.0154	-1.79	0.107		0.126	

Region1:Group1	0.0968	0.106	-0.102	0.294	0.91	0.371		0.368	
Region2:Group1	-0.0268	0.106	-0.228	0.159	-0.252	0.803		0.798	
Region3:Group1	-0.0448	0.106	-0.262	0.149	-0.422	0.677		0.673	
Region1:Group2	0.036	0.106	-0.186	0.238	0.338	0.738		0.748	
Region2:Group2	0.0515	0.106	-0.132	0.267	0.484	0.632		0.647	
Region3:Group2	-0.101	0.106	-0.29	0.122	-0.953	0.349		0.357	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.00603	0.0777	0	0.235	6.25	0.658		R2M / R2C	0.191 / 0.242
Residual	0.0904	0.301	0.215	0.355	93.7	NA		AIC/BIC	69.5 / 95.7
Rs.N/CA (Rs.N. OPD.CA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.319	0.0428	0.239	0.402	7.44	0	***	0.999	
Region1	-0.0188	0.0742	-0.159	0.128	-0.253	0.802		0.79	
Region2	-0.122	0.0742	-0.267	0.026	-1.64	0.11		0.102	
Region3	0.00598	0.0742	-0.131	0.161	0.0807	0.936		0.931	
Group1	-0.0845	0.0606	-0.199	0.0281	-1.4	0.171		0.262	
Group2	0.0235	0.0606	-0.0873	0.143	0.388	0.7		0.742	
Region1:Group1	0.0533	0.105	-0.162	0.263	0.508	0.615		0.595	
Region2:Group1	0.0503	0.105	-0.148	0.245	0.479	0.635		0.616	
Region3:Group1	0.00378	0.105	-0.216	0.201	0.036	0.971		0.977	
Region1:Group2	0.121	0.105	-0.071	0.331	1.15	0.258		0.256	
Region2:Group2	-0.178	0.105	-0.402	0.0119	-1.7	0.0979	.	0.0863	.
Region3:Group2	-0.0838	0.105	-0.301	0.131	-0.799	0.43		0.414	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	0.214	0	1		R2M / R2C	0.219 / 0.219
Residual	0.0881	0.297	0.208	0.353	100	NA		AIC/BIC	76.4 / 103
a.Rm.Cr/RA (a.Rm.Cr. OPD.RA) (#/mm^2)	Lambda = 0.75								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	11.1	0.583	9.97	12.3	19	0	***	0.996	
Region1	0.976	0.587	-0.107	2.18	1.66	0.108		0.12	
Region2	-1.13	0.587	-2.28	0.0142	-1.93	0.0643	.	0.0688	.
Region3	0.297	0.587	-0.859	1.55	0.507	0.616		0.617	
Group1	-0.276	0.824	-1.8	1.36	-0.336	0.745		0.741	
Group2	-0.747	0.824	-2.32	1.02	-0.906	0.388		0.4	
Region1:Group1	-0.176	0.83	-1.73	1.36	-0.212	0.834		0.834	
Region2:Group1	1.99	0.83	0.422	3.44	2.4	0.0236	*	0.0239	*

Region3:Group1	-1.04	0.83	-2.73	0.476	-1.25	0.222		0.23	
Region1:Group2	1.04	0.83	-0.696	2.62	1.25	0.222		0.239	
Region2:Group2	-1.88	0.83	-3.32	-0.203	-2.27	0.0315	*	0.0305	*
Region3:Group2	0.702	0.83	-0.771	2.45	0.846	0.405		0.409	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	2.7	1.64	0.465	3.04	32.9	0.0323	*	R2M / R2C	0.211 / 0.47
Residual	5.51	2.35	1.69	2.81	67.1	NA		AIC/BIC	313 / 339
T.On/RA (T.On. OPD.RA) (#/mm^2)	Lambda = 0.625								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	7.21	0.321	6.6	7.86	22.5	0	***	0.993	
Region1	0.533	0.334	-0.0838	1.22	1.6	0.122		0.135	
Region2	-0.606	0.334	-1.26	0.0468	-1.81	0.081	.	0.0811	.
Region3	0.169	0.334	-0.49	0.884	0.505	0.617		0.618	
Group1	-0.108	0.454	-0.954	0.79	-0.239	0.817		0.811	
Group2	-0.449	0.454	-1.32	0.533	-0.99	0.348		0.358	
Region1:Group1	-0.0845	0.473	-0.967	0.791	-0.179	0.86		0.861	
Region2:Group1	1.08	0.473	0.183	1.9	2.28	0.0309	*	0.033	*
Region3:Group1	-0.604	0.473	-1.57	0.259	-1.28	0.212		0.216	
Region1:Group2	0.515	0.473	-0.473	1.42	1.09	0.286		0.306	
Region2:Group2	-0.978	0.473	-1.79	-0.0214	-2.07	0.0482	*	0.0456	*
Region3:Group2	0.463	0.473	-0.376	1.46	0.98	0.336		0.35	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.79	0.889	0.2	1.67	30.7	0.045	*	R2M / R2C	0.202 / 0.446
Residual	1.79	1.34	0.96	1.6	69.3	NA		AIC/BIC	313 / 339
C.On/RA (C.On. OPD.RA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	11.3	0.793	9.72	12.9	14.2	2E-07	***	0.997	
Region1	1.92	1.08	-0.108	4.06	1.78	0.0856	.	0.0835	.
Region2	-2.39	1.08	-4.49	-0.244	-2.22	0.0354	*	0.0347	*
Region3	0.759	1.08	-1.23	3.01	0.704	0.487		0.493	
Group1	0.0268	1.12	-2.16	2.26	0.0239	0.981		0.998	
Group2	-1.7	1.12	-3.93	0.558	-1.52	0.163		0.174	
Region1:Group1	0.78	1.52	-2.34	3.82	0.512	0.613		0.604	
Region2:Group1	1.81	1.52	-1.07	4.64	1.19	0.246		0.245	
Region3:Group1	-1.38	1.52	-4.57	1.48	-0.905	0.374		0.372	
Region1:Group2	1.17	1.52	-1.61	4.23	0.768	0.449		0.453	
Region2:Group2	-2.5	1.52	-5.74	0.267	-1.64	0.113		0.107	

Region3:Group2	0.173	1.52	-2.98	3.29	0.114	0.91		0.91	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	2.91	1.71	0	3.85	13.5	0.354		R2M / R2C	0.229 / 0.333
Residual	18.6	4.31	3.02	5.19	86.5	NA		AIC/BIC	273 / 300
F.On/RA (F.On. OPD.RA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	12.9	1.04	10.7	15	12.3	6E-07	***	0.989	
Region1	1.11	0.915	-0.612	2.92	1.22	0.235		0.238	
Region2	-0.583	0.915	-2.37	1.24	-0.637	0.53		0.517	
Region3	0.0318	0.915	-1.66	1.94	0.0348	0.973		0.962	
Group1	-0.89	1.47	-3.68	2	-0.604	0.561		0.582	
Group2	-0.529	1.47	-3.42	2.4	-0.359	0.728		0.74	
Region1:Group1	-1.39	1.29	-4.04	1.19	-1.08	0.291		0.292	
Region2:Group1	3.69	1.29	1.25	6.09	2.85	0.00819	**	0.00772	**
Region3:Group1	-1.8	1.29	-4.51	0.621	-1.4	0.174		0.175	
Region1:Group2	1.31	1.29	-1.05	3.91	1.01	0.319		0.332	
Region2:Group2	-2	1.29	-4.75	0.347	-1.54	0.134		0.13	
Region3:Group2	1.98	1.29	-0.695	4.62	1.53	0.137		0.135	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	9.71	3.12	1.3	5.41	42	0.00658	**	R2M / R2C	0.167 / 0.517
Residual	13.4	3.66	2.57	4.4	58	NA		AIC/BIC	270 / 296
sL.On/RA (sL.On. OPD.RA) (#/mm^2)	Lambda = 0.725								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	2.75	0.325	2.14	3.42	8.44	1.43E-05	***	0.998	
Region1	0.686	0.252	0.222	1.2	2.73	0.0111	*	0.0123	*
Region2	-0.377	0.252	-0.87	0.114	-1.5	0.145		0.138	
Region3	-0.104	0.252	-0.6	0.435	-0.412	0.684		0.686	
Group1	-0.327	0.46	-1.17	0.554	-0.711	0.495		0.529	
Group2	0.0504	0.46	-0.872	0.976	0.11	0.915		0.929	
Region1:Group1	-0.0601	0.356	-0.724	0.599	-0.169	0.867		0.869	
Region2:Group1	0.991	0.356	0.319	1.61	2.79	0.00961	**	0.00982	**
Region3:Group1	-0.924	0.356	-1.65	-0.275	-2.6	0.015	*	0.0123	*
Region1:Group2	0.225	0.356	-0.518	0.902	0.632	0.533		0.559	
Region2:Group2	-0.907	0.356	-1.52	-0.187	-2.55	0.0168	*	0.0182	*
Region3:Group2	0.856	0.356	0.224	1.6	2.41	0.0232	*	0.0242	*
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit

Sample	1.02	1.01	0.513	1.73	50.1	0.00118	**	R2M / R2C	0.209 / 0.605
Residual	1.01	1.01	0.723	1.21	49.9	NA		AIC/BIC	227 / 253
dL.On/RA (dL.On. OPD.RA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	6.34	0.458	5.48	7.26	13.8	2E-07	***	0.995	
Region1	0.573	0.721	-0.785	2	0.795	0.433		0.409	
Region2	-1.41	0.721	-2.82	0.0212	-1.96	0.0604	.	0.0568	.
Region3	0.642	0.721	-0.69	2.15	0.89	0.381		0.378	
Group1	0.523	0.648	-0.695	1.71	0.807	0.44		0.477	
Group2	-1.74	0.648	-3.05	-0.399	-2.69	0.0249	*	0.0305	*
Region1:Group1	0.805	1.02	-1.28	2.84	0.789	0.437		0.416	
Region2:Group1	-0.0245	1.02	-1.95	1.87	-0.024	0.981		0.984	
Region3:Group1	0.414	1.02	-1.72	2.33	0.406	0.688		0.678	
Region1:Group2	0.756	1.02	-1.11	2.8	0.742	0.465		0.456	
Region2:Group2	-0.896	1.02	-3.07	0.953	-0.879	0.387		0.37	
Region3:Group2	-1.39	1.02	-3.5	0.697	-1.36	0.185		0.181	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.436	0.66	0	2.17	4.98	0.722		R2M / R2C	0.278 / 0.314
Residual	8.32	2.88	2.02	3.45	95	NA		AIC/BIC	242 / 268
tL.ON/RA (tL.On. OPD.RA) (#/mm^2)	Lambda = 0.55								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	2.95E-05	4.9E-06	1.99E-05	3.96E-05	6.03	0.000195	***	0.979	
Region1	-2.7E-06	7.69E-06	-1.7E-05	1.31E-05	-0.348	0.73		0.716	
Region2	-9.2E-06	7.69E-06	-2.4E-05	5.8E-06	-1.2	0.241		0.236	
Region3	5.22E-06	7.69E-06	-9.9E-06	2.17E-05	0.679	0.503		0.481	
Group1	8.96E-06	6.93E-06	-4.2E-06	0.000022	1.29	0.228		0.25	
Group2	-1.2E-05	6.93E-06	-2.6E-05	1.54E-06	-1.8	0.106		0.125	
Region1:Group1	8.6E-06	1.09E-05	-1.2E-05	2.87E-05	0.791	0.436		0.433	
Region2:Group1	1.15E-06	1.09E-05	-1.9E-05	2.01E-05	0.106	0.917		0.906	
Region3:Group1	-5.6E-06	1.09E-05	-2.8E-05	1.42E-05	-0.515	0.611		0.613	
Region1:Group2	4.1E-06	1.09E-05	-1.9E-05	2.48E-05	0.377	0.709		0.72	
Region2:Group2	1.22E-06	1.09E-05	-1.8E-05	2.32E-05	0.112	0.912		0.927	
Region3:Group2	-9E-06	1.09E-05	-2.8E-05	1.38E-05	-0.83	0.414		0.424	

Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0.00001	0	2.35E-05	NaN	0.713		R2M / R2C	0.152 / 0.196
Residual	0	0.00003	2.19E-05	3.63E-05	NaN	NA		AIC/BIC	1.027027
Rs.N/RA (Rs.N. OPD.RA) (#/mm^2)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	0.918	0.123	0.689	1.15	7.49	3.74E-05	***	0.997	
Region1	-0.0127	0.202	-0.393	0.387	-0.0629	0.95		0.944	
Region2	-0.233	0.202	-0.627	0.168	-1.16	0.257		0.243	
Region3	0.0603	0.202	-0.312	0.482	0.299	0.767		0.769	
Group1	-0.152	0.173	-0.467	0.171	-0.874	0.405		0.456	
Group2	0.103	0.173	-0.232	0.46	0.592	0.568		0.607	
Region1:Group1	-0.0254	0.285	-0.609	0.543	-0.0892	0.93		0.943	
Region2:Group1	0.316	0.285	-0.223	0.845	1.11	0.278		0.264	
Region3:Group1	0.0755	0.285	-0.522	0.61	0.265	0.793		0.794	
Region1:Group2	0.496	0.285	-0.0255	1.07	1.74	0.0936	.	0.0902	.
Region2:Group2	-0.66	0.285	-1.27	-0.143	-2.32	0.0284	*	0.02	*
Region3:Group2	-0.274	0.285	-0.864	0.308	-0.961	0.345		0.334	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0178	0.134	0	0.591	2.67	0.847		R2M / R2C	0.203 / 0.224
Residual	0.65	0.806	0.566	0.961	97.3	NA		AIC/BIC	149 / 176
T.On / Rs.N	Lambda = -0.175								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	-0.579	0.01	-0.597	-0.557	-57.6	0	***	0.986	
Region1	0.0182	0.0174	-0.0139	0.0539	1.05	0.302		0.296	
Region2	-0.0288	0.0174	-0.0628	0.00518	-1.65	0.107		0.1	
Region3	0.003	0.0174	-0.0313	0.0402	0.173	0.864		0.864	
Group1	0.0165	0.0142	-0.011	0.0419	1.16	0.253		0.307	
Group2	-0.0317	0.0142	-0.061	-0.00361	-2.23	0.0321	*	0.0544	.
Region1:Group1	-0.0114	0.0246	-0.0574	0.0341	-0.465	0.645		0.654	
Region2:Group1	0.0184	0.0246	-0.0281	0.0613	0.749	0.459		0.476	
Region3:Group1	-0.0288	0.0246	-0.0791	0.016	-1.17	0.249		0.251	
Region1:Group2	0.0027	0.0246	-0.0487	0.0495	0.11	0.913		0.926	
Region2:Group2	-0.0105	0.0246	-0.053	0.0392	-0.429	0.671		0.669	
Region3:Group2	0.032	0.0246	-0.0117	0.0837	1.3	0.201		0.208	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0	0	0	0.0489	0	1		R2M / R2C	0.19 / 0.19

Residual	0.00484	0.0696	0.05	0.0819	100	NA		AIC/BIC	393 / 419
On.MAR.I.dL (um/day)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	1.75	0.102	1.54	1.95	17.2	0	***	0.992	
Region1	0.0571	0.0795	-0.0927	0.215	0.718	0.479		0.466	
Region2	-0.0931	0.0795	-0.248	0.065	-1.17	0.252		0.243	
Region3	0.0141	0.0795	-0.133	0.18	0.177	0.86		0.855	
Group1	0.11	0.144	-0.161	0.392	0.761	0.466		0.505	
Group2	-0.126	0.144	-0.417	0.168	-0.876	0.404		0.449	
Region1:Group1	-0.124	0.112	-0.354	0.1	-1.1	0.281		0.28	
Region2:Group1	-0.0478	0.112	-0.26	0.161	-0.425	0.674		0.669	
Region3:Group1	0.109	0.112	-0.126	0.32	0.973	0.339		0.333	
Region1:Group2	0.16	0.112	-0.0458	0.385	1.42	0.167		0.175	
Region2:Group2	-0.0753	0.112	-0.315	0.129	-0.67	0.509		0.5	
Region3:Group2	0.011	0.112	-0.222	0.241	0.0978	0.923		0.92	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0995	0.315	0.149	0.537	49.6	0.00134	**	R2M / R2C	0.0982 / 0.545
Residual	0.101	0.318	0.223	0.383	50.4	NA		AIC/BIC	95.8 / 122
On.MAR.I (um/day)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	1.67	0.104	1.46	1.88	16.1	1E-07	***	0.992	
Region1	0.0645	0.0966	-0.117	0.256	0.668	0.51		0.501	
Region2	-0.146	0.0966	-0.335	0.0458	-1.52	0.141		0.15	
Region3	-0.00301	0.0966	-0.181	0.199	-0.0312	0.975		0.987	
Group1	0.0957	0.147	-0.183	0.388	0.652	0.531		0.55	
Group2	-0.0968	0.147	-0.382	0.197	-0.659	0.526		0.554	
Region1:Group1	-0.172	0.137	-0.452	0.1	-1.26	0.218		0.221	
Region2:Group1	-0.0558	0.137	-0.314	0.198	-0.409	0.686		0.684	
Region3:Group1	0.0464	0.137	-0.24	0.303	0.34	0.736		0.74	
Region1:Group2	0.198	0.137	-0.0516	0.472	1.45	0.159		0.173	
Region2:Group2	-0.0118	0.137	-0.303	0.236	-0.0864	0.932		0.929	
Region3:Group2	0.0651	0.137	-0.217	0.344	0.477	0.637		0.645	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0921	0.303	0.116	0.534	38.2	0.0134	*	R2M / R2C	0.115 / 0.453
Residual	0.149	0.386	0.271	0.465	61.8	NA		AIC/BIC	107 / 133
On.MAR.C (um/day)	Lambda = None								

Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	1.85	0.0987	1.64	2.05	18.7	0	***	0.998	
Region1	0.0303	0.103	-0.164	0.234	0.294	0.771		0.768	
Region2	-0.166	0.103	-0.368	0.0385	-1.62	0.118		0.122	
Region3	0.0507	0.103	-0.14	0.266	0.492	0.626		0.633	
Group1	0.123	0.14	-0.14	0.401	0.882	0.4		0.409	
Group2	-0.213	0.14	-0.482	0.0664	-1.53	0.161		0.175	
Region1:Group1	-0.125	0.146	-0.423	0.166	-0.857	0.399		0.411	
Region2:Group1	0.0412	0.146	-0.234	0.312	0.283	0.779		0.775	
Region3:Group1	0.00639	0.146	-0.299	0.28	0.0438	0.965		0.973	
Region1:Group2	0.238	0.146	-0.0278	0.531	1.64	0.113		0.122	
Region2:Group2	-0.0625	0.146	-0.373	0.202	-0.429	0.671		0.662	
Region3:Group2	0.0114	0.146	-0.29	0.309	0.0785	0.938		0.938	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	0.0745	0.273	0.049	0.5	30.5	0.0462	*	R2M / R2C	0.157 / 0.414
Residual	0.17	0.412	0.289	0.496	69.5	NA		AIC/BIC	109 / 135
σ_f dL (days)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	20.9	0.98	18.9	22.8	21.4	0	***	0.981	
Region1	-1.72	1.14	-3.87	0.544	-1.51	0.144		0.148	
Region2	0.487	1.14	-1.74	2.76	0.427	0.673		0.67	
Region3	0.256	1.14	-1.85	2.64	0.224	0.824		0.824	
Group1	-2.32	1.39	-4.94	0.445	-1.68	0.128		0.138	
Group2	2.54	1.39	-0.187	5.32	1.83	0.1		0.0993	.
Region1:Group1	3.12	1.61	-0.186	6.34	1.93	0.0638	.	0.0579	.
Region2:Group1	-2.56	1.61	-5.61	0.437	-1.59	0.124		0.109	
Region3:Group1	0.292	1.61	-3.09	3.32	0.181	0.858		0.868	
Region1:Group2	-0.572	1.61	-3.52	2.67	-0.354	0.726		0.729	
Region2:Group2	3.4	1.61	-0.0418	6.32	2.1	0.0449	*	0.0435	*
Region3:Group2	-3.71	1.61	-7.05	-0.41	-2.3	0.0296	*	0.0319	*
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	6.3	2.51	0	4.84	23.2	0.123		R2M / R2C	0.277 / 0.445
Residual	20.8	4.57	3.2	5.5	76.8	NA		AIC/BIC	280 / 307
σ_f I (days)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	22.3	1.23	19.7	24.7	18.2	0	***	0.996	
Region1	-2.17	1.43	-4.86	0.669	-1.51	0.142		0.146	

Region2	0.995	1.43	-1.8	3.84	0.695	0.493		0.485	
Region3	0.379	1.43	-2.26	3.37	0.265	0.793		0.796	
Group1	-1.99	1.73	-5.26	1.48	-1.14	0.282		0.295	
Group2	1.87	1.73	-1.54	5.35	1.08	0.309		0.308	
Region1:Group1	3.76	2.02	-0.389	7.79	1.86	0.0743	.	0.0695	.
Region2:Group1	-3.31	2.02	-7.13	0.451	-1.63	0.114		0.0982	.
Region3:Group1	0.778	2.02	-3.46	4.57	0.384	0.704		0.701	
Region1:Group2	-0.685	2.02	-4.38	3.38	-0.338	0.738		0.744	
Region2:Group2	2.81	2.02	-1.5	6.48	1.39	0.176		0.185	
Region3:Group2	-4.43	2.02	-8.62	-0.297	-2.19	0.0374	*	0.0375	*
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	9.87	3.14	0	6.06	23.1	0.124		R2M / R2C	0.211 / 0.394
Residual	32.8	5.72	4.02	6.89	76.9	NA		AIC/BIC	297 / 323
σ_f C (days)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	20.1	1.14	17.8	22.4	17.7	0	***	0.999	
Region1	-1.56	1.18	-3.78	0.781	-1.32	0.198		0.205	
Region2	1.29	1.18	-1.02	3.64	1.09	0.285		0.287	
Region3	-0.236	1.18	-2.42	2.23	-0.2	0.843		0.847	
Group1	-2.09	1.61	-5.13	1.13	-1.29	0.228		0.243	
Group2	3.07	1.61	-0.0366	6.3	1.9	0.0894	.	0.0926	.
Region1:Group1	3.6	1.67	0.183	6.93	2.16	0.04	*	0.0333	*
Region2:Group1	-3.59	1.67	-6.75	-0.485	-2.15	0.0408	*	0.0365	*
Region3:Group1	0.917	1.67	-2.58	4.05	0.549	0.587		0.587	
Region1:Group2	-1.73	1.67	-4.78	1.62	-1.03	0.31		0.305	
Region2:Group2	3.62	1.67	0.0652	6.65	2.17	0.0391	*	0.0365	*
Region3:Group2	-3.3	1.67	-6.75	0.113	-1.98	0.0585	.	0.0614	.
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	10	3.17	0.646	5.79	31	0.0426	*	R2M / R2C	0.267 / 0.494
Residual	22.3	4.72	3.31	5.68	69	NA		AIC/BIC	285 / 311
Ac.F.I.dL (#/mm^2/year)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	70.3	6.69	56.3	83.5	10.5	2.4E-06	***	0.978	
Region1	10.2	6.96	-2.92	24	1.47	0.154		0.159	
Region2	-24.7	6.96	-38.3	-10.9	-3.56	0.00141	**	0.0014	**
Region3	0.0397	6.96	-12.8	14.6	0.0057	0.995		0.982	
Group1	4.35	9.46	-13.5	23.2	0.46	0.656		0.669	

Group2	-20	9.46	-38.3	-1.07	-2.12	0.0634	.	0.0751	.
Region1:Group1	1.86	9.84	-18.3	21.5	0.189	0.852		0.83	
Region2:Group1	6.8	9.84	-11.8	25.1	0.691	0.496		0.491	
Region3:Group1	-9.04	9.84	-29.7	9.41	-0.919	0.366		0.367	
Region1:Group2	1.32	9.84	-16.7	21.1	0.134	0.894		0.898	
Region2:Group2	-10.2	9.84	-31.1	7.64	-1.04	0.309		0.311	
Region3:Group2	10	9.84	-10.3	30.1	1.02	0.316		0.328	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	344	18.5	3.54	33.9	30.7	0.0445	*	R2M / R2C	0.307 / 0.52
Residual	774	27.8	19.5	33.5	69.3	NA		AIC/BIC	413 / 439
Ac.F.I (#/mm^2/year)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	67.7	7.03	53.1	81.7	9.63	4.9E-06	***	0.986	
Region1	9.73	7.24	-3.91	24.1	1.34	0.19		0.198	
Region2	-26.5	7.24	-40.6	-12.1	-3.66	0.00109	**	0.0014	**
Region3	-1.37	7.24	-14.7	13.8	-0.189	0.851		0.855	
Group1	4.36	9.95	-14.4	24.2	0.439	0.671		0.68	
Group2	-19	9.95	-38.2	0.899	-1.91	0.0879	.	0.0965	.
Region1:Group1	1.57	10.2	-19.4	22	0.154	0.879		0.858	
Region2:Group1	4.74	10.2	-14.6	23.7	0.463	0.647		0.637	
Region3:Group1	-9.81	10.2	-31.3	9.38	-0.959	0.346		0.348	
Region1:Group2	2.98	10.2	-15.7	23.5	0.292	0.773		0.78	
Region2:Group2	-8.15	10.2	-29.9	10.4	-0.796	0.433		0.427	
Region3:Group2	12.4	10.2	-8.8	33.3	1.21	0.238		0.24	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	384	19.6	4.31	35.7	31.4	0.0401	*	R2M / R2C	0.302 / 0.522
Residual	838	28.9	20.3	34.8	68.6	NA		AIC/BIC	416 / 442
Ac.F.C (#/mm^2/year)	Lambda = None								
Fixed Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	t	p-value	Sig	pMCMC	Sig
(Intercept)	76.5	7.65	60.5	91.2	10	3.6E-06	***	0.989	
Region1	10.5	8.95	-6.41	28.2	1.17	0.253		0.26	
Region2	-29.2	8.95	-46.7	-11.4	-3.26	0.00301	**	0.00351	**
Region3	-0.768	8.95	-17.3	17.9	-0.0858	0.932		0.935	
Group1	5.58	10.8	-14.9	27.2	0.516	0.619		0.632	
Group2	-24.7	10.8	-46	-2.99	-2.28	0.0483	*	0.0481	*
Region1:Group1	4.54	12.7	-21.4	29.8	0.359	0.723		0.717	
Region2:Group1	10.6	12.7	-13.4	34.1	0.835	0.411		0.411	

Region3:Group1	-14.1	12.7	-40.6	9.67	-1.11	0.276		0.287	
Region1:Group2	3.1	12.7	-20	28.5	0.245	0.808		0.812	
Region2:Group2	-8.7	12.7	-35.7	14.2	-0.687	0.498		0.492	
Region3:Group2	11.5	12.7	-14.7	37.3	0.905	0.374		0.387	
Random Factor	Estimate	Std. Error	CI (2.5%)	CI (97.5%)	ICC (%)	p-value	Sig	Model Test	Model Fit
Sample	382	19.5	0	37.8	22.9	0.128		R2M / R2C	0.309 / 0.468
Residual	1280	35.8	25.1	43.1	77.1	NA		AIC/BIC	429 / 455

Regional Femur: Post-Hoc Tests for Significant Fixed Effects

Cortical Area (mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	5.63E-13	***	5.636394	Large	100%
Region		Anterior > Medial	4.47E-12	***	5.159157	Large	
Region		Lateral < Posterior	1.03E-11	***	4.975593	Large	
Region		Medial < Posterior	1.00E-10	***	4.498356	Large	
Remodeling Area (mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.000204	***	2.01311	Large	100%
Region		Anterior < Posterior	0.023713	*	1.251913	Large	
Region		Lateral < Medial	0.065476	.	1.066047	Large	
Region		Lateral < Posterior	7.89E-08	***	3.265023	Large	
Region		Medial < Posterior	6.09E-05	***	2.198976	Large	
RA/CA (%)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.058156	.	1.088727	Large	100%
Region		Anterior < Posterior	0.027373	*	1.22664	Large	
Region		Lateral < Medial	0.021711	*	1.267314	Large	
Region		Lateral < Posterior	2.87E-05	***	2.315367	Large	
Region		Medial < Posterior	0.071848	.	1.048053	Large	
Group		Fentanyl < Morphine	0.047669	*	1.123637	Large	62%
Percent Porosity (%)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.005843	**	1.487467	Large	100%
Region		Lateral < Medial	0.004999	**	1.512756	Large	
Region		Lateral < Posterior	0.000112	***	2.104676	Large	
Pore Density CA (1/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.0065	**	1.470098	Large	100%
Region		Lateral < Medial	0.004583	**	1.526793	Large	
Region		Lateral < Posterior	8.28E-05	***	2.151806	Large	
Total Pore Number	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	1.77E-05	***	2.390373	Large	100%
Region		Anterior > Medial	0.064508	.	1.068914	Large	
Region		Lateral < Medial	0.015856	*	1.321459	Large	
Region		Lateral < Posterior	1.93E-07	***	3.114728	Large	
Region		Medial < Posterior	0.000847	***	1.793269	Large	
Total Pore Area (um^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.00034	***	1.934344	Large	100%
Region		Lateral < Medial	0.025506	*	1.239117	Large	
Region		Lateral < Posterior	7.45E-06	***	2.525082	Large	
Region		Medial < Posterior	0.019497	*	1.285965	Large	

Mean Pore Area (um^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral < Medial	0.098138	.	0.985979	Large	84%
Mean Pore Perimeter (um)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral < Medial	0.091019	.	1.001222	Large	87%
Mean Pore Circularity	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.050414	.	1.115655	Large	76%
Mean Pore Min Feret Diameter (um)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral < Medial	0.025769	*	1.237305	Large	90%
Mean Pore Aspect Ratio	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior < Lateral	0.001728	**	1.681996	Large	95%
Region		Lateral > Medial	0.053206	.	1.105545	Large	
Mean Pore Roundness	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.032986	*	1.193379	Large	78%
Region		Anterior > Posterior	0.070687	.	1.051225	Large	
T.On	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.000146	***	2.064309	Large	100%
Region		Lateral < Medial	0.025929	*	1.236214	Large	
Region		Lateral < Posterior	1.13E-05	***	2.46036	Large	
Region		Medial < Posterior	0.027762	*	1.224146	Large	
C.On	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.000937	***	1.777678	Large	100%
Region		Lateral < Medial	0.078936	.	1.029605	Large	
Region		Lateral < Posterior	0.000196	***	2.018992	Large	
Region		Medial < Posterior	0.096507	.	0.989386	Large	
F.On	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	9.31E-05	***	2.133814	Large	100%
Region		Lateral < Medial	0.02624	*	1.234117	Large	
Region		Lateral < Posterior	3.14E-06	***	2.661384	Large	
Region		Medial < Posterior	0.008437	**	1.427267	Large	
Group | Region	Control	Anterior > Lateral	0.029361	*	1.62686	Large	63%
Group | Region	Control	Anterior > Medial	0.078435	.	1.293442	Large	
Group | Region	Control	Lateral < Posterior	0.005793	**	2.118969	Large	
Group | Region	Control	Medial < Posterior	0.017745	*	1.785551	Large	
Group | Region	Fentanyl	Anterior > Lateral	0.000133	***	3.147674	Large	
Group | Region	Fentanyl	Lateral < Medial	0.001661	**	2.470213	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.000136	***	3.140633	Large	
Group | Region	Morphine	Anterior > Lateral	0.029357	*	1.626906	Large	
Group | Region	Morphine	Lateral < Posterior	0.000652	***	2.72455	Large	

Group | Region	Morphine	Medial < Posterior	0.015565	*	1.825829	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.01209	*	2.159971	Large	
Region | Group	Medial	Control < Morphine	0.095903	.	1.391141	Large	
Region | Group	Posterior	Control < Morphine	0.087074	.	1.431419	Large	
Region | Group	Posterior	Fentanyl < Morphine	0.039252	*	1.743887	Large	
sL.On	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.004662	**	1.524044	Large	98%
Region		Lateral < Posterior	0.018544	*	1.294618	Large	
Group | Region	Control	Anterior > Lateral	0.093576	.	1.229068	Large	53%
Group | Region	Control	Anterior > Medial	0.025535	*	1.671532	Large	
Group | Region	Control	Medial < Posterior	0.081809	.	1.278231	Large	
Group | Region	Fentanyl	Anterior > Lateral	0.009756	**	1.966509	Large	
Group | Region	Fentanyl	Lateral < Medial	0.021855	*	1.720695	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.062038	.	1.376556	Large	
Group | Region	Morphine	Anterior > Lateral	0.062038	.	1.376556	Large	
Group | Region	Morphine	Lateral < Posterior	0.025535	*	1.671532	Large	
Group | Region	Morphine	Medial < Posterior	0.093576	.	1.229068	Large	
Region | Group	Medial	Control < Fentanyl	0.046259	*	1.81902	Large	
dL.On	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.002813	**	1.604948	Large	100%
Region		Lateral < Medial	0.049868	*	1.117693	Large	
Region		Lateral < Posterior	0.000119	***	2.095948	Large	
Group		Fentanyl < Morphine	0.011879	*	1.316491	Large	94%
tL.On	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral < Posterior	0.099108	.	0.983974	Large	67%
tL.On Mean Outer Label (um)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group		Fentanyl < Morphine	0.061652	.	0.990729	Large	73%
C.On Mean Wall Thickness (W.Th.C) (um)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Control	Lateral < Medial	0.017209	*	1.794999	Large	92%
Group | Region	Morphine	Anterior < Medial	0.015526	*	1.826605	Large	
Group | Region	Morphine	Anterior < Posterior	0.026287	*	1.662287	Large	
sL.On Mean Wall Thickness (W.Th.sL) (um)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior > Lateral	0.007221	**	2.055043	Large	90%
Group | Region	Fentanyl	Lateral < Posterior	0.003283	**	2.280761	Large	
Group | Region	Morphine	Anterior < Medial	0.027846	*	1.643881	Large	
Group | Region	Morphine	Anterior < Posterior	0.049921	*	1.4514	Large	
Region | Group	Lateral	Control > Fentanyl	0.040296	*	1.948381	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.035223	*	2.005518	Large	

Region | Group	Medial	Fentanyl < Morphine	0.087261	.	1.603159	Large	
dL.On Mean Wall Thickness (W.Th.dL) (um)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Control	Anterior > Lateral	0.020723	*	1.737344	Large	64%
Group | Region	Control	Lateral < Medial	0.012582	*	1.890432	Large	
Group | Region	Control	Lateral < Posterior	0.023678	*	1.695468	Large	
Region | Group	Lateral	Control < Fentanyl	0.049668	*	2.18912	Large	
tL.On Mean Wall Thickness (W.Th.tL) (um)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Morphine	Anterior < Medial	0.028643	*	1.634818	Large	61%
Group | Region	Morphine	Lateral < Medial	0.067685	.	1.345982	Large	
Region | Group	Medial	Control < Morphine	0.085787	.	1.365916	Large	
Region | Group	Medial	Fentanyl < Morphine	0.012052	*	2.048526	Large	
T.On Mean Area (um^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral < Medial	0.011504	*	1.375694	Large	97%
Region		Lateral < Posterior	0.01373	*	1.345911	Large	
T.On Mean Circularity	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral > Medial	0.026267	*	1.233935	Large	82%
Region		Lateral > Posterior	0.053608	.	1.104126	Large	
T.On Mean Solidity	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral > Medial	0.017062	*	1.308925	Large	82%
Region		Lateral > Posterior	0.054742	.	1.100183	Large	
C.On Mean Area (um^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral < Medial	0.004307	**	1.536795	Large	98%
Region		Lateral < Posterior	0.013092	*	1.353948	Large	
Group | Region	Control	Anterior > Lateral	0.015804	*	1.821169	Large	73%
Group | Region	Control	Lateral < Medial	0.001716	**	2.461289	Large	
Group | Region	Control	Lateral < Posterior	0.009674	**	1.968994	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.087949	.	1.251876	Large	
Group | Region	Morphine	Anterior < Medial	0.024859	*	1.680054	Large	
Group | Region	Morphine	Anterior < Posterior	0.036416	*	1.556815	Large	
Region | Group	Anterior	Control > Morphine	0.015823	*	2.515044	Large	
Region | Group	Medial	Control > Fentanyl	0.058452	.	1.920095	Large	
C.On Mean Circularity	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral > Medial	0.021254	*	1.271019	Large	79%
Region		Lateral > Posterior	0.075429	.	1.038543	Large	
C.On Mean Aspect Ratio	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Lateral < Medial	0.038369	*	1.539597	Large	55%
Group | Region	Fentanyl	Medial > Posterior	0.06237	.	1.374696	Large	

Region | Group	Medial	Fentanyl > Morphine	0.065737	.	1.342088	Large	
C.On Mean Roundness	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Lateral > Medial	0.041215	*	1.515868	Large	58%
Group | Region	Fentanyl	Medial < Posterior	0.046389	*	1.476244	Large	
Region | Group	Medial	Fentanyl < Morphine	0.088377	.	1.238497	Large	
C.On Mean Solidity	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior < Lateral	0.046134	*	1.132176	Large	83%
Region		Lateral > Medial	0.012682	*	1.359309	Large	
Region		Lateral > Posterior	0.03525	*	1.181412	Large	
F.On Mean Area (um^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.079884	.	1.027248	Large	93%
Region		Lateral < Medial	0.043901	*	1.141346	Large	
Region		Lateral < Posterior	0.011585	*	1.374519	Large	
F.On Mean Aspect Ratio	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior < Posterior	0.093775	.	0.995206	Large	91%
Group | Region	Control	Anterior < Medial	0.008139	**	2.020011	Large	82%
Group | Region	Fentanyl	Anterior < Posterior	0.035776	*	1.562637	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.005904	**	2.113464	Large	
Group | Region	Fentanyl	Medial < Posterior	0.072929	.	1.319519	Large	
F.On Mean Roundness	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Medial	0.069622	.	1.054175	Large	95%
Group | Region	Control	Anterior > Medial	0.010362	*	1.948598	Large	76%
Group | Region	Fentanyl	Anterior > Posterior	0.048898	*	1.458435	Large	
Group | Region	Fentanyl	Lateral > Medial	0.031604	*	1.603073	Large	
Group | Region	Fentanyl	Lateral > Posterior	0.002109	**	2.404323	Large	
Region | Group	Lateral	Control < Fentanyl	0.072161	.	1.617422	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.064106	.	1.668505	Large	
sL.On Mean Area (um^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral < Medial	0.068392	.	1.05763	Large	96%
Region		Lateral < Posterior	0.00853	**	1.425452	Large	
Group | Region	Control	Lateral < Medial	0.066752	.	1.35088	Large	88%
Group | Region	Fentanyl	Anterior > Lateral	0.004738	**	2.176689	Large	
Group | Region	Fentanyl	Anterior > Medial	0.058461	.	1.397208	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.002581	**	2.348149	Large	
Group | Region	Fentanyl	Medial < Posterior	0.035124	*	1.568669	Large	
Group | Region	Morphine	Anterior < Medial	0.043325	*	1.499202	Large	
Group | Region	Morphine	Anterior < Posterior	0.05986	.	1.389002	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.07371	.	1.502996	Large	
Region | Group	Lateral	Control > Fentanyl	0.070323	.	1.521977	Large	

Region | Group	Medial	Control > Fentanyl	0.014931	*	2.093377	Large	
Region | Group	Medial	Fentanyl < Morphine	0.09611	.	1.393415	Large	
sL.On Mean Solidity	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior > Lateral	0.093467	.	1.229499	Large	64%
Region | Group	Lateral	Control > Fentanyl	0.020417	*	1.927082	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.017361	*	1.981817	Large	
dL.On Mean Area (um^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.044908	*	1.137161	Large	99%
Region		Lateral < Medial	0.000529	***	1.866165	Large	
Region		Lateral < Posterior	0.005247	**	1.504938	Large	
dL.On Mean Circularity	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Lateral > Medial	0.027888	*	1.223339	Large	79%
Region		Lateral > Posterior	0.098798	.	0.984613	Large	
dL.On Mean Aspect Ratio	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Lateral < Medial	0.053466	.	1.427983	Large	51%
Group | Region	Fentanyl	Medial > Posterior	0.03637	*	1.557227	Large	
Region | Group	Posterior	Control > Fentanyl	0.086849	.	1.326609	Large	
dL.On Mean Roundness	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior > Medial	0.088936	.	1.247789	Large	76%
Group | Region	Fentanyl	Medial < Posterior	0.017449	*	1.790746	Large	
Region | Group	Medial	Fentanyl < Morphine	0.060742	.	1.426948	Large	
Region | Group	Posterior	Control < Fentanyl	0.058883	.	1.437908	Large	
dL.On Mean Solidity	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior < Lateral	0.027596	*	1.225206	Large	90%
Region		Lateral > Medial	0.019057	*	1.289913	Large	
Region		Lateral > Posterior	0.040383	*	1.156692	Large	
Group | Region	Fentanyl	Anterior < Lateral	0.079393	.	1.289067	Large	50%
Group | Region	Fentanyl	Lateral > Medial	0.003292	**	2.279974	Large	
Group | Region	Fentanyl	Lateral > Posterior	0.046481	*	1.475576	Large	
Group | Region	Morphine	Anterior < Lateral	0.056402	.	1.4096	Large	
Group | Region	Morphine	Lateral > Posterior	0.093805	.	1.228166	Large	
Region | Group	Anterior	Control < Fentanyl	0.095443	.	1.511101	Large	
Region | Group	Lateral	Control < Fentanyl	0.046728	*	1.823218	Large	
Rs.N	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior > Lateral	0.032281	*	1.596195	Large	77%
Group | Region	Fentanyl	Lateral < Posterior	0.003518	**	2.261276	Large	
Group | Region	Fentanyl	Medial < Posterior	0.032281	*	1.596195	Large	
Region | Group	Posterior	Control < Fentanyl	0.047247	*	1.463178	Large	
a.Rm.C	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power

Region		Anterior > Lateral	0.00037	***	1.921446	Large	100%
Region		Lateral < Medial	0.080297	.	1.026227	Large	
Region		Lateral < Posterior	2.17E-05	***	2.358139	Large	
Region		Medial < Posterior	0.014912	*	1.331912	Large	
a.Rm.Cr/CA (a.Rm.Cr. OPD.CA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.014712	*	1.334197	Large	99%
Region		Lateral < Medial	0.033298	*	1.191684	Large	
Region		Lateral < Posterior	0.000907	***	1.782663	Large	
T.On/CA (T.On. OPD.CA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.016918	*	1.310377	Large	98%
Region		Lateral < Medial	0.040059	*	1.158166	Large	
Region		Lateral < Posterior	0.001443	**	1.710218	Large	
C.On/CA (C.On. OPD.CA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.015675	*	1.32341	Large	99%
Region		Lateral < Medial	0.031911	*	1.199322	Large	
Region		Lateral < Posterior	0.002732	**	1.609581	Large	
Group		Fentanyl < Morphine	0.029875	*	1.103955	Large	83%
F.On/CA (F.On. OPD.CA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.047444	*	1.126976	Large	97%
Region		Lateral < Posterior	0.003079	**	1.590553	Large	
sL.On/CA (sL.On. OPD.CA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.039573	*	1.160395	Large	93%
Group | Region	Control	Anterior > Medial	0.049734	*	1.452676	Large	75%
Group | Region	Fentanyl	Anterior > Lateral	0.024167	*	1.688998	Large	
Group | Region	Fentanyl	Lateral < Medial	0.005243	**	2.147655	Large	
Group | Region	Morphine	Lateral < Posterior	0.084105	.	1.268184	Large	
Region | Group	Medial	Control < Fentanyl	0.018449	*	2.164125	Large	
Region | Group	Medial	Control < Morphine	0.077677	.	1.582829	Large	
dL.On/CA (dL.On. OPD.CA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.065233	.	1.066763	Large	98%
Region		Lateral < Medial	0.042066	*	1.149207	Large	
Region		Lateral < Posterior	0.004174	**	1.54184	Large	

Group		Fentanyl < Morphine	0.015314	*	1.265629	Large	94%
Rs.N/CA (Rs.N. OPD.CA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior > Lateral	0.066195	.	1.35383	Large	73%
Group | Region	Fentanyl	Lateral < Posterior	0.010669	*	1.939899	Large	
Region | Group	Posterior	Control < Fentanyl	0.097712	.	1.202248	Large	
a.Rm.Cr/RA (a.Rm.Cr. OPD.RA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior > Lateral	0.005347	**	2.142013	Large	75%
Group | Region	Fentanyl	Lateral < Medial	0.022593	*	1.71025	Large	
Group | Region	Fentanyl	Lateral < Posterior	0.080228	.	1.28529	Large	
Region | Group	Lateral	Control > Fentanyl	0.0411	*	1.850553	Large	
Region | Group	Lateral	Fentanyl < Morphine	0.091333	.	1.510566	Large	
T.On/RA (T.On. OPD.RA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior > Lateral	0.009674	**	1.968994	Large	72%
Group | Region	Fentanyl	Lateral < Medial	0.026647	*	1.657952	Large	
Region | Group	Lateral	Control > Fentanyl	0.043908	*	1.791688	Large	
C.On/RA (C.On. OPD.RA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.091663	.	0.999803	Large	79%
F.On/RA (F.On. OPD.RA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Control	Lateral > Medial	0.069913	.	1.334531	Large	82%
Group | Region	Fentanyl	Anterior > Lateral	0.063613	.	1.367793	Large	
Group | Region	Fentanyl	Lateral < Medial	0.087172	.	1.255123	Large	
sL.On/RA (sL.On. OPD.RA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.068688	.	1.056793	Large	96%
Group | Region	Control	Anterior > Medial	0.027854	*	1.643788	Large	91%
Group | Region	Control	Lateral > Medial	0.028886	*	1.632108	Large	
Group | Region	Fentanyl	Anterior > Lateral	0.004654	**	2.181807	Large	
Group | Region	Fentanyl	Anterior > Posterior	0.081094	.	1.281406	Large	
Group | Region	Fentanyl	Lateral < Medial	0.008023	**	2.024197	Large	
Region | Group	Medial	Control < Fentanyl	0.044377	*	2.1444	Large	
dL.On/RA (dL.On. OPD.RA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group		Fentanyl < Morphine	0.063595	.	1.025701	Large	85%

Rs.N/RA (Rs.N. OPD.RA) (#/mm^2)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior > Lateral	0.02283	*	1.706964	Large	85%
Group | Region	Fentanyl	Lateral < Posterior	0.012904	*	1.882809	Large	
Region | Group	Anterior	Fentanyl > Morphine	0.086303	.	1.264039	Large	
Region | Group	Posterior	Control < Fentanyl	0.075254	.	1.313055	Large	
σ_f dL(days)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior < Lateral	0.066531	.	1.352048	Large	83%
Group | Region	Fentanyl	Lateral > Medial	0.031279	*	1.606428	Large	
Group | Region	Morphine	Anterior < Medial	0.020619	*	1.738927	Large	
Region | Group	Lateral	Control < Fentanyl	0.006225	**	2.368771	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.067428	.	1.529324	Large	
Region | Group	Posterior	Control < Fentanyl	0.083182	.	1.444671	Large	
σ_f I (days)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Lateral > Medial	0.062649	.	1.373138	Large	82%
Group | Region	Fentanyl	Medial < Posterior	0.088694	.	1.248789	Large	
Group | Region	Morphine	Anterior < Medial	0.030011	*	1.619798	Large	
Region | Group	Lateral	Control < Fentanyl	0.038556	*	1.742859	Large	
σ_f C (days)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Group | Region	Fentanyl	Anterior < Lateral	0.020872	*	1.73511	Large	85%
Group | Region	Fentanyl	Lateral > Medial	0.017635	*	1.787465	Large	
Region | Group	Lateral	Control < Fentanyl	0.004672	**	2.618196	Large	
Region | Group	Lateral	Fentanyl > Morphine	0.065522	.	1.632303	Large	
Region | Group	Posterior	Control < Fentanyl	0.07278	.	1.587686	Large	
Ac.F.I.dL (#/mm^2/year)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.023229	*	1.255523	Large	91%
Region		Lateral < Posterior	0.009322	**	1.410754	Large	
Ac.F.I (#/mm^2/year)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.023976	*	1.249985	Large	94%
Region		Lateral < Posterior	0.004248	**	1.539037	Large	
Ac.F.C (#/mm^2/year)	Interaction Contrast	Comparison	p-value	Sig.	Cohen's D	Effect Group	Power
Region		Anterior > Lateral	0.052764	.	1.107114	Large	89%
Region		Lateral < Posterior	0.012697	*	1.359112	Large	

Regional Femur: All Directional Trends

Cortical Area (mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Posterior > Medial > Lateral
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Anterior > Posterior > Lateral > Medial
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Remodeling Area (mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
RA/CA (%) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Percent Porosity (%) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral

Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Pore Density CA (1/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Pore Density RA (1/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Fentanyl > Control
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Medial > Lateral > Anterior > Posterior
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Total Pore Number Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Fentanyl > Control
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Total Pore Area (um^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral

Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Mean Pore Area (um^2) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Fentanyl > Morphine
Mean Pore Perimeter (um) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Anterior > Lateral
Group	NA	Control > Fentanyl > Morphine
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Medial > Lateral > Posterior > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Fentanyl > Morphine
Mean Pore Circularity Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Lateral > Medial > Posterior
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl

Mean Pore Max Feret Diameter (um) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Lateral > Anterior
Group	NA	Control > Fentanyl > Morphine
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Medial > Lateral > Posterior > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Fentanyl > Morphine
Mean Pore Min Feret Diameter (um) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Anterior > Lateral
Group	NA	Control > Fentanyl > Morphine
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Fentanyl > Morphine
Mean Pore Aspect Ratio Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Posterior > Medial > Anterior
Group	NA	Fentanyl > Control > Morphine
Group | Region	Cortical	Lateral > Posterior > Medial > Anterior
Group | Region	Cortical	Lateral > Posterior > Medial > Anterior
Group | Region	Cortical	Lateral > Posterior > Medial > Anterior
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Fentanyl > Control > Morphine
Mean Pore Roundness Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Lateral > Medial > Posterior
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl

Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
Mean Pore Solidity Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Lateral > Medial > Posterior
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Total Osteon Count (T.On) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
Complete Osteon Count (C.On) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Forming Osteon Count (F.On) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral

Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
Single-Labeled Osteon Count (sL.On) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Posterior > Medial > Lateral
Group	NA	Morphine > Fentanyl > Control
Group | Region	Cortical	Anterior > Posterior > Lateral > Medial
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
Double-Labeled Osteon Count (dL.On) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Fentanyl > Control
Triple-Labeled Osteon Count (tL.On) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
dL.On Mean Inner Label (um) Trends		
Factor	Contrast	Trend

Region	NA	Anterior > Posterior > Medial > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Lateral > Anterior > Medial
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
tL.On Mean Inner Label (um) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
tL.On Mean Outer Label (um) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Combined Mean Inner Label (um) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Anterior > Medial > Lateral > Posterior
Group | Region	Cortical	Posterior > Anterior > Lateral > Medial
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Fentanyl > Morphine

Region | Group	Posterior	Control > Morphine > Fentanyl
Combined Mean Label (um) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
C.On Mean Wall Thickness (W.Th.C) (um) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Lateral > Anterior
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Posterior > Lateral > Anterior > Medial
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Fentanyl > Control
sL.On Mean Wall Thickness (W.Th.sL) (um) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Lateral > Medial > Anterior
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Fentanyl > Control
dL.On Mean Wall Thickness (W.Th.dL) (um) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Posterior > Lateral > Anterior > Medial
Group | Region	Cortical	Posterior > Medial > Lateral > Anterior

Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Fentanyl > Morphine > Control
tL.On Mean Wall Thickness (W.Th.tL) (um) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Fentanyl > Control
T.On Mean Area (um^2) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Fentanyl > Morphine
T.On Mean Circularity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Group | Region	Cortical	Anterior > Lateral > Posterior > Medial
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
T.On Mean Aspect Ratio Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Lateral > Anterior
Group	NA	Control > Morphine > Fentanyl

Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Lateral > Posterior > Medial > Anterior
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Fentanyl > Control > Morphine
T.On Mean Roundness Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Lateral > Posterior > Medial
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Anterior > Lateral > Posterior > Medial
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
T.On Mean Solidity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Fentanyl > Control > Morphine
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Control > Fentanyl > Morphine
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Morphine > Control > Fentanyl
C.On Mean Area (um^2) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
C.On Mean Circularity Trends		

Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
C.On Mean Aspect Ratio Trends		
Factor	Contrast	Trend
Region	NA	Medial > Anterior > Posterior > Lateral
Group	NA	Control > Fentanyl > Morphine
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Control > Morphine > Fentanyl
C.On Mean Roundness Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Posterior > Anterior > Medial
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Fentanyl > Morphine > Control
C.On Mean Solidity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Lateral > Medial > Anterior > Posterior
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control

Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
F.On Mean Area (um^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Control > Fentanyl > Morphine
F.On Mean Circularity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Fentanyl > Control > Morphine
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Group | Region	Cortical	Anterior > Lateral > Posterior > Medial
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Control > Morphine > Fentanyl
F.On Mean Aspect Ratio Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Lateral > Anterior
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Medial > Lateral > Posterior > Anterior
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Lateral > Posterior > Medial > Anterior
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Fentanyl > Morphine > Control
F.On Mean Roundness Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Lateral > Posterior > Medial
Group	NA	Fentanyl > Control > Morphine
Group | Region	Cortical	Anterior > Lateral > Posterior > Medial
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior

Group | Region	Cortical	Anterior > Lateral > Posterior > Medial
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Control > Morphine > Fentanyl
F.On Mean Solidity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Control > Fentanyl > Morphine
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Group | Region	Cortical	Anterior > Lateral > Posterior > Medial
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Control > Fentanyl > Morphine
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Control > Morphine > Fentanyl
sL.On Mean Area (um^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Fentanyl > Morphine
sL.On Mean Circularity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
sL.On Mean Aspect Ratio Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Posterior > Medial > Lateral

Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Anterior > Medial > Lateral > Posterior
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Fentanyl > Morphine > Control
sL.On Mean Roundness Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Lateral > Medial > Anterior
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Medial > Lateral > Anterior
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Fentanyl > Morphine
sL.On Mean Solidity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
dL.On Mean Area (um^2) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Anterior > Lateral
Group	NA	Control > Fentanyl > Morphine
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Control > Fentanyl > Morphine

dL.On Mean Circularity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Anterior > Posterior > Medial
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Lateral > Medial > Anterior > Posterior
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Group | Region	Cortical	Lateral > Medial > Posterior > Anterior
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
dL.On Mean Aspect Ratio Trends		
Factor	Contrast	Trend
Region	NA	Medial > Anterior > Posterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Medial > Anterior > Lateral > Posterior
Group | Region	Cortical	Anterior > Lateral > Posterior > Medial
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Fentanyl > Control > Morphine
Region | Group	Posterior	Control > Morphine > Fentanyl
dL.On Mean Roundness Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Lateral > Anterior > Medial
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Group | Region	Cortical	Posterior > Anterior > Lateral > Medial
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Control > Morphine
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Fentanyl > Morphine > Control
dL.On Mean Solidity Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Posterior > Anterior > Medial
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Lateral > Medial > Posterior > Anterior
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Lateral > Medial > Posterior > Anterior
Region | Group	Anterior	Fentanyl > Morphine > Control

Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Fentanyl > Morphine > Control
tL.On Mean Area (um^2) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Posterior > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
tL.On Mean Circularity Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Lateral > Medial
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
tL.On Mean Aspect Ratio Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
tL.On Mean Roundness Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Lateral > Medial

Group | Region	Cortical	Posterior > Lateral > Anterior > Medial
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
tL.On Mean Solidity Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Lateral > Anterior
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Posterior > Anterior > Lateral > Medial
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Fentanyl > Control
Unlabeled Resorption Space Count (Rs.N) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Lateral > Anterior
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Active Remodeling Centers (a.Rm.Cr) (T.On + Rs.N) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
a.Rm.Cr/CA (a.Rm.Cr.OPD.CA) (#/mm^2) Trends		
Factor	Contrast	Trend

Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
T.On/CA (T.On.OPD.CA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
C.On/CA (C.On.OPD.CA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
F.On/CA (F.On.OPD.CA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control

Region | Group	Posterior	Morphine > Control > Fentanyl
sL.On/CA (sL.On.OPD.CA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Posterior > Medial > Lateral
Group	NA	Morphine > Fentanyl > Control
Group | Region	Cortical	Anterior > Posterior > Lateral > Medial
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
dL.On/CA (dL.On.OPD.CA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Fentanyl > Control
tL.ON/CA (tL.On.OPD.CA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Rs.N/CA (Rs.N.OPD.CA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Morphine > Fentanyl > Control
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Medial > Lateral > Anterior

Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
a.Rm.Cr/RA (a.Rm.Cr.OPD.RA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
T.On/RA (T.On.OPD.RA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Lateral > Anterior > Medial > Posterior
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
C.On/RA (C.On.OPD.RA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Lateral > Medial > Posterior
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Fentanyl > Control
F.On/RA (F.On.OPD.RA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Fentanyl > Control

Group | Region	Cortical	Lateral > Anterior > Posterior > Medial
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Fentanyl > Control
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Control > Fentanyl
sL.On/RA (sL.On.OPD.RA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Medial > Posterior > Lateral
Group	NA	Morphine > Fentanyl > Control
Group | Region	Cortical	Anterior > Lateral > Posterior > Medial
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Fentanyl > Morphine > Control
Region | Group	Posterior	Morphine > Fentanyl > Control
dL.On/RA (dL.On.OPD.RA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Medial > Anterior > Posterior > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Morphine > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Fentanyl > Control
tL.ON/RA (tL.On.OPD.RA) (#/mm^2) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Rs.N/RA (Rs.N.OPD.RA) (#/mm^2) Trends		

Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Medial > Lateral > Anterior > Posterior
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Fentanyl > Morphine > Control
T.On / Rs.N Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Posterior > Medial > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Lateral > Medial
Group | Region	Cortical	Medial > Anterior > Posterior > Lateral
Group | Region	Cortical	Anterior > Posterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Control > Morphine > Fentanyl
On.MAR.I.dL (dL Inner Labels) (um/day) Trends		
Factor	Contrast	Trend
Region	NA	Anterior > Posterior > Medial > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Medial > Posterior > Anterior > Lateral
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Lateral > Anterior > Medial
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
On.MAR.I (Inner Labels) (um/day) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Anterior > Medial > Lateral > Posterior
Group | Region	Cortical	Posterior > Anterior > Lateral > Medial
Region | Group	Anterior	Fentanyl > Morphine > Control
Region | Group	Lateral	Morphine > Control > Fentanyl

Region | Group	Medial	Control > Fentanyl > Morphine
Region | Group	Posterior	Control > Morphine > Fentanyl
On.MAR.C (Combined Labels) (um/day) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Medial > Anterior > Lateral
Group	NA	Control > Morphine > Fentanyl
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Medial > Anterior > Lateral
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Control > Morphine > Fentanyl
Region | Group	Medial	Control > Morphine > Fentanyl
Region | Group	Posterior	Control > Morphine > Fentanyl
Osteon Formation Time (W.Th.C / On.MAR.I.dL) (days) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Lateral > Medial > Anterior
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Group | Region	Cortical	Medial > Posterior > Lateral > Anterior
Region | Group	Anterior	Fentanyl > Control > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Osteon Formation Time (W.Th.C / On.MAR.I) (days) Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Posterior > Medial > Anterior
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial
Group | Region	Cortical	Medial > Lateral > Posterior > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Fentanyl > Morphine > Control
Osteon Formation Time (W.Th.C / On.MAR.C) (days) Trends		
Factor	Contrast	Trend
Region	NA	Lateral > Posterior > Medial > Anterior
Group	NA	Fentanyl > Morphine > Control
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Lateral > Posterior > Anterior > Medial

Group | Region	Cortical	Medial > Lateral > Posterior > Anterior
Region | Group	Anterior	Control > Fentanyl > Morphine
Region | Group	Lateral	Fentanyl > Morphine > Control
Region | Group	Medial	Morphine > Fentanyl > Control
Region | Group	Posterior	Fentanyl > Morphine > Control
Ac.F.I.dL ((C.On.OPD.CA/OFT.I.dL)*365) (#/mm^2/year) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Ac.F.I ((C.On.OPD.CA/OFT.I)*365) (#/mm^2/year) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Anterior > Medial > Posterior > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl
Ac.F.C ((C.On.OPD.CA/OFT.C)*365) (#/mm^2/year) Trends		
Factor	Contrast	Trend
Region	NA	Posterior > Anterior > Medial > Lateral
Group	NA	Morphine > Control > Fentanyl
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Group | Region	Cortical	Posterior > Anterior > Medial > Lateral
Region | Group	Anterior	Morphine > Control > Fentanyl
Region | Group	Lateral	Morphine > Control > Fentanyl
Region | Group	Medial	Morphine > Control > Fentanyl
Region | Group	Posterior	Morphine > Control > Fentanyl

The University of Akron Research Vivarium (UARV)

New User Checklist

Individuals wishing to obtain access to the UARV must complete all items on this checklist and provide the necessary documentation to the UARV Manager before access is granted. **Documentation must be submitted to the UARV Manager as a printed copy – not electronic.** You may either deliver your documentation in person, or place it in the UARV mailbox in the Biology Department.

IMPORTANT NOTICE: All information provided by the applicant to the UARV will be maintained in confidentiality by The University of Akron. If you have questions or concerns, please contact Beth Kenaga, UARV Manager at bkenaga@uakron.edu or 330-972-5845.

* * * * * * * * * * * * * *

Name: ____Janna M. Andronowski_____

Date	
N/A	IACUC Personnel Form complete with PI signature
12/19/18	Completion Reports for required CITI Program courses
12/19/18	UARV Zip Card Entry Request form with Advisor signature
12/19/18	UARV Health Assessment form
12/19/18	UARV Allergen and Zoonoses Statement form
12/19/18	UARV Policies for Vivarium Users form
12/19/18	Personal Protective Equipment (PPE) Information
12/19/18	UARV Policy on Laboratory Safety Standard
N/A	Student Employee Confidentiality Agreement
N/A	Student Employee Manual (**UARV Staff Only** - Handout & Posting location)
_____	Complete a vivarium tour with UARV Manager • Current Manager is: Beth Kenaga • Please email to set up an appointment: bkenaga@uakron.edu
_____	Documentation complete & reviewed Initial: _____
_____	UARV Access requested
_____	Added to Access List
_____	Added to UARV list-serve

THE UNIVERSITY OF AKRON: REQUIRED CURRICULUM

In order to successfully pass a course, you must **complete all elective modules** and **achieve an average score of 80% on all quizzes.** You may retake quizzes as many times as necessary to achieve a passing score; there is no limit. Individuals will have to complete courses relating to the IACUC and species-specific research. Individuals intending to perform survival surgery during their research will be required to complete a course on aseptic surgery techniques.

* * * * * * * * * * * * * *

☒ "Working with the IACUC (Investigators, Staff, and Students)"
ALL STUDENTS, FACULTY, AND STAFF are required to take this course.

☐ "Aseptic Surgery"
ALL STUDENTS, FACULTY, AND STAFF intending to perform survival surgery during their research are required to take this course, regardless of species studied.

SPECIES-SPECIFIC COURSES

All students, faculty, and staff are required to take courses relating to the species studied in their research. Select from the following options; if your research involves a species of animal not currently listed, please contact the UARV Supervisor at 330-972-5845 for further instructions.

RATS

☐ "Reducing Pain and Distress in Laboratory Mice and Rats"
☐ "Working with Rats in Research Settings"

MICE

☐ "Reducing Pain and Distress in Laboratory Mice and Rats"
☐ "Working with Mice in Research Settings"

REPTILES

☐ "Working with Reptiles in Research Settings"

AMPHIBIANS

☐ "Working with Amphibians in Research Settings"

FISH

☐ "Working with Fish in Research Settings"
☐ **Zebrafish Users Only**: Instead of the above course, you need to complete the "Working with Zebrafish (Danio rerio) in Research Settings" course.

DOGS

☐ "Working with Dogs in Research Settings"

Once you have selected the appropriate curriculum for your circumstances, your registration or affiliation with The University of Akron is complete. Click "Finalize registration" or "Submit" to finish the process and begin your coursework. When you first enter a course, the only item available will be "The Integrity Assurance Statement". Completing this will unlock modules and quizzes within the course. Print your completion report, and return it to the UARV Supervisor with the rest of your UARV New User Forms.

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CITI PROGRAM

Completion Date 19-Dec-2018
Expiration Date 18-Dec-2021
Record ID 29784144

This is to certify that:

Janna Andronowski

Has completed the following CITI Program course:

Working with the IACUC (Curriculum Group)
Investigators, Staff and Students (Course Learner Group)
1 - Basic Course (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?wc8137923-aeff-40d0-a086-8af096f6d966-29784144

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CITI PROGRAM

Completion Date 19-Dec-2018
Expiration Date 18-Dec-2021
Record ID 29784145

This is to certify that:

Janna Andronowski

Has completed the following CITI Program course:

Working with Rabbits in Research Settings (Curriculum Group)
Working with Rabbits in Research Settings (Course Learner Group)
1 - Basic Course (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?w42680ebd-5931-4d6a-9049-0c42592f02c8-29784145

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The University of Akron Research Vivarium (UARV)

Zip Card Entry Request

Individuals wishing to obtain access to the UARV must complete this form in full and submit it to the UARV Supervisor. Zip Card activation usually occurs within 24-72 hours after the submission of all requisite forms. Fill in fields neatly and legibly using blue or black ink. Do not use pencil. If you have questions, please contact the UARV Supervisor at 330-972-S845.

* * * * * * * * * * * * * *

Full Name: Janna M. Andronowski

UA ID Number (on back of Zip Card): 3172338

** if you are a visiting researcher, you will be issued a Guest Pass – please leave the above field blank*

Phone Number (circle one: home / cell / campus): 330-217-9634

University/Professional E-mail Address: jandronowski@uakron.edu

Status (circle one): undergraduate student graduate student staff faculty visiting researcher

Department: Biology When do you require access to the UARV? (Circle one. *Students, check with your advisor.*)

Weekdays 7AM-5PM 24/7

If you are an undergraduate student requiring 24/7 access, please provide justification below:

List the animal species you will be working with: New Zealand White Rabbits

Estimate the average number of hours of animal contact per week: 8-10

Applicant Signature: [signature] Date: Dec. 19/18

Supervisor/Collaborator Printed Name: _____

Supervisor/Collaborator Signature: _____ Date:

UARV OFFICE USE ONLY

Code(s): _____ Date Granted: _____ Initials: _____ Date Voided: _____ Initials: _____

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The University of Akron Research Vivarium (UARV)

Janna Andrzejewski

Health Assessment

This document is intended to help a professional healthcare provider determine whether or not an individual applying for Zip Card access ("Applicant") to the UARV should perform research with animals in the UARV. **Only the last page of this form, Section D: Healthcare Provider Statement, is turned into the UARV Supervisor.** The Applicant should fill out this assessment and consult with their supervisor/advisor if necessary so that accurate information is communicated to the professional healthcare provider. Health care records from other institutions or organizations are not transferrable to the UARV.

A health assessment is available for any Applicant – student, faculty, or staff – by a professional healthcare provider at Student Health Services (SHS), or by the Applicant's personal physician. Appointments can be scheduled at SHS by calling 330-972-7808 and requesting a "Biology Health Screening". Health screenings are generally covered under a personal health insurance plan. Follow-up health assessments are not required by the UARV, but the Applicant is strongly recommended to abide by follow-up instructions given to them by the professional healthcare provider.

* * * * * * * * * * * * * *

SECTION A: JOB ACTIVITY AND LENGTH OF RESEARCH

Applicant Job Title Assistant Professor Estimated Start

Date 01/01/2019 Estimated End Date 31/12/2020

SECTION B: EXPOSURES AND CONTACT

1. Which species of animal you will be researching in the UARV? New Zealand White Rabbits
2. Is the nature of animal research direct contact, observational, or both? Both direct contact and observational.
3. Estimate the average number of hours of animal contact per week: 8-10
4. Will your job involve the use of...? Check "Yes" or "No".

NOTE: Your healthcare provider may ask you for specification if you answer "yes" to any of these. Consult with your supervisor/advisor if you are not sure how to answer.

	Yes	No
Anesthetic gases		No
Animal cells, blood, or tissues		No
Anti-neoplastic compounds		No
Carcinogens		No
Formaldehyde, paraformaldehyde, glutaraldehyde, or other preservatives	Yes	
Latex gloves	Yes	
Infectious agents (bacteria, viruses, fungi, etc.)		No
Radiation or radionuclides	Yes	
Respirator/mask	Yes	
Toxins or venoms		No
Other toxic or hazardous chemicals.	Yes	

5. Have you had prior animal contact in a laboratory or research setting? YES NO
   If you circled yes, with what species:

6. Are you currently in contact with animals as part of a job or volunteering? YES NO
   If you circled yes, with what species:

7. List any pets in your home. Two domestic cats.

8. Have you experienced any symptoms that you or a professional healthcare provider suspect may be related to working with animals? If so, please explain: N/A

SECTION C: MEDICAL AND IMMUNIZATION HISTORY

1. Do you have...? Check "Yes" or "No".

	Yes	No
Asthma		X
Eczema		X
Hand dermatitis		X
Latex allergies		X
Immunodeficiencies		X
Heart conditions		X
Diabetes		X
Hypoglycemia		X
Respiratory problems		X

- **NOTE: Your healthcare provider may ask you for clarification if you answer "yes" to any of these.**

2. List any known allergies and reactions: N/A

3. Immunizations -- have you had...?

	Yes	No	Date
Hepatitis A vaccine			
Hepatitis B vaccine			
Measles/Mumps/Rubella booster			
Rabies vaccine			
TB test			
Tetanus booster*			
Varicella vaccine			

* **For admittance to the UARV, tetanus booster must be current within the last 10 years.**

Vaccine recommendations from Applicant's supervisor/advisor:

Additional comments:

SECTION D: HEALTHCARE PROVIDER STATEMENT

Full Name of UARV Applicant (print): Janna M. Andronowski

Signature of UARV Applicant: [signature]

Full Name of Supervisor/Advisor of UARV Applicant (print): Beth Kenaga .

* * * * * * * * * * * * * *

I have reviewed the Health Assessment form provided by the Applicant and have determined the Applicant is permitted to work with the animal species listed on the Health Assessment form in the UARV. I confirm that the Applicant has a tetanus immunization that is current within the last ten (10) years, as required by the UARV.

Full Name of Healthcare Provider (print): ALMA OLSON, ARNP, CNP

Signature of Healthcare Provider: Alma Olson, CNP Date: 12/19/18

Healthcare Provider Facility (print): STUDENT HEALTH SERVICES
UNIVERSITY OF AKRON
382 CARROLL ST. STE.260
AKRON, OHIO 44325-1101
(330) 972-7808

awaiting vaccine record.

* * * * * * * * * * * * * *

UARV OFFICE USE ONLY

Reviewed By (print) _____ on (date) _____

Signature _____

The University of Akron Research Vivarium (UARV)

Allergen and Zoonoses Statement

SECTION A: VOLUNTARY DISCLOSURE OF ALLERGIES

Information voluntarily provided by the individual applying for Zip Card access to the UARV ("Applicant") to the UARV in this section is provided to the University of Akron Police Department (UAPD) in response to a medical emergency involving the Applicant.

The Applicant:

☐ declines to disclose allergy information to the UARV.

☐ elects to disclose allergy information to the UARV.

If Applicant elects to disclose allergy information to the UARV, please list allergies and related symptoms: Pertussis allergy; febrile reaction, loss of consciousness, hospitalization

SECTION B: ALLERGEN AND ZOONOTIC DISEASE STATEMENTS

Allergen Statement

Allergies (also known as allergic reactions) are an overreaction of the body's natural immune system in response to a substance that is usually harmless (such as dust, pollen, dander, or a medicine). These substances are known as *allergens*. Symptoms of allergies vary from person to person and can range from mild to severe. Mild to moderate symptoms commonly include a rash or hives, itching and swelling of the skin, itching and watery eyes, a runny nose, and trouble breathing. Severe, whole-body allergic reactions (anaphylaxis) can include swelling of the face/nose/lips/throat to the point of asphyxiation, heart palpitations, low blood pressure, dizziness, and unconsciousness. **An anaphylactic reaction can be life-threatening and is always a medical emergency; call UAPD at 330-972-2911 immediately for medical attention.** Allergies can develop after one exposure to an allergen, or can develop after repeated exposure to an allergen over a period of time. Individuals who work in a lab setting with animals often develop allergies in response to the following allergens: latex, animal urine, animal fur or dander. If you develop an allergy, please contact your medical provider for advice on continuing work with animals and alert the UARV Supervisor.

Zoonotic Diseases Statement

Zoonotic diseases (also known as *zoonoses*) are caused by bacteria, viruses, parasites, or fungi and are spread between animals and humans. Individuals more prone to contracting zoonoses include people with a weakened immune system and pregnant women. The transmission of zoonoses from laboratory animals is uncommon due to animal vendors providing specific pathogen-free animals, and maintaining vivarium facility cleanliness. Researchers participating in field studies in which they are in contact with wild animals can be exposed to zoonoses not commonly seen in laboratory animals. Individuals can help prevent the transmission of zoonoses by participating in and encouraging routine sanitation of lab spaces, wearing personal protective equipment (PPE) while handling animals, and washing their hands before and after handling animals. Please contact the UARV Supervisor if you have questions regarding a specific zoonoses; information will be provided to you.

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SECTION C: DISCLOSURE OF ADDITIONAL ANIMAL HANDLING

The UARV requires a disclosure of animals with which the Applicant interacts with regularly outside of the facility. This information allows the UARV Supervisor to provide recommendations to the Applicant and their Supervisor regarding specific personal protective equipment (PPE) and other required measures necessary to reduce the potential of pathogen transmission between research and personal animals.

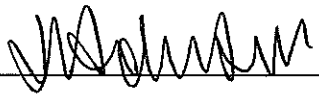
Species you are studying in the UARV (all): _____New Zealand White Rabbit_____

Species you have regular contact with outside the UARV (including volunteer work, pets, etc.): Domestic cats

SECTION D: ACKNOWLEDGMENT AND SIGNATURE

The Applicant understands that information provided in Section A is voluntary and disclosure is not required to obtain UARV access. The Applicant has read, disclosed, and understands required information in Sections B and C. The Applicant acknowledges that if their allergen status changes at any time after submitting this form, they may contact the UARV Supervisor to amend Section A. This form is kept on file with your Zip Card Access record in the UARV and is maintained in confidentiality. You may contact the UARV Supervisor at 330-972-5845 at any time if you have questions or amendments.

Full Name of UARV Applicant (print): _____Janna M. Andronowski_____

Signature of UARV Applicant: 

Date: Dec. 19/18

UA ID Number (on back of Zip Card): _____3172338_____

****

UARV OFFICE USE ONLY

Reviewed By (print) _____ on (date) _____

Signature _____

The University of Akron Research Vivarium (UARV)

Policies for Vivarium Users

All policies established for the UARV are for your safety, and to protect the welfare of animals housed in the facility. **Your privilege to access the vivarium will be terminated if you violate these policies.** Additionally, if you are a student, your advisor will be notified. If you have questions or concerns, please contact the UARV Supervisor at 330-972-5845.

* * * * * * * * * * * * * *

1. Zip Card access. Do **not** lend out your Zip Card to anyone for any reason. If your Zip Card is stolen or misplaced, notify the UARV Supervisor immediately, as Zip Cards are easily deactivated and replaced. Each time you use your Zip Card to enter the facility, your name, current date, and time of entry will be recorded.

2. Authorized users only. Visitors are **not permitted** to enter the UARV without prior permission from the UARV Supervisor. Upon entry to the UARV, authorized visitors may be required to provide identification containing their full legal name and date of birth (e.g. state-issued driver license/ID, military ID), and will sign a guest entry form.

3. Photo and videos. Photos and videos are **not permitted** to be taken within the vivarium without prior permission from the UARV Supervisor. If permission is obtained, use of photos and videos are subject to review by the UARV Supervisor. No identifying information about the facility, personnel, or location is permitted in photos and videos.

4. Food, drink, cosmetics, etc. Eating, drinking, smoking (or any variety of tobacco use), handling contact lenses, applying cosmetics, and storing food for human consumption is **not permitted** on the 2nd and 3rd floors of the UARV. This is in accordance with Occupational Safety and Health Administration (OSHA) and Centers for Disease Control and Prevention (CDC) safety guidelines and regulations. Room B103 on the 1st floor is the UARV break room. The room contains a fridge and freezer for food and beverage intended for human consumption, and these activities are permitted within this room only. The break room is intended for temporary use and storage, and is not a personal locker. Be courteous of other users and clean up after yourself.

5. Animal transportation and external animals. When transporting animals back and forth between the UARV and your experimental labs, always cover the animal cage(s) with a protective layer (e.g. spare labcoat, PPE gown, towel). This protects the animal from external stressors; it also protects students, faculty, and staff members in the building who may not feel comfortable with a certain species of animal or may not agree with animal experimentation in research. Animals are **not permitted** to leave the campus unless previously approved by the UARV Supervisor. No unapproved, external animals (pets or other) are to enter the UARV at any time for any reason.

6. Doors and locks. Doors to animal rooms are to be kept closed at all times. Doors will only be locked with permission from the UARV Supervisor. **Make sure the door closes behind you when you exit an animal room, as the doors don't always close completely on their own!** You are only permitted to enter the rooms in which your animals are housed or you are experimenting, as well as common areas (bathrooms, break room, food prep, etc.) – **do not** enter rooms in which you have no direct business.

7. Injuries and animal bites. If you are injured in the UARV or bitten by an animal – regardless of how it happened – contact the UARV Supervisor immediately for first aid. If your injury is beyond first aid, call the UAPD immediately (330-972-2911) and instruct them to contact the UARV Supervisor while you receive treatment. File an Injury Report form in case you need to receive treatment for the injury/bite; the form is located in a file folder on the desk outside of the UARV Supervisor's office (B101).

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8. "Five Senses Rules"
    a. Light. All animal rooms have timer-controlled lights. Automatic timer settings are **not** to be changed without UARV Supervisor permission. Most rooms have an additional light switch that can be flipped on to provide more additional light to researchers while they are in the room, but must be switched off after leaving (the switch emits an orange glow when the switch is off).
    b. Sound. Excessive noise (music, yelling, etc.) is **not permitted** in the vivarium, as it is disruptive to animals and can cause undue stress.
    c. Smell. Do **not** wear strong perfume, aftershave, lotion, deodorant, or other strong scents (like cleaning products and chemicals) into the animal rooms.
    d. Taste. **No** food, drink, chewing gum, or other such products are allowed on the 2nd and 3rd floors of the UARV (see Policy Item #4). **No** chewing tobacco (or any tobacco products) are permitted in the UARV.
    e. Touch. You are only allowed to handle your animals and equipment, and common use items (clean cages, water bottles, racks). Do **not** touch animals or equipment belonging to other researchers, or the UARV (e.g. cagewasher).

9. Emergencies, maintenance, and security.
    a. **Minor non-emergency**. Examples include small water leaks or dripping faucets, lights burnt out in hallways, etc. Contact the UARV Supervisor so that a work request can be placed.
    b. **Major emergency**. Examples include escaped animals, A/C or heating breakdowns, necropsy freezer failure, anything that negatively affects animal health and safety (such as burnt out lights), etc. Contact the UARV Supervisor immediately for assistance.
    c. **Security emergency**. Examples include animal activist groups on campus, a person exhibiting threatening behavior, etc. Contact the UAPD immediately (330-972-2911).
    d. **Chemical/biohazard emergency**. Examples include biohazard waste spills or leaks, chemical injury or spills, etc. Contact UAPD immediately (330-972-2911) for medical attention, and instruct them to notify Environmental and Occupational Health and Safety (EOHS) to handle the issue while you receive treatment. The EOHS emergency line is 330-972-7123.

10. Trash and cleaning. The University of Akron custodial staff does not enter animal rooms, personal labs and offices, or the surgical suite. When you are done using a room, place the trash can into the hallway so it will be emptied overnight. **Always dispose of sharps in a biohazard container, never in the trash!** There are brooms and dustpans in each animal room; mops, buckets, and cleaning supplies can be found on each floor. If you can't find something, ask UARV staff for assistance. Dirty clothing such as scrubs or labcoats that belong to the UARV can be placed in the hamper in the laundry room (B115).

If you ever have questions or need assistance, please talk with the UARV staff or supervisor for help.
We are here to ensure you have the best experience with animal care and safety!

* * * * * * * * * * * * * *

I Joanna Andronowski (print name) have thoroughly read and understand the UARV Policies for Vivarium Users outlined in this form. I understand that I will be held accountable for my actions with regard to these rules, and that I must follow them at all times. I acknowledge that failure to comply with these policies will result in termination of my access to the UARV facility, and additional consequences may be incurred if my actions are determined to be severe. I confirm that I have been given a UARV tour by the UARV Supervisor.

User Signature: _____ Date: Dec. 19/18

UARV Manager Signature: _____ Date: _____

CITI PROGRAM

Completion Date 24-Aug-2016
Expiration Date 24-Aug-2019
Record ID 20586330

This is to certify that:

Reed Davis

Has completed the following CITI Program course:

Working with the IACUC (Curriculum Group)
Investigators, Staff and Students (Course Learner Group)
1 - Basic Course (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?wa48e88f6-7776-481a-90b2-e6b68e9f956b-20586330

501

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CITI PROGRAM

Completion Date 31-Oct-2018
Expiration Date 30-Oct-2021
Record ID 29296716

This is to certify that:

Reed Davis

Has completed the following CITI Program course:

Working with Rabbits in Research Settings (Curriculum Group)
Working with Rabbits in Research Settings (Course Learner Group)
1 - Basic Course (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?w7962ab76-7ca3-4849-92e0-9107d2d8e92f-29296716 502

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CITI PROGRAM

Completion Date 10-Jan-2019
Expiration Date 09-Jan-2022
Record ID 30001783

This is to certify that:

Adam Schuller

Has completed the following CITI Program course:

Working with the IACUC (Curriculum Group)
Investigators, Staff and Students (Course Learner Group)
1 - Basic Course (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?wd8da8494-0097-44a6-80cc-94d542c95547-30001783

503

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CITI PROGRAM

Completion Date 10-Jan-2019
Expiration Date 09-Jan-2022
Record ID 30001785

This is to certify that:

Adam Schuller

Has completed the following CITI Program course:

Working With Animals In Biomedical Research - Refresher Course (Curriculum Group)
Working With Animals In Biomedical Research - Refresher Course (Course Learner Group)
1 - Lab Animal Research (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?wbf0ac1ac-1995-40cf-a537-71afc0f684c7-30001785

504

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CITI PROGRAM

Completion Date 10-Jan-2019
Expiration Date 09-Jan-2022
Record ID 30001784

This is to certify that:

Adam Schuller

Has completed the following CITI Program course:

Working with Rabbits in Research Settings (Curriculum Group)
Working with Rabbits in Research Settings (Course Learner Group)
1 - Basic Course (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?wb2dd626d-6abc-47b4-9387-ac48936b9dd3-30001784

505

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The University of Akron Research Vivarium (UARV)

New User Checklist

Individuals wishing to obtain access to the UARV must complete all items on this checklist and provide the necessary documentation to the UARV Manager before access is granted. **Documentation must be submitted to the UARV Manager as a printed copy – not electronic.** You may either deliver your documentation in person, or place it in the UARV mailbox in the Biology Department.

IMPORTANT NOTICE: All information provided by the applicant to the UARV will be maintained in confidentiality by The University of Akron. If you have questions or concerns, please contact Beth Kenaga, UARV Manager at bkenaga@uakron.edu or 330-972-5845.

* * * * * * * * * * * * * *

Name: Gina Tubo

Date

_____ IACUC Personnel Form complete with PI signature

_____ Completion Reports for required CITI Program courses

_____ UARV Zip Card Entry Request form with Advisor signature

_____ UARV Health Assessment form

_____ UARV Allergen and Zoonoses Statement form

_____ UARV Policies for Vivarium Users form

_____ Personal Protective Equipment (PPE) Information

_____ UARV Policy on Laboratory Safety Standard

_____ Student Employee Confidentiality Agreement

_____ Student Employee Manual (**UARV Staff Only** - Handout & Posting location)

_____ Complete a vivarium tour with UARV Manager
- Current Manager is: Beth Kenaga
- Please email to set up an appointment: bkenaga@uakron.edu

_____ Documentation complete & reviewed Initial: _____

_____ UARV Access requested

_____ Added to Access List

_____ Added to UARV list-serve

ADDITION OF PERSONNEL - REQUEST TO USE ANIMALS

Please add the participant named below to my approved protocol # entitled, .

PRINCIPAL OR CO- INVESTIGATOR :

PRINCIPAL OR CO-INVESTIGATOR SIGNATURE — DATE: 01/09/19

PARTICIPANT NAME:

PARTICIPANT TITLE:

PARTICIPANT SIGNATURE — DATE: 1/9/19

RESPONSIBILITIES ON THE PROTOCOL :

Day to day dosage and monitoring of rabbits
Dissection of rabbits to procure femora & tibiae for uCT & confocal imaging

EXPERIENCE/QUALIFICATIONS:

experience with aseptic survival surgery of rats in foundations of physiology I lab

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
Working with the IACUC	online	1-3-19
Working with Rabbits in Research Settings	online	1-3-19

IACUC APPROVAL (*approval signature requirements vary according to institution*):

IACUC Member
Date:

Attending Veterinarian
Date:

IACUC Chairperson
Date:

The University of Akron Research Vivarium (UARV)

Zip Card Entry Request

Individuals wishing to obtain access to the UARV must complete this form in full and submit it to the UARV Supervisor. Zip Card activation usually occurs within 24-72 hours after the submission of all requisite forms. Fill in fields neatly and legibly using blue or black ink. Do not use pencil. If you have questions, please contact the UARV Supervisor at 330-972-5845.

* * * * * * * * * * * * * *

Full Name: Gina Tubo

UA ID Number (on back of Zip Card): 4045107

** if you are a visiting researcher, you will be issued a Guest Pass – please leave the above field blank*

Phone Number (circle one: home / (cell) / campus): 330-705-7798

University/Professional E-mail Address: grt13@zips.uakron.edu

Status (circle one): (undergraduate student) graduate student staff faculty visiting researcher

Department: Biology

When do you require access to the UARV? (Circle one. *Students, check with your advisor.*)

Weekdays 7AM-5PM (24/7)

If you are an undergraduate student requiring 24/7 access, please provide justification below:
Our rabbits require routine monitoring at any hour

List the animal species you will be working with: New Zealand White Rabbit

Estimate the average number of hours of animal contact per week: 5

Applicant Signature: Gina Tubo Date: 1/7/19

Supervisor/Collaborator Printed Name: Jana M. Andronowski

Supervisor/Collaborator Signature: Jana M Date: 1/9/19

UARV OFFICE USE ONLY

Code(s): _____ Date Granted: _____ Initials: _____ Date Voided: _____ Initials: _____

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SECTION D: HEALTHCARE PROVIDER STATEMENT

Full Name of UARV Applicant (print): Gina Tubo

Signature of UARV Applicant: Gina Tubo

Full Name of Supervisor/Advisor of UARV Applicant (print): Janna M. Andronowski

* * * * * * * * * * * * * *

I have reviewed the Health Assessment form provided by the Applicant and have determined the Applicant is permitted to work with the animal species listed on the Health Assessment form in the UARV. I confirm that the Applicant has a tetanus immunization that is current within the last ten (10) years, as required by the UARV.

Full Name of Healthcare Provider (print): Jason Springer DO

Signature of Healthcare Provider: [signature] Date: 1/14/19

Healthcare Provider Facility (print): Tri-County Medical Services

TRI-COUNTY EMERGENCY
MEDICAL SERVICES, INC.
855 W. MAPLE, STE. 120
HARTVILLE, OH 44632
(330) 877-6613

* * * * * * * * * * * * * *

UARV OFFICE USE ONLY

Reviewed By (print) _____ on (date) _____

Signature _____

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The University of Akron Research Vivarium (UARV)

Allergen and Zoonoses Statement

SECTION A: VOLUNTARY DISCLOSURE OF ALLERGIES

Information voluntarily provided by the individual applying for Zip Card access to the UARV ("Applicant") to the UARV in this section is provided to the University of Akron Police Department (UAPD) in response to a medical emergency involving the Applicant.

The Applicant:

☐ declines to disclose allergy information to the UARV.

☑ elects to disclose allergy information to the UARV.

If Applicant elects to disclose allergy information to the UARV, please list allergies and related symptoms:

none known

SECTION B: ALLERGEN AND ZOONOTIC DISEASE STATEMENTS

Allergen Statement

Allergies (also known as allergic reactions) are an overreaction of the body's natural immune system in response to a substance that is usually harmless (such as dust, pollen, dander, or a medicine). These substances are known as *allergens*. Symptoms of allergies vary from person to person and can range from mild to severe. Mild to moderate symptoms commonly include a rash or hives, itching and swelling of the skin, itching and watery eyes, a runny nose, and trouble breathing. Severe, whole-body allergic reactions (anaphylaxis) can include swelling of the face/nose/lips/throat to the point of asphyxiation, heart palpitations, low blood pressure, dizziness, and unconsciousness. **An anaphylactic reaction can be life-threatening and is always a medical emergency; call UAPD at 330-972-2911 immediately for medical attention.** Allergies can develop after one exposure to an allergen, or can develop after repeated exposure to an allergen over a period of time. Individuals who work in a lab setting with animals often develop allergies in response to the following allergens: latex, animal urine, animal fur or dander. If you develop an allergy, please contact your medical provider for advice on continuing work with animals and alert the UARV Supervisor.

Zoonotic Diseases Statement

Zoonotic diseases (also known as *zoonoses*) are caused by bacteria, viruses, parasites, or fungi and are spread between animals and humans. Individuals more prone to contracting zoonoses include people with a weakened immune system and pregnant women. The transmission of zoonoses from laboratory animals is uncommon due to animal vendors providing specific pathogen-free animals, and maintaining vivarium facility cleanliness. Researchers participating in field studies in which they are in contact with wild animals can be exposed to zoonoses not commonly seen in laboratory animals. Individuals can help prevent the transmission of zoonoses by participating in and encouraging routine sanitation of lab spaces, wearing personal protective equipment (PPE) while handling animals, and washing their hands before and after handling animals. Please contact the UARV Supervisor if you have questions regarding a specific zoonoses; information will be provided to you.

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SECTION C: DISCLOSURE OF ADDITIONAL ANIMAL HANDLING

The UARV requires a disclosure of animals with which the Applicant interacts with regularly outside of the facility. This information allows the UARV Supervisor to provide recommendations to the Applicant and their Supervisor regarding specific personal protective equipment (PPE) and other required measures necessary to reduce the potential of pathogen transmission between research and personal animals.

Species you are studying in the UARV (all): New Zealand White Rabbit

Species you have regular contact with outside the UARV (including volunteer work, pets, etc.):

SECTION D: ACKNOWLEDGMENT AND SIGNATURE

The Applicant understands that information provided in Section A is voluntary and disclosure is not required to obtain UARV access. The Applicant has read, disclosed, and understands required information in Sections B and C. The Applicant acknowledges that if their allergen status changes at any time after submitting this form, they may contact the UARV Supervisor to amend Section A. This form is kept on file with your Zip Card Access record in the UARV and is maintained in confidentiality. You may contact the UARV Supervisor at 330-972-5845 at any time if you have questions or amendments.

Full Name of UARV Applicant (print): Gina Tubo

Signature of UARV Applicant: Gina Tubo Date: 1/7/19

UA ID Number (on back of Zip Card): 4045107

****

UARV OFFICE USE ONLY

Reviewed By (print) _____ on (date) _____

Signature _____

8. "Five Senses Rules"
    a. Light. All animal rooms have timer-controlled lights. Automatic timer settings are **not** to be changed without UARV Supervisor permission. Most rooms have an additional light switch that can be flipped on to provide more additional light to researchers while they are in the room, but must be switched off after leaving (the switch emits an orange glow when the switch is off).
    b. Sound. Excessive noise (music, yelling, etc.) is **not permitted** in the vivarium, as it is disruptive to animals and can cause undue stress.
    c. Smell. Do **not** wear strong perfume, aftershave, lotion, deodorant, or other strong scents (like cleaning products and chemicals) into the animal rooms.
    d. Taste. **No** food, drink, chewing gum, or other such products are allowed on the 2nd and 3rd floors of the UARV (see Policy Item #4). **No** chewing tobacco (or any tobacco products) are permitted in the UARV.
    e. Touch. You are only allowed to handle your animals and equipment, and common use items (clean cages, water bottles, racks). Do **not** touch animals or equipment belonging to other researchers, or the UARV (e.g. cagewasher).

9. Emergencies, maintenance, and security.
    a. **Minor non-emergency**. Examples include small water leaks or dripping faucets, lights burnt out in hallways, etc. Contact the UARV Supervisor so that a work request can be placed.
    b. **Major emergency**. Examples include escaped animals, A/C or heating breakdowns, necropsy freezer failure, anything that negatively affects animal health and safety (such as burnt out lights), etc. Contact the UARV Supervisor immediately for assistance.
    c. **Security emergency**. Examples include animal activist groups on campus, a person exhibiting threatening behavior, etc. Contact the UAPD immediately (330-972-2911).
    d. **Chemical/biohazard emergency**. Examples include biohazard waste spills or leaks, chemical injury or spills, etc. Contact UAPD immediately (330-972-2911) for medical attention, and instruct them to notify Environmental and Occupational Health and Safety (EOHS) to handle the issue while you receive treatment. The EOHS emergency line is 330-972-7123.

10. Trash and cleaning. The University of Akron custodial staff does not enter animal rooms, personal labs and offices, or the surgical suite. When you are done using a room, place the trash can into the hallway so it will be emptied overnight. **Always dispose of sharps in a biohazard container, never in the trash!** There are brooms and dustpans in each animal room; mops, buckets, and cleaning supplies can be found on each floor. If you can't find something, ask UARV staff for assistance. Dirty clothing such as scrubs or labcoats that belong to the UARV can be placed in the hamper in the laundry room (B115).

If you ever have questions or need assistance, please talk with the UARV staff or supervisor for help. We are here to ensure you have the best experience with animal care and safety!

* * * * * * * * * * * * * *

I Gina Tubo (print name) have thoroughly read and understand the UARV Policies for Vivarium Users outlined in this form. I understand that I will be held accountable for my actions with regard to these rules, and that I must follow them at all times. I acknowledge that failure to comply with these policies will result in termination of my access to the UARV facility, and additional consequences may be incurred if my actions are determined to be severe. I confirm that I have been given a UARV tour by the UARV Supervisor.

User Signature: Gina Tubo Date: 1/7/19

UARV Manager Signature: _____ Date: _____

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STUDENT'S STATEMENT

All students working in any chemical laboratory must read the **Standard Operating Procedure (SOP)** and sign the student's statement. The department or college may opt to have students sign this statement annually or at the beginning of each semester, therefore ensuring that the SOP is reviewed consistently. The Department of Environmental and Occupational Health and Safety (EOHS) will provide Laboratory Safety Training when requested.

"I have read, or have had read to me, the **SOP**. I understand the requirements and contents of the **SOP**. I have received training for conducting the laboratory activities assigned to me through the Hazard Communication Standard with special focus on Laboratory Safety. I am familiar with the required tools and protective equipment as well as associated safety and health hazards. I agree to follow the **SOP** and abide by safety rules and procedures it contains. I will discuss with the right university official prior to deviating from rules and safety measures instituted in the **SOP**. I will review the **SOP** as frequently as required by the department or by my advisor."

STUDENT'S NAME	STUDENT'S SIGNATURE	UAKRON STUDENT ID
Gina Tubo	Gina Tubo	4045107

The University of Akron

Student Employee Confidentiality Agreement

I, Gina Tubo (PRINT NAME), understand and accept the following conditions and responsibilities of my employment at the University of Akron as a student assistant:

1. In the performance of my duties, I may have access to confidential information, which includes records of other students, faculty, or staff; business information, correspondence and reports. All of these types of information are considered confidential.

2. I shall treat ALL information accessible to me in the performance of my duties as Confidential Information, regardless of its format (e.g., electronic, paper, oral), unless and until advised otherwise by my supervisor.

3. **I agree to not access Confidential Information unless I am authorized to do so, and I agree to maintain the confidentiality and privacy of Confidential Information during and after my period of student employment with the University**. I shall not, directly or indirectly, communicate **orally, in writing, or by e-mail**, any Confidential Information to any unauthorized person, including, without limitation, other students, work colleagues, family members, etc.

4. I may gain access to sensitive or confidential information and records that may be protected from disclosure by federal or state law. Examples include education records protected under the Family Educational Rights and Privacy Act of 1974 (FERPA). I understand that unauthorized disclosure of such Information can adversely impact the University, individual persons, or affiliated organizations.

5. I shall use my access to Confidential Information for the sole purpose of performing my job duties. I shall not disclose Information to ANYONE without prior authorization from my supervisor.

6. I shall not permit myself or any other person to copy, reproduce, alter, delete, or enter any Information other than what is required in the regular performance of my job duties.

7. I am aware that any breach of this agreement, release of Confidential Information, or any abuse of my position, may result in disciplinary action through The University of Akron Code of Conduct or otherwise, including possible termination of my position, prosecution through appropriate University judicial processes, expulsion from the University, and civil and criminal legal sanctions.

8. The provisions contained in this agreement are considered conditions of my participation in programs and employment offered by the University.

I have reviewed and read this document. I understand its terms and its legal effect.

Gina Tubo	ID Number 4045107	Gina Tubo	1/7/19
Student Employee Name (Print)		Signature	Date
Janna M. Andronowski			1/9/19
Supervisor Name (Print)		Signature	Date

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CITI PROGRAM

Completion Date 03-Jan-2019
Expiration Date 02-Jan-2022
Record ID 29898812

This is to certify that:

Gina Tubo

Has completed the following CITI Program course:

Working with Rabbits in Research Settings (Curriculum Group)
Working with Rabbits in Research Settings (Course Learner Group)
1 - Basic Course (Stage)

Under requirements set by:

The University of Akron

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?we4e3c416-90bd-4723-8677-6ee18c15bce7-29898812 515

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ADDITION OF PERSONNEL - REQUEST TO USE ANIMALS

Please add the participant named below to my approved protocol # entitled, .

PRINCIPAL OR CO- INVESTIGATOR :

PRINCIPAL OR CO-INVESTIGATOR SIGNATURE — DATE: 02/06/19

PARTICIPANT NAME: Abigail LaMarca

PARTICIPANT TITLE: Research Assistant

PARTICIPANT SIGNATURE — DATE: 2/5/19

RESPONSIBILITIES ON THE PROTOCOL :

daily check of animals

EXPERIENCE/QUALIFICATIONS:

Rabbitt training will be done @ NEOMED with Dr. Dan Miller

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
CITI Training	online	2/1/19
Rabbitt CITI Training	Online	2/1/19

IACUC APPROVAL (*approval signature requirements vary according to institution*):

IACUC Member
Date:

Attending Veterinarian
Date:

IACUC Chairperson
Date:

The University of Akron

Student Employee Confidentiality Agreement

I, Abigail LaMarca (PRINT NAME), understand and accept the following conditions and responsibilities of my employment at the University of Akron as a student assistant:

1. In the performance of my duties, I may have access to confidential information, which includes records of other students, faculty, or staff; business information, correspondence and reports. All of these types of information are considered confidential.

2. I shall treat ALL information accessible to me in the performance of my duties as Confidential Information, regardless of its format (e.g., electronic, paper, oral), unless and until advised otherwise by my supervisor.

3. **I agree to not access Confidential Information unless I am authorized to do so, and I agree to maintain the confidentiality and privacy of Confidential Information during and after my period of student employment with the University**. I shall not, directly or indirectly, communicate **orally, in writing, or by e-mail**, any Confidential Information to any unauthorized person, including, without limitation, other students, work colleagues, family members, etc.

4. I may gain access to sensitive or confidential information and records that may be protected from disclosure by federal or state law. Examples include education records protected under the Family Educational Rights and Privacy Act of 1974 (FERPA). I understand that unauthorized disclosure of such Information can adversely impact the University, individual persons, or affiliated organizations.

5. I shall use my access to Confidential Information for the sole purpose of performing my job duties. I shall not disclose Information to ANYONE without prior authorization from my supervisor.

6. I shall not permit myself or any other person to copy, reproduce, alter, delete, or enter any Information other than what is required in the regular performance of my job duties.

7. I am aware that any breach of this agreement, release of Confidential Information, or any abuse of my position, may result in disciplinary action through The University of Akron Code of Conduct or otherwise, including possible termination of my position, prosecution through appropriate University judicial processes, expulsion from the University, and civil and criminal legal sanctions.

8. The provisions contained in this agreement are considered conditions of my participation in programs and employment offered by the University.

I have reviewed and read this document. I understand its terms and its legal effect.

Abigail LaMarca	ID Number 4178964	[signature]	1/28/19
Student Employee Name (Print)		Signature	Date
Jana Andrzejewski		[signature]	01/28/19
Supervisor Name (Print)		Signature	Date

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The University of Akron Research Vivarium (UARV)

Allergen and Zoonoses Statement

SECTION A: VOLUNTARY DISCLOSURE OF ALLERGIES

Information voluntarily provided by the individual applying for Zip Card access to the UARV ("Applicant") to the UARV in this section is provided to the University of Akron Police Department (UAPD) in response to a medical emergency involving the Applicant.

The Applicant:

☐ declines to disclose allergy information to the UARV.

☑ elects to disclose allergy information to the UARV.

If Applicant elects to disclose allergy information to the UARV, please list allergies and related symptoms:

Dairy, eggs, nuts, seafood - anaphylaxis

SECTION B: ALLERGEN AND ZOONOTIC DISEASE STATEMENTS

Allergen Statement

Allergies (also known as allergic reactions) are an overreaction of the body's natural immune system in response to a substance that is usually harmless (such as dust, pollen, dander, or a medicine). These substances are known as *allergens*. Symptoms of allergies vary from person to person and can range from mild to severe. Mild to moderate symptoms commonly include a rash or hives, itching and swelling of the skin, itching and watery eyes, a runny nose, and trouble breathing. Severe, whole-body allergic reactions (anaphylaxis) can include swelling of the face/nose/lips/throat to the point of asphyxiation, heart palpitations, low blood pressure, dizziness, and unconsciousness. **An anaphylactic reaction can be life-threatening and is always a medical emergency; call UAPD at 330-972-2911 immediately for medical attention.** Allergies can develop after one exposure to an allergen, or can develop after repeated exposure to an allergen over a period of time. Individuals who work in a lab setting with animals often develop allergies in response to the following allergens: latex, animal urine, animal fur or dander. If you develop an allergy, please contact your medical provider for advice on continuing work with animals and alert the UARV Supervisor.

Zoonotic Diseases Statement

Zoonotic diseases (also known as *zoonoses*) are caused by bacteria, viruses, parasites, or fungi and are spread between animals and humans. Individuals more prone to contracting zoonoses include people with a weakened immune system and pregnant women. The transmission of zoonoses from laboratory animals is uncommon due to animal vendors providing specific pathogen-free animals, and maintaining vivarium facility cleanliness. Researchers participating in field studies in which they are in contact with wild animals can be exposed to zoonoses not commonly seen in laboratory animals. Individuals can help prevent the transmission of zoonoses by participating in and encouraging routine sanitation of lab spaces, wearing personal protective equipment (PPE) while handling animals, and washing their hands before and after handling animals. Please contact the UARV Supervisor if you have questions regarding a specific zoonoses; information will be provided to you.

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The University of Akron Research Vivarium (UARV)

Health Assessment

This document is intended to help a professional healthcare provider determine whether or not an individual applying for Zip Card access ("Applicant") to the UARV should perform research with animals in the UARV. **Only the last page of this form, Section D: Healthcare Provider Statement, is turned into the UARV Supervisor.** The Applicant should fill out this assessment and consult with their supervisor/advisor if necessary so that accurate information is communicated to the professional healthcare provider. Health care records from other institutions or organizations are not transferrable to the UARV.

A health assessment is available for any Applicant – student, faculty, or staff – by a professional healthcare provider at Student Health Services (SHS), or by the Applicant's personal physician. Appointments can be scheduled at SHS by calling 330-972-7808 and requesting a "Biology Health Screening". Health screenings are generally covered under a personal health insurance plan. Follow-up health assessments are not required by the UARV, but the Applicant is strongly recommended to abide by follow-up instructions given to them by the professional healthcare provider.

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SECTION A: JOB ACTIVITY AND LENGTH OF RESEARCH

Applicant Job Title Research Assistant

Estimated Start Date 02/2019 Estimated End Date 12/2021

SECTION B: EXPOSURES AND CONTACT

1. Which species of animal you will be researching in the UARV? New Zealand white rabbit
2. Is the nature of animal research direct contact, observational, or both? both
3. Estimate the average number of hours of animal contact per week: 10
4. Will your job involve the use of...? Check "Yes" or "No".
   NOTE: Your healthcare provider may ask you for specification if you answer "yes" to any of these. Consult with your supervisor/advisor if you are not sure how to answer.

	Yes	No
Anesthetic gases		X
Animal cells, blood, or tissues	X	
Anti-neoplastic compounds		X
Carcinogens		X
Formaldehyde, paraformaldehyde, glutaraldehyde, or other preservatives	X	
Latex gloves	X	
Infectious agents (bacteria, viruses, fungi, etc.)		X
Radiation or radionuclides		X
Respirator/mask		X
Toxins or venoms	X	
Other toxic or hazardous chemicals.		X

5. Have you had prior animal contact in a laboratory or research setting? YES (NO)
   If you circled yes, with what species:

6. Are you currently in contact with animals as part of a job or volunteering? YES (NO)
   If you circled yes, with what species:

7. List any pets in your home.
   Dogs & Cats

8. Have you experienced any symptoms that you or a professional healthcare provider suspect may be related to working with animals? If so, please explain:
   no

SECTION C: MEDICAL AND IMMUNIZATION HISTORY

1. Do you have...? Check "Yes" or "No".

	Yes	No
Asthma	X	
Eczema	X	
Hand dermatitis		X
Latex allergies		X
Immunodeficiencies		X
Heart conditions		X
Diabetes		X
Hypoglycemia		X
Respiratory problems		X

- **NOTE: Your healthcare provider may ask you for clarification if you answer "yes" to any of these.**

2. List any known allergies and reactions:
   Dairy, eggs, nuts, seafood. – anaphalaxis

3. Immunizations -- have you had...?

	Yes	No	Date
Hepatitis A vaccine		X	—
Hepatitis B vaccine	X		12/16/2000
Measles/Mumps/Rubella booster	X		5/20/05
Rabies vaccine		X	—
TB test	X		09/2018
Tetanus booster*	X		08/16/2011
Varicella vaccine	X		7/17/07

* **For admittance to the UARV, tetanus booster must be current within the last 10 years.**

Vaccine recommendations from Applicant's supervisor/advisor:

Additional comments:

CITI PROGRAM

Completion Date 01-Feb-2019
Expiration Date 31-Jan-2022
Record ID 30411118

This is to certify that:

Abigail LaMarca

Has completed the following CITI Program course:

Working with the IACUC (Curriculum Group)
Investigators, Staff and Students (Course Learner Group)
1 - Basic Course (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?w5009ae9b-8c80-408f-b742-d667c088bd5b-30411118

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CITI PROGRAM

Completion Date 01-Feb-2019
Expiration Date 31-Jan-2022
Record ID 30411119

This is to certify that:

Abigail LaMarca

Has completed the following CITI Program course:

Working with Rabbits in Research Settings (Curriculum Group)
Working with Rabbits in Research Settings (Course Learner Group)
1 - Basic Course (Stage)

CITI
Collaborative Institutional Training Initiative

Under requirements set by:

The University of Akron

Verify at www.citiprogram.org/verify/?wbfa18fba-82f8-46f7-97a9-acd47779d607-30411119

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Appendix XXVIII: Calcein Protocol

November 24, 2017

SOP for making Clacein solutions:

- Animals will be dosed with calcein (10 mg/kg at a 20mg/ml concentration) on two consecutive days subcutaneously.
- Keep all solutions in a dark container, and refrigerated.
- 35 rabbits*2days*4.25kg*10mg=2.975g is needed for each 2 days period: we will need 3 batches of 1gram in 50mL (for each time-point). Concentration would be 20mg/mL. **Dose: inject 0.5mL/kg**
- Add 500mg Sodium Bicarbonate and 1g Calcein to a 50mL tube and fill up to 50mL with 0.9% sterile saline. After adding the saline to Sodium Bicarbonate reacts with Calcein, which leads to dissolution of Calcein, but also it foams, so close the tube cap to avoid spilling. You can also add only 40mL of saline at first and then make the solution to 50mL. Mix the solution for a few minutes until Calcein dissolves.
- The pH is likely around 6.0, so raise the pH by adding NaOH (1N) solution (I have made enough). **15 drops** should bring the pH to 7.5, but **DO NOT** add all 15 drops at once, go gradually, as it might overshoot the pH. pH of 7.2-7.6 is acceptable.
- Check the pH before injection, if solution is pre-made.
- If you are preparing needles at once and before injections, they need to be covered by aluminum foil.

Note: ideally Calcein needs to made fresh, however, since in the past in our lab it was made and stocked and still the signal was detectable in the bones, we will make 50mL volumes on or before the injection day. That should be enough for dosing 12 rabbits for 2 days.

- Have lab coat and gloves on, the Calcein is messy, and also NaOH can cause skin irritation.

Appendix XXIX: Andronowski Lab Rabbit Daily Check Log

Date: 4/16/19
Personnel: JMA, Reed, Gina, Adam, Beth
Notetaker: JMA
Time In: 12:15 PM

Tasks Performed (Dosing, Exercise, etc.): N/A - Acclimation

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: Ear tattoos are scabbed over and ears somewhat bruised -- this is consistent for all animals.

Vet Checks Requested?: N/A

Any Additional Notes:

- Arrival day!
- Animals arrived at 11:45 A.M.
- Unboxed the animals and weighed each one.
- Spinach was provided for enrichment/since no rabbit diet is provided on day one.
- Basic health checks performed. Looked for discharge from eyes/nose, brightness of eyes, condition of fur, any scratches on ears, and behavioral changes.
    - Ear tattoos are scabbed over and ears somewhat bruised -- this is consistent for all animals.
- 01 appeared more stressed than the others. Sclera showing, rapid breathing, crouched in corner.

Date: 4/17/19
Personnel: JMA, Adam, Gina, Abbie
Notetaker: JMA
Time In: 8:10 AM
Tasks Performed (Dosing, Exercise, etc.): N/A - Acclimation

Temperature: 71.4
Humidity: 43%

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: #17, Left rear 1st digit inflammation

Vet Checks Requested?: #17 for L 1st rear digit inflammation and overgrooming. Text message sent to Stan with photo.

Any Additional Notes:

- Issue with HVAC chiller being shut off overnight. Temperature jumped to 76 degrees in rabbit room with no alert. Facilities were notified. Dr. A to follow up.
- Any leftover spinach removed, and new spinach provided.
- 01 had minimal fecal output but his rapid breathing has subsided, did not consume all spinach and drank minimal water.
- 08 had minimal fecal output and cecotropes fell through cage.
- 10 had minimal fecal output.
- 13 had normal fecal output (a large amount).
- 14 is very active.
- 15 appears to have water bottle leakage.
- 16 low fecal output.
- 17 no urine and minimal fecal output, minimal water consumed, minimal spinach/hay eaten. Appears somewhat stressed, elevated breathing, in corner of cage.
- 20 minimal food intake, normal fecal and urine output.
- Update: Beth contacted JMA and Adam at 12:10 P.M. and indicated that 17 was excessively grooming its left hind limb. JMA, Adam, Reed responded immediately to assess the animal. Found inflammation of first digit, some hair had been groomed away.
- 17 also urinated and ate both rabbit diet/spinach.

Date: 4/18/19
Personnel: Tubo, Schuller, Andronowski
Notetaker: Tubo
Time In: 8:20
Tasks Performed (Dosing, Exercise, etc.): N/A - Acclimation

Temperature: 68.4 F
Humidity: 47%

Changes in Behavior (Lethargy, Hyperactivity, etc.): 09 was chewing the plastic in his pan, 11 was a little timid

Health Concerns: 15 had signs of dried foreign brown material on cage pan and on tail (not fresh, it is old and dried), 17 fur chewed on rear left toe (contacted vet 4-17-19), 20 had minimal fecal output (attributed to stress from novel environment)

Vet Checks Requested?: No

Any Additional Notes: Enrichment given in the form of papaya tablets, spinach, and metal bowls. Most rabbits seem to like to play with the rabbit bowls**.**

JMA 2:45 P.M. update: Rabbits 7,8,9,10,11,13,16,17,18,19,20 flipped their bowls and so the papaya tablets fell through the cage bottom. The UARV staff disposed of them. I gave each of these rabbits a new tablet in their food feeders. We should place the tablets in the food feeders from now on so they do not fall through.

UARV staff also asked us to make sure we fill in the food enrichment log each day. The binder is on the cart with the others.

Date: 4/19/19
Personnel: Reed and JMA
Notetaker: Reed
Time In: 8:00
Tasks Performed (Dosing, Exercise, etc.): N/A - Acclimation

Temperature: 69.4
Humidity: 58%

Changes in Behavior (Lethargy, Hyperactivity, etc.):
#11 still a little timid
Health Concerns:
#7 has weirdly shaped feces. Normal quantity, but seem small or weirdly shaped.
#11 also has weirdly shaped feces. Same notes as #7.
#20 has dark urine, still normal amounts. Also has dried feces on cage, not as much as #15.

Vet Checks Requested?: N/A

Any Additional Notes: Vet check completed for #17. Swollen posterior ⅔ of left rear 2nd digit. No treatments as of now.
#15 drinking a lot. His water spilled through the night. Maybe just parched?

Two fire alarms in the afternoon - 2:45 and 4:00 P.M. Rabbits seem stressed when we checked on them (frozen in cages).

Date: 4/20/19
Personnel: Reed and Adam
Notetaker: Reed
Time In: 8:00
Tasks Performed (Dosing, Exercise, etc.): Weighed all rabbits, Acclimation

Temperature: 65.5
Humidity: 45%

Changes in Behavior (Lethargy, Hyperactivity, etc.):
#5 and #7 and shedding, possibly stressed

Health Concerns:
More dried diarrhea on #15 and in #15's cage than yesterday. Now dried diarrhea on his nose.
Adam: do you think we should report this to Stan for a vet check? JMA

#20 has cecotropes in cage that he didn't eat.

Vet Checks Requested?: N/A

Any Additional Notes: #14 is the witchling. Ripped both gloves to shreds when removing for weighing. Aggressive. #17's toe fur is growing back.

Do you mean #17?! JMA - Yes. Adam and I were talking about #15 as I was writing this. Sorry. (RAD)

Date: 4/21/19
Personnel: Reed and Adam
Notetaker: Reed
Time In: 8:01
Tasks Performed (Dosing, Exercise, etc.): Enrichment food, health checks.

Temperature: 66.0
Humidity: 43%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Any Additional Notes: MacBook no longer working. Restarted itself overnight and it is trying to install updates which fail. Can use Reed's old laptop if needed. Don't believe pads were changed yesterday.

#5 does not eat the papaya tablets. Just licks them and leaves them alone.

Date: 4/22/19
Personnel: JMA, Adam, Abbie
Notetaker: JMA, Abbie filling in logs
Time In: 8:00 A.M.
Tasks Performed (Dosing, Exercise, etc.): First day of exercise, enrichment foods, health checks, nail trimming.

Temperature: 65.5
Humidity: 47.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):
#11 still more timid than others.

Health Concerns:
Dried diarrhea on #15's cage pan. Does not seem to be any worse than previous days, however.

Vet Checks Requested?: No

Any Additional Notes:
A spreadsheet will be created today to track the weights of the animals over time.
All rabbits seem to be shedding -- is consistent among the animals. Could be due to stress or temperature adjustment.

Group 1: Animals 01-07. #01 placed at 8:20 A.M. and each rabbit followed about 4 minutes later. Dumbbells from their cages placed with them.

All seem to be adjusting to the exercise pen and exploring, eating some shavings. #2 especially active, did try to jump out of the top once. #3 eats a lot of shavings, he also found a large stick in the shavings and we discarded this.

Group 2: Animals 08-14. #08 placed at 9:10 A.M. Not as active as the group 1 rabbits. #10 almost fell asleep.

Group 3: Animals 15-21. #15 placed at 10:00 A.M. Also not as active as group 1 rabbits. All are relaxed, just not moving around as much.

Date: 4/23/19
Personnel: Reed, Gina
Notetaker: Reed, Gina filling in logs

Time In: 8:03 A.M.
Tasks Performed (Dosing, Exercise, etc.): Health checks and enrichment food. UARV cleaning batteries.

Temperature: 66.0
Humidity: 53.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.):
#11 still more timid than others.

Health Concerns:
Two spots of diarrhea on #15's pad per Beth. Does not seem to be any worse than previous days, however.
#1 and #5 have urine with high protein content. The type that congeals in the corners.

Vet Checks Requested?: No

Any Additional Notes:
#4 Had a little bit of food leftover. Only about 10 pellets.

#13 pushed new water bottle out of holder and shattered it on the floor. Reed cleaned up broken glass and mopped the water. Replaced with plastic bottle, may need more. Proceeded to knock it out 3 more times, it is now inside his cage.

Beth also suggested extra water bottles in the exercise pens otherwise it is a restriction and we have to report it in a protocol change to USDA and IACUC.
----------Date: 4/24/19
Personnel: Adam, Abbie
Notetaker: Abbie
Time In: 8:00 A.M.
Tasks Performed (Dosing, Exercise, etc.): Exercise, enrichment foods, health checks.

Temperature: 67.0
Humidity: 44.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):
None

Health Concerns:
A lot of dried diarrhea on #15's cage pan and noted diminished flesh condition and rough fur condition.

Vet Checks Requested?: Yes, vet check on cage

Any Additional Notes: The following animals all had either a little (#03, 04, 05, 08, 10, 17) or a lot (#13, 19, 21) of food left in their trough.

Control: Animals- control group. #04 placed at 8:15 A.M. and each rabbit followed about 3 minutes later. Jingle balls placed in cages.

Fentanyl: Animals-fentanyl group . #01 placed at 9:05 A.M. Animal #03 did attempt to jump out of the exercise pen one time.

Morphine: Animals- morphine group. #08 placed at 9:55 A.M. Animal #12 sprayed urine out of the cage onto the floor.

**NOTE: the same jingle balls were in the exercise pens for all three groups as the only enrichment device in the cage was a rattle.

All rabbits acting normal in cages and playing with their toys, all rabbits water was transported to the exercise pen with them; however, none of them drank any of their water while in the exercise pens and the springs were difficult to use to attach water bottles to exercise pens.

Date: 4/25/19
Personnel: Adam, Reed
Notetaker: Adam (logs), Reed (PC)
Time In: 8:00 A.M.
Tasks Performed (Dosing, Exercise, etc.): Enrichment foods, health checks.

Temperature: 65.5
Humidity: 47.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):
All rabbits a little timid after cages being changed into new arrangement (separated into groups and then re-arranged to correct mistake last night)

Health Concerns:
Animal #15 - ongoing diarrhea and diminished flesh and fur condition

Vet Checks Requested?:
Same as previous day - see Dr. Dannemiller's email for details.

Any Additional Notes:
All rabbits given approximately ⅓ of a carrot as enrichment (first time this enrichment food has been administered) all rabbits expressed interest in this and are either eating or smelling the carrot (some are

also tossing it around like a toy). We will continue to monitor the intake of this novel enrichment food tomorrow.

Date: 4/26/19
Personnel: Adam and Reed
Notetaker: Reed (logs) and Adam (computer)
Time In: 8:00 A.M.
Tasks Performed (Dosing, Exercise, etc.): Exercise, enrichment foods, health checks.

Temperature: 65.5 *F
Humidity: 66%

Changes in Behavior (Lethargy, Hyperactivity, etc.):
Number 03 did seem a bit more lethargic than he has previously. Noted that he had almost a full serving of food remaining. We will monitor this closely.

Health Concerns:
04, 14, 15 are all exhibiting signs of diarrhea. (14 is very slight so we will watch him for now). 04 had a fair amount of soiling on his fur so we placed a vet check card in his cage as well as the recurring one in number 15's cage.

Vet Checks Requested?: 04 (as well as recurring 15)

Any Additional Notes:
Carrots seemed to be better received today compared to yesterday. Many rabbits now have orange stains on the fur from eating this. #06 repetitive sneezing with no substantive nasal discharge.

Control: Placed #04 at 8:10 A.M. and each subsequent animal 1 minute after this. All animals showed signs of activity and enjoyed playing in the pen with jingle balls. Number 06 ate a fair amount of shavings. All animals exhibiting digging behaviors. 14 did drink a little from his water bottle.

Morphine: Placed #21 at 9:05 A.M. and each subsequent animal 1 minute after this. Number 12 is very energetic when it comes to the playpen and tried to jump out once.

Fentanyl: Placed #01 at 9:55 A.M. and each subsequent animal 1 minute after this. Number 02 attempted to jump out two times. Number 03 showed much more interest in moving than he did in his cage. Number 13 very energetic and active

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Date: 4/27/19
Personnel: Gina and Abbie
Notetaker: Gina
Time In: 8:00 am

Tasks Performed (Dosing, Exercise, etc.): Exercise, enrichment foods, health checks.

Temperature: 65.5 F
Humidity: 45 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):
N/A

Health Concerns:
04, 07, 10, 14, 15 are all exhibiting signs of diarrhea. (14 is very slight so we will watch him for now). Vet check cards still on 15 and 4.

Vet Checks Requested?:
4 and 15 - still in from previous day
Any Additional Notes: 13 had low water, not sure if he knocked it down again or he has high water intake

Control: 04 - low food intake and some diarrhea present, 06 - sneezing (as noted in previous days) and little food intake, 07 - little diarrhea, 14 - little food intake

Fentanyl: 01 - little food intake, 13 - lots of food left and low water (possibly because of bottle leakage),

Morphine: 10 - little food left, some diarrhea, 21 - little diarrhea
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Date: 4/28/19
Personnel: Reed and Abbie
Notetaker: Abbie
Time In: 8:00 am
Tasks Performed (Dosing, Exercise, etc.): Enrichment foods, health checks.

Temperature: 65.0 F
Humidity: 45.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):
N/A

Health Concerns:
15 still has ongoing diarrhea and 3 still is not eating very much.

Vet Checks Requested?:
4 and 15 - still in from previous day
Any Additional Notes:

13's water was empty, it leaked, we have made a note to the vivarium asking for a new bottle since this problem is ongoing
Control:4,5 6 had lots of food left, 15 still has diarrhea

Fentanyl: 1-lots of food left, 3- almost all food left, 13 has a medium amount of food left.

Morphine: 10 lots of food left, 11 little food left 17 has a small amount of secatrop, 21 has a little food left

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Date: 4/29/19
Personnel: Reed, Abbie, JMA
Notetaker: Abbie
Time In: 8:00 am
Tasks Performed (Dosing, Exercise, etc.): Exercise, Weighing, Enrichment foods, health checks.

Temperature: 66.0 F
Humidity: 45.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):
#3 ate substantially more than the previous couple days. Still had food left, but not his entire ration.

Lots of food left: 1, 4, 5, 6, 10, 14
Little food left: 3, 13, 21

17 had less than average fecal output, but doesn't appear shockingly less. Monitor.

Health Concerns:
15 still has ongoing diarrhea and it appears to be more liquid now, 15 also has dark urine and 3 still is not eating very much.

Vet Checks Requested?:
4 and 15 - still in from previous day

Any Additional Notes:

Control: First rabbit went into exercise pen at 8:10, last rabbit in at 8:15

Fentanyl: First rabbit into exercise pen at 9:40, last in at 9:45

Morphine: First rabbit went into exercise pen at 8:55, last in at 9:00

All rabbits acting normal in exercise pens and are still enjoying the jingle balls.

Date: 4/30/19
Personnel: Reed, Adam, Gina
Notetaker: Reed
Time In: 8:00 am
Tasks Performed (Dosing, Exercise, etc.): Enrichment (kale), clipping, health checks

Temperature: 65.5 F
Humidity: 48.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: Ongoing #15 diarrhea, #3 still ate more than he has previously, but there is still a lot of food left in his trough.

Vet Checks Requested?: Cathy requested one for #3 not eating.

Any Additional Notes: Finished the rest of the kale, still have carrots and carrot tops.

Control: The patch controls were shaved today
Fentanyl: Animals were shaved today. #2 and #3 are not fans of the clippers.
Morphine: **none**

Date: 5/01/19
Personnel: Reed, Adam, Gina, Abbie, JMA *everyone present since it is the first dosing day.
Notetaker: JMA
Time In: 8:00 am
Tasks Performed (Dosing, Exercise, etc.): Enrichment (carrots), clipping #15, health checks, first narcotic dosing day.

Temperature: 66 F
Humidity: 65.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: #15 ongoing diarrhea, #6 lower fecal output, #3 ate all pellets and enrichment and fecal output appears normal.

#s 11, 2, 3, 20 appear most heavily sedated from the first treatments. Most notably 3 (fentanyl) and 11 (morphine). All animals monitored by Beth's team around noon, and Andronowski Lab at 2:00 and 4:00 P.M. By 4:00, the animals seemed more alert.

Vet Checks Requested?: N/A

Any Additional Notes: Still have carrots and carrot tops. Spinach and kale purchased by JMA and Adam today. Need more papaya tablets.

#13 water bottle still leaking and he did not have water this morning. Bottle and sipper were replaced.
Rabbit 2 jumping around has a lot of energy after patch placement, seems to not like it.
Rabbit 3 began exhibiting effects such as lethargy aprox 20 minutes after patch placement. Rabbit 16 is also exhibiting some of this very relaxed behavior.
Rabbit 10 exhibited a large amount of eflux from the injection.
Rabbit 17 exhibited a small amount of eflux from the injection.
#2 consistent chewing of tegaderm around patch. Decided to replace patch at 10:40.
#18 patch control panicking during patch placement. Took 4 attempts to adhere tegaderm. Removed patch by noon. Will reapply tomorrow.

We will shave all morphine and control rabbits tomorrow to ensure this does not happen again.

Exercising:

Control: First rabbit placed at 9:05. Activity level comparable to previous days.
Fentanyl: First rabbit #1 in pen at 8:10. Exercising this group first since today is their first dosing day. Activity level comparable to previous days (since not dosed beforehand).
Morphine: First rabbit placed at 9:55. Number 11 did exhibit less playing behavior than normal - unsure if this is due to the dosing or not. Number 08 is exhibiting the same type of behavior. Number 20 exhibiting this behavior as well. All rabbits eventually exhibited behavior like this with the exception of 10 and 17 who we believed did not receive the full dose.

Date: 5/02/19
Personnel: Reed, Adam, JMA delivered food enrichment
Notetaker: Adam
Time In: 8:10 am
Tasks Performed (Dosing, Exercise, etc.): Enrichment (Spinach), clipping #04, 05, 08, 10, 11, 12, 14, 17, 20, 21, health checks, morphine dosing day, patch readministration.

Temperature: 68 F
Humidity: 61.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): Rabbits returned to normal behavior after departure from this yesterday (given first day of dosing). All morphine rabbits are very lethargic post-dosing.

Health Concerns: #15 ongoing diarrhea, #3 low fecal output.

#10, 17 appear most heavily sedated from their first full treatments. After about a half hour, they appear less heavily sedated. All animals exhibiting similar effects.
#17 scratched a lot at his shaved area, leaving abrasions.
#11 appears heavily sedated

Vet Checks Requested?: N/A

Any Additional Notes: Need more papaya tablets.
Rabbit #02 had fentanyl patch readministered. All control tegaderm rabbits had this treatment readministered. All rabbits are now shaved. #2 is still annoyed by his patch. #18 still hates his tegaderm.

UPDATE (4:30 PM) All rabbits appear to be back to normal activity and behavior. All the controls have removed their Tegaderm patches. #2 once again removed his patch and it was lying on the floor of his cage. Reed removed it and disposed of it in the biohazard bin. Reed then readministered another patch to #2 a bit closer to his neck, but still well in the shaved, scapular region and covered with Tegaderm. Cathy came in to check on the rabbits water saw 13 fling his sipper from side to side to dislodge it.

Date: 5/03/19
Personnel: Gina, Adam
Notetaker: Adam
Time In: 8:35 am
Tasks Performed (Dosing, Exercise, etc.): Enrichment (Spinach), health checks, morphine dosing day, patch readministration, exercise.

Temperature: 66 F
Humidity: 68.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): Rabbits returned to normal behavior after departure from this yesterday (given first day of dosing). All morphine rabbits are very lethargic post-dosing.

Health Concerns: #15 ongoing diarrhea, #3 low fecal output, #7 and #17 received ointment administration.

Vet Checks Requested?: N/A

Any Additional Notes: Rabbit #02 had fentanyl patch readministered because he had eaten most of his previous patch (we found about ½ of a patch in the cage).

Exercising:

Control: #04 placed at 8:45 A.M. with each subsequent animal being placed approximately 1 minute after this. All rabbits seemed fairly active. Water and bone transferred from cage.

Morphine: #08 placed at 9:45 A.M. with each rabbit placed around 1 minute after this. All rabbits very active and energetic. Water and bone transferred from cage.

Fentanyl: #01 placed at 10:35 A.M. with each rabbit placed shortly after. All rabbits energetic and moving around except #02 who had just received patch readministration. Water and bone enrichment transferred from cage. #16 tried to escape one time.

All Morphine rabbits appear very lethargic and exhibit slowed activity and breathing at 11:21 A.M. about one hour after dosing was complete.

5:10 PM update: JMA went to NEOMED to retrieve rabbit jackets from Dr. Dannemiller. JMA and Reed placed jacket on number 2. He is not happy, but will control patch disturbance. Will need to monitor him.

All other animals have returned to normal after dosing.

Date: 5/04/19
Personnel: Gina, Adam
Notetaker: Gina
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (kale and papaya tablets), health checks, morphine dosing day, fentanyl dosing day

Temperature: 65.5 F
Humidity: 55.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: 05 and 11 did not receive water overnight (it leaked out),

Vet Checks Requested?: N/A

Any Additional Notes: 02 seems upset about the jacket (otherwise, behavior is normal)

Exercising: no exercising

Control: 05 had an empty bottle (appeared to leak overnight), 05 had a little food leftover
Morphine: 11 had an empty bottle (appeared to leak overnight): 08, 10, 11, 17, 21 had most of their food leftover, 08 also did not eat the spinach enrichment food
Rabbit #21 had a fair amount of eflux out of the injection site. All rabbits showing signs of sedation by approximately 20 minutes after dosing, maintained until we left the rabbit room.

Fentanyl: 01, 02 had most of their food leftover
All fentanyl rabbits were trying to scratch off the tegaderm so we put a vest on all of them. The rabbits do not appear to be showing the signs of sedation previously demonstrated on day one of dosing.

Date: 5/05/19
Personnel: JMA, Abbie
Notetaker: Abbie
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (kale and papaya tablets), health checks, morphine dosing day, saline controls
Temperature: 66.5 F
Humidity: 55.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: 01, 09, 05, and 20 did not receive water overnight (it leaked out), they started drinking a lot when it was refilled this morning. A buildup of food and water was also noted at the bottom of 01's food trough, this is likely from the water leaking, it was cleaned with a cavi wipe. 09 has diarrhea and 15 still has diarrhea. 20 did not eat his enrichment kale yesterday, it was replaced with a carrot today. 18 managed to wiggle one leg out of his vest, we put the vest back on him properly. Rabbit 02 has low fecal output. Rabbits 02, and 03, have lots of food left from yesterday. Rabbit 16 seems nervous.

Vet Checks Requested?: N/A

Any Additional Notes:

Exercising: no exercising

Control: All vests were checked and zippers fixed if needed.
Morphine: First injection given at 8:57, rabbits began showing effects such as slight sedation within 20 minutes. 10 eflux noted upon dosing.
Fentanyl: All vests checked and zippers fixed if needed.

Date: 5/06/19
Personnel: Gina, Adam
Notetaker: Gina
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (carrot and papaya tablets), health checks, morphine dosing day, saline controls, exercise, weight checks
Temperature: 66.0 F
Humidity: 48.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): morphine rabbits very active in pens prior to dosing, 15 slipped out of vest (placed a new vest on him), 17 sprayed urine on the floor

Health Concerns:.

Vet Checks Requested?: N/A

Any Additional Notes:

Exercising: all rabbits exercised for 45 minutes, control group in at 8:30, morphine group in at 9:30, fentanyl group in at 10:20

Control: 15 slipped front feet out of vest, all rabbits active and playing with shavings.
Morphine: 17 sprayed urine on floor, all rabbits fairly active.
Fentanyl: 09 diarrhea on vest, 19 right eye clear ophthalmic discharge and fur loss on rear left leg (vet check filled out), #s 02 and 03 both slipped a foot out of the vest in the exercise pen. All rabbits chewing on vests in pens and not really moving.

Date: 5/07/19
Personnel: Gina, Reed
Notetaker: Reed
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (spinach and papaya tablets), health checks, morphine dosing day, saline controls, fentanyl patches
Temperature: 66.0 F
Humidity: 57.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): All rabbits displaying normal behavior before dosing and the morphine group is exhibiting their usual post-dose lethargy.

Health Concerns: #7 healing scratches nicely. Flaky scabs, but inflammation is down

Vet Checks Requested?: N/A

Exercising: N/A

Any Additional Notes: 18 is VERY energetic in trying to get out of the vest and off table when reapplying patches. 18 was reshaved with the 40 blade to allow patch to stick better. 2 also is fighting us on

---

Date: 5/08/19
Personnel: Abbie, Adam
Notetaker: Abbie

Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (spinach and papaya tablets), health checks, morphine dosing day, saline controls, and exercise.
Temperature: 65.5 F
Humidity: 44.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): All rabbits displaying normal behavior before dosing and the morphine group is exhibiting their usual post-dose lethargy.

Health Concerns: #02 and 19 water bottles empty, leaked again so springs seem to not help this issue.

Vet Checks Requested?: N/A

Exercising: all rabbits exercised for 45 minutes. First rabbit from control group placed at 8:05. First rabbit from morphine group placed at 8:55. First rabbit from fentanyl group placed at 10:00 A.M.

Any Additional Notes:
Control: #04, 06, 14, 18 had a little food left, #05 had minimal urine/fecal output, #15 still has diarrhea

Ethernet port installed from 1:00-3:00 PM in 212.

Morphine: #08, 10, 11, 12, 17, 20, 21 had a little food left, #08 and 21 also had low fecal output. Rabbit #10 does have some urine on his right side fur (dried) on the shoulder. He appears to have been sprayed by one of the other rabbits (perhaps while in the exercise pens).
Fentanyl: #03 had a little food left

Date: 5/09/19
Personnel: Reed, Adam
Notetaker: Adam
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (kale and papaya tablets), health checks, morphine dosing day, saline controls.
Temperature: 67.0 F
Humidity: 66.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: 19 still has ophthalmic discharge, no worse than previous days.

Vet Checks Requested?: N/A

Exercising: N/A

Any Additional Notes:

Control: 5 had low fecal and lots of food left. 6 had slightly low fecal output. 14 had a lot of food and low fecal and urine output. Water bottle sipper was stuck and he couldn't get water overnight. This was remedied with a spring today.
Morphine: 8, 10 low fecal. 12 had no water overnight, the bottle is now lifted with a spring. 17 had slightly low fecal output
Fentanyl: 2 had a leaky bottle, which has been fixed.

Date: 5/10/19
Personnel: Reed, Gina
Notetaker: Reed
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (kale and papaya tablets), health checks, morphine dosing day, fentanyl, saline controls, exercise
Temperature: 65.5 F
Humidity: 65.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): No changes in behavior. Rabbits seem to prefer the new jackets, still chewing, but not as determined.

Health Concerns: 19 still has ophthalmic discharge, no worse than previous days.

Vet Checks Requested?: N/A

Exercising: Controls: Placed at 8:30. Removed at 9:15… Morphine: Placed at 9:40. Removed at 10:25… Fentanyl: Placed at 10:45

Any Additional Notes:
Reed installed shelf for AVTECH system in afternoon. System working now.

18 had many cecotropes in his cage that he didn't eat

---**Date:**
5/11/19
Personnel: Reed, Gina
Notetaker: Gina
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (spinach and papaya tablets), health checks, morphine dosing day, saline controls
Temperature: 65.0 F
Humidity: 43.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): No changes in behavior. Rabbits have been chewing the new vests (07, 01, 06, and 19 especially). The vests can slide if not tight enough underneath (exposing the patch). Rotated some of the vests

Health Concerns: 19 still has ophthalmic discharge, no worse than previous days.

Vet Checks Requested?: N/A

Exercising: N/A
Any Additional Notes:
11 and 13 had empty bottles upon arrival. 13's bottle was on the ground and 11's leaked out. A lot of rabbits have been biting the sipper. Many bottles are completely full (indicating inability to drink). I (Gina) have noticed that the rabbits do not like kale (prefer spinach and carrot). Additionally, many do not eat the chow and eat the enrichment food right away.

Date: 5/12/19
Personnel: Reed, JMA
Notetaker: JMA
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (spinach and papaya tablets), health checks, morphine dosing day, saline controls

Temperature: 66 F
Humidity: 53.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): No changes in behavior. Rabbits seem to prefer the new jackets, still chewing, but not as determined (except for #2)

Health Concerns: 19 still has ophthalmic discharge, no worse than previous days. #3 slight opthalmic discharge. 15 still has diarrhea on cage pan.

Vet Checks Requested?: N/A

Any Additional Notes:
#2 twisted new vest around and chewed it almost through. Patch still on animal but loosened around edges and not well adhered. Replaced his vest with a jacket from NEOMED and his fentanyl patch.

#3 vest partially chewed. Should be replaced with a new one during fentanyl patch application tomorrow.

Remnants of adhesive on fentanyl animals. Need to reshave application area.

#4 and #11 water bottles completely empty overnight. Need to remedy this issue ASAP.

#10, 11, 17 had a lot of food leftover. #21 had a moderate amount. All morphine animals.

Morphine animals exhibiting usual post-injection lethargy.

Date: 5/13/19
Personnel: Reed, Gina
Notetaker: Gina
Time In: 8:00 AM
Tasks Performed (Dosing, Exercise, etc.): Enrichment (spinach and papaya tablets), health checks, morphine dosing day, saline controls, fentanyl, exercise, weighing
Temperature: 65.5 F
Humidity: 53.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: 19 and 3 still have ophthalmic discharge, no worse than previous days.

Vet Checks Requested?: N/A

Exercising: Controls places at 8:10. Removed at 8:55. Morphine placed at 9:10, removed at 9:55. Fentanyl placed at 10:05, removed at 10:50.

Any Additional Notes:
No empty bottles today. 13 and 17 tried to jump out of exercise pens. All rabbits have very long nails, may need trimmed soon. All morphine rabbits took injections well, all are lounging but don't seem too sedated. All the patch controls rubbed their patches off without removing the vests. All the fentanyl rabbits still had patches. Tried the new Tegaderm on the controls and smaller patches to see if this solves the problem.

All fentanyl rabbits reshaved as best as possible.Gummed up fur made it difficult.

Date: 5/14/19
Personnel: JMA, Adam
Notetaker: JMA
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment (spinach and papaya tablets), health checks, morphine dosing day, saline controls
Temperature: 66 F
Humidity: 45.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: #15 still exhibiting diarrhea and a messy cage pan, #19 still has watery eye discharge, consistent with the past week. #3 eye has cleared up. #2 nas chewed his fur around the forelimbs, but the area does not look irritated or inflamed.

Vet Checks Requested?: N/A

Any Additional Notes:
Slightly low fecal output for #6, #9, #11, #18
#21 quite low fecal output
#20 dark urine colour
#18 cecotropes fell through cage
#3 and #19 had to be placed in jackets since they chewed through the straps of the Amazon vests.

Rabbits erratic behaviour during calcein injections, especially the patch control animals. All have been difficult to handle, and some need to be held while injection is being administered.

#3 panicked during calcein injection and kicked things off the table, was not happy.

Date: 5/15/19
Personnel: JMA, Adam
Notetaker: Adam and JMA
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment (spinach and papaya tablets), health checks, morphine dosing day, saline controls, calcein #2 dosing day
Temperature: 66.0 F
Humidity: 44.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: #19 ophthalmic discharge. #15 recurring diarrhea.

Vet Checks Requested?: N/A

Any Additional Notes:

Most rabbits exhibited fluorescent orange urine following the calcein injections. This turned green in certain cases where the water bottles leaked.

Rabbit #06 had a fair amount of eflux of calcein during injection (due to large flinch) so we readministered 0.4mL of calcein on the contralateral side. Fur slightly stained orange.

#7 did not tolerate calcein injection well -- flinched very hard and calcein was expelled. Tried a second injection of 0.6 mL and he also flinched and it was expelled. May not have much that was successfully injected for day 2 of calcein. Please check urine tomorrow to see if orange to assess dosage. Fur is also stained orange.

#21 also fought calcein injection. Had to administer this in two parts, but there was minimal eflux.

Remaining food for: #1 (little), 3 (little), 5 (little), 6 (little), 8 (little). 9 (little), 10 (little), 11 (a lot) and papaya tablet, 16 (little), 17 (a lot), 20 (little), 21 (lots).

Very low fecal output for #21, second day in a row. Keep an eye on this.

Rabbits are also exhibiting signs of sexual maturity -- more aggression, urine spraying in the exercise pens, and #7 had an erection while we were administering injections.

Exercise: Placed the first group at 8:15 A.M. starting with rabbit #04
Placed the second group at 9:05 A.M. starting with rabbit #08
Placed the third group at 10:00 A.M. starting with rabbit #01
Transferred enrichment bowls instead of the rattles**.** Most rabbits seemed to enjoy the pens and played in the aspen, chewed it, etc.

Date: 5/16/19
Personnel: Abbie, Reed
Notetaker: Abbie
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, TD patches, saline controls, patch controls

Temperature: 65.0 F
Humidity: 55.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: 3 and 19 both still have their ophthalmic discharge, but not worse than the previous days. The rubbing of fur from the vests appears no worse than previous. No irritated or inflamed skin on 19 or 2 where the rubbing of the vest. Andronowski team was never informed where vet tape was going to be left for us to apply to these rabbits' vests. Could not apply today for this reason.

Vet Checks Requested?: N/A

Any Additional Notes: Attempted to shave the fentanyl rabbits where there was gummed up fur. It worked for a good number of them, however some got too stressed and fought so we had to stop before we could get all the old adhesive off. There was enough new fur to get the clippers underneath the gummed fur.

All rabbits were a bit jumpy from needles, but no issues with administering the dose to them. Just more timid of the needles, likely due to the calcein injections the previous two days. Most rabbits have bright orange urine from the calcein. The rest appear to have little urine output. Noted in log book.

Little food left: 1, 9, 12, 20
Lot food left: 3, 10, 11, 17, 21
Low fecal: 6, 17, 20, 21

JMA: I figured they would be a little jumpy today with the needles post-calcein injection (especially the controls). Did #6/7 have orange urine?
RAD: 6- somewhat orange. 7 VERY orange.
Thanks, Reed! This is good because it indicates at least some dye was successfully injected (especially 7).

Date: 5/17/19
Personnel: Abbie, Adam
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, exercise, saline controls, enrichment foods

Temperature: 66.0 F
Humidity: 67.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): 12 especially hyperactive today and is pacing.

Health Concerns: 3 and 19 both still have their ophthalmic discharge, but not worse than the previous days. Andronowski team was never informed where vet tape was going to be left for us to apply to these rabbits' vests and still could not be applied today for this reason.

Rabbit 02 ingested his fentanyl patch and is displaying extreme lethargy as well as respiratory distress, elevated heart rate, and abdominal distension. Dr. Dannemiller was was called 6 times and a voicemail was left. Dr. Lou was contacted and advised that the rabbit is displaying signs of an overdose and it is unlikely he will survive and to euthanize the rabbit. JMA and Reed arrived by ~11:30. Upon calling Dr. Lou a second time to update that the rabbit's health is slightly improving he advised to continue to observe the rabbit and if he worsens again to euthanize. Dr. A made the decision to wait until Dr. Dannemiller calls us back and can do a physical assessment of the rabbit before proceeding with

euthanizing. After about 3 hours post injection rabbit 02 seems to be acting normal and is not exhibiting as great affects from the patch injection. His sclera is noted to be red, likely from stress. Treatment plan is to observe him and give him extra enrichment foods to increase intestinal motility. Dr. Dannemiller says that the amount of fentanyl ingested is not enough to be fatal. We will continue to observe rabbit 02 to make sure there is no intestinal blockage.

We are also unable to access the drugs in the safe due to the safe battery likely being dead, for this reason morphine dosing was delayed today. Rabbits were dosed at 12:45pm. Dr. Bagatto brought the back-up key so we could open the safe until the battery can be replaced.

Vet Checks Requested?: N/A

Exercise: Placed the first group at 8:15 A.M. starting with rabbit #04
Placed the second group at 9:00 A.M. starting with rabbit #08
Placed the third group at 9:45 A.M. starting with rabbit #01
Transferred enrichment bowls instead of the rattles**.**

Any Additional Notes:
15 still experiencing some diarrhea**.**
Little food left: 05, 10, 11, 12, 16, 17, 21
Lot food left: 08
Low fecal 05, 06

Date: 5/18/19
Personnel: Gina, Reed
Notetaker: Gina
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, saline controls, enrichment foods, health checks

Temperature: 65.5 F
Humidity: 55.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: Monitoring 2 for passing of patch. Nothing yet, but fecal output is normal as is behavior.

Vet Checks Requested?: N/A

Exercise: N/A

Any Additional Notes:

15 still experiencing some diarrhea**.**
Little food left: 5, 8, 9, 10, 12, 16, 17,
Lot food left: 7, 11, 18, 20, 21
Low fecal: 5, 6, 18 (slight)
4 and 16 pulled the sippers out of their bottles and were without water overnight. We refilled and replaced.
6 chewed the neck portion of his harness off, so we replaced with a new one.

Date: 5/19/19
Personnel: Adam, Reed
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, fentanyl dose, saline controls, enrichment foods, health checks

Temperature: 67.0 F
Humidity: 67.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: Monitoring 2 for passing of patch. Nothing yet, but fecal output is normal as is behavior.

Vet Checks Requested?: N/A

Exercise: N/A

Any Additional Notes:
16 pulled his sipper out of his bottle and was without water overnight. Even with zip ties.
Replaced harnesses with vests on rabbits 1 and 9 because they chewed through it.
Replaced harnesses on 15 and 18 because they chewed through the neck straps. They are each wearing new pink vests. We are now out of replacement vests for the controls. All that's left are the NEOMED jackets.

All the fentanyl rabbits were reshaved.

Date: 5/20/19
Personnel: Adam, Gina

Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, saline controls, enrichment foods, health checks, weighing

Temperature: 66.0 F
Humidity: 65.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: Recurring monitoring of #02 for passing of patch. Nothing yet, fecal output is normal, as is behavior.

Vet Checks Requested?: N/A

Exercise:
Controls went in at 8:15 A.M. starting with rabbit #04. The enrichment bowls were transferred as toys for all rabbits.
Morphine rabbits went in at 9:15 A.M. starting with rabbit #08.
Fentanyl rabbits went in at 10:10 A.M. starting with rabbit #01.

Any Additional Notes:
New springs are here. We used these on the exercise pens and on some of the cages. No bottles had leaked. All rabbits same health condition as previous day. Still no patch remnants in #02 feces. Rabbit #03 posture has normalized some. Rabbit #20 was humping the table during dosing. Rabbit #08 sprayed out of his exercise pen on the floor. Rabbit #15 had escaped his vest so this was reattached. Rabbit #10 is still fairly aggressive when handled. We filled up the last pink slip for Morphine bottle #2, put this on Beth's door and started a new slip.

Some rust was noted on the morphine group battery at the top of the cage, and also on #8's cage latch.

JMA 1:00 P.M. update: Rabbit #2 is now able to remove the fentanyl patch from underneath the jacket. Cathy noted it to be off during pan cleaning. It appears he is chewing the forelimb holes enough to loosen the vest and then is somehow getting underneath it. Reed and I re-shaved the patch area and replaced the patch and secured vet wrap around the abdomen to add an additional layer. We also replaced his jacket for one that was not extensively chewed. Will need to monitor him closely until pillow collar arrives on 5/21/19.

AC unit was also installed by PFOC and seems to be lowering humidity/temp already. Will need to empty the water reservoir daily.

Date: 5/21/19
Personnel: Abbie, Reed
Notetaker: Reed
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, saline controls, enrichment foods, health checks

Temperature: 64.5 F
Humidity: 44.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: Recurring monitoring of #02 for passing of patch. Nothing yet, fecal output is normal, as is behavior.

Vet Checks Requested?: N/A

Exercise: N/A

Any Additional Notes: Vet wrap is still on 2. He seems to be less irritated by it, or at least to have claimed defeat with it. All rabbits acting normally. Morphine rabbits calm, but not heavily sedated looking.

Little food left: 10, 11, 20, 21
Lot food left: 3
Low fecal: 3
Low urine: 11

JMA afternoon update: Pillow collar arrived for #2 as did the extra jackets from Lomir. Reed and I placed the collar on #2 at 2:30 P.M. We observed him eating/drinking with the collar, even though he was displeased with it. We will observe him closely to make sure he continues to eat/drink, etc.

Date: 5/22/19
Personnel: Abbie, Adam
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, saline controls, enrichment foods, health checks, exercising, fentanyl dosing

Temperature: 65.0 F
Humidity: 45.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: 01 has an abrasion where his patch is usually placed, Dr. Lou has been contacted. The abrasion is approx an inch in length and is an open sore.

Vet Checks Requested?: 01 for abrasion

Exercise: Cage Card holders are still missing from all cages except pen #1.
Controls: #04 placed at 8:10 A.M.
Morphine: #08 placed at 9:00 A.M. (glass water bottles fall off exercise pens so thick/tight springs must be used.)
Fentanyl: #02 placed at 9:50 A.M. (rabbit #01 originally not placed until 10:15 A.M. - removed at 11 A.M.)

JMA: I would inform Beth this morning about the missing card holders*
ARL: I just slacked her, thank you
Update: cage cards placed on pens at 8:40

Rabbit #01 had an abrasion on the dorsal interscapular region. Consulted with Dr. Lou the attending veterinarian via text message and he advised to remove patch and treat with topical antibiotic ointment. We were instructed to keep the vest and all coverings off of the rabbit and leave this open to heal. Will check later in the day to ensure that this is not being subsequently scratched. Rabbit seems fine after being placed back into cage.

Rabbit #03 is performing a strange behavior with his ears being pinned at the sides of his head and moving his head from side to side in a "scanning" manner. Adam performed a health check, removed jacket, looked in ears/genitals, palpated abdomen and all appears normal.

Any Additional Notes: #02 does appear to have a slight bulge to his eyes. This is attributed likely to the collar administration. Attempted to remove feces from collar using cavi wipe.

Low fecal: 03, 21,05,06, 08
Little food left: 21,05,04,14,12,17
Lots of food left: 03, 08, 10,11

Date: 5/23/19
Personnel: Abbie, JMA
Notetaker: Abbie
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, saline controls, enrichment foods, and health checks.
Temperature: 67.0 F
Humidity: 69.0% at 8:00 A.M. after adjusting AC unit the humidity went down to 60% at 9:30.

Changes in Behavior (Lethargy, Hyperactivity, etc.): Rabbit 19 urinates in his enrichment food bowl almost everyday.

Health Concerns: 01's abrasion is looking better than yesterday and seems to be healing well, we treated with ointment again today per Dr. Lou's orders. A patch was able to be placed today above the abrasion and vet wrap was placed over the patch but not over the abrasion. No Jacket was placed on 01 so that the abrasion is able to be left open to air for additional healing.
02 still has red eyes, likely from stress.
15 still has his usual amount of ongoing diarrhea
07's vest was twisted, this may have been why he did not eat his usual amount, a new vest was placed on him and his behavior went back to normal.
03 was observed to flop his ears around from side to side, looks somewhat dazed. We will continue to keep an eye on him.
Little food left: 01, 03, 06, 10, 11, 14, 17, 21
Lots of food left: 07
Low fecal: 06, 21
2cd vial of morphine finished off today, vial 3 was used to dose 20 and 21.

Vet Checks Requested?: 01 for abrasion

Exercise: N/A

Date: 5/24/19
Personnel: Adam, Reed
Notetaker: Reed
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, saline controls, enrichment foods, exercise, and health checks.
Temperature: 67.0 F
Humidity: 57.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): 2 kicked his collar off as we were removing him from the exercise pen. Reapplied the collar. He is not pleased, but it's staying on so far.

Health Concerns: 3 has recurring low fecal output. We should monitor. #1's abrasion is looking much better. Applied ointment and a new patch as he had curled it off himself. Re-vet taped and put him in a vest to deter chewing.

Vet Checks Requested?: No new ones.

Exercise: Controls placed at 8:10 A.M. beginning with #04, removed at 8:55. Morphine placed at 9:00 A.M. beginning with #08. Removed at 9:45. Fentanyl placed at 9:55 A.M. beginning with #01. Removed at 10:40

Additional Notes: 4 ripped the sipper out of his bottle and had no water overnight. Refilled at 8:05.

Date: 5/25/19
Personnel: Adam, JMA
Notetaker: JMA
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, saline controls, enrichment foods, fentanyl patches, and health checks.
Temperature: 67.5 F
Humidity: 66.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: 3's behaviour appears normal today, and a higher fecal output. #1's abrasion is looking much better. Applied ointment as per Dr. Lou.

All fentanyl rabbits except 2 and 19 had very gummy fur and mild skin inflammation. Ointment applied to all others and patch adhered to an alternative area that was unaffected.

#2 and 9 had dry and flaky skin underneath patch areas.Could be from lack of self grooming.

Vet Checks Requested?: No new ones.

Exercise: N/A

Additional Notes: 4 pulled out rubber plunger around 8:30. Refilled at 8:35. #2 feces on collar, cleaned with cavi-wipe.

Reshaved all controls/fentanyl animals and trimmed gummy hair as best as we could.

A lot of food left: 3
Little food left: 1, 6, 8, 9, 10, 11, 17, 21
Low fecal: N/A

Date: 5/26/19
Personnel: Adam, Gina
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Morphine dose, saline controls, enrichment foods, and health checks.
Temperature: 66.5 F
Humidity: 71%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: No new ones.

Exercise: N/A

Additional Notes: #04 pulled out rubber plunger overnight and had no access to water. Refilled at 8:10 A.M. He did drink quite a bit of water right away.

#07 unzipped his vest and had this completely off. We re-zipped this and made sure clips were fastened.

Checked on the rabbits with ointment administration yesterday. All skin looks very good in condition. We trimmed some gummy hair off of each of the rabbits with this.

A lot of food left: 03, 09,
Little food left: 02, 05, 06, 10, 11, 16, 18, 21
Low fecal: 03, 06

Date: 5/27/19
Personnel: JMA, Reed
Notetaker: Both
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): exercise, morphine, saline controls.
Temperature: 64.5
Humidity: 64.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): 3 flattening ears and dragging cranial half of body against cage floor. Fur condition is not great. We created a vet check form and JMA will follow up with Stan.

Health Concerns: 3 displaying unusual behavior.

Vet Checks Requested?: Yes, for 3.

Exercise:
Controls weighed and went into pens at 8:10.
Removed at 8:55.
Morphine weighed and went into pens at 9:55.
Fentanyl group weighed and placed at 10:05

Additional Notes: 21's papaya tablet fell through the bottom of the cage.
3 has lost weight and continues to display a change in behavior. Changed his vest since he almost chewed through the new one.

Date: 5/28/19
Personnel: JMA, Reed, Adam, Abbie
Notetaker: Reed/Abbie
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.):
Temperature: 66.5 F
Humidity: 72.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): 03 has diminished health and is acting lethargic

Health Concerns: Rabbit 03 is diminished in flesh condition and very rough in coat condition suggesting poor overall health. Still demonstrating stress behaviors and diminished appetite as well as fecal output. He is receiving twice the normal amount of enrichment food per Dr. Dannemiller's email response. All fentanyl rabbits received ointment on their red skin.
15 still has ongoing diarrhea
Little food left: 06, 08, 09, 11, 12, 13, 21, 03
Low fecal: 05, 14
Lots of food left: 17

Vet Checks Requested?: Dr. Dannemiller contacted yesterday via email and we requested that he come evaluate 03, however he responded this morning stating that he might be unavailable today.

Exercise: N/A

Additional Notes: Animals that had eflux after calcein dosing: 08, 15, 09
All animals seem more lethargic then they have been recently post morphine dosing.

New NEOMED vest for #19 since he chewed through the new Lomir one already.

Date: 5/29/19
Personnel: Adam,Abbie
Notetaker: Adam/Abbie
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Calcein, exercise, enrichment foods,health checks, morphine doses and saline controls.
Temperature: 65F
Humidity: 69.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): 03 seems more lethargic than yesterday. Holding one ear up and one ear down and head is between two front legs laying on cage bottom.

Health Concerns:
04 and 05 did not have water when we arrived due to leaking, refilled at 8:20 A.M.
01 still has very bulgy eyes.

Vet Checks Requested?: 03 requested still waiting for Dr. Dannemiller to come on Thursday or Friday.
01 requested today for ocular proptosis.

Exercise:
Control group placed at 8:15 starting with 04
Morphine group placed at 9:05 starting with 08
Fentanyl group placed at 9:55 starting with 01
All rabbits had jingle ball, water bottle, and cage card moved to exercise pen.

Additional Notes:
Low fecal: 01, 03, 05, 06, 14, 16, 17, 21.
Little food left: 08, 09, 10, 11, 12, 13, 17, 19, 21.
Lots of food left: 16, 03.
15 has ongoing diarrhea, 17 has a small amount of cecotropes on the cage floor.

All control and morphine rabbits received full dose of calcein and had no efflux.

JMA spoke with Dr. Dannemiller at 10:15 A.M regarding rabbit #3. He prescribed 50-100 mL of sterile saline to be administered via SubC injection, and additional enrichment foods. He will also be coming to UA to do a health check between 4:00-6:00 P.M. today.
Upon trying to inject 03 with the saline we pushed 30mL of saline however some efluxed out due to it being such a large volume transdermally, the rabbit probably got approx 20mL of saline. We tried three different sites to push the saline. A spot was shaved near the animals rear for one of the spots. This animal also had a large amount of calcein efflux.

Rabbit #03 was re-weighed in the presence of Dr. Dannemiller and his new weight was 2.66 kg.

Date: 5/30/19
Personnel: Adam, Reed
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods, health checks, morphine dosing and saline controls
Temperature: 66.5 F
Humidity: 61.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): 03 seems to be doing better today. He did flip the bowl with extra enrichment food over and spilled most of this through the bottom of the cage

Health Concerns: N/A

Vet Checks Requested?:

Additional Notes:
Low fecal: 1, 3 (Slightly), 5
Little food left: 1, 3, 5, 11, 16, 17, 21
Lots of food left:
15 has ongoing diarrhea
7 displayed a hunched posture for the duration of the time we observed him, could be due to the vest

Trimmed nails on all morphine rabbits and saline control rabbits.
Rabbit 17 does now have chewed hair again on his front paws.

Date: 5/31/19
Personnel: Adam, Reed
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods, health checks, morphine dosing and saline controls, exercise, fentanyl dosing
Temperature: 65.0 F
Humidity: 61.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): 03 seems to be doing better today, normal fecal output and food consumption. 07 still a little lethargic and displaying hunched posture.

JMA: What is up with 07? Adam, can you give him a quick health assessment? Sometimes he twists himself up weirdly in his vest.
He just seems overall very upset with the vest. We looked him over an found no abnormalities, he just seems depressed. Thank you!

Health Concerns: N/A

Vet Checks Requested?:

Additional Notes:
Control rabbits started at 8:10, removed at 8:55. Morphine rabbits placed at 9:05, removed at 9:50. Fentanyls placed at 10:00, removed at 10:50

All control patch rabbits had nails trimmed and all morphine rabbits were re-shaved.
All fentanyl rabbits reshaved. There was a small nick on rabbit #03 from attempting to remove the adhesive.

We also did have one patch that was pulled up by the application of the tegaderm and this rabbit had to have a second patch applied. #16 was this rabbit.

Low fecal: 07
Little food left: 11, 13, 17, 21
Lots of food left: 07

Date: 6/1/19
Personnel: Adam, Gina
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods, health checks, morphine dosing and saline controls
Temperature: 65.0 F
Humidity: 61.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): #07

Health Concerns: 07 is very lethargic today and is not eating or drinking. He has very low fecal and urine output. We will put more enrichment food in his pen for him. I think that he is having the same type of symptoms as #03 when he was in the brunt of his issues.

Vet Checks Requested?: #07

Additional Notes:
Low fecal: 07 (very low fecal and urine output), 16, 21 (slightly)
Little food left: 01, 08, 10, 11, 17, 19, 21
Lots of food left: 07, 13, 16

Date: 6/2/19
Personnel: Adam, Reed
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods, health checks, morphine dosing and saline controls
Temperature: 65.5 F
Humidity: 60.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: #07 has very low fecal output for the second day in a row. He was given a large amount of enrichment food yesterday to attempt to entice him to eat; however, this does not appear to have been touched. Upon inspection today, we observed a large abnormality in the gum tissue on this animal's left lower lip and in the mouth surrounding lower left incisor. Documented and sent to veterinary staff.

Vet Checks Requested?:

Additional Notes:
Low fecal: 05, 07 (very low)
Little food left: 03, 05, 08, 09, 10, 11, 13, 16, 17, 20, 21
Lots of food left: 07

Date: 6/3/19
Personnel: Adam, Gina, Mary Beth
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods, health checks, morphine, fentanyl dosing and saline controls, exercise, weekly weights
Temperature: 64.5 F
Humidity: 44.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): #07 has perked up, eaten his food, and passed normal amount of fecal output.
Health Concerns:

Vet Checks Requested?:

Additional Notes:
Low fecal:
Little food left:
Lots of food left:

Exercise:
Control: Starting with animal #04 placed at 8:10 A.M. Moved cage card, jingle ball, and water bottle with each rabbit. Animal #18 was chewing on the AC cord sticker. In future exercise sessions, make sure his cage is moved out from this.
#12 chewed his jingle ball and there are rough edges.
Morphine: Starting with animal #08 placed at 9:00 A.M.
Fentanyl: Starting with animal #01 placed at 9:50 A.M.

Rabbit #19 was very aggressive today. He did attempt to bite at the researchers.
Handle with caution!!

Update: #2 appears to have eaten his fentanyl patch as he was without one and the patch could not be located when researchers checked. He appeared more heavily sedated than usual after new patch placement. Two new collars and vet wrap were used to secure the new patch. AJS and JMA checked on #2 at 2:30 P.M. as per the UARV staff request. The tightness of the wrap was checked and adjusted. He appeared sedated but fine.

Date: 6/4/19
Personnel: Reed, MBC
Notetaker: Reed
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods, health checks, morphine, saline controls
Temperature: 66.0 F
Humidity: 46.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): #1 has cecotropes that he didn't eat.

Health Concerns: #7's gums look like they're healing from lesion. Fecal output was normal and ate all his chow. #2 still has patch on. Seems more docile, maybe still high from eating the other patch? Still behaving normally, grooming, eating, drinking, etc.

JMA: Thank you! We should keep an eye on #2 today since his fecal is low/some food left.

Vet Checks Requested?: N/A

Additional Notes:
Low fecal: 2, 14, 11 (slightly), 14, 17 (slightly)
Little food left: 2, 3, 5, 11, 17, 20, 21
Lots of food left: NO ONE!

Date: 6/5/19
Personnel: Gina, Mary Beth
Notetaker: Mary Beth
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (celery, papaya tablet), health checks, morphine dosing and saline controls, exercise
Temperature: 65.0 F
Humidity: 66.0%

Changes in Behavior (Lethargy, Hyperactivity, etc.): None

Health Concerns: #1 did not eat cecotropes again (same as yesterday 6/4); #15 has two small patches of diarrhea (same as 6/1 and 6/2)

Vet Checks Requested?: None

Additional Notes:
Low fecal: #11, #14, #17, #21
Little food left: #3, #5, #6, #10, #11, #17, #20, #21
Lots of food left: None

Exercise:
Control: Starting with animal #04 placed at 8:40 A.M. Moved cage card, jingle ball, and water bottle with each rabbit.
Morphine: Starting with animal #08 placed at 9:40 A.M.
Fentanyl: Starting with animal #01 placed at 10:35 A.M.

#6 unzipped his jacket (zippers locked - they must have unzipped together) and ate the control patch - replaced with new control patch
Update - he started to unzip it in the exercise pen - jacket replaced with backpack NEOMED jacket to reduce ability to unzip

#2 removed one pink collar by chewing off the plastic bobble. He chewed the bobble off the other but the second collar remained around his front legs. Fentanyl patch checked and is holding fined under the green vet wrap. Both bobbles were found in the fecal tray (probably just fell through, didn't appear eaten or encased in feces) - one was too far back in the back left corner to retrieve. Both collars were replaced via velcro and their strings tied together over the collars. #2 behavior normal although disliked replacement of collars and stood on table, wanting to jump around. His eyes are bulging and red as previously. Replaced pink collar with large white collar.
Update 10:33 am - after placement of white collar, #2 seems calm and his eyes are no longer bulging or red

#19 chewed through neck strap on jacket - replaced with new jacket. Old jacket laundered to be returned. Fentanyl patch in place.

Date: 6/6/19
Personnel: Reed, Adam
Notetaker: Reed
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, papaya tablet, hay cube), health checks, morphine dosing and saline controls, fentanyl patches/control patches

Temperature: 65.0 F
Humidity: 63.5%

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: None

Additional Notes:
#2 has very dry skin whenever reapplying patches. Use the big tegaderm on him as it sticks better. Using the custom cut tegaderm, it doesn't stick to to his skin and pulls the patch up so it has to be wasted.

Low fecal: N/A
Little food left: N/A
Lots of food left: N/A

Date: 6/7/19
Personnel: JMA, MBC
Notetaker: JMA
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, papaya tablet, hay cube), health checks, morphine dosing and saline controls, exercise

Temperature: 63.5 F
Humidity: 66.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:
4, 5, 2 water bottles leaked but not to the point of being empty.

We checked 2 to see if his patch is holding. He displaced his vet wrap and the patch, but it was still adhered. Patch replaced and vet wrap replaced as well.

17 had some fur chewed off on his left front paw, similar to what was noted for him previously.

19 still has opthalmic discharge.

20 has two scrabs from past injections but these are healing.

21 had papaya tablet left over from yesterday.

Exercise:

Controls in the pens at 8:15, removed at 9:00
Morphine in the pens at 9:10 and removed at 9:55
Fentanyl in the pens at 10:00 and removed at 10:45

Little food left: 5, 6, 8, 10, 11, 15, 17, 21
Lots of food left: None
Slightly low fecal: 6, 17

Date: 6/8/19
Personnel: JMA, AJS
Notetaker: JMA
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, papaya tablet, hay cube), health checks, morphine dosing and saline controls

Temperature: 65.0 F
Humidity: 60 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:

#21 has a puncture injury from 06/07/19 from an attempted morphine injection. The area has a small contusion surround the site. Antibiotic ointment was administered. We will keep an eye on this.

#2 and #3 patches are still on and vet wrap is secured.

Morphine and saline control rabbits need to be reshaved prior to calcein

Little food left: 9, 11, 15, 17
Lots of food left: None.
Low fecal: None.

Date: 6/9/19
Personnel: Reed, Gina
Notetaker: Reed
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, papaya tablet, raisins), health checks, morphine dosing and saline controls, fentanyl placement

Temperature: 65.0 F

Humidity: 61.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: 10 jumped out of GIna's arms when putting back into cage, hit his head against back wall of his cage. Did a neurologic assessment, does not appear to be any damage. No trauma visible. No changes in behavior or gait.

Vet Checks Requested?: N/A

Additional Notes: All rabbits shaved in preparation for calcein dosing. #13's patch was not well adhered again, tried to shave much closer to the skin this time and applied a patch. Unsure if he is getting his full dose.

Little food left: 1, 2, 3, 5, 6, 8, 10, 11, 12, 14, 17, 20
Lots of food left: 9, 21
Low fecal: 11 (Slightly), 17 (slightly), 18 (slightly), 20 (slightly), 21

Date: 6/10/19
Personnel: Mary Beth, Gina
Notetaker: Mary Beth
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (raisin, dried apricot, papaya tablet), health checks, morphine dosing and saline controls, exercise, weighing

Temperature: 65.5 F
Humidity: 69.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

#21 bit Mary Beth (before even being touched) but did not break skin - did not want to be taken out of cage for exercise - had moderate amount of food left and slightly low fecal so maybe is constipated?
JMA: He often does not eat all of his food/has low fecal. Please assess his puncture wound to make sure this is not worse -- could be a reason for a change in behaviour.

#21's puncture wound has healed, no blood or redness.

Health Concerns: N/A

Vet Checks Requested?: N/A

Exercise:

Controls in the pens at 8:15, removed at 9:00
Morphine in the pens at 9:05 and removed at 9:50
Fentanyl in the pens at 9:50 and removed at 10:35

Additional Notes:

#1 Checked - patch in place, tegaderm replaced to prevent edges from curling up
#2 Checked - patch and vet wrap in place
#3 Checked - patch and vert wrap in place, ointment applied to vet wrap stuck to skin
#9 Checked - patch in place, tegaderm replaced to prevent edges from curling up
#13 was reshaved and a new fentanyl patch (#113) was applied. His patch was not attached at all but still present underneath his jacket. Vet wrap was placed to secure it.
#16 was reshaved and a new fentanyl patch (#112) applied. His patch was found to be half adhered during a check.
#19 Checked - patch in place, tegaderm replaced to prevent edges from curling up

Little food left: #2, #5, #9, #11, #15, #17, #19, #20, #21
Lots of food left: #3
Low fecal: #3 (slightly), #11 (slightly), #12 (slightly), #17 (slightly), #18 (slightly), #20 (slightly - did not eat some cecotropes), #21 (slightly)

Date: 6/11/19
Personnel: Mary Beth, Reed, Adam
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (dried apples, banana chips, papaya tablet), health checks, morphine dosing and saline controls, calcein injections, patch checks

Temperature: 65.5 F
Humidity: 51.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes: Abrasion on 21 completely healed. 6 was reshaved and new tegaderm applied. 10 may have effluxed some of his calcein because he was fighting against us. Retained most of the his dose though. 3 fought the needle for calcein and effluxed a bit of his dose.

Little Food Left: #11, 15

Date: 6/12/19
Personnel: Mary Beth, JMA, Gina, Adam
Notetaker: Mary Beth/JMA
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (toasted O's, apricots, papaya tablet), health checks, morphine dosing and saline controls, calcein injections, patch application, exercise

Temperature: 64.5 F
Humidity: 47.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:

#1 did not eat his apple or apricot from previous enrichment
#14 did not eat banana chips
#17 had three banana chips left but ate most of his food
#9 and #10 has bruised interscapular region and really fought the calcein injection
#13 had to be poked multiple times prior to a successful calcein injection. Bruised area in interscapular region. Antibiotic ointment was applied to the area.
#5 had slight calcein eflux
#15's abdomen feels slightly bloated, but not stiff. Fecal output normal (usual diarrhea) and he ate all of his food.

Exercise:

Controls went into exercise pens at 8:22 am and were removed at 9:07 am
Morphine went into exercise pens at 9:15 am and were removed at 10:00 am
Fentanyl rabbits 1,2,3,9 into exercise pens at 10:10 am and were removed at 10:55 am
*Delay for the others due to patch placement and calcein injections

Fentanyl rabbit 13 in at 10:20 am, removed 11:05
16 in at 10:33 am, removed at 11:18
19 in at 10:41 am, removed at 11:25

Date: 6/13/19
Personnel: Reed, Adam
Notetaker: Reed
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (dried pineapple, banana chips, papaya tablet), health checks, morphine dosing and saline controls, patch checks

Temperature: 65.5 F
Humidity: 63.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:

Little Food Left: #01, 03, 05, 11, 13, 16, 17, 20, 21
Lot of Food Left: #09
Low Fecal: #03, 05, 09 (no fecal or urine), 11

Date: 6/14/19
Personnel: Mary Beth, Reed
Notetaker: Mary Beth
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (banana chips, apricots, papaya tablet), health checks, morphine dosing and saline controls, patch checks, exercise

Temperature: 65.5 F
Humidity: 47.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns:

#9 again barely consumed food or water, and has no urine and very low and small diameter feces output. However, behavior seems normal and he began eating enrichment foods immediately upon placement in bowl.

JMA removed his vet wrap and checked to make sure he does not have infection, worsening of abrasions, etc. ← Done for #9. Bruising has healed and area is looking better. Gummy fur will need to be trimmed tomorrow.

#11 and #16 seem to be in the same lot of food / low fecal / low urine state. However, it is not as low as previously (all had multiple discrete feces, just a lower amount)

#15 seems less bloated than previous days, but not entirely without bloat. Couldn't get a great examination because he was biting.

Vet Checks Requested?: N/A

Additional Notes:

Little Food Left: #01, 05, 08, 10, 13, 14, 17, 20
Lot of Food Left: #03, 09, 11, 16
Low Fecal: #03, 05 (slightly), 09, 10 (slightly), 11, 13, 14 (slightly), 16, 17 (slightly), 18 (slightly), 20 (slightly), 21

Many rabbits seem to have low fecal today
*Does anything seem different in the room? Is it warm/cold, noisy from construction? Any differences?
No room changes

#4 dumped water into cage overnight, without water for undetermined time. Water refilled immediately.

#8 did not eat dried pineapple from yesterday (2 pieces) - all other rabbits consumed enrichment foods

#9 Given extra enrichment food since he is not eating his regular food.

Exercise:

Controls went into exercise pens at 8:20 am and were removed at 9:05 am
Morphine went into exercise pens at 9:15 am and were removed at 10:00 am
Fentanyl rabbits into exercise pens at 10:05 am and were removed at 10:50 am

Fentanyl Patch Checks:

No patches needed to be replaced

#1 - patch in place, no bruising or abrasion

#2 - patch in place. Margin of patch and vet wrap was red but skin not broken - antibiotic ointment applied

#3 got totally out of his vest except the neck band and was chewing it, but fentanyl patch was intact under the vet wrap.

#9 checked - patch in place, bruising coloration close to normal skin. Re-vet wrapped and the original vet wrap may have been tight

#13 - patch in place, no bruising or abrasion

#16 - patch in place, no bruising or abrasion

#19 - patch in place, no bruising or abrasion

Date: 6/15/19
Personnel: Adam, MBC, Abbie
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (celery, banana, papaya), health checks, morphine dosing and saline controls, fentanyl patch application, all patch animals shaved today.

Temperature: 67.5 F
Humidity: 55.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: 07 has an abrasion under his tegaderm, likely from the calcein injection, ointment applied.18 also has an abrasion under tegaderm, ointment applied. 03 has matted fur on the sternal region, ointment applied.

#09 evaluation: Fecal and urine output low but present and has a little food left. 09 Ate all of his enrichment food today within five minutes of it being given. Extensive health check revealed an empty stomach upon palpation. Abdomen is tender, no blockage present, gums/teeth look normal, no signs of malocclusion.

#16 evaluation: Fecal and urine output are normal, but 16 has a small amount of food left. A right interscapular contusion adjacent to the patch and contusions under the patch have also been noted. Bilateral contusion adjacent to maxilla present. Fluorescent yellow discharge has also been noted on the back corner of the right eye. This is likely calcein mixed with the clear efflux of eye fluid.

Animals have less vet wrap applied today in order to avoid contusions and abrasions.
09 and 11 are heavily sedated today.

Vet Checks Requested?: N/A

Fentanyl Patch Checks:

All patches replaced today for normal dosing

#1 - Patch replaced. Abrasions noted under patch, ointment applied.

#2 - Patch replaced. Raw skin noted under patch, ointment applied.

#3- Patch replaced. Abrasion noted under patch, ointment applied.

#9- Patch replaced. Large contusion noted under where the patch was, he was chewing on his vet wrap and sliding it around which us what may have caused the contusion.

#13 - Patch replaced. No abrasions noted but ointment applied to degum fur.

#16 - Patch replaced. Contusions on and around patch sight, ointment applied.

#19 - Patch replaced. No abrasions noted but ointment applied to degum fur.

Additional Notes:
Low Fecal: 03, 09, 11, 17
Little Food Left: 03, 05, 08, 09, 10, 11, 14, 16, 17, 21

Date: 6/16/19
Personnel: JMA, Abbie
Notetaker: JMA
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (celery, apricot, papaya tablet), health checks, morphine dosing and saline controls

Temperature: 66 F
Humidity: 63.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:

#1 and #2 water bottles switched again. Made another note for UARV staff.
#14 did not eat his enrichment celery.
#14 has gross mashed up hay cube in the corner (not feces)
#9, 11, 16 still have lower fecal output and food left. Continue to monitor them.
*Morphine #4 vial is almost empty. Will likely need a new one for tomorrow. JMA will request a second vial for safe.

Morphine/saline rabbits need to be reshaved. Adam/Gina, can this be done tomorrow?

Patch checks:

#1 - chewed off some vet wrap around forelimb, this was trimmed back. Patch still adhered well.
#2 - patch remains adhered.
#3 - fully removed both his vest and the vet wrap. His patch remained adhered, but new tegaderm was applied. His jacket was reapplied, but we left the wrap off as he seems to fight more with this.
#9 - trimmed vet wrap, patch remains adhered.
#13 - patch was loose and there was hair regrowth. He was reshaved and a new patch was applied. He appears sedated.
#16 - patch remains adhered. Vet wrap not surrounding forelimbs due to matted fur.
#19 - patch adhered but not well on the sides. May need new patch applied tomorrow. A new tegaderm was applied.

Little food: 3, 7, 8, 10, 17
Lots of food: 9, 11, 16
Low fecal: 9, 11
Slightly low fecal: 5, 14, 17, 21

Date: 6/17/19
Personnel: Adam, Gina
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (toasted O's, papaya tablets), health checks, morphine dosing and saline controls, exercise

Temperature: 66 F

Humidity: 67.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): Rabbit #01 peed right into his mouth and was drinking his urine. Very strange behavior that I have never seen before in rabbits.

Rabbit #3 had a fit where he threw his enrichment bowl around the cage and tried to remove his vest. JMA was notified and she and MBC came to check this. Behavior had returned to normal by the time they arrived.

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:
Exercise:
Controls placed at 8:15 starting with #04.
Morphine placed at 9:10 starting with #08.
Fentanyl placed at 10:10 starting with #01.

All patch rabbits checked and patches still adhered. Shaved morphine and saline rabbits. Re-fastened all vests and secured the snaps. Rabbit #03 received a new vest because he had chewed through the leg holes. We are going to come back up after the rabbit meeting and check him to make sure he is okay.

Rabbits #15, 02, 09, and 03 had their vests unzipped when we came in. The snaps were not hooked together on the vests in the fentanyl group with the exception of rabbit #01.

Yes, we did this on purpose for 2,3,9 thinking they may not be able to use the zippered connection to pull them off? But I guess this was worse! Now we know.

Ahh, this makes sense. I am not sure how they keep getting these unzipped? It is very strange!

Yes, and wasn't a problem like this until recently! Not sure how to keep them in.

We can try to zip and lock the clasps and see what happens. We could always remove vet wrap if this is potentially what is bothering them? But it wouldn't explain why 15 is behaving like this.

JMA - 06/18/19 -- let me know if there are issues with temp/humidity today. There were issues last night/early this morning with the chiller. Also, I did not receive notifications via our RoomAlert and this concerns me. Reed, can you check this today and make sure it is online? RAD - Checked both the unit itself and the website. Both would indicate the unit is online and working.

Date: 6/18/19
Personnel: Reed, MBC
Notetaker: Both
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (toasted O's, sunflower seeds, papaya tablets), health checks, morphine dosing, controls, patches

Temperature: 66.0 F
Humidity: 66.5 %

*Humidity has been over our 65% set-point for ~two days.

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:
Low fecal: #13, #21
Lots of food left: #21

#3 wriggled out of his vest and was chewing on his patch this morning. New vest applied and vet tape reapplied now that he's chewing on the patch.

#2 scratched himself when applying a new patch and gave himself a couple lacerations from his claws. We applied antibiotic ointment and applied a patch not over the new lacerations.

#9 and #16 had pre-existing abrasions from previous patches, so we applied a new patch away from these abrasions and treated them with antibiotic ointment.

RAD: Dr. A, we will need more patches (we have enough for one more patch day) and one more bottle of morphine in the safe (only one in the safe now that bottle 4 is empty). Could you please email Beth with the order? We also only have cheerios and sunflower seeds left for enrichment food. Enough for tomorrow, but we will need more. Not sure who wants to go get some.

JMA: Thanks for letting me know. I sent an email to request 15 fentanyl patches/1 vial of morphine.

Radio will not turn on. Power light is on, but no sound and no display. Switched to playing radio on the laptop in the room. Update: We got the radio to work. Not sure what the issue was.

3:45 pm check (MBC): All patched rabbits still jacketed and seem calm; no chewing or scratching

---

Date: 6/19/19
Personnel: Adam, MBC
Notetaker: Adam
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (toasted O's, sunflower seeds, papaya tablets), health checks, morphine dosing, saline controls, exercise, patch checks

Temperature: 65.5 F
Humidity: 64.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Exercise:

Control Rabbits placed at 8:15 starting with #04.
Morphine Rabbits placed at 9:05 starting with #08.
Fentanyl Rabbits placed at 10:05 starting with #01.

Patch checks:

#1 - Patch intact - vet wrap replaced - some hair / old patches trimmed
#2 - Patch intact - vet wrap replaced - scratch healing well, ointment applied
#3 - Patch and vet wrap intact
#9 - Patch intact, vet wrap replaced, some hair / old patches trimmed - three old patch abrasions, ointment applied
#13 - Patch intact, no vet wrap existing or applied. Crescent chips in teeth from chewing water bottle sipper and cage. Slight hair loss on nose.
#16 - Patch intact, vet wrap intact. Some hair trimmed behind vet wrap. Health exam performed - teeth and gums healthy, no stomach abnormalities
#19 - Patch intact, no vet wrap existing or applied

Additional Notes:

Rabbit #16 did not eat his enrichment food.
Rabbit #18 nipped crook of arm above kevlar when carried to exercise pen

Little Food Left: 05, 06, 08, 10, 11,

Lot of Food Left: 03, 09, 13, 16, 21
Slightly Low Fecal: 05, 06, 08
Very Low Fecal: 03, 09, 13, 16, 21

All fentanyl rabbits appear to be behaving normally, with unexplained lack of appetite and fecal output reflecting this (except #01, 02, 19). We will keep them under close monitoring. Maybe they will be enticed to eat by exercising?

Update: #13 and #16 had especially low fecal - we noticed #13 biting his water bottle sipper and banging it against the cage. Upon inspection, for both rabbits, the sipper needed to be pushed down in the bottle further for gravity to allow rabbits to drink freely. Lack of water may have contributed to low fecal output and reduced feeding. #3 and #9 also had low fecal, but not as low as #13 and #16, and their water bottles were normal.

Thank you - Adam just left a note. Thanks. Were any bottles switched today? Or any other issues with them? I am emailing Beth. MBC: No water bottles switched today

---

Date: 6/20/19
Personnel: Adam, Reed
Notetaker: Reed
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, papaya tablets), health checks, morphine dosing, saline controls, patch checks

Temperature: 67.0 F
Humidity: 64.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Patch checks:

Rabbits #02 and 09 received triple antibiotic ointment in attempts to treat abrasions. Rabbit #02 lacerations look very good, almost completely healed. Rabbit #16 had loose vet wrap. We trimmed this and applied a new jacket since his old one was fairly chewed. All patches were adhered and no new patches were applied.

Additional Notes:

Little Food Left: 09, 11, 16, 17, 21

All morphine rabbits appear fairly sedated. They are exhibiting normal breathing and postural stability, but minimal activity and some do have eyes partly shut.
JMA: If more sedated than usual, please keep an eye on them for a bit.
We are doing this. I was planning to stay for about 20 more minutes or until they seem to be up and moving around. They were dosed around 9:00

Update: 9:37 A.M. All morphine rabbits have been observed up and taking a few hops. Breathing rate is normal and all seem alert and healthy. Great, thanks!

Adam: We think the fecal output was better on 13 and 16 but are not 100% sure because the cage batteries were changed out prior to our arrival in the room at 8:00 A.M.

JMA: I will email Beth and ask, and request that they tell us if batteries need to be changed before 8 and if so the staff should fill in the fecal log.

Adam: Thank you, it was also a little frustrating having to wait due to batteries in the doorway, etc. Only one more week! Other than this, all dosing went smoothly and everyone seems to be doing well.

Date: 6/21/19
Personnel: Gina, MBC, JMA (to assist with patches)
Notetaker: MBC and JMA
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach/spring mix, papaya tablets), health checks, morphine dosing, saline controls, patch application

Temperature: 65 F
Humidity: 66%

Changes in (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Exercise:

Control rabbits put in at 8:25 and taken out at 9:10
Morphine rabbits put in 9:18 at and taken out at 10:03

Fentanyl rabbits put in at 10:10 and taken out at 10:55 - #19 in at 10:28

Additional Notes:

Little food left: #1, #5, #6, #9, #10, #16, #17
Lot of food left: #21
Low fecal: #5 (slightly), #14 (slightly), #17 (slightly), #21 (slightly)

JMA assisted with patch application due to ongoing issues with gummy fur/poor skin condition. All animals reshaved and gummy hair trimmed back. Ointment applied to reddened skin as needed.

#1 skin looks good, no abrasions. Sausage-style vet wrap applied.
#2 abrasion from scratching almost fully healed. Ointment applied. Vet wrap applied, since tegaderm did not stick very well.
#3 vet wrapped - very small scratch treated with ointment
#9 has bad skin, gummy fur, redness, and is angry - just getting a sausage wrap due to agitation - **he should be checked tomorrow**
#15 has bad skin - small abrasion treated with ointment
#19 has gummy hair - abrasion treated with ointment

#21 biting

One CaviWipes container is empty and the other is also empty - we need more

Syringe and Needle Count:

Small Box
Bag of 72 smaller (saline/morphine) syringes
32 needles

Large Box
100 (1 full box) needles
100 (1 full box) needles
Bag of 102 smaller (saline/morphine) syringes
Bag of 92 smaller (saline/morphine) syringes
Bag of larger (calcein) syringes

Totals:
232 needles
226 smaller (saline/morphine) syringes
82 larger (calcein) syringes)

Minimum Needed for Remaining Study
5 days remaining x 10 saline/morphine per day = 50 small syringes
(2 calcein days x 21 rabbits) + 21 large syringes for fatal plus = 63 large syringes
113 needles

Date: 6/22/19
Personnel: Abbe, Reed
Notetaker: Abbie
Time In: 8:00 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, papaya tablets), health checks, morphine dosing, saline controls, patch checks

Temperature: 65.5 F
Humidity: 61.5 %

Changes in Behavior (Lethargy, Hyperactivity, etc.): #9 a little lethargic. Was annoyed when I re-vested him, but didn't feel anything abnormal (bloating, fur quality, etc.) I think he's just having a fit about the vet wrap.

Health Concerns: #3 appears to be having the same ophthalmic discharge that #19 was having. Discharge is clear and there doesn't appear to be a lot of fluid. Right eye does not appear cloudy or have any other abnormalities. Likely just irritated (allergies?). No change in behavior.

Vet Checks Requested?: N/A

Patch checks: All animals' patches intact. Only #9 unzipped his jacket, but the vet wrap was intact. Skin looks better, but not completely without redness and gummy fur. He was less irritated.

Additional Notes: A few logs were not filed out/filled out incorrectly, UARV staff left some notes about this, as well as a note about leaving dirty bowls in the sink. Logs have been corrected.

JMA: Can you guys let me know which logs were incorrect?
Health check log. #11 was filled out twice, #16 wasn't filled out at all.

Little Food Left: #3, #11, #17, #19
Lots of food left: #9, #13, #16, #21
Low Fecal: #9, #13, #21

Date: 6/23/19
Personnel: Abbie, MBC
Notetaker: MBC
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, apricot, papaya tablets), health checks, morphine dosing, saline controls, patch checks

Temperature: 63.5 F
Humidity: 62.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns:
#3-- eyes look fine today, no discharge noted.
#9-- Skin around patch has greatly improved, much less red

Vet Checks Requested?: N/A

Patch checks:
All fentanyl patches were checked and adhered with vet wrap covering (except #16, see below). None of the rabbits had significant abrasion or skin reddening around the patch area under the vet wrap.

#3 unzipped jacket and it is hanging around the collar - vet wrap intact and unchewed. The jacket was in good condition so it was put back on.

Additional vet wrap was added to #16 - his sausage had slipped down below the patch so that the patch was not covered. Cross-leg pieces were added.

#19 (control with orange vest) had no Tegaderm patch. His vest was chewed off around the neck, leaving only the buckled portion so the patch either fell off or was eaten. His hair had grown too much to adhere a patch so he was shaved, and a new Tegaderm patch applied. A new orange jacket was applied.

Additional Notes:

Little Food Left: #8, #9, #10, #12, #13, #16, #17
Lots of food left: #11, #21
Low Fecal: #13, #21

Kevlar sleeves are missing - maybe they were laundered. Will check laundry room. **Update:** Kevlar sleeves being returned by UARV staff

Enrichment foods - spinach is at half bag - do we want more fresh, or use up the dry food?

#21 had low fecal and lots of food left - he was observed sucking his sipper without bubbling, and very high water level in the water bottle. We pushed sipper back down into the water bottle until it bubbled when touched on the end. He is now drinking a lot of water, so hopefully this should assist his digestion. It's a good idea to check sippers if they appear to have not been used (~1 inch below top)

All logs double checked by both individuals; no issues

Date: 6/24/19
Personnel: Reed, MBC
Notetaker: Reed
Time In: 8:05 AM

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, prune, papaya tablets), health checks, weighing, morphine dosing, saline controls, patches

Temperature: 62.5 F
Humidity: 72.0 %
*JMA: The humidity has been very high today. Beth called Energy Systems and they switched us to the University Chiller (as opposed to the UARV specific chiller) without prior notification. She requested that it be switched back ASAP and remain on the UARV chiller for the remainder of the summer.

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Exercise: Controls placed at 8:30, removed at 9:15
Morphine placed at 9:40, removed at 10:25
Fentanyl rabbits placed at 10:30, removed at 11:15

Additional Notes:

All animals consumed the totality of enrichment foods from yesterday.

All control and fentanyl rabbits were shaved both in the patched region and at the nape of the neck (if needed) to prepare for calcein tomorrow. All rabbits had regions that could just be shaved to apply the patch - there was no extensive removal of gummy hair. The fentanyl rabbits were all very combative towards further removal of non-shavable gummy hair via scissors or pulling.

The scratch on #2 was fully healed. Some ointment was applied to a more caudal region reddened from the collar/jacket rubbing.

#3 had unzipped his jacket by pulling it against the grain, so it was difficult to rezip. His collar and front legs were in proper position. He had pulled on the vet wrap through the shoulder region and exposed the patch. We applied the new patch far enough back to only need to sausage wrap him. He seems to be getting out of the jacket partially through leveraging the cross-pieces on the shoulders, which he continually pulls on and chews. Hopefully by only placing a sausage wrap, he will not gain the leverage needed to unzip his jacket. Fortunately, #3 seems to have no interest in removing his patch, although he is frequently able to unzip it.

Fentanyl rabbit skin was in much improved condition today. The only rabbit with a dark purple abrasion was #13, who had a bad abrasion under the patch removed today. We treated it with antibiotic ointment.

All fentanyl rabbits were vet wrapped during the previous patch day, and this vet wrap was replaced in the same arrangement (except #3, who did not have shoulder crosspieces put on as he continues to pull on them). No control rabbits were vet wrapped.

There were no observed issues with water bottle drippage or severely low fecal output today.

Lots of food left: #21
Low Fecal: #21 (slightly)

Date: 6/25/19
Personnel: Reed, MBC, Adam
Notetaker: Reed
Time In: 8:05 AM

Good morning, Team! Good luck and let me know if you need anything.

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach, papaya tablets), health checks, morphine dosing, saline controls, calcein, patch checks

Temperature: 65.0 F
Humidity: 71.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns:

Vet Checks Requested?: N/A

Additional Notes:

Little food left: 1, 5, 8, 9, 10, 11, 12, 14, 16,
Lots of food left: 17, 19, 20, 21
Low fecal: 19, 21

Rabbit #07 chewed all hair off of his hind limbs. Most of the hair on the anterior aspect of both hind limbs. His legs are by no means completely bare. Maybe rubbed off from the rear leg loops of the vest?

#1's sipper was not in the stopper well, so he couldn't get water and is a bit dehydrated. We fixed the sipper and tested it. He now has access to water again and is drinking normally.

#3 scratched himself while administering calcein. Now has a small laceration lateral to the patch site. We applied ointment and re-vet wrapped him.

#5 effluxed a few drops of calcein due to pulling his skin tight.
#7 jerked while administering calcein and lost just the last bit of his dose. Will need to make sure he has full dose tomorrow.
#9 being extremely difficult during calcein dosing, has an abrasion from fighting the injection. We applied ointment and re-vet wrapped him. We did administer a full dose with Adam doing his special hold.
#13 also being difficult, required 3 jabs to get the full dose, effluxed a small amount.
#16 had a small amount of efflux and is jumping like crazy. Highly agitated from the calcein injection.
#19 effluxed a couple drops from jerking away from the needle.

*If any of them lose a lot, please try a second time with the approximate dose that was efluxed. But do not try more than twice. RAD: Will do. JMA: Thank you. I know this is difficult and appreciate your efforts.

Dehumidifier bucket was not emptied, nor was it draining itself so it was not running. We emptied the bucket and reattached the drain hose, hopefully this will help with the humidity issues in the room.

JMA: Definitely leave a note for UARV staff regarding dehumidifier/sippers. I have already discussed the sipper issues with Beth and its importance.

Patch Checks:

All patches intact. #3, #19 needed re-vet wrapped due to chewing.

Date: 6/26/19
Personnel: Adam, MBC
Notetaker: Both
Time In: 8:22 AM (after calcein preparation took a bit of extra time - we ran out of saline and had to get extra from Beth Kenaga)

Final day of experimental treatments

None of the rabbits had S-hooks on their cages when we came into the room this morning after enrichment devices were changed by UARV staff last night. Aspen bin was empty. Not all exercise pens had springs.

I hope you told Beth this when you went to get saline -- if not, please do as this is unacceptable
We did not, as we had not been up to the room. We had to get more saline for calcein preparation. I will let her know when we return the pink slips.

Okay. I will email her now. Did any rabbits open cages or anything like that?

Thankfully not, however, three of them did have poop on the S hook so we had to get new ones. Were they on the cages just not secured? Some were, but a lot were on the floor of the room or in the rabbit's cages. Thank you -- I have emailed Beth. Also, Reed is on his way. See you around 10!

Ok, thank you. Sounds good, hopefully your meeting goes well.

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (spinach and papaya tablets), health checks, morphine dosing, saline controls, calcein, patch checks

Temperature: 65.5 F
Humidity: 67.0 %

Changes in Behavior (Lethargy, Hyperactivity, etc.):

Health Concerns:

Vet Checks Requested?:

Additional Notes:
Little food left: 11, 20, 21
Low fecal: 21

#3 removed vet wrap but not patch. Wrap (sausage-style) was replaced. #3 had to be poked multiple times (4-5 times) due to jerking during the injections. Ointment was placed on the injection sites.

S-hooks were not replaced on cages by UARV staff and were found on the room floor/floors of their cages. UARV staff was notified.

Exercise:

Exercise - Controls placed at 8:30 A.M. starting with rabbit #04
Morphine placed at 9:30 starting with rabbit #8 and removed at 10:15
Fentanyl placed at 10:21 starting with #1 and removed at 11:06

Date: 6/27/19
Personnel: Abbie, Adam, JMA, MBC
Notetaker: JMA
Time In: 8:00 AM

Euthanasia Day #1

Tasks Performed (Dosing, Exercise, etc.): Enrichment foods (papaya tablets, spinach, toasted o's), health checks, patch removal, euthanasia

Temperature: 65.5 F
Humidity: 71.5**%**

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:

Rabbits #4,5,6,7,14 (controls) were euthanized between 8:00-11:00 A.M. in the UARV necropsy suite. Dissections of the left hindlimb followed in D410.

Rabbits # 15,18 (controls),8,10,11,12 (morphine) were euthanized between 2:00-5:30 P.M. in the UARV necropsy suite. Dissections of the left hindlimb followed in D410.

Adam and JMA removed fentanyl patches from all fentanyl rabbits, provided enrichment foods to the remaining rabbits, completed/corrected logs, took photographs of patch areas between 5:30-6:30 P.M.

Dissection continued in D410 until 7:30 P.M.

Date: 6/28/19
Personnel: Abbie, Adam, JMA, MBC
Notetaker: JMA
Time In: 8:00 A.M.

Euthanasia Day #2

Tasks Performed (Dosing, Exercise, etc.): Euthanasia

Temperature: F
Humidity:

Changes in Behavior (Lethargy, Hyperactivity, etc.): N/A

Health Concerns: N/A

Vet Checks Requested?: N/A

Additional Notes:

Rabbits #17,20,21(morphine), and 1,2 (fentanyl) were euthanized between 8:00-10:30 A.M. in the UARV necropsy suite. Dissections of the left hindlimb followed in D410.

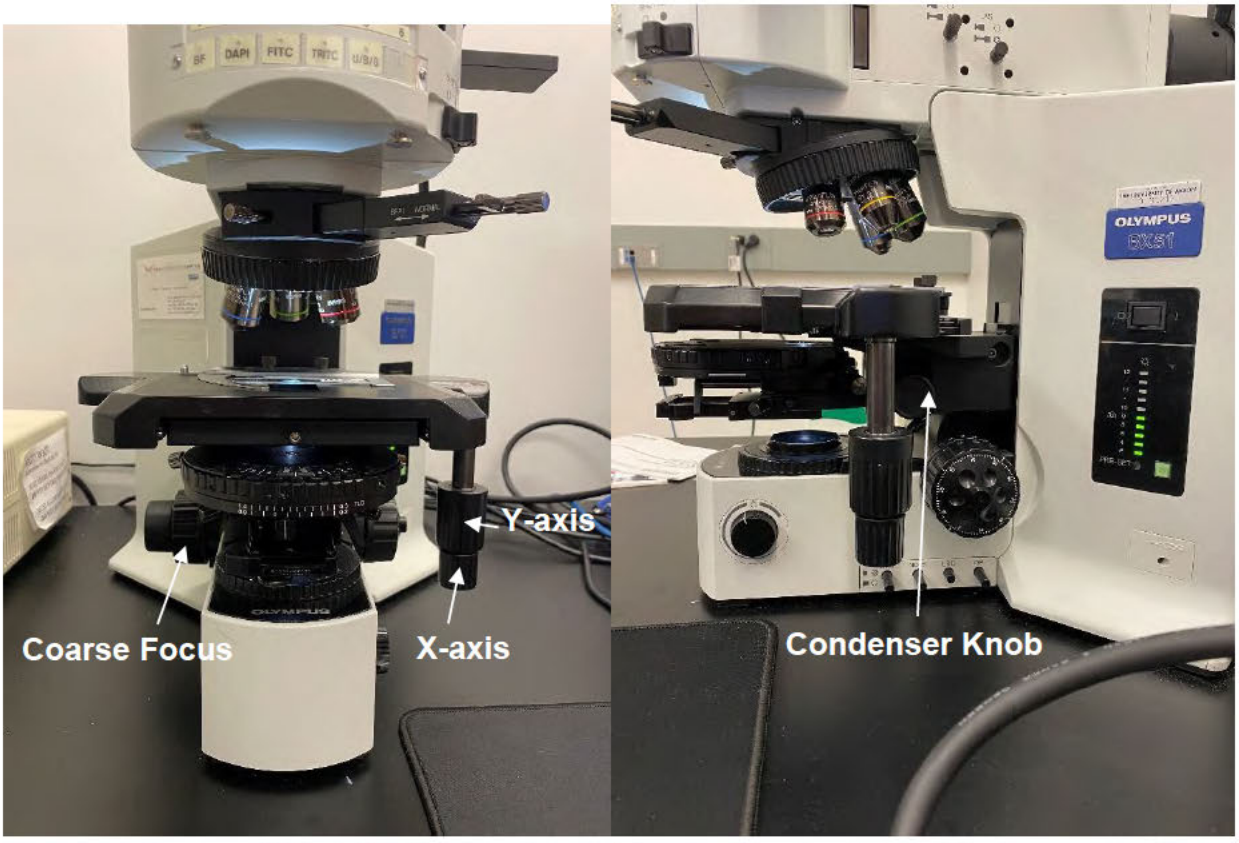
Rabbits # 3,9,13,16,19 (fentanyl) were euthanized between 1:30-4:00 P.M. in the UARV necropsy suite. Dissections of the left hindlimb followed in D410.

Appendix XXX: Olympus BX51 Microscope Imaging SOP

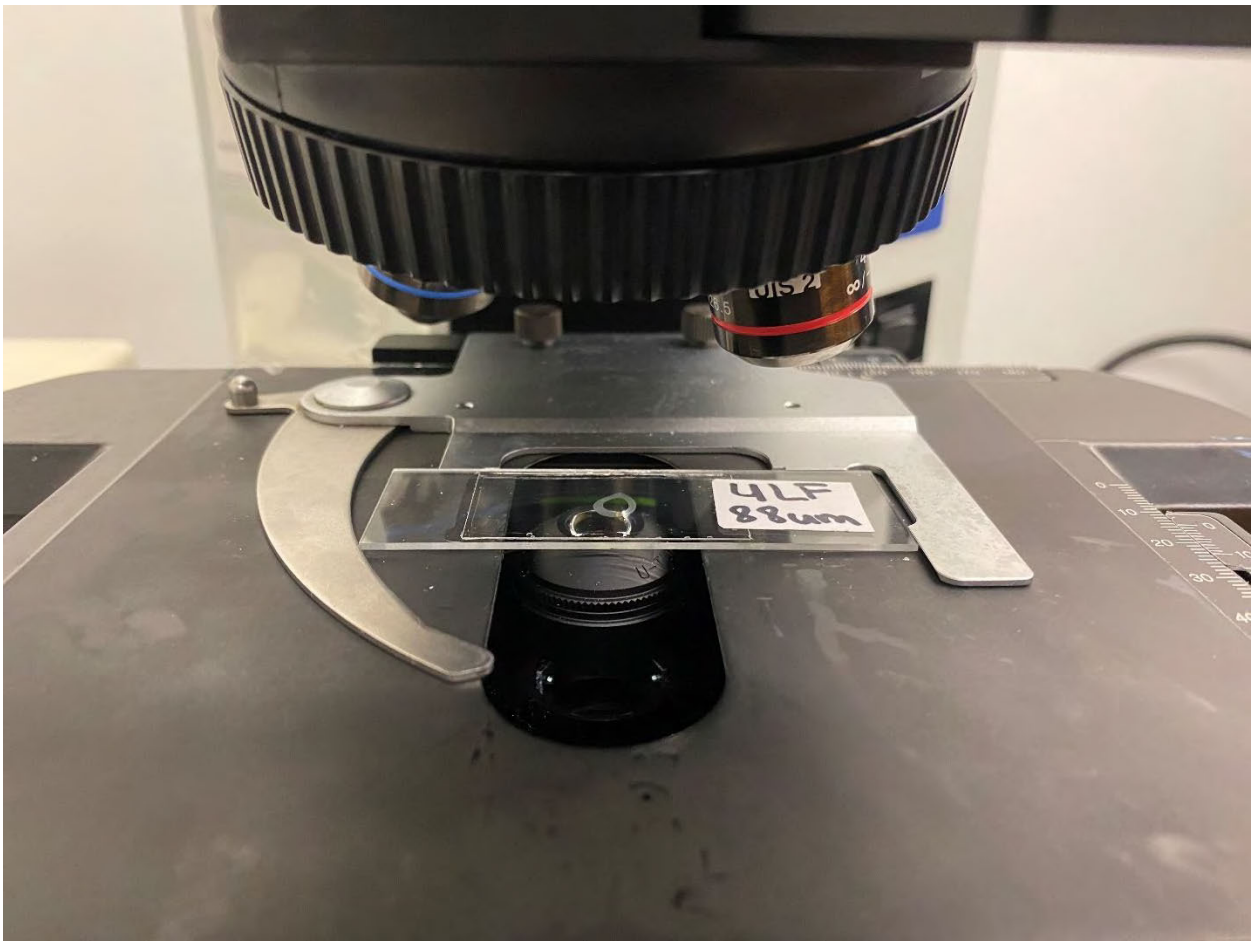
Never use Kimwipes or other tissue to clean the lamp, condenser lens, objectives, eyepiece, or polarizer lenses! Only use lens wipes!

Microscope Setup – Do when starting a new slide

1. Remove microscope dust cover
2. Flip the switch on the right side from O to | to turn the microscope on
3. **Loading the slide**
    a. The safest way to load the slide is to rotate the **objective nose** to the position that does not have an objective. This way you avoid scraping the objective
    b. Use the **coarse focus adjustment knob** to lower the entire stage away from the objectives, to about the halfway point between the objectives and lamp
    c. Lower the condenser slightly using the **condenser height adjustment knob**, to avoid hitting the condenser lens with the slide in the open part of the stage
    d. Move the arm of the slide holder out, position the slide in the upper right corner, and clip the slide into place. **The coverslip should face up.**
    e. Use the X-axis and Y-axis knob to move the stage around and make sure it stays in the clip and does not stick to the stage. If the slide is wet it may stick.

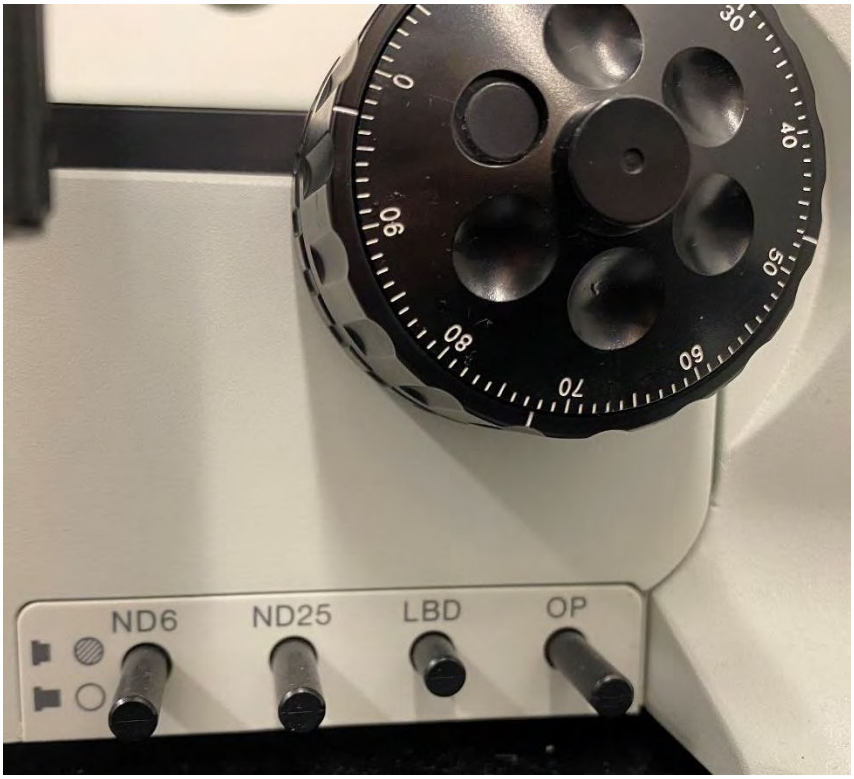


Safest objective / condenser / stage position for slide loading



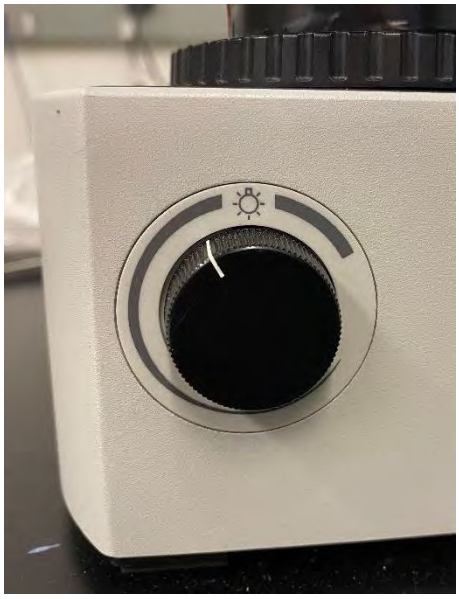
Slide clipped into place – coverslip facing up!

4. **Adjust filter knobs**

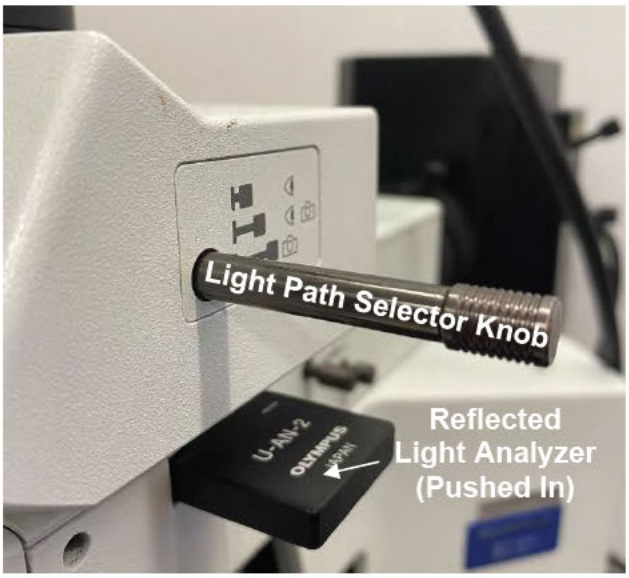


a. Look at the right side of the microscope under the coarse adjustment knob
b. LBD should be **pushed in.**
This is a color balancing daylight filter
c. ND6, ND25, and OP should **not be pushed in.**
These are neutral density filters that limit light transmittance

5. **Adjust lamp brightness**
    a. Make sure the button next to Pre-Set is not lit. If it is lit, press the button to remove the pre-set. This will allow you to adjust brightness intensity.
    b. On the right side of the microscope, adjust the brightness intensity knob for the lamp until the lamp voltage indicator for **9** lights up on the microscope body. This is the optimum brightness for photography with the LBD filter.

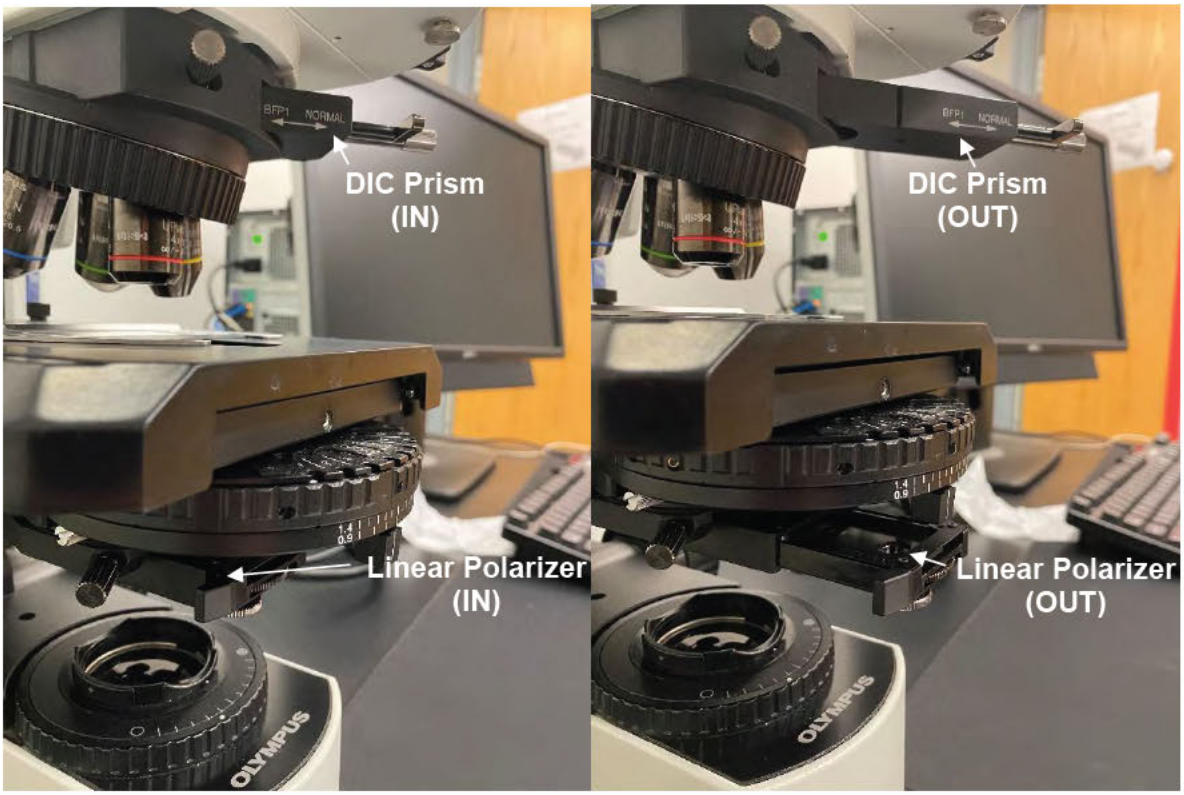


6. **Remove polarizing filters**



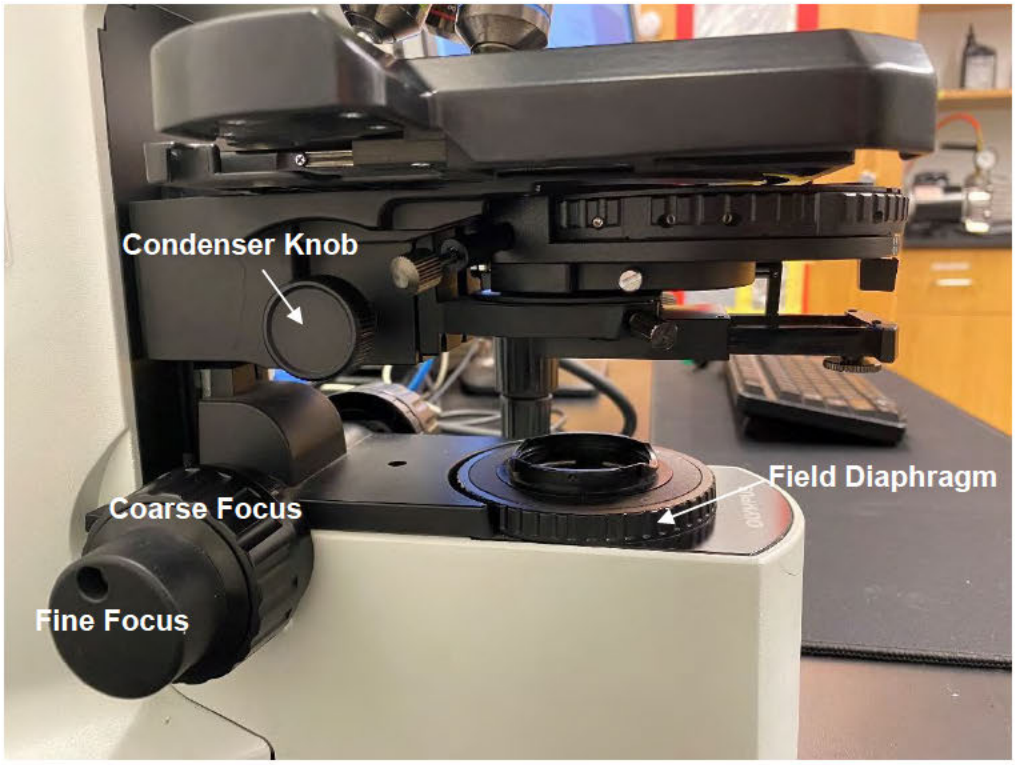
a. Make sure there is a light path to the eyepiece by pushing the **light path selector knob** to the right of the eyepiece to either the **first stop** (eyepiece only) or **second stop** (eyepiece plus camera). The third stop blocks light to the eyepiece.
b. I like to keep the **reflected light analyzer** (U-AN-2) in during setup because the lamp is extremely bright without it, and hard to look at through the eyepiece. We will slide it to the out position before circular polarization and fluorescence imaging.

c. Remove the **circular polarizer cover** from the lamp, if applicable
d. Slide the **linear polarizer** away from the condenser until it clicks to the "out" position
e. Slide the **cartridge just above the objective nose** (contains either U-DICTS DIC prism or the circular polarization analyzer) away from the microscope until it clicks to the "out" position. You may need to loosen the associated screw to move the cartridge.



7. **Focus specimen**

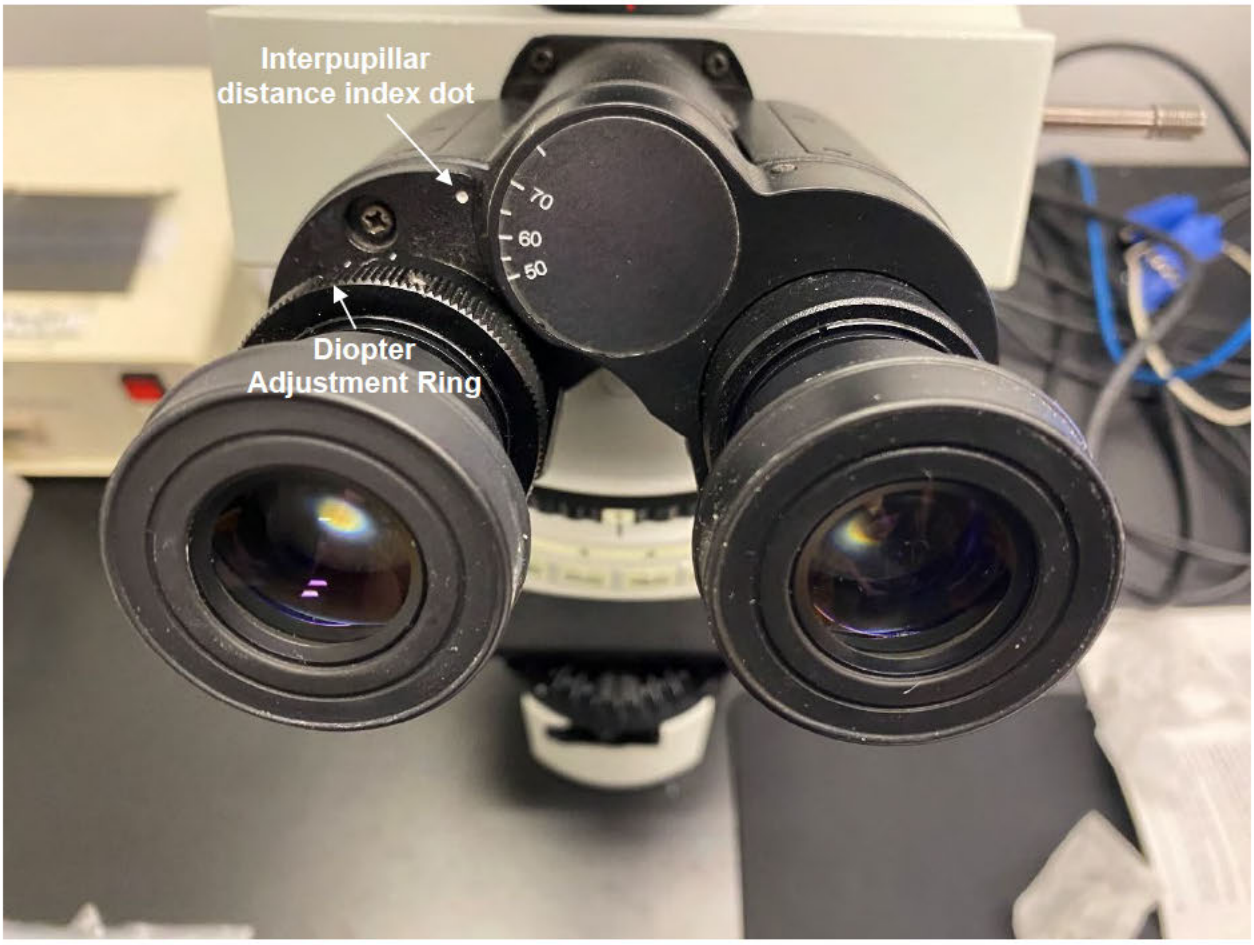
a. Microscopes are parfocal, meaning that the specimen and condenser will remain (mostly!) in focus even when you rotate objectives. It's easier to set the focus roughly at a lower objective because you can see more of the bone.
b. Rotate the objective nose to either the **4x** (red) or **10x** (yellow) objective.
c. Not looking through the eyepiece, use the X-Y axis knobs to position the bone section over the dot of light visible on the slide
d. Use the coarse focus knob to raise the stage and bring the specimen into view
e. Use the fine focus knob to focus the specimen more precisely
f. **If you can't see the specimen** the condenser is probably out of focus.
    i. Rotate the **field diaphragm** all the way to the **right**
    ii. If the condenser is out of focus, you will just see a vaguely gray field
    iii. Use the **condenser knob** to raise the condenser until you see a decagon shape come into focus through the eyepiece
    iv. Open the field diaphragm and try to re-focus the specimen using the coarse and fine focus knobs



g. Rotate the objective nose until the **20x** objective (**green ring**) is on top of the specimen.
h. Use the fine focus knob to perfect the focus at 20x.

8. Eyepiece adjustment

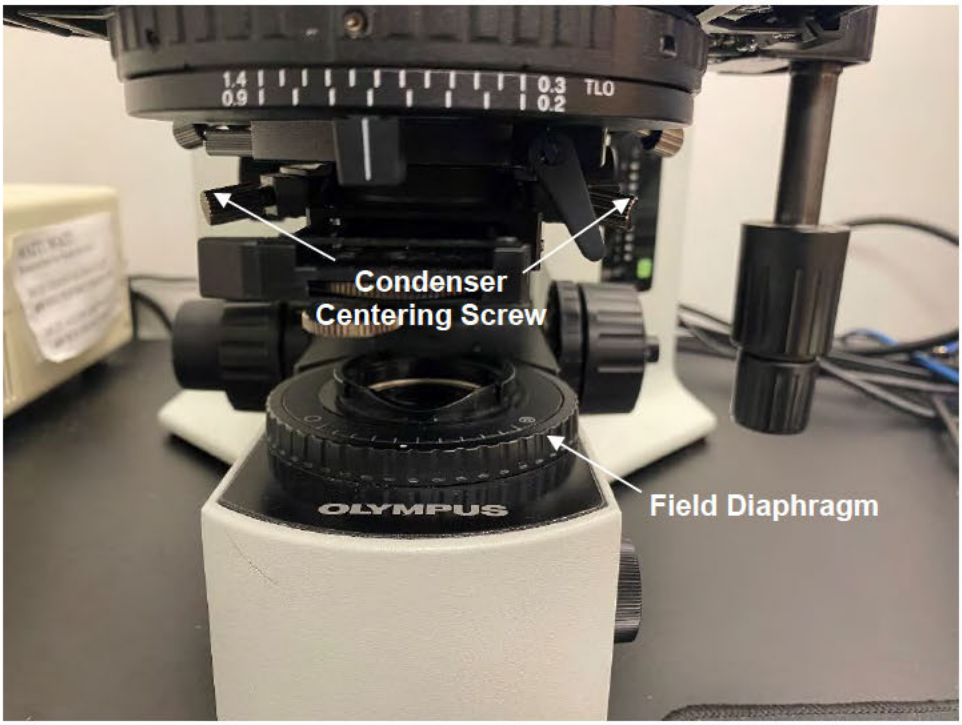
a. **Interpupillar distance:** Looking through the eyepieces, grasp and move the binoculars until you see a unified dot of light (not double images). You can note the position of the index dot so you can easily adjust this in the future.
b. **Diopter adjustment**
    i. While looking through the eyepieces, close your **left** eye. Focus the specimen well using the fine focus knob
    ii. Now close your **right** eye. The specimen may be slightly out of focus. Turn the **diopter adjustment ring** on the **left eyepiece** to bring the specimen into focus. Now it should be in focus for both eyes.

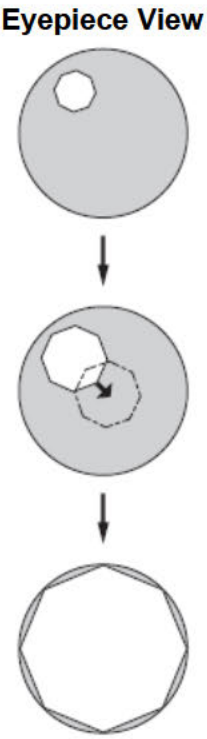


My interpupillar distance is 65

9. Kohler Illumination (Adjusts condenser position)

a. Make sure specimen is focused, as above
b. Rotate the **field diaphragm** all the way to the **right**
c. Use the **condenser knob** to raise the condenser to the top
d. Look through the eyepiece.
e. Lower the condenser using the condenser knob until you see a **decagon shape**. Make the edges of the shape **as sharp as possible.**
f. Use the **condenser centering screws** to position the decagon shape in the center of the field of view.
    i. It can be useful to open the field diaphragm so it is just inside the field of view for centering

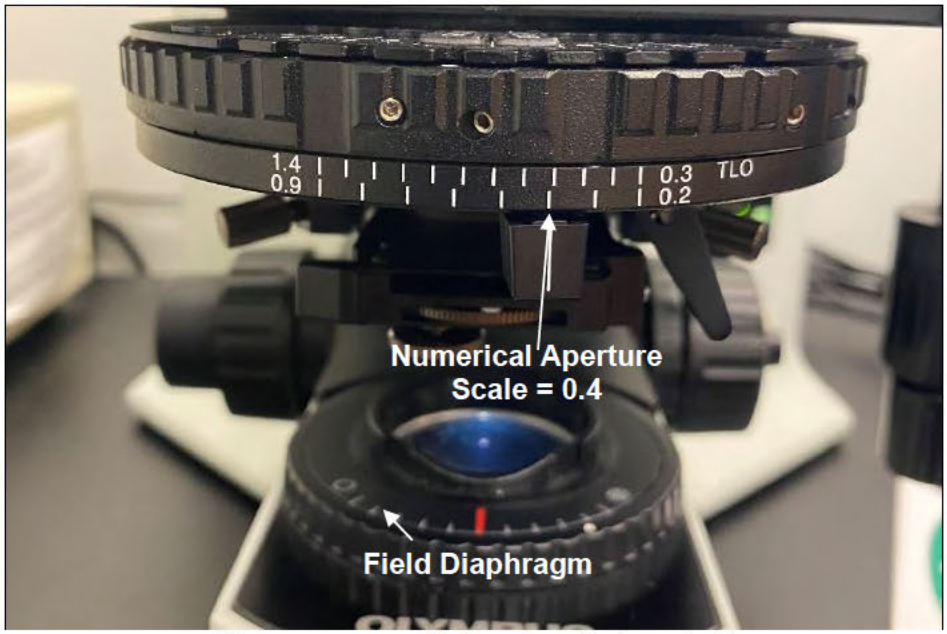




10. Set Numerical Aperture Scale

a. Look at the **bottom** scale (0.2 – 0.9) of the **numerical aperture (N.A.)** scale. We want to set it at **0.4** which is 80% of the the N.A. of the **20x** eyepiece (0.5)

b. Open the field diaphragm to about **70-80%** of the field of view. This will help reduce out-of-focus light. **You need to adjust the field diaphragm aperture whenever you change objectives.**

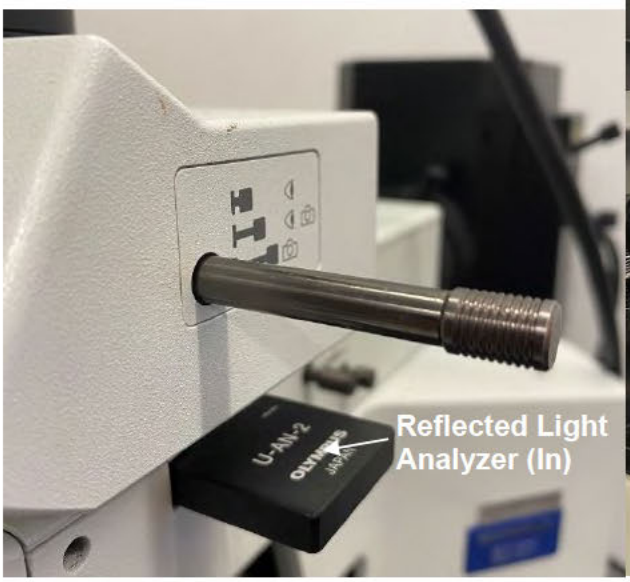


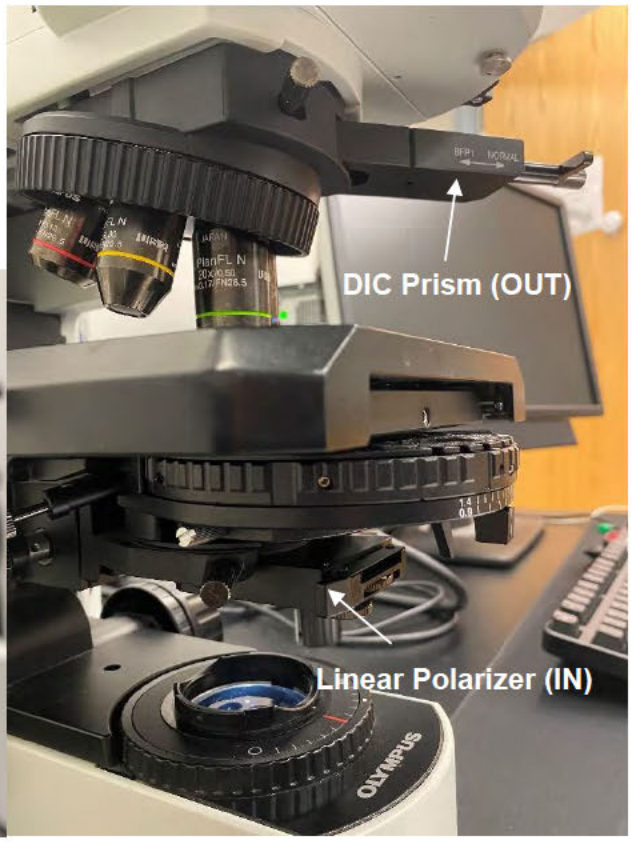
Now you are ready to set up for imaging!

Normanski Differential Interference Contrast (DIC) Observation

DIC Setup

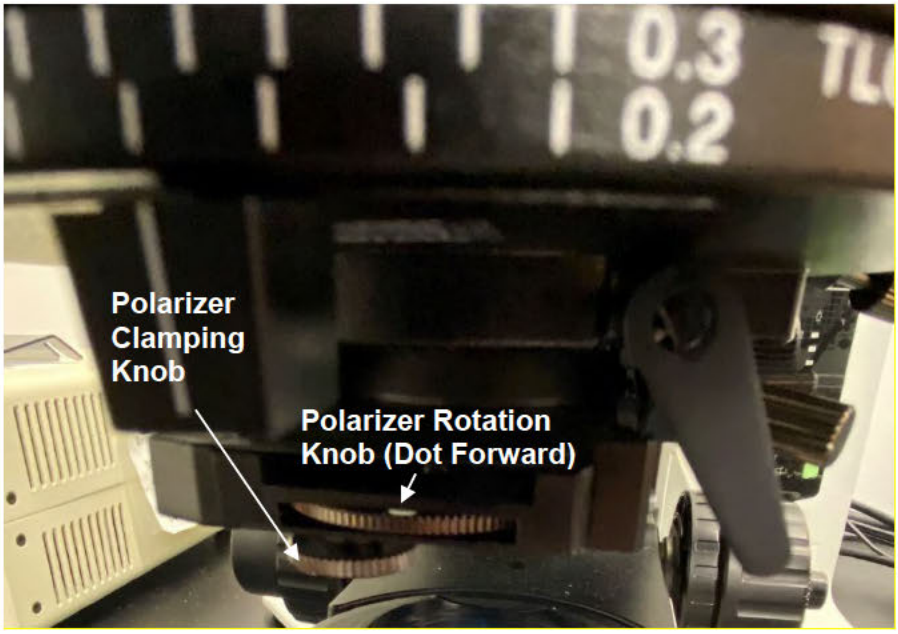
1. **You must have completed all microscope setup steps to proceed**. Make sure the specimen is still in focus at 20x and that the aperture iris diaphragm is still at 70-80%
2. Make sure the **reflected light analyzer (U-AN-2)** is pushed into the slot just underneath the light path selector knob
3. Push **in** the **linear polarizer** underneath the condenser until it clicks to its "in" position
4. Pull **out** the **DIC prism (U-DICT5)** from the cartridge slot above the objective nose until it clicks to its "out" position.





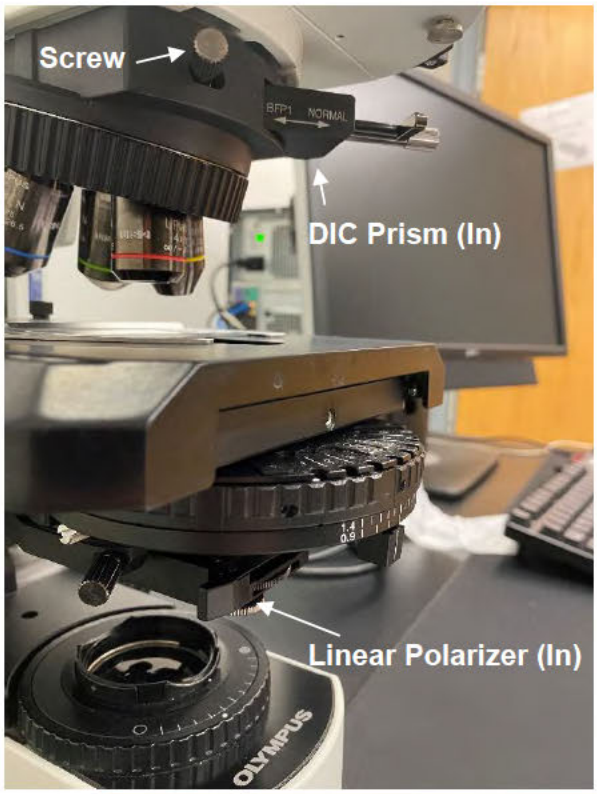
5. Rotate the turret under the stage until the dot matches up with **1.** This is the brightfield light path.

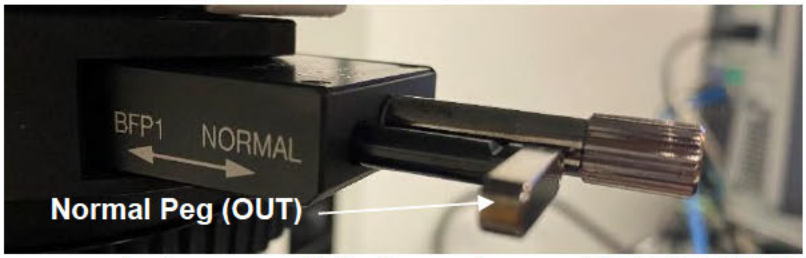
6. Look through the eyepiece. On the **linear polarizer**, rotate the **polarizer rotation knob** until the field of view is **as dark as possible**. At this position, the white dot on the polarizer rotation knob faces the front of the microscope.
   a. You may need to loosen the **polarizer clamping knob**.
   b. It may help to move to a non-bone area of the slide so that you cannot see the (white) collagen fibers.
   c. Once you have determined the darkest position, tighten the polarizer clamping knob by rotating it **counter-clockwise (left).**



7. Push **in** the **DIC prism** until it clicks to its "in" position. Tighten the screw to hold it in the cartridge slot.

a. The BFP1 ←→ Normal peg should be pulled **out.**

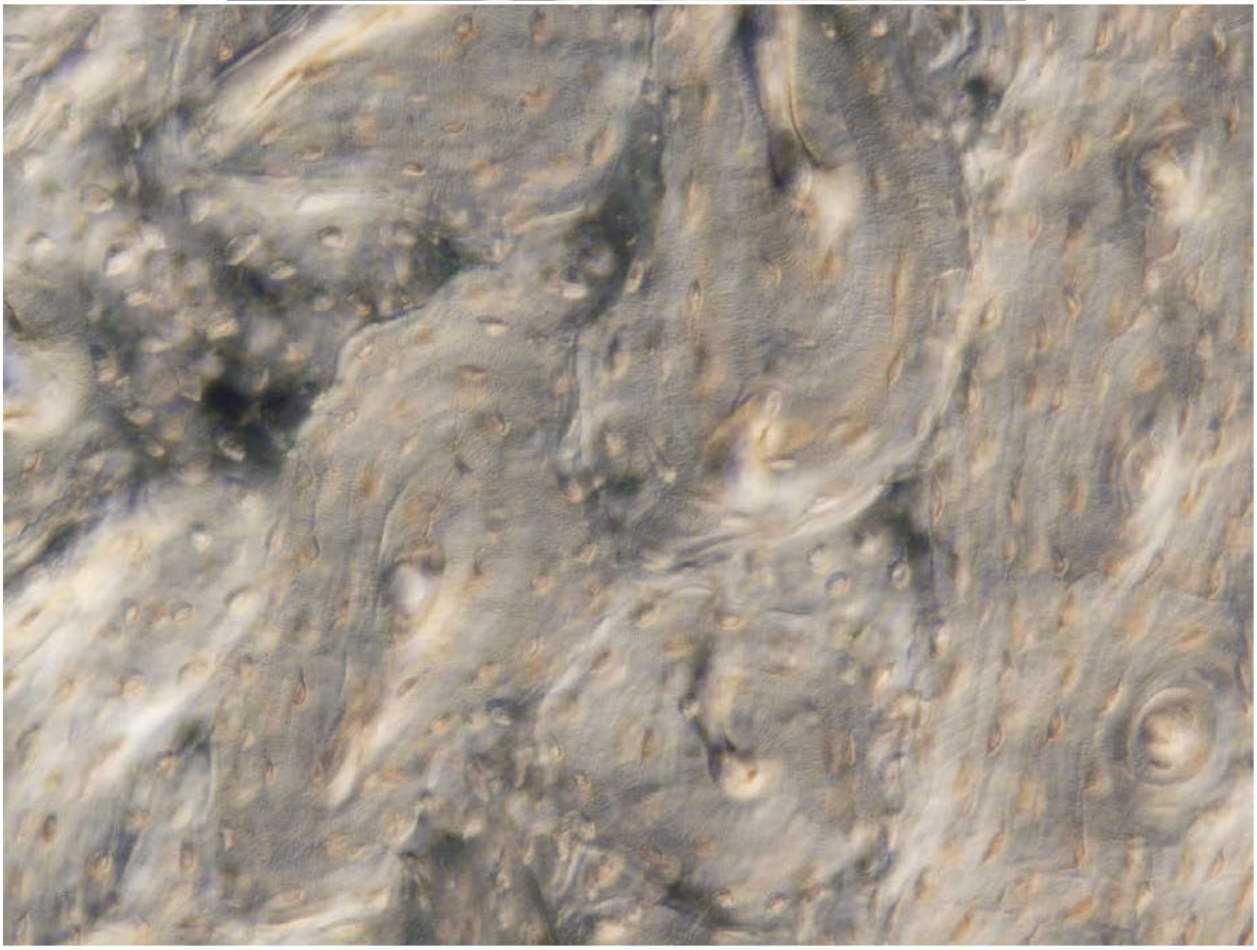




8. Rotate the turret under the stage until the dot matches up with **DIC20.** This is the DIC light path for the 20x objective.

9. Rotating the prism control knob produces, from **left to right**:
   a. Furthest left: Flat yellow

b. Dome-shaped (convex) osteons
c. Sensitive gray
d. Cup-shaved (concave) osteons
e. Furthest right: Flat yellow
f. Rotate the prism control knob to the near-furthest left so it produces **cup-shaved (concave) osteons**

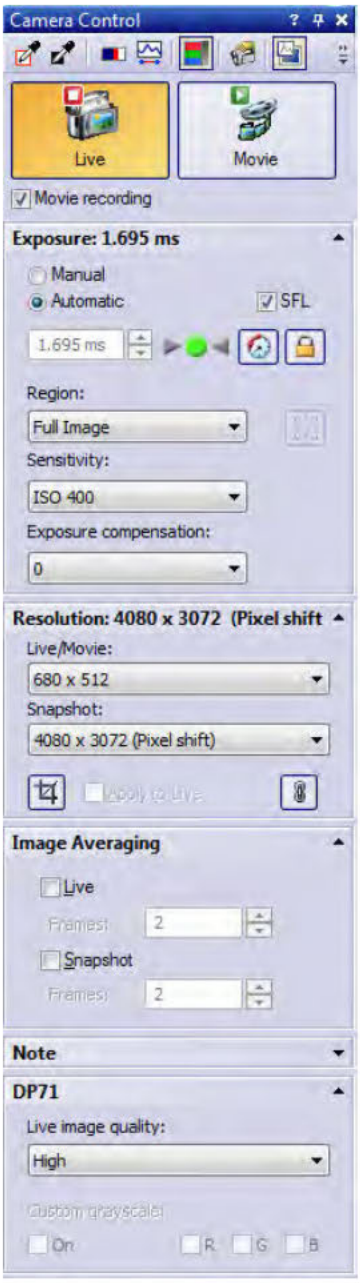


Sharp cement lines in the 3D-like DIC image

DIC Imaging Setup

1. Open cellSens Entry from the Desktop

2. Click Live to turn on Live image
   a. When live, a red rectangle appears on the icon
   b. When stopped, a green triangle appears on the icon
3. Pull the light path adjuster knob out to the third stop so light no longer travels to the eyepiece, and all light is diverted to the camera
4. Due to the physics of optics, the focal plane of a camera lens is slightly different than the focal plane of your eyeball lens. Use the fine focus knob to finely adjust the image. **Look for sharp cement lines.**
5. **Top Left toolbar:**
   a. Select objective **20x** to set the scale properly
   b. Unselect **scalebar** so it does not appear on screen
   c. Select Toggle RGB/Grayscale mode so image is in **color**



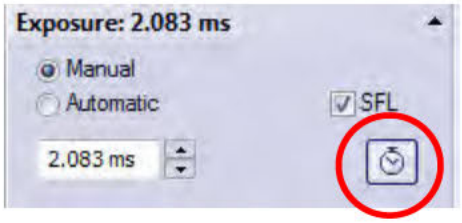
4. **Camera Control** (left) should have the following defaults:
   a. **Exposure**
      i. **Region:** Full Image
      ii. **Sensitivity**: ISO 400
      iii. **Exposure** Compensation: 0
   b. **Resolution:**
      i. **Live/Movie:** 680 x 512

1. Any higher and the image will "seize"
      ii. **Snapshot:** 4080 x 3072 (Pixel Shift)
   c. **Image Averaging**
      i. **Live** unchecked
      ii. **Snapshot** unchecked
   d. **DP71**
      i. **Live image quality:** High

6. **Set the white balance**
   a. Use the X-axis and Y-axis buttons to move to a region of the slide that does **not** have the sample, but is still under the coverslip (e.g. inside the marrow cavity)
   b. Select the **White Balance on ROI** (white eyedropper) under Camera Control
   c. Click on the white region of the Live Image, avoiding obvious trash/defects
   d. This pixel value is automatically set to white

7. **Set the exposure**

a. Use the X-axis and Y-axis adjustors to move to a representative intracortical area that includes osteons
b. Under Exposure, select Manual and then the clock icon to conduct a One-Time Auto Exposure
c. The collected auto-exposure value will appear, and Exposure will be automatically changed to manual
d. **Record the Exposure value on this spreadsheet:** https://docs.google.com/spreadsheets/d/1bCUXlHeUb77umeDTyggK8aZp3Nm01BjlowNvjxVjxyw/edit#gid=0



8. **Image Acquisition Settings**

a. Click the hand on camera icon under Camera Control
b. **Document: Snapshot**
   i. Change Text to the image name in the format: **NumberLF_Slide_DIC**
   ii. E.g. 4LF_88um_DIC for Rabbit 4 Left femora, 88 um slide
   iii. Reset Counter Start to 0 and Counter digits to 4
c. **Saving: Snapshot**
   i. **File Type**: **.bmp** (.tif does not save correctly)
   ii. **Check**: Close after save (keeps images from piling up in the window)
      a. I recommend checking your settings (white balance and exposure) by keeping this box unchecked, take a test image in an **osteon-heavy region**, and look at it in the popup tab. Then delete that test image from the folder and check the box so your real images are not saved in the cellSens window.
   iii. **Path**: File path to a new folder on your external USB / Hard Drive (we will transfer images to a better computer for photomerging)
      a. **Save to a subfolder called "Left" as you will start on the left side**
d. **Camera: General**
   i. **Bit depth:** 10-bit RGB color
   ii. **Mirror:** Unchecked
e. **Camera: Adjustment** (Keep Defaults)
   i. Gamma: 1
   ii. Sharpness: 2
   iii. Contrast: 2
   iv. Shading: Flatfield
f. **Camera: Color**
   i. Copy the **R, G, B** values (from your white balance) to this spreadsheet: https://docs.google.com/spreadsheets/d/1bCUXlHeUb77umeDTyggK8aZp3Nm01BjlowNvjxVjxyw/edit#gid=0
   ii. **Black Balance**: 0

iii. Uncheck Device dependent white balance

g. Click OK to exit

DIC Serpentine Left – Top – Right – Bottom Technique

1. Move to an intracortical area with secondary osteons and fine focus to make the **cement lines as sharp as possible.**
    a. If you find yourself accidentally adjusting the coarse focus knob a lot, you can temporarily lock it using the clamp on the coarse condenser knob

2. In order to facilitate easy photomerging, you will start with the **left side**.
    a. Use the X-axis adjustor to move to the far left of the specimen (on screen – it is reversed on the stage)
    b. Check with the Y-axis adjustor up and down that you are at the very left edge of the sample
    c. Use the Y-axis adjustor to move to the very top left of the sample
9. Hit **F8** on the keyboard to take a snapshot
    a. This image will take a moment to expose and average, will briefly appear in the toolbar, and then will save to your folder and disappear. I recommend keeping a File Explorer window open of your folder to assure yourself that the image saved.
10. Move **down** so that you have about **20-25% overlap** with the previous image. Look for distinct structures (osteons, periosteum/endosteum, etc.)
11. If needed, fine focus the new image, and then hit F8 again to snapshot the next image. However, try not to modify the fine focus unless the Haversian canals or secondary osteon cement lines look out of focus.
12. Continue moving about 75-80% down, fine focus, F8 snapshot until you reach the bottom of the sample

13. Now move to the **right**, leaving about **20-25% overlap** with the previous column. Move the rest of the way down to the **bottom** of this new column (if needed). Then you're your way **up** the column to the top in the same fashion.
14. Continue in this serpentine fashion until you reach the marrow cavity at the top, denoted by a **blank space** below your column. Now you will start on the **top** section.
    a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Top"**
    b. You can begin resizing the images from the "Left" folder at this point.
    c. In the top section, image only from the top of the bone to the marrow cavity in a serpentine fashion.
15. Continue in this serpentine fashion until you reach the right side of the marrow cavity, denoted by bone continuing from the top to the bottom of the sample. You are now in the **right** section.
    a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Right"**
    b. You can begin resizing the images from the "Top" folder at this point.
    c. In the right section, image all the way from the top to the bottom of the bone in a serpentine fashion.
16. On the very **last** image in the **right side** section, **turn back on the scale bar** by clicking on its icon on the top toolbar. A scale bar should appear in the lower right hand corner and be saved with the final image.

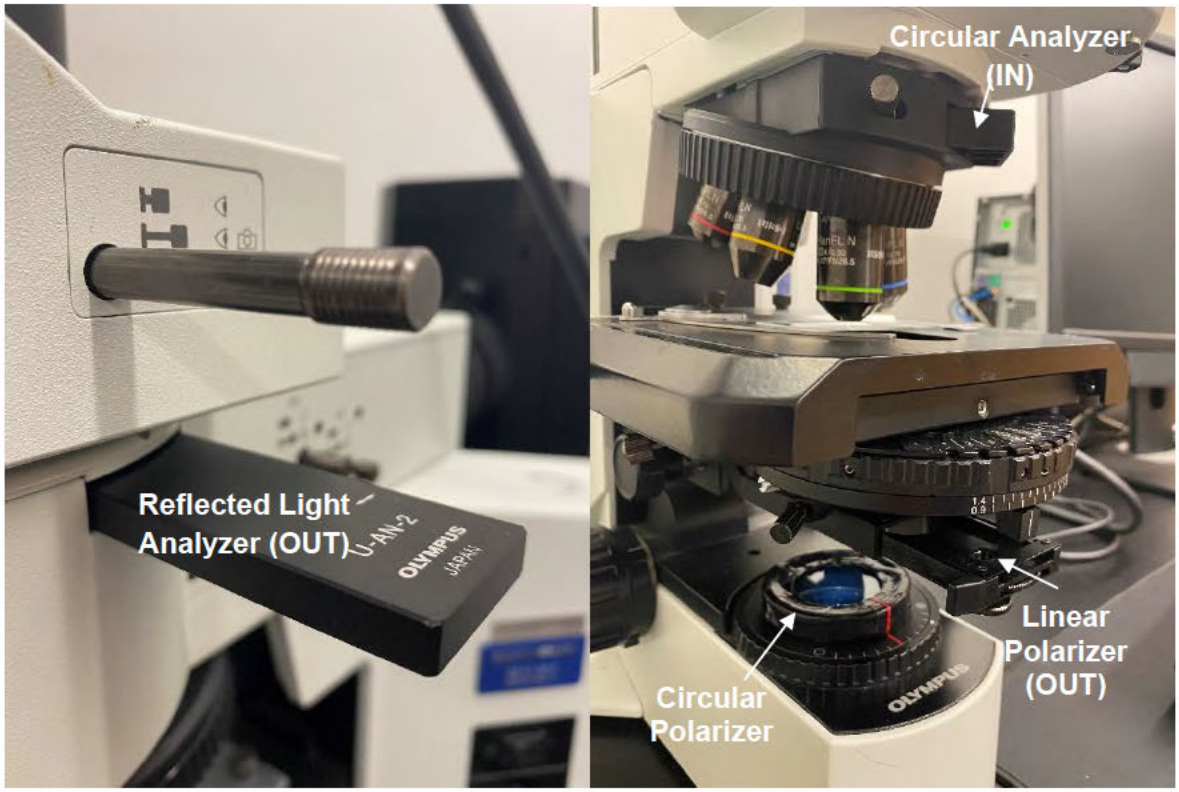
17. Turn the scale bar back off
18. Now you will image the **bottom** section. Go all the way back over to the **left** rim of the marrow cavity, until you see bone from the top to the bottom of the bone. Move over slightly so that you have just barely entered the region where the marrow cavity (blank space) is above you.
    a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Bottom"**
    b. You can begin resizing the images from the "Right" folder at this point.
    c. In the bottom section, image only from the marrow cavity to the bottom of the bone in a serpentine fashion.
    d. After finishing the bottom section, resize its folder.
19. **Resize photos after imaging**
    a. Photomerging is difficult with full sized images. Navigate to the folder and click the first image, hold down Shift, and click the last image to select all images.
    b. Right-click on one of the selected images and select **Resize Images** from the pop-up menu
        i. Note: This is a special plugin installed on both the BX51 computer and FOOTE
    c. Select **Small** from the Resize Images popup
    d. **Be patient! Resizing takes several minutes and there is no evidence that it is running. Do not click inside the File Explorer window while it is working.**
    e. Once the images resize, the original images will be highlighted. Right-click and Cut the selected images, and move to a new subfolder labeled **Original.** Put the resized small images in a new subfolder labeled **Small**.

f. If you clicked inside the File Explorer window while Resize Images was running, nothing will be selected once it finishes. Search for (Small) in the File Explorer window, and you will be able to select only the Small images and move them to a subfolder labeled **Small**.

Circular Polarization

Circular Polarization Setup

1. You must have completed all microscope setup steps to proceed. Make sure the specimen is still in focus at 20x and that the aperture iris diaphragm is still at 70-80%
2. The microscope lamp needs to have been on for **at least 30 minutes** to allow its intensity to stabilize
3. Pull the **reflected light analyzer** out until it clicks into its "out" position
4. Pull the **linear polarizer** out until it clicks into its "out" position
5. Pull the DIC prism U-DICTS out of the cartridge slot completely and **carefully** place it in the padded box
6. Insert the **circular analyzer cartridge** carefully into that same slot – smooth side **up**
    a. Be gentle – the circular analyzer film is held in place only with double sided tape
    b. Push it in past the first "out" click to the second "in" click
    c. Tighten the screw to hold it in place
7. Place the **circular polarizer lens** over the lamp. Match the red line on the circular polarizer with the red line on the field diaphragm



8. Rotate the turret under the stage until the dot matches up with **1.** This is the brightfield light path.

9. Look through the eyepiece. Adjust the rotation of the circular polarizer lens until the background is as dark as possible. This should correspond with alignment of the red lines on the circular polarizer lens and the field diaphragm

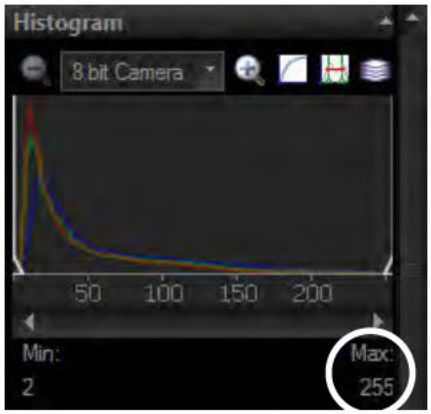
Circular Polarizer Imaging Setup

1. Open cellSens Entry from the Desktop
2. Click Live to turn on Live image
   a. When live, a red rectangle appears on the icon
   b. When stopped, a green triangle appears on the icon
3. Pull the light path adjuster knob out to the third stop so light no longer travels to the eyepiece, and all light is diverted to the camera
4. **Top Left toolbar:**
   a. Select objective **20x** to set the scale properly
   b. Unselect **scalebar** so it does not appear on screen
   c. Select Toggle RGB/Grayscale mode so image is in **color**

5. **Set the white balance on a brightfield image (if you have not already done so for DIC on this sample, on this day)**
   a. Pull the circular analyzer cartridge out to its "out" stop
   b. Use the fine focus knob to finely adjust the image as it appears on the Live image
   c. Use the X-axis and Y-axis buttons to move to a region of the slide that does **not** have the sample, but is still under the coverslip (e.g. inside the marrow cavity)
   d. Pull the light path adjuster knob out to the third stop so light no longer travels to the eyepiece, and all light is diverted to the camera
   e. Select the **White Balance on ROI** (white eyedropper) under Camera Control
   f. Click on the white region of the Live Image, avoiding obvious trash/defects
   g. This pixel value is automatically set to white
   h. Push the circular analyzer cartridge back in to its "in" stop

6. **Set the dark balance on the circularly polarized image**
   a. Select the **Dark Balance on ROI** (black eyedropper) under Camera Control
   b. Move to a perfectly dark region (e.g. the marrow cavity)
   c. Click on the dark region of the Live Image, avoiding obvious trash/defects
   d. This pixel value is automatically set to black

7. **Set the exposure to a 0 – 255 range**
    a. Move to an area that is primarily populated by **secondary osteons**
    b. Set Exposure to Automatic to find a good starting point
    c. Set Exposure to Manual to lock the Automatic value
    d. Look at the Histogram range. Use the Exposure slider, and then the arrows, to adjust the exposure until it has **just** reached the 0 – 255 range.
        i. It's okay if the bottom value is slightly above zero, but the max should be 255
        ii. Generally, this range is around **3 – 5 ms** (on samples I tested)
    e. Move to a few other secondary osteon areas and confirm that you stay in the max-255 range
        i. The maximum will be lower in primary bone areas
    f. Copy the Exposure value to the spreadsheet: https://docs.google.com/spreadsheets/d/1bCUXlHeUb77umeDTyggK8aZp3Nm01BjlowNvjxVjxyw/edit#gid=0



8. **Camera Control** (left) should have the following defaults:
   e. **Exposure**
      i. **Region:** Full Image
      ii. **Sensitivity**: ISO 400
      iii. **Exposure** Compensation: 0
   f. **Resolution:**
      i. **Live/Movie:** 680 x 512 - Any higher and the image will "seize"
      ii. **Snapshot:** 4080 x 3072 (Pixel Shift)
   g. **Image Averaging**
      i. **Live** unchecked
      ii. **Snapshot** unchecked
   h. **DP71**
      i. **Live image quality:** High

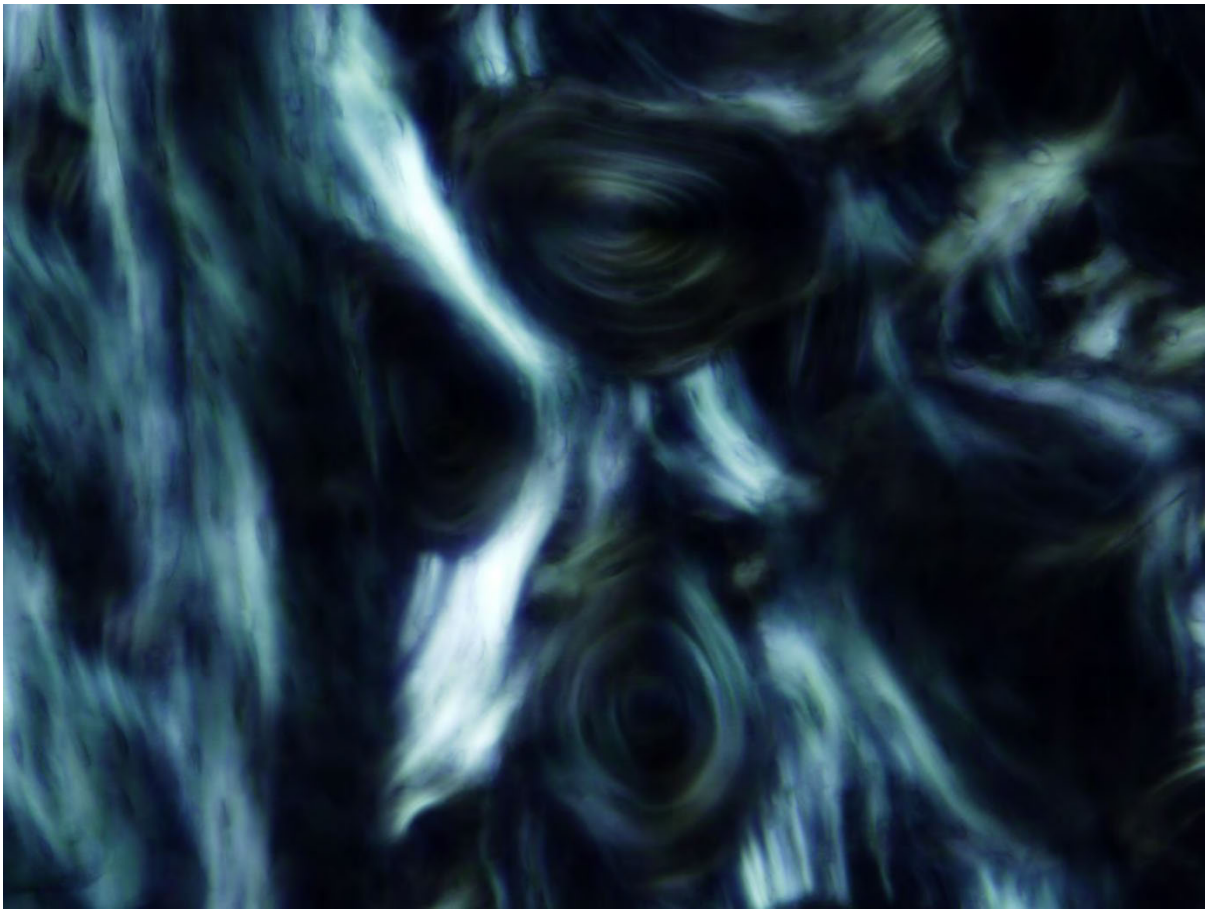
9. Image Acquisition Settings

   a. Click the hand on camera icon under Camera Control
   b. **Document: Snapshot**
      i. Change Text to the image name in the format: **NumberLF_Slide_Pol**
      ii. E.g. 4LF_88um_Pol for Rabbit 4 Left femora, 88 um slide
      iii. Reset Counter Start to 0 and Counter digits to 4
   c. **Saving: Snapshot**
      i. **File Type**: **.bmp** (.tif does not save correctly)
      ii. **Check**: Close after save (keeps images from piling up in the window)

a. I recommend checking your settings (white balance and exposure) by keeping this box unchecked, take a test image, and look at it in the popup tab. Then delete that test image from the folder and check the box so your real images are not saved in the cellSens window.
    iii. **Path**: File path to a new folder on your external USB / Hard Drive (we will transfer images to a better computer for photomerging)
d. **Camera: General**
    i. **Bit depth:** 10-bit RGB color
    ii. **Mirror:** Unchecked
e. **Camera: Adjustment** (Keep Defaults)
    i. Gamma: 1
    ii. Sharpness: 2
    iii. Contrast: 2
    iv. Shading: Flatfield
f. **Camera: Color**
    i. Copy the **R, G, B** values (from your white balance) to this spreadsheet: https://docs.google.com/spreadsheets/d/1bCUXlHeUb77umeDTyggK8aZp3Nm01BjlowNvjxVjxyw/edit#gid=0
    ii. Copy the **Black Balance** value to the spreadsheet
    iii. Uncheck Device dependent white balance
g. Click OK to exit

Circular Polarizer Serpentine Left – Top – Right – Bottom Technique

1. Move to an intracortical area with secondary osteons and fine focus to **make the fibers inside the (bright) collagen fibers as sharp as possible.**
    a. If you find yourself accidentally adjusting the coarse focus knob a lot, you can temporarily lock it using the clamp on the coarse condenser knob



2. In order to facilitate easy photomerging, you will start with the **left side**.
    a. Use the X-axis adjustor to move to the far left of the specimen (on screen – it is reversed on the stage)
    b. Check with the Y-axis adjustor up and down that you are at the very left edge of the sample
    c. Use the Y-axis adjustor to move to the very top left of the sample
20. Hit **F8** on the keyboard to take a snapshot
    a. This image will take a moment to expose and average, will briefly appear in the toolbar, and then will save to your folder and disappear. I recommend keeping a File Explorer window open of your folder to assure yourself that the image saved.
21. Move **down** so that you have about **20-25% overlap** with the previous image. Look for distinct structures (osteons, periosteum/endosteum, etc.)
22. If needed, fine focus the new image, and then hit F8 again to snapshot the next image. However, try not to modify the fine focus unless the Haversian canals or secondary osteon cement lines look out of focus.
23. Continue moving about 75-80% down, fine focus, F8 snapshot until you reach the bottom of the sample
24. Now move to the **right**, leaving about **20-25% overlap** with the previous column. Move the rest of the way down to the **bottom** of this new column (if needed). Then you're your way **up** the column to the top in the same fashion.
25. Continue in this serpentine fashion until you reach the marrow cavity at the top, denoted by a **black space** below your column. Now you will start on the **top** section.

a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Top"**
b. You can begin resizing the images from the "Left" folder at this point.
c. In the top section, image only from the top of the bone to the marrow cavity in a serpentine fashion.

26. Continue in this serpentine fashion until you reach the right side of the marrow cavity, denoted by bone continuing from the top to the bottom of the sample. You are now in the **right** section.
    a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Right"**
    b. You can begin resizing the images from the "Top" folder at this point.
    c. In the right section, image all the way from the top to the bottom of the bone in a serpentine fashion.
27. On the very **last** image in the **right side** section, **turn back on the scale bar** by clicking on its icon on the top toolbar. A scale bar should appear in the lower right hand corner and be saved with the final image.

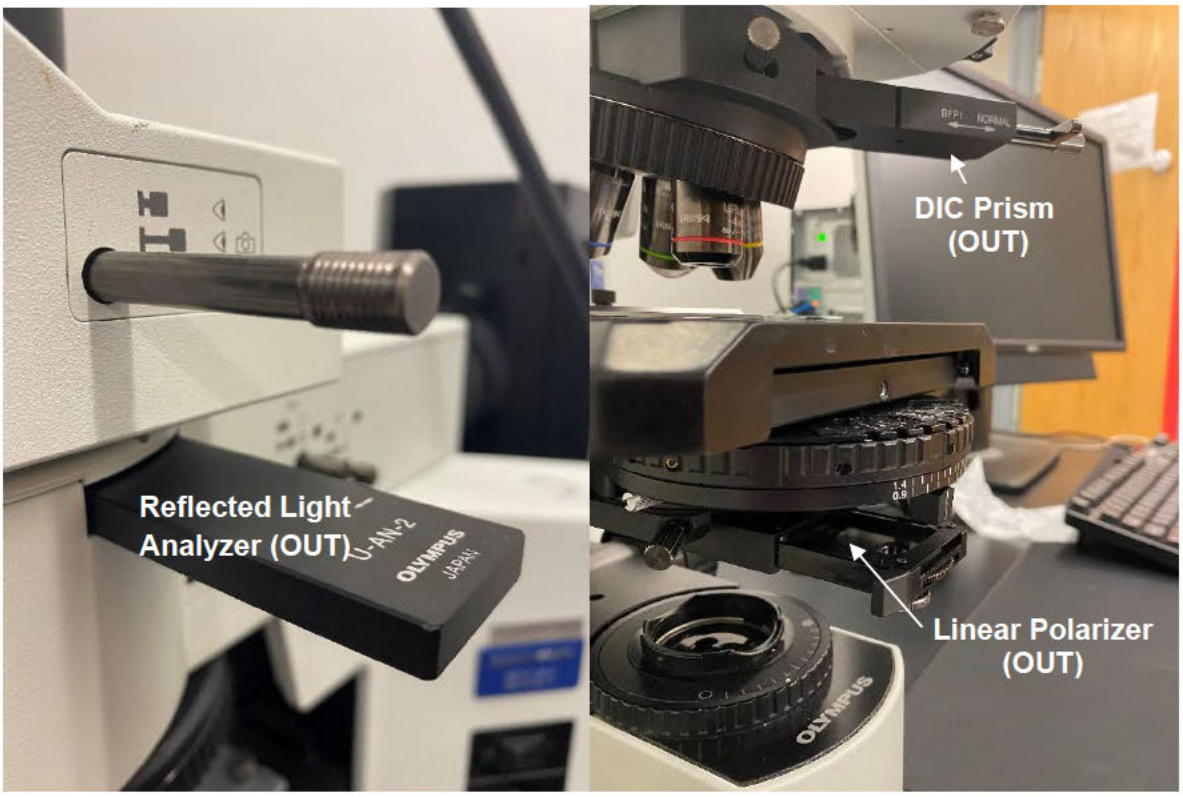
28. Turn the scale bar back off
29. Now you will image the **bottom** section. Go all the way back over to the **left** rim of the marrow cavity, until you see bone from the top to the bottom of the bone. Move over slightly so that you have just barely entered the region where the marrow cavity (blank space) is above you.
    a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Bottom"**
    b. You can begin resizing the images from the "Right" folder at this point.
    c. In the bottom section, image only from the marrow cavity to the bottom of the bone in a serpentine fashion.
    d. After finishing the bottom section, resize its folder.
30. **Resize photos after imaging**
    a. Photomerging is difficult with full sized images. Navigate to the folder and click the first image, hold down Shift, and click the last image to select all images.
    b. Right-click on one of the selected images and select **Resize Images** from the pop-up menu
        i. Note: This is a special plugin installed on both the BX51 computer and FOOTE
    c. Select **Small** from the Resize Images popup
    d. **Be patient! Resizing takes several minutes and there is no evidence that it is running. Do not click inside the File Explorer window while it is working.**
    e. Once the images resize, the original images will be highlighted. Right-click and Cut the selected images, and move to a new subfolder labeled **Original.** Put the resized small images in a new subfolder labeled **Small**.
    f. If you clicked inside the File Explorer window while Resize Images was running, nothing will be selected once it finishes. Search for (Small) in the File Explorer window, and you will be able to select only the Small images and move them to a subfolder labeled **Small**.

Fluorescence Imaging

Fluorescence Setup

1. You must have completed all microscope setup steps to proceed. Make sure the specimen is still in focus at 20x and that the aperture iris diaphragm is still at 70-80%
2. Turn on the mercury lamp and reset the timer, which counts in fractions of hours
    a. The lamp must run **at least 40 minutes** (~0.7) once you turn it on
        i. Wait 5-10 minutes for it to stabilize before capturing images
    b. The lamp must rest **at least 40 minutes** after you turn it off, before more use

3. Pull the **reflected light analyzer** out until it clicks into its "out" position
4. Pull the **linear polarizer** out until it clicks into its "out" position
5. Pull the DIC prism U-DICTS until it clicks into its "out" position



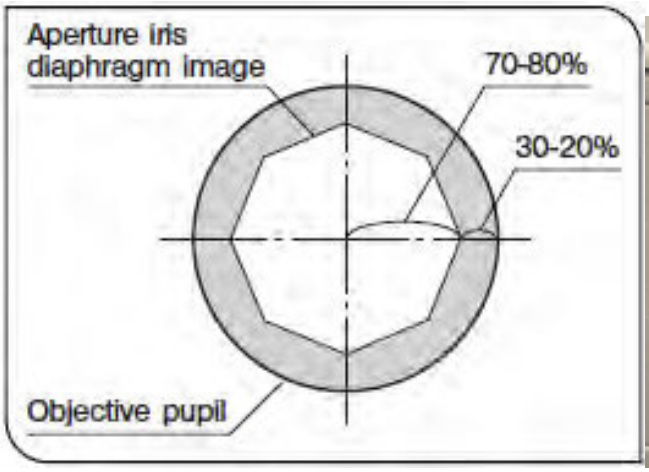
6. Rotate the turret under the stage until the dot matches up with **1.** This is the brightfield light path.

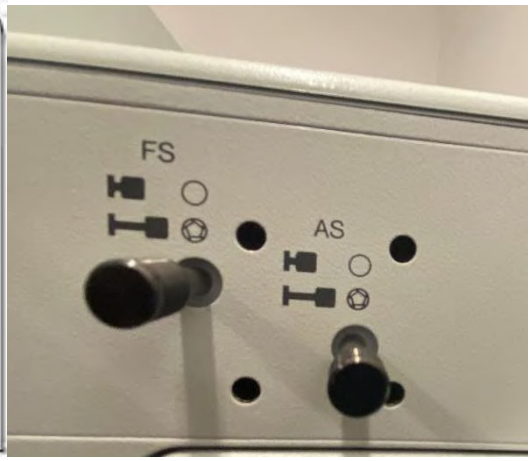
7. Turn the lamp intensity all the way off
8. **Turn off the room light and close the door**. You can use the red LEDs to see better.
9. Rotate the filter cube to **3 (FITC)**

10. Turn the shutter from the black dot (off) to the white dot (on)

Avoid photobleaching! Close the shutter when you are not actively imaging!

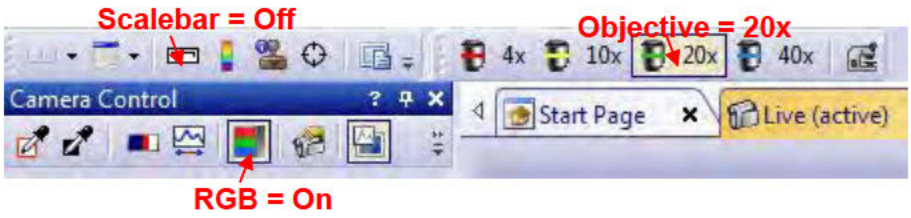
11. **Adjust field iris and aperture iris**
    a. Looking through the eyepiece, rotate the **pull out** the **field iris diaphragm knob (FS)** (on the right side of the microscope above the objectives) until the lighted space takes up 70-80% of the field of view
    b. Pull the **aperture iris diaphragm knob (AS)** all the way **out**. This should reduce background fluorescence.





Fluorescence Imaging Setup

1. Open cellSens Entry from the Desktop
2. Click Live to turn on Live image
   a. When live, a red rectangle appears on the icon
   b. When stopped, a green triangle appears on the icon
3. Pull the light path adjuster knob out to the third stop so light no longer travels to the eyepiece, and all light is diverted to the camera
4. **Top Left toolbar:**
   a. Select objective **20x** to set the scale properly
   b. Unselect **scalebar** so it does not appear on screen
   c. Select Toggle RGB/Grayscale mode so image is in **color**

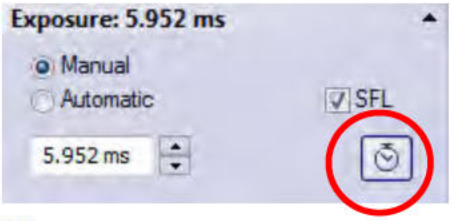


5. **Set the white balance on a brightfield image (if you have not already done so for DIC on this sample, on this day)**
   a. Close the shutter, rotate the filter cube back to 1, and return the lamp intensity to 9
   b. Use the fine focus knob to finely adjust the image as it appears on the Live image
   c. Use the X-axis and Y-axis buttons to move to a region of the slide that does **not** have the sample, but is still under the coverslip (e.g. inside the marrow cavity)
   d. Pull the light path adjuster knob out to the third stop so light no longer travels to the eyepiece, and all light is diverted to the camera
   e. Select the **White Balance on ROI** (white eyedropper) under Camera Control
   f. Click on the white region of the Live Image, avoiding obvious trash/defects
   g. This pixel value is automatically set to white
   h. Rotate the filter cube back to 3, turn the lamp intensity to zero, and open the shutter

6. **Set the dark balance on the fluorescence image**
   a. Select the **Dark Balance on ROI** (black eyedropper) under Camera Control
   b. Move to a perfectly dark region (e.g. the marrow cavity)
   c. Click on the dark region of the Live Image, avoiding obvious trash/defects
   d. This pixel value is automatically set to black

6. **Camera Control** (left) should have the following defaults:
    i. **Exposure**
        i. **Region:** Full Image
        ii. **Sensitivity**: ISO 400
        iii. **Exposure** Compensation: 0
    j. **Resolution:**
        i. **Live/Movie:** 680 x 512
1. Any higher and the image will “seize”
        ii. **Snapshot:** 4080 x 3072 (Pixel Shift)
    k. **Image Averaging**
        i. **Live** unchecked
        ii. **Snapshot** unchecked
    l. **DP71**
        i. **Live image quality:** High

7. **Set the exposure**
    a. Use the X-axis and Y-axis adjustors to move to the **linea aspera**, which includes both the periosteal rim and secondary osteons as fluorescing structures
    b. Under Exposure, select Manual and then the clock icon to conduct a One-Time Auto Exposure
    c. The collected auto-exposure value will appear, and Exposure will be automatically changed to manual
    d. **Record the Exposure value on this spreadsheet:** https://docs.google.com/spreadsheets/d/1bCUXlHeUb77umeDTyggK8aZp3Nm01BjlowNvjxVjxyw/edit#gid=0



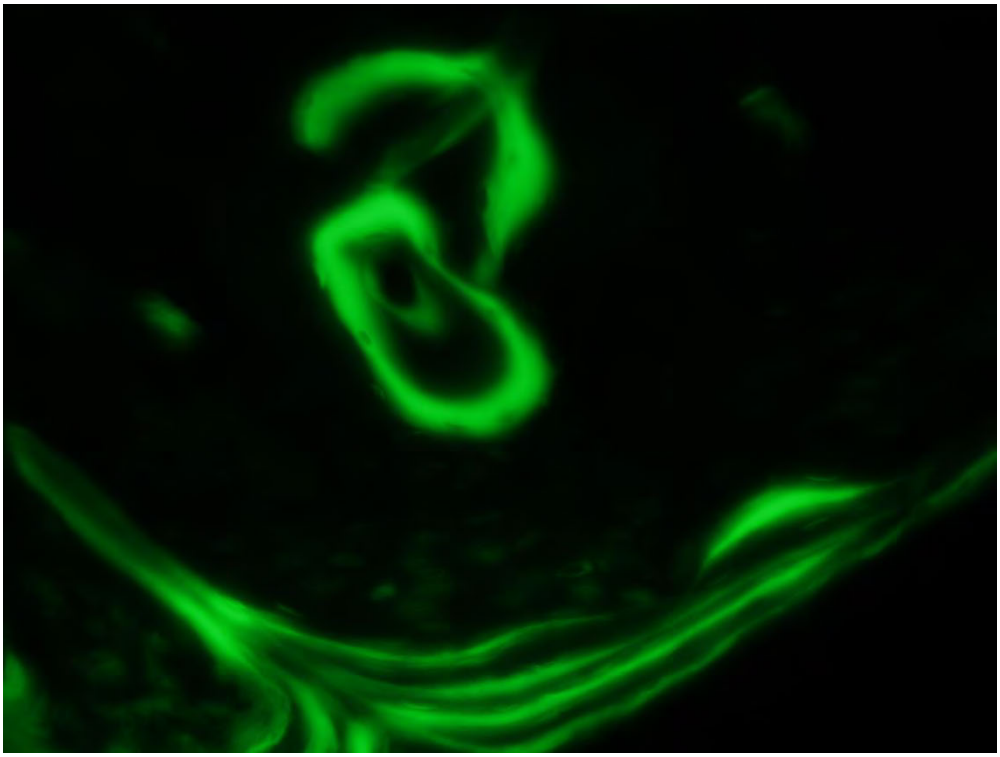
8. **Image Acquisition Settings**

    a. Click the hand on camera icon under Camera Control
    b. **Document: Snapshot**
        i. Change Text to the image name in the format: **NumberLF_Slide_Flo**
        ii. E.g. 4LF_88um_Flo for Rabbit 4 Left femora, 88 um slide
        iii. Reset Counter Start to 0 and Counter digits to 4
    c. **Saving: Snapshot**
        i. **File Type**: **.bmp** (.tif does not save correctly)
        ii. **Check**: Close after save (keeps images from piling up in the window)
            a. I recommend checking your settings (white balance and exposure) by keeping this box unchecked, take a test image, and look at it in the popup tab. Then delete that test image from the folder and check the box so your real images are not saved in the cellSens window
        iii. **Path**: File path to a new folder on your external USB / Hard Drive (we will transfer images to a better computer for photomerging)
    d. **Camera: General**
        i. **Bit depth:** 10-bit RGB color
        ii. **Mirror:** Unchecked
    e. **Camera: Adjustment** (Keep Defaults)
        i. Gamma: 1
        ii. Sharpness: 2
        iii. Contrast: 2
        iv. Shading: Flatfield
    f. **Camera: Color**
        i. Copy the **R, G, B** values (from your white balance) to this spreadsheet: https://docs.google.com/spreadsheets/d/1bCUXlHeUb77umeDTyggK8aZp3Nm01BjlowNvjxVjxyw/edit#gid=0
        ii. Copy the **Black Balance** value to the spreadsheet
        iii. Uncheck Device dependent white balance

g. Click OK to exit

Fluorescence Serpentine Left – Top – Right – Bottom Technique

1. Move to an intracortical area with secondary osteons and fine focus to **make the osteons and periosteal lines as sharp as possible** (minimize background glow)
    a. If you find yourself accidentally adjusting the coarse focus knob a lot, you can temporarily lock it using the clamp on the coarse condenser knob



2. In order to facilitate easy photomerging, you will start with the **left side**.
    a. Use the X-axis adjustor to move to the far left of the specimen (on screen – it is reversed on the stage)
    b. Check with the Y-axis adjustor up and down that you are at the very left edge of the sample
    c. Use the Y-axis adjustor to move to the very top left of the sample
31. Hit **F8** on the keyboard to take a snapshot
    a. This image will take a moment to expose and average, will briefly appear in the toolbar, and then will save to your folder and disappear. I recommend keeping a File Explorer window open of your folder to assure yourself that the image saved.
32. Move **down** so that you have about **20-25% overlap** with the previous image. Look for distinct structures (osteons, periosteum/endosteum, etc.)
33. If needed, fine focus the new image, and then hit F8 again to snapshot the next image. However, try not to modify the fine focus unless the Haversian canals or secondary osteon cement lines look out of focus.
34. Continue moving about 75-80% down, fine focus, F8 snapshot until you reach the bottom of the sample
35. Now move to the **right**, leaving about **20-25% overlap** with the previous column. Move the rest of the way down to the **bottom** of this new column (if needed). Then you're your way **up** the column to the top in the same fashion.
36. Continue in this serpentine fashion until you reach the marrow cavity at the top, denoted by a **black space** below your column. Now you will start on the **top** section.
    a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Top"**
    b. You can begin resizing the images from the "Left" folder at this point.
    c. In the top section, image only from the top of the bone to the marrow cavity in a serpentine fashion.

37. Continue in this serpentine fashion until you reach the right side of the marrow cavity, denoted by bone continuing from the top to the bottom of the sample. You are now in the **right** section.
    a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Right"**
    b. You can begin resizing the images from the "Top" folder at this point.
    c. In the right section, image all the way from the top to the bottom of the bone in a serpentine fashion.
38. On the very **last** image in the **right side** section, **turn back on the scale bar** by clicking on its icon on the top toolbar. A scale bar should appear in the lower right hand corner and be saved with the final image.

39. Turn the scale bar back off
40. Now you will image the **bottom** section. Go all the way back over to the **left** rim of the marrow cavity, until you see bone from the top to the bottom of the bone. Move over slightly so that you have just barely entered the region where the marrow cavity (blank space) is above you.
    a. Under Image Acquisition Settings, change the subfolder for saving to a new subfolder **"Bottom"**
    b. You can begin resizing the images from the "Right" folder at this point.
    c. In the bottom section, image only from the marrow cavity to the bottom of the bone in a serpentine fashion.
    d. After finishing the bottom section, resize its folder.
41. **Resize photos after imaging**
    a. Photomerging is difficult with full sized images. Navigate to the folder and click the first image, hold down Shift, and click the last image to select all images.
    b. Right-click on one of the selected images and select **Resize Images** from the pop-up menu
        i. Note: This is a special plugin installed on both the BX51 computer and FOOTE
    c. Select **Small** from the Resize Images popup
    d. **Be patient! Resizing takes several minutes and there is no evidence that it is running. Do not click inside the File Explorer window while it is working.**
    e. Once the images resize, the original images will be highlighted. Right-click and Cut the selected images, and move to a new subfolder labeled **Original.** Put the resized small images in a new subfolder labeled **Small**.
    f. If you clicked inside the File Explorer window while Resize Images was running, nothing will be selected once it finishes. Search for (Small) in the File Explorer window, and you will be able to select only the Small images and move them to a subfolder labeled **Small**.

Photomerging: All Images

I highly recommend that you mark on the slide label the direction the slide was facing (e.g. arrow towards the microscope) so that it is easy to replace if you miss a spot.

Attempt First: Image Composite Editor

Image composite editor is good at photomerging clear images, but you cannot adjust any of the components if they merge incorrectly. Try it first and see if you are lucky to have a clean merge. It will run very quickly.

1. Move the hard drive with your resized images to FOOTE
2. Open Image Composite Editor
3. New Panorama from Images → Navigate to the **Small** folder of one of your four regions (Top, Bottom, Right, Left)
4. Simple Panorama
    a. Camera Motion = Planar Motion
    b. Click Next
5. If the stitching is successful, click next
6. Crop:
    a. Do not use Auto Complete
    b. Select No Crop
    c. Click Next
7. Export
    a. Image Size Scale = 100%
    b. Image File Format = Windows Bitmap
    c. Export to Disk → Save in desired file location
8. If the image merges well (no distorted internal regions) but has large regions that are disconnected, try merging ½ or 1/3 of the images and then pasting the sections together in photoshop. Look for a clean top and bottom so you end your sub-section on a complete column.

Attempt Second: Photoshop CS3 Photomerge

Photoshop CS3 is old, but it allows you to move components that have incorrectly merged and "snap" them into place. It can only handle about 150 of the 200x images at a time. It may take several hours to overnight to run a whole region.

1. Search for Adobe Bridge CS3 (not the most recent version) on FOOTE
2. Navigate to the **Small** folder of one of your four regions (Top, Bottom, Right, Left)
3. Select all images by clicking the first image, holding Shift, and clicking the last image.
4. Tools → Photoshop → Photomerge
5. Adobe Photoshop CS3 will open
6. Select Layout option **Interactive Layout**
7. Check box **Blend Images Together**
8. Click OK
9. Photomerge may take quite some time to run – leave it alone
10. **Manually fixing merge errors:**
    a. Images that photoshop could not merge appear in the top panel. You can leave them in the panel if you don't want them (e.g. completely white or black) and they will be deleted
    b. Zoom in and out using the mountain icon slider

c. To move an incorrectly merged image, just drag it to the correct location with your mouse. If it matches well, it should "snap" into place. You can also just leave it there to force it into place.
d. Click OK when finished

11. Image → Layer → Flatten Image
12. Save As → Save as a BMP with default settings

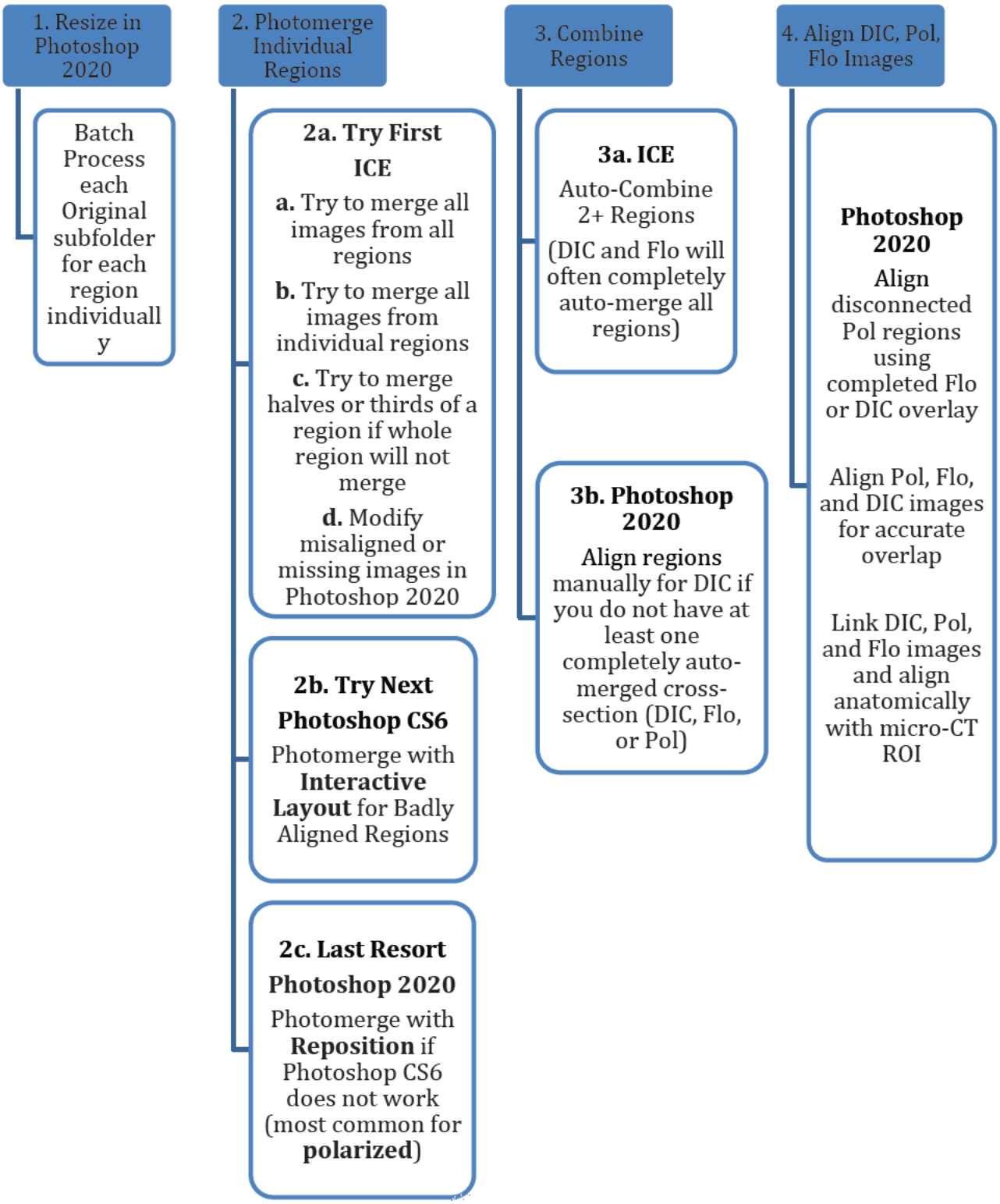
Combining The Four Regions in Photoshop

1. This is easier in Photoshop 2019 or 2020 (on FOOTE)
2. Open the first region using File → Open
3. If you merged with Image Composite Editor, you will need to make the black background transparent
    a. On the left-side toolbar, hold down the eraser tool  and select Magic Eraser Tool from the popup menu
    b. Click on the black background regions to make them transparent
4. Expand canvas to accommodate the next region using Image → Canvas Size
5. Repeat steps 3 and 4 for the next region, which will open in a new tab
6. Go to the tab for the region you want to place, and select Edit → Copy
7. Go to the tab for the region with the expanded canvas and select Edit → Paste. The region will paste as a new layer
8. On the Layers panel, select the region you just pasted. Click Control – T to free transform, or use the Edit → Transform menu to rotate and flip the selected region
9. Use the magnifying glass from the left-side tools panel to zoom in and make sure the regions fit well together
10. Layer → Flatten Image to combine
11. Crop excess white space out of the image using Image → Trim
12. File → Save As and save the final image as a BMP (uncompressed)

I Missed a Spot!

1. Make sure the microscope is set up for the appropriate imaging setup and the slide is in the same position on the stage
2. In cellSens Entry:
    a. Image Acquisition: Camera Color, enter your recorded values for white balance (R, G, B) and black balance (if appropriate)
    b. Exposure: Enter your recorded value for exposure
3. Navigate to the missed region using the landmarks on your photomerged image and re-take the image
4. Resize the image to Small as before
5. If you needed to take multiple images, merge them into a small region using Image Composite Editor or Photoshop CS3
6. Paste and Flatten the small region onto your main photomerge as before.

Appendix XXXI: Photomerging SOP



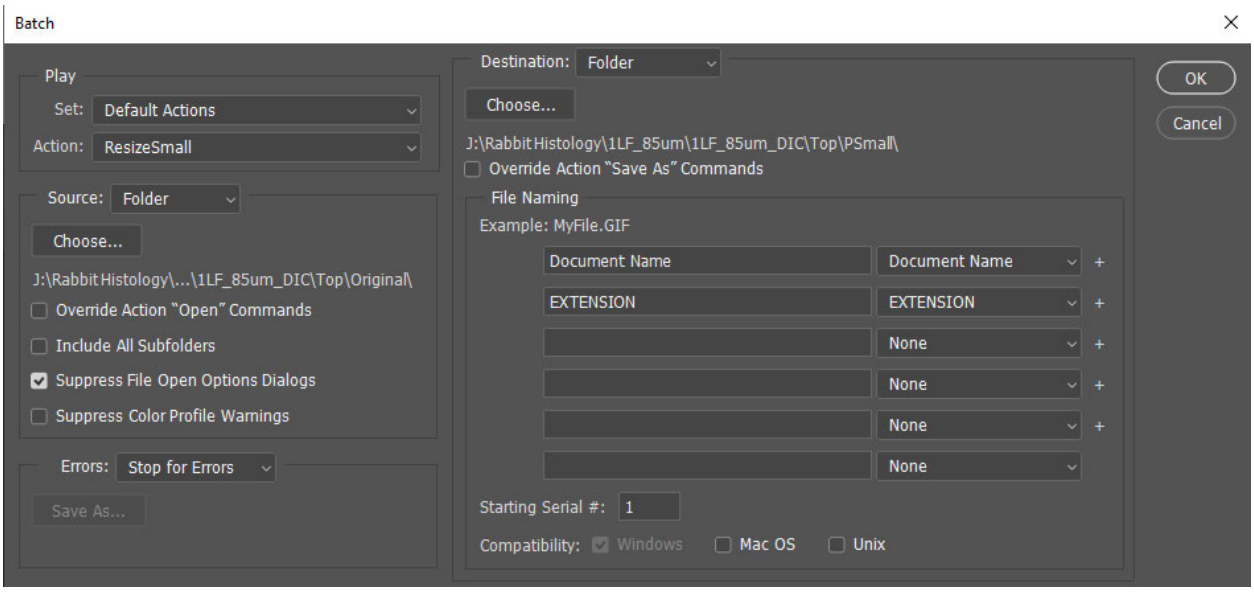
1. **Resize in Photoshop**

We previously resized in Windows File Explorer, but this creates a rim of light around photo edges that interferes with ICE. We will now resize in Photoshop using a custom macro. We are resizing to a quarter of original size (1020 x 736 pixels) to layer-based export from ICE and manual correction in photoshop
How to set up the resize macro the first time in Photoshop 2020 (needs to be done if you are using a new computer or installation – it is already on FOOTE):

1. Open any original-sized microscopic image in photoshop
2. Go to Window --> Actions
3. Select the + button to create a new action (I named mine ResizeQuarter) then click Record. The red button will be lit in the actions panel indicating you are recording.
4. Select Image --> Image Size
5. In the Image Size popup, change Width to 1020 pixels. The Height should automatically change to 768 pixels. Keep the other defaults (Resolution 300 Pixels/Inch, Resample Automatic).
6. Click OK
7. Click the white square stop button on the Actions panel to stop recording
8. Now your macro should be available under File --> Automate --> Batch in the Action dropdown menu

How to resize images after setting up the macro:

1. Move the hard drive with the original-sized images to FOOTE
2. Open Photoshop 2020 (search Photoshop in toolbar to find it)
    a. Note that Photoshop 2019 and CS3 are also installed
3. File → New → Create → Accept defaults
4. File → Automate → Batch
    a. Set: Default Actions
    b. Action: ResizeQuarter
    c. Source: Folder → Choose your Original folder
        i. Checkbox: Suppress File Open Options Dialogs
        ii. **Note: It will show you the last folder you were in within photoshop – make sure you are in the correct folder**
    d. Destination: Folder → Make a new output folder to hold the resized images (I have been calling it PSmall)
        i. **Note: It will show you the last folder you were in within photoshop – make sure you are in the correct folder**
    e. Leave all other defaults
    f. Click OK; images will automatically load, resize, save, and close in turn



Batch Processing Dialog in Photoshop 2019

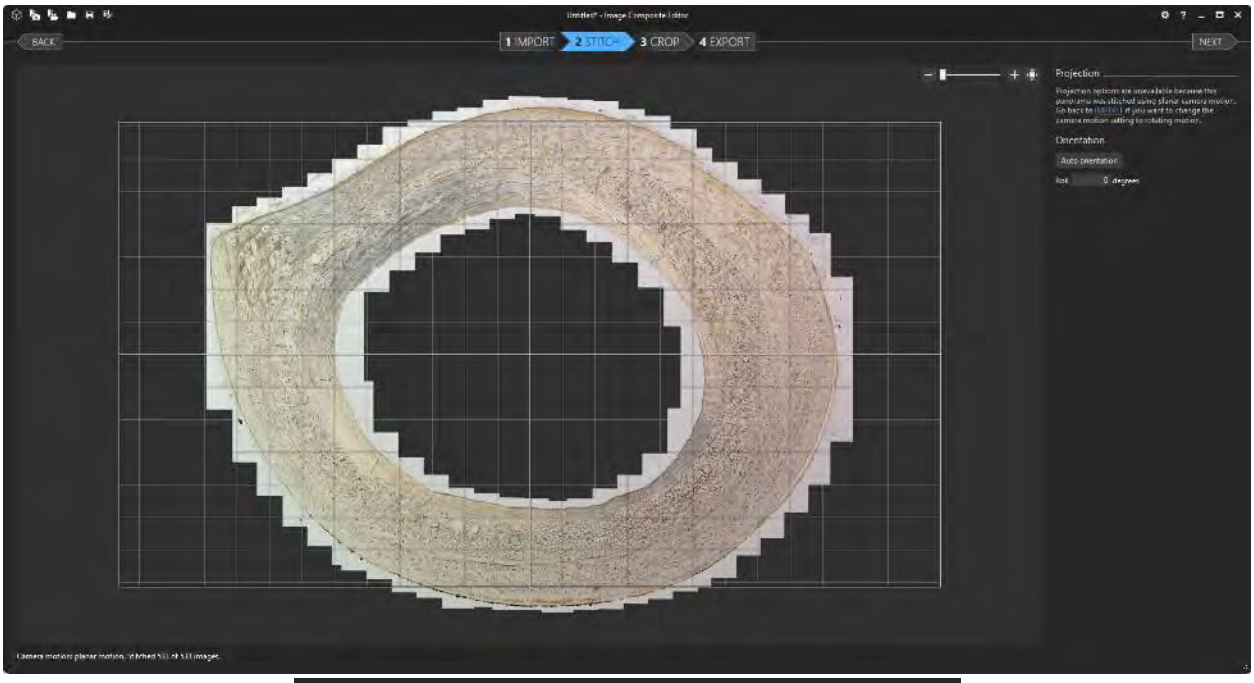
2. Photomerge Each Region

2a. Image Composite Editor (ICE)

If you get lucky, sometimes all images from your regions will merge into a single composite. You may try throwing **images from all four regions** in to ICE first (before merging individual regions) to see if this works. If this does not work, **restrict the input to one region at a time.**

ICE Download: https://www.microsoft.com/en-us/research/product/computational-photography-applications/image-composite-editor/

1. Install ICE if on a new computer (it is already on Foote)
2. Search ICE in the toolbar to open Image Composite Editor
3. Select New Panorama from Images
4. In the Select Overlapping Images popup, select the **photoshop resized** version of one of your regional folders (e.g. Bottom Small)
    a. Ctrl + A to highlight all images
    b. Click Open
5. If you want to try merging **all** regions, click **Add Images** and repeat step 4 to load the small versions of images from the other three regions
6. Select Simple Panorama
7. Under Camera Motion, change to Planar Motion
8. Click Next and wait for ICE to run (it runs fairly quickly – usually a few minutes)
9. Check the number of images merged at the bottom (e.g. "Stitched X of X Images")



ICE could combine all Regions

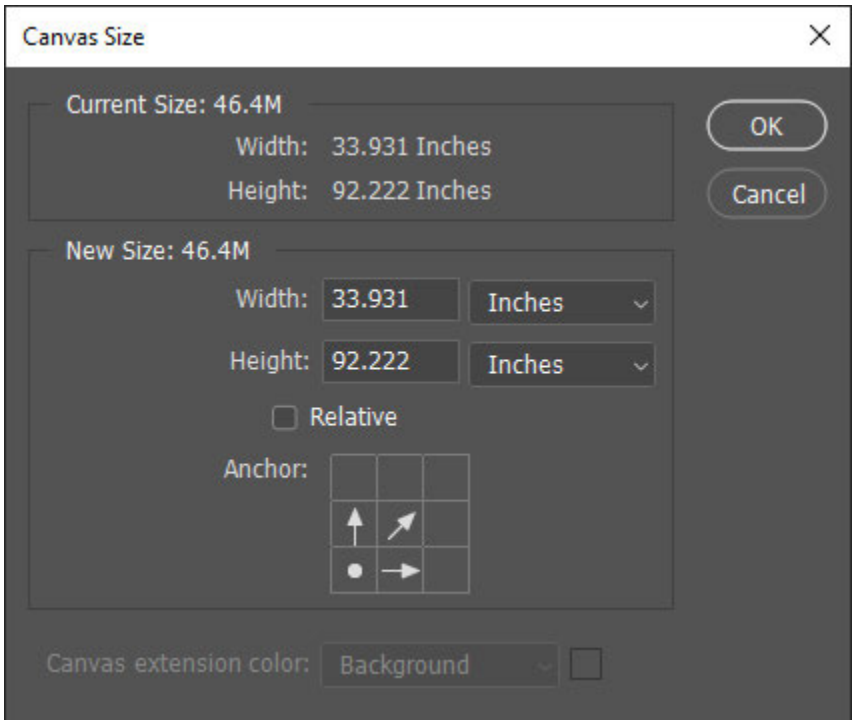
10. **If you were merging multiple regions and they merged badly or missed many images:** Click the New panorama icon in the upper left hand corner  and try combining only the regions that merged well (or try one region at a time)
11. "2 Stitch" tab displays; do not select any settings; just click Next
12. "3 Crop" tab displays; do not select any settings; just click Next
13. "4 Export" tab displays; keep Image scale at default 100%
14. **If all images were merged:** Export as TIFF file (single composite images)
    a. File format = TIFF Image
    b. Alpha = Check box to include Alpha channel
    c. Export to Disk = Save in new ICE subfolder for merged regions
        i. Call it by region name e.g. "Bottom"
15. **If some images are missing or just slightly incorrectly merged:** Export as Photoshop layers for manual correction (the composite will be made of the layers of individual images)
    a. File format = Adobe Photoshop
    b. Layers = All layers
    c. Check box to Maximize compatibility
    d. Export to disk = Save in new ICE subfolder for merged regions to modify
        i. Denote it needs to be fixed in photoshop e.g. "BottomPS"
    e. Note that merges of multiple regions may be too large to export in Photoshop – in this case, merge the slightly incorrect region individually and export it for individual correction in photoshop
16. **If the individual region is very badly merged or has many images missing**
    a. Redo by loading just half of the region (look for the natural break of the top or bottom of a column) and merging it alone.

i. To select a subset of images (e.g. try to photomerge only half of the region), click the first image you want, hold Shift, and click the last image you want.

b. If this works, repeat with the other half

Correcting ICE Merge Mistakes of Individual Images in Photoshop

1. Open Photoshop 2020
2. Use View → Uncheck "Extras" to turn off grid for greater visibility
3. Drag and drop the .psd file exported from ICE, or File → Open
4. Make sure Snap to Layer is activated under the top toolbar View → Snap.
    a. If you do not see a checkmark, click Snap
    b. Check Snap To and make sure only Layers is checked
5. Scroll down to the bottom of the layers panel and click on the layer "Composite." This is the composite image than cannot be modified. Right click → Delete Layer
6. **Expand Canvas Size:** If you need to expand the size of the Canvas to accommodate missing or misaligned images, use Image → Canvas Size
    a. Anchor the current region in the corner or side opposing the corner/side you want to expand, using the arrow keys
    b. Ballpark how much you want to expand (e.g. double the width or height) – you will crop the excess later, so it's fine to use more than you will need
    c. Click OK



Anchoring a Canvas in the lower left corner to expand it to the top and right sides

7. Manually Move Incorrectly Merged Images
    a. With the **move tool** selected from the left hand toolbar  , click the misaligned image. It will be highlighted on the right-side **Layers** toolbar.

   i. To move multiple connected images, use the move tool to draw a box around the connected images
b. Use hotkeys **Ctrl + Shift +]** on the keyboard (easier on the right side) to bring the selected image to the front, or use Layer → Arrange → Bring to Front
c. Move the image / images to the larger section of the image that is correctly merged. Use visual similarly to match the image.
   i. If you're not sure where an image goes, click on the incomplete region of the larger, correctly merged section. The layer will be selected on the Layer panel and you can check the number. Look for a free-floating or misaligned image with a similar number.
d. Gently move the image towards the visual similarity on the larger, correctly merged image. When the visual similarities match, the moving image will "snap" or slightly settle into place
   i. Be aware that (especially on polarized images), images do sometimes incorrectly snap together if they are visually homogenous
   ii. If the visual similarity is not clear enough for the two images to be "snapped", just manually move it into place

8. **Add Missing Adjacent Images**
   a. Try this when a whole column or section of adjacent columns is missing, but you think they may merge to each other
   b. File → Scripts → Load Files into Stack
   c. In the Load Layers popup, Browse and select the adjacent missing images composing the column(s) from your **photoshop resized** folder of that region
      i. Click the first image you want, hold Shift, and click the last image you want. **Only select images adjacent to each other.**
      ii. Click OK. The file names appear in the Load Layers window
      iii. Check box for "Attempt to Automatically Align Source Images"
      iv. Click OK
   d. The mini-photomerge appears in a **new tab** at the top of the page.
   e. If the photomerge was successful, it will appear in whole on the page.
   f. If the photomerge was not successful, click the first Layer in the Layers pane, hold down shift, and click the last Layer in the layers pane to highlight them all
      i. Select Edit → Auto-Align Layers
      ii. In the Auto-Align Layers popup, click **Reposition**
      iii. Click OK and the mini-photomerge will be attempted
   g. Once the mini-photomerge is acquired, merge the layers down so they operate as a single layer
      i. Click the first Layer in the Layers pane, hold down shift, and click the last Layer in the layers pane to highlight them all
      ii. Merge them using hotkey **Ctrl + E**, or else select Layer → Merge Down.
   h. Copy the merged layer by clicking it then **Ctrl + C**
   i. Paste the merged layer onto your main photoshop tab using **Ctrl + P**

9. **Add Missing Isolated Images**
   a. Try this if you need to paste one or more missing images that are not connected (or if the mini-photomerge in the previous step fails or excludes some images)
   b. Open a File Explorer window and navigate to your photoshop resized folder of that region. Identify the missing images.

i. You can click on an unfinished edge or section of the current photoshop image and it will be highlighted in the layers panel. Look at the number in the filename to see what adjacent number to select.

c. In File Explorer, highlight the missing image you want to paste
   i. To highlight multiple disconnected images, click Ctrl and click each one in turn
d. Drag and drop the select image(s) onto the Photoshop window
e. You will need to click Enter for each pasted image, which appears in a single stack with a blue X over the top. The X is removed when all images have been pasted via the Enter key.
f. If you pasted more than one image, move the pasted images out of a single stack with the move tool  and snap them to their correct location as before.

10. **(Only if Necessary) Merge Completed Sections:** If there are one or more chunks of the image that are well-merged, but you keep accidentally moving them, you can lock them into a single layer so they will not be accidentally modified. I would only do this for **internal** regions you are sure are complete, because edge regions may not actually be aligned with the connecting images and you cannot undo this step.
    a. Select the **move tool** from the left hand toolbar 
    b. Click and draw a box around the completed section you want to merge
    c. Use hotkey **Ctrl + E**, or else select Layer → Merge Down. Now this section will behave as a single layer
11. **Flattening and Exporting**
    a. Image → Trim → Based on Transparent Pixels
    b. Layer → Flatten Image
    c. File → Save As → Save on Your Computer
    d. Change Save as Type to .tiff
    e. In the .tiff options popup, choose Image Compression = None. Leave all other defaults and click OK.

2b. <u>Photomerge with Interactive Layout in Photoshop CS6 – for images that won't merge recognizably in ICE</u>

Photoshop CS6 is old, but it has an Interactive Layout feature that more easily allows you to move components that have incorrectly merged and "snap" them into place. It can only handle about 150 of the 200x images at a time. It may randomly freeze during operation – in which case, move on to Photoshop 2020.

13. Search for Adobe Bridge CS6 (not the most recent version) on FOOTE
14. Navigate to the **photoshop resized** folder of one of your four regions (Top, Bottom, Right, Left)
15. Select all images with Ctrl+A.
    a. To select a subset of images (e.g. try to photomerge only half of the region), click the first image you want, hold Shift, and click the last image you want.
16. Tools → Photoshop → Photomerge
17. Adobe Photoshop CS6 will open
18. Select Layout option **Interactive Layout**
19. Check box **Blend Images Together**
20. Click OK

21. Photomerge may take a while to run – leave it alone
22. **Manually fixing merge errors:**
    a. Images that photoshop could not merge appear in the top panel. You can leave them in the panel if you don't want them (e.g. completely white or black) and they will be deleted
    b. Zoom in and out using the mountain icon slider
    c. To move an incorrectly merged image, just drag it to the correct location with your mouse. If it matches well, it should "snap" into place. You can also just leave it there to force it into place.
    d. **Be aware that images may be incorrectly merged behind other images – look for blurry merged regions and move the top image out of the way**
    e. Click OK when finished

23. Image → Layer → Flatten Image
24. Save As → Save as a BMP with default settings

2c. <u>Photomerge with Reposition in Photoshop 2020</u>

If ICE badly merges images, and Photoshop CS6 freezes, you can photomerge a region or sub-region with a manual repositioning component in Photoshop 2020. Recent versions of photoshop do not include the Interactive Layout option.

1. Search for Adobe Bridge 2020 on FOOTE
2. Navigate to the **photoshop resized** folder of one of your four regions (Top, Bottom, Right, Left)
3. Select all images with Ctrl+A.
    a. To select a subset of images (e.g. try to photomerge only half of the region), click the first image you want, hold Shift, and click the last image you want.
4. Tools → Photoshop → Photomerge
5. Adobe Photoshop 2020 will open
6. In the Photomerge Popup, select Layout option **Reposition**
    a. **UNCHECK** box **Blend Images Together**

b. If the images blend together, the layers will separate along features within the image
c. Click OK
d. Wait for the photomerge to finish

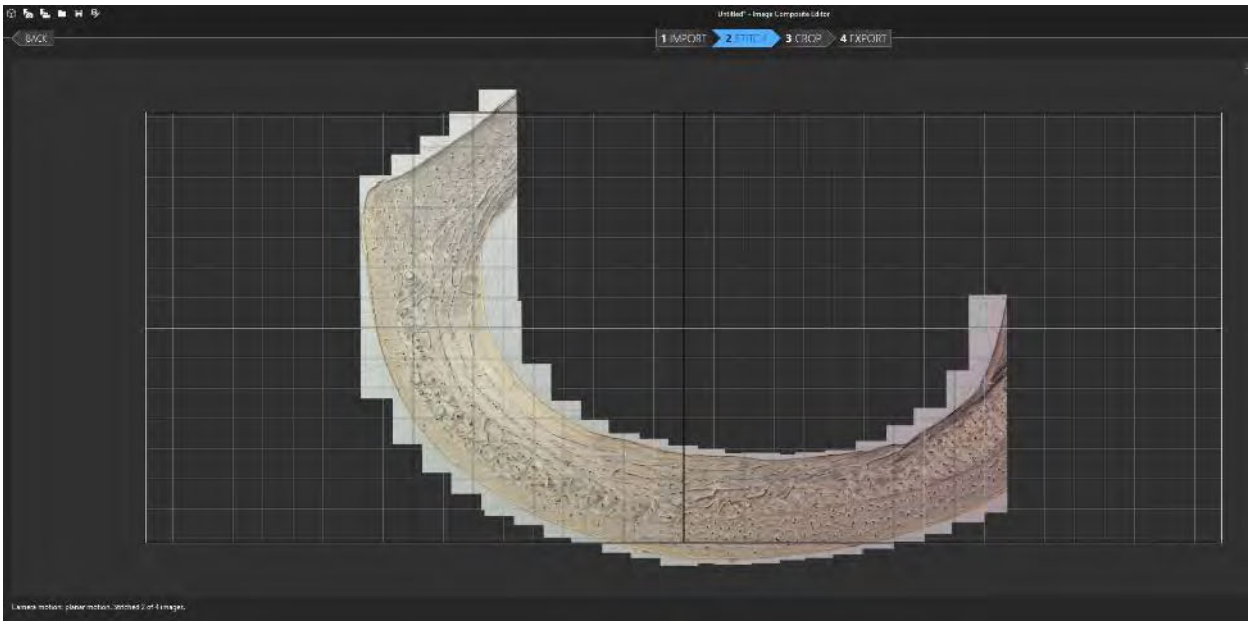
7. Use View → Uncheck "Extras" to turn off grid for greater visibility
8. **Manually Move Incorrectly Merged Images**
   - **a.** With the **move tool** selected from the left hand toolbar  , click the misaligned image. It will be highlighted on the right-side **Layers** toolbar.
     - **i.** To move multiple connected images, use the move tool to draw a box around the connected images
   - **b.** Use hotkeys **Ctrl + Shift +]** on the keyboard (easier on the right side) to bring the selected image to the front, or use Layer → Arrange → Bring to Front
   - **c.** Move the image / images to the larger section of the image that is correctly merged. Use visual similarly to match the image.
     - **i.** If you're not sure where an image goes, click on the incomplete region of the larger, correctly merged section. The layer will be selected on the Layer panel and you can check the number. Look for a free-floating or misaligned image with a similar number.
   - **d.** Gently move the image towards the visual similarity on the larger, correctly merged image. When the visual similarities match, the moving image will "snap" or slightly settle into place
     - **i.** Be aware that (especially on polarized images), images do sometimes incorrectly snap together if they are visually homogenous
     - **ii.** If the visual similarity is not clear enough for the two images to be "snapped", just manually move it into place
9. **(Only if Necessary) Merge Completed Sections:** If there are one or more chunks of the image that are well-merged, but you keep accidentally moving them, you can lock them into a single layer so they will not be accidentally modified. I would only do this for **internal** regions you are sure are complete, because edge regions may not actually be aligned with the connecting images and you cannot undo this step.
   - **a.** Select the **move tool** from the left hand toolbar 
   - **b.** Click and draw a box around the completed section you want to merge
   - **c.** Use hotkey **Ctrl + E**, or else select Layer → Merge Down. Now this section will behave as a single layer
10. **Flattening and Exporting**
    a. Image → Trim → Based on Transparent Pixels
    b. Layer → Flatten Image
    c. File → Save As → Save on Your Computer
    d. Change Save as Type to .tiff
    e. In the .tiff options popup, choose Image Compression = None. Leave all other defaults and click OK.

3. Combine Regions

3a. Combine regions in Image Composite Editor

Sometime multiple regions will auto-merge in ICE. Follow the same protocol as ICE merging within regions.

1. Search ICE in the toolbar on FOOTE to open Image Composite Editor
2. Select New Panorama from Images
3. In the Select Overlapping Images popup, select the folder containing all four of your photomerged regions
    a. Ctrl + A to highlight all images
    b. Click Open
4. Select Simple Panorama
5. Under Camera Motion, change to Planar Motion
6. Click Next and wait for ICE to run (it runs fairly quickly – usually a few minutes)
7. Check the number of images merged at the bottom (e.g. "Stitched X of X Images")
8. "2 Stitch" tab displays; do not select any settings; just click Next
9. "3 Crop" tab displays; do not select any settings; just click Next
10. 4 Export tab displays; keep Image scale at default 100%
11. Export as TIFF file (single composite images)
    a. File format = TIFF Image
    b. Alpha = Check box to include Alpha channel
    c. Export to Disk = Save in new ICE subfolder for merged regions
        i. Call it by region names e.g. "LeftBottom"
12. If not all regions combined (common with Pol images), we will do it in Photoshop (see below)



ICE could combine the bottom and left regions, but not the top and right regions

3b. <u>What to do if there is NO complete auto-merge of DIC, Pol, or Flo</u>

In most cases, the DIC and/or Flo image will completely auto-merge. You can use this as a template for aligning regions on the other images. If you do not have a complete auto-merge of ANY image for a sample (DIC, Pol, or Flo), you will need to manually align one of them in Photoshop 2020. I recommend aligning the DIC image manually because it has the most reference structures and is most likely to at least partially have combined regions in ICE.

1. Open Photoshop 2020

2. Use View → Uncheck "Extras" to turn off grid for greater visibility
3. Open the first region (or ICE-merged regional composite) of the DIC image using File → Open. I like to open the Left side first.
4. Expand canvas to accommodate the next region using Image → Canvas Size. Ballpark how much bigger the space will need to be and increase the pixels by that factor. It's fine to oversize – we will trim later.
5. From File Explorer, drag and drop the next adjacent region. It will appear with a blue X. Click Enter to paste.
6. Click on the pasted Layer in the Layers panel and choose Convert to Layer
7. Click on the region and press **Ctrl + T** to open free transform. Move the pasted region into place overlapping the existing region.
    a. ICE sometimes rotates layers slightly, so you may need to rotate the layer (look for the curved arrow when you move to the side of the image)
8. Try to Auto-Align the regions tightly by holding Ctrl and clicking both layers in the Layers panel. Use Edit → Auto Align Layers and choose Reposition
9. If Auto-Align layers doesn't work due to insufficient overlap, just move the layers into place manually
    a. You can toggle layer transparency to assist in aligning layers by clicking on the layer in the Layers panel and reducing its Opacity. Just remember to put back to 100% before flattening the layers.
    b. It is okay if regions don't perfectly align, as long as they are mostly aligned.
10. Repeat steps 4 – 8 until all regions (and sub-regions) are aligned
11. **Get rid of color seams:** ICE creates a brightness gradient for DIC images. Equalize this gradient by selecting all regions in the Layers panel and clicking Edit → Auto Blend Layers
12. **Flattening and Exporting**
    a. Image → Trim → Based on Transparent Pixels
    b. Layer → Flatten Image
    c. File → Save As → Save on Your Computer
    d. Change Save as Type to .tiff
    e. In the .tiff options popup, choose Image Compression = None. Leave all other defaults and click OK.
13. **Save the finalized cross-section to cloud storage**

4. Align and Anatomically Orient DIC, Flo, and Pol in Photoshop 2020

4a. Align Flo and Pol images with DIC image

We want all three images to be as precisely aligned as possible, so that we can use Flo markers for the DIC and Pol structures. For this step, you will need at least one fully aligned cross-section. If you don't, go back to step 3b and manually align the DIC cross-section.

This SOP assumes that DIC and Flo are fully auto-merged (as is typical), and Pol is not fully merged, but you can also apply it to other variations. For example, you can use DIC to align the Flo regions, and then make a Flo overlay for the Pol regions.

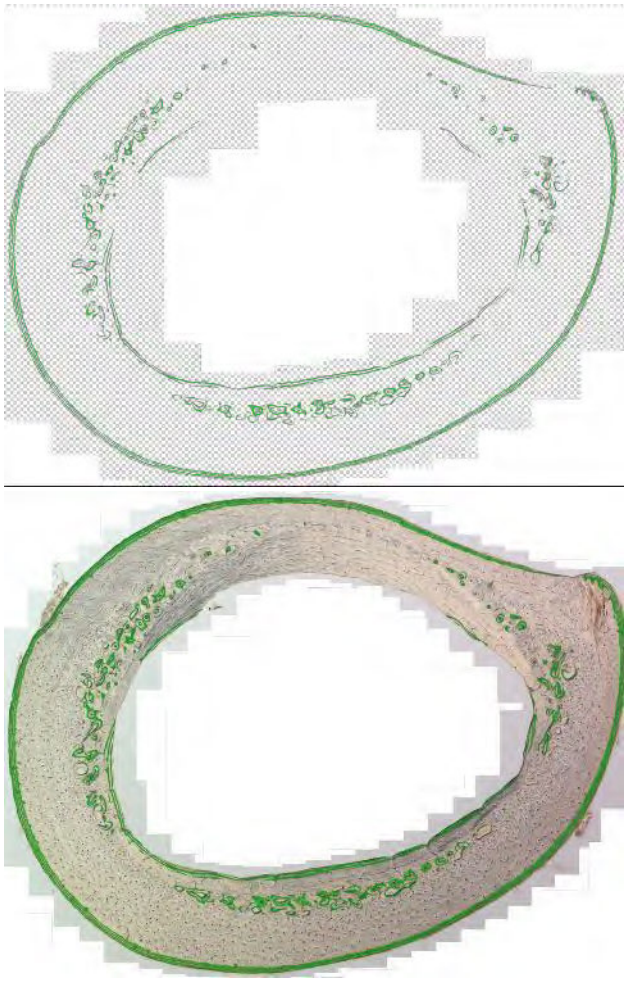
1. Open DIC image in Photoshop 2020
    a. Layer → Rename Layer → "DIC"
2. Drag and drop Flo image.
    a. You must be resized on DIC for full size for Flo to drop into the middle; otherwise it will drop where you are zoomed

b. Layer → Rename Layer → "Flo"

3. Because it is 100x, Flo needs to be resized.
   a. Ctrl+T to resize
   b. At top of screen, change W and H to 200%
4. Next we will make a Flo overlay for DIC. Click the Flo layer and select Layer → Duplicate
   a. Select copied layer and Layer → Rename Layer → "Flo"
   b. Click copied layer and Layer → Rasterize → Layer
5. Now we convert the copied Flo layer into an overlay
   a. Turn off visibility for original Flo and DIC layer in the layer panel by clicking the eye icon
   b. Select → Color Range
   c. Click on the black area in the middle of Flo
   d. Set fuzziness to 200
   e. Click the eyedropper minus icon 

Color Range Settings

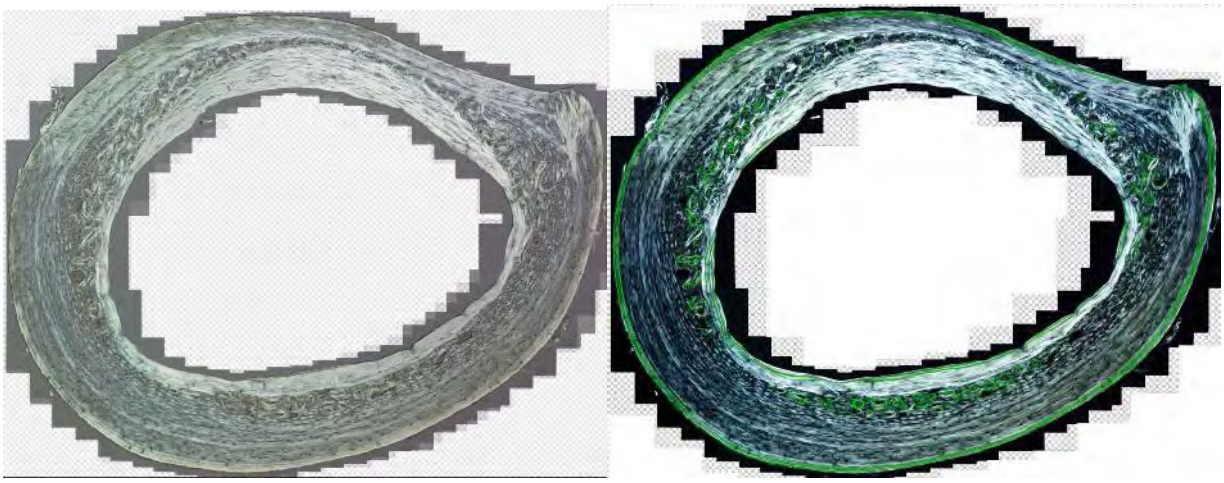
   f. Click ok
   g. Black areas will now be highlighted. Backspace to delete them from Flo copy
   h. Select → Deselect
6. Link the original and Flo layer copy together by selecting them both and clicking the  icon at the bottom of the layer panel. The same icon should appear next to both layers. Now the layers will transform in sync
7. Move the Flo copy overlay to the top of the layer panel
8. Turn on visibility for the Flo overlay and for DIC



Flo Overlay (left) Superimposed on DIC (right)

9. DIC will be the base layer, which we do not move. We will superimpose Flo and eventually Pol on this layer.
10. Click on the Flo overlay image to select. Ctrl + T to move and rotate
    a. First align the outer periosteal ring with the border of the DIC image. Then align the fluorescent overlays with cortical pores as closely as possible.
    b. Use hotkey Z to change to zoom tool so you can zoom in and out
    c. Use hand tool (hotkey H) to move around while zoomed
    d. You may need to very slightly scale the Flo overlay (due to imprecisions in 100x – 200x transition). Do this only after it is well-position, in case it does not exactly fit to DIC pores
11. Typically, Pol images will not merge all in one piece. We will merge them piece by piece, checking against DIC and the finalized Flo overlay.
    a. **Note:** Even if two or more regions combine in ICE, they may not be merged correctly due to the similar patterning of polarized images. You may still need to load all four regions separately in photoshop if you can't make them match the Flo and DIC overlays.
    b. Turn off the Flo layer but leave DIC layer on
    c. Drop the largest Pol piece onto DIC
    d. Set opacity of DIC layer to 50% - 60% and move it on **top** of the layer stack

e. Ctrl+T to move the Pol image into alignment with DIC image. You may need to slightly rotate the Pol Overlay
f. Turn off DIC and turn on Flo overlay to check fit.
g. Drop the next Pol piece onto DIC and repeat placement using steps a – g
h. You may also need to toggle the opacity of adjacent Pol pieces

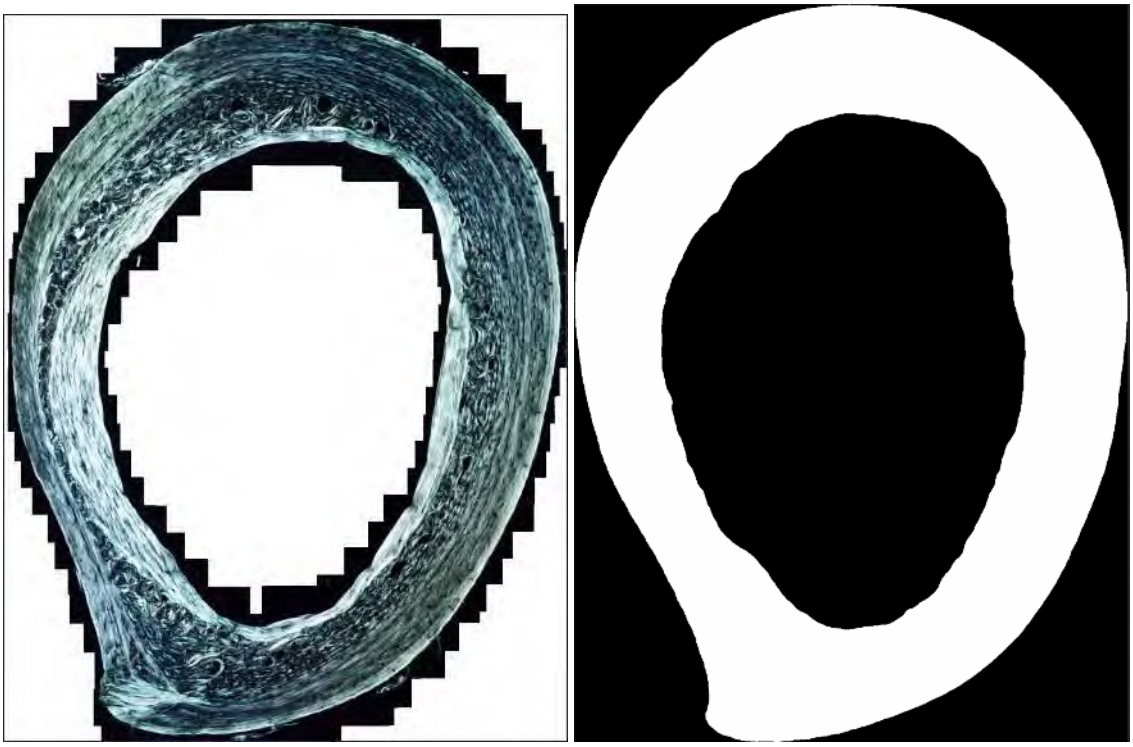


50% Opacity DIC Superimposed on Pol (left) and Flo Superimposed on Pol (right)

12. Merging Oriented Pol Layers
    a. Highlight all the components of the cross-section in the layers panel and Layer → Duplicate Layers
    b. Highlight all of these copies and Layer → Rasterize → Layer
    c. Highlight all of the copied layers and Edit → Auto-Blend Layers → Panorama
        i. Check Seamless Tones and Colors
        ii. Uncheck Content aware fill transparent area
    d. Highlight all of the copied layers and Layer → Merge Layers
    e. Layer → Rename Layer → "Pol"
13. Exporting Oriented Layers
    a. Turn on visibility **only** for the finalized DIC, Flo, and Pol layers
    b. If needed, set opacity back to 100%
    c. If any layers are unrasterized they will have this icon:  Rasterize with Layer → Rasterize → Layer
        i. Note: If you get a blank image output, the image needs to be rasterized
    d. File → Export → Layers to Files
        i. Make a new destination folder like "Oriented"
        ii. Append Prefix with slide name (e.g. 18lf_91um_)
        iii. Check box for visible layers only
        iv. File Type: TIFF
        v. Image Compression: None
    e. Click Run

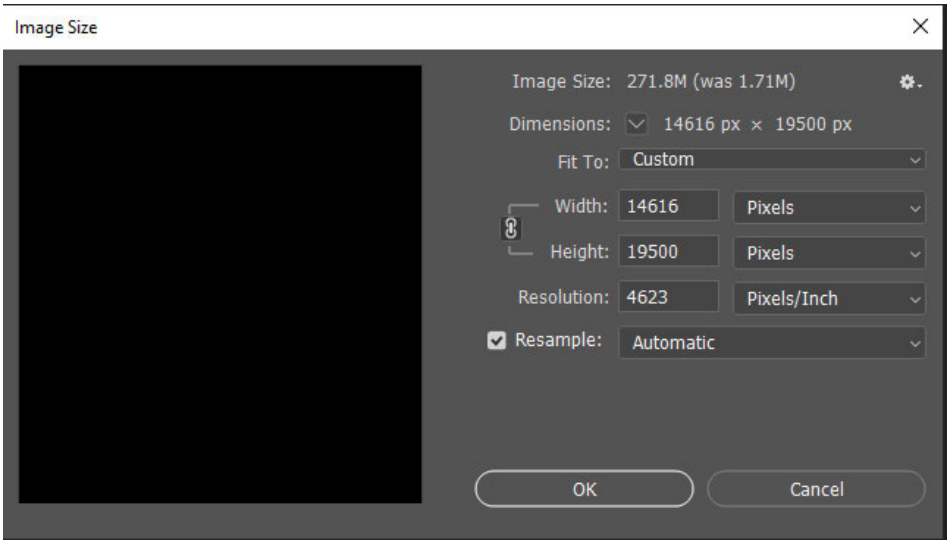
4b. <u>Align all images anatomically</u>

1. Download the **first** ROI image from the micro-CT scan of the associated rabbit femoral midshaft
    - **a.** Hard copies are located on FOOTE under FOOTE1 (D:) → Rabbit Opioid Scans Backup – Femur → **(Sample Name)** → Anatomical Rotation → Centerline Anatomical Tomo → ROI
    - **b.** Zipped copies are located on Andronowski Lab Data Storage → Rabbit Opioid Project → microCT Scan Backup → Finalized Data Processing Backup → Femur Diaphysis → **(Sample Name)** → ROI.7z
        - **i.** https://drive.google.com/drive/u/1/folders/1uIEZTg1GiA4ZTRaQZQweGyG8QfWV3phq
        - ii. You will need to download the free software 7zip to extract these files: https://www.7-zip.org/
2. Open Photoshop 2020
3. Use File → Open to load DIC image
    a. Click the lock icon on the layer panel to unlock DIC layer from the background
    b. Layer → Rename Layer → "DIC"
4. Drag and drop Flo image. Press Enter to paste.
    a. Layer → Rename Layer → "Flo"
5. Drag and drop Flo image. Press Enter to paste.
    a. Layer → Rename Layer → "Pol"
6. Use File → Open to open the ROI in a new tab
    a. To crop to the bone boundaries, select Image → Trim → Based on top left pixel color
7. Return to the tab with the DIC, Pol, and Flo images.
    a. Select all three images in the Layers panel with Ctrl + Click.
    b. Right click → Link layers (so that the images transform together)
    c. Use Image → Image Rotation to rotate to the same **rough** orientation as the ROI
        i. Stick to rotating the image by 90° increments and flipping horizontally or vertically. Arbitrary rotation will be completed in the next step.



Stack of Roughly Transformed Histological Images (Left) and ROI (Right)

8. The micro-CT ROI is much smaller than the histological images. To find the pixel dimensions of the histological images, use Image → Image Size and record the **Width** and **Height** of the histological images.
    a. In this example for rabbit #18, the histological images are 15096 (W) x 19500 (H) after the rough transformation. The ROI is 1158 (W) x 1545 (H) after cropping.
9. Switch to the ROI tab and go to Image → Image Size
    a. Type in the Width and Height from the histological images. Make sure that width and height are linked. The linked height may not exactly match the linked width.
    b. In this case, the ROI was resized to 14616 (W) x 19500 (H)

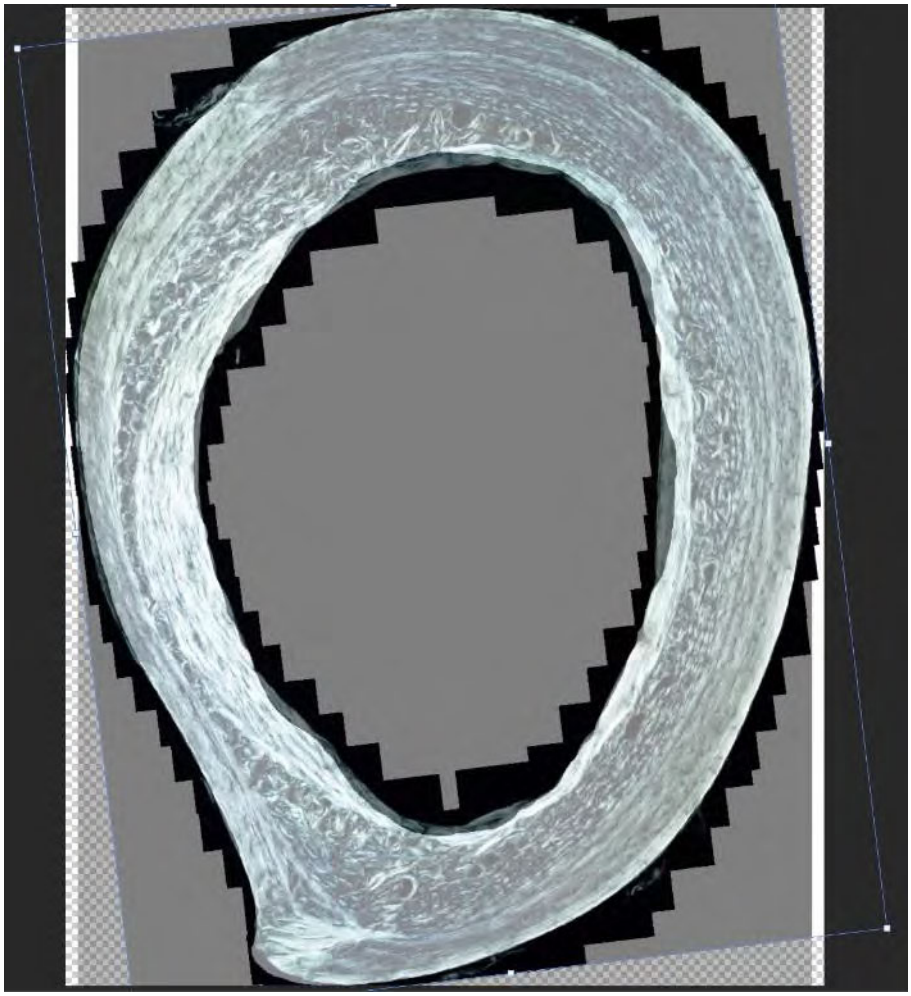


10. On the ROI tab, use Select → All (or Ctrl + A) and Edit → Copy (or Ctrl + C) to copy the ROI.

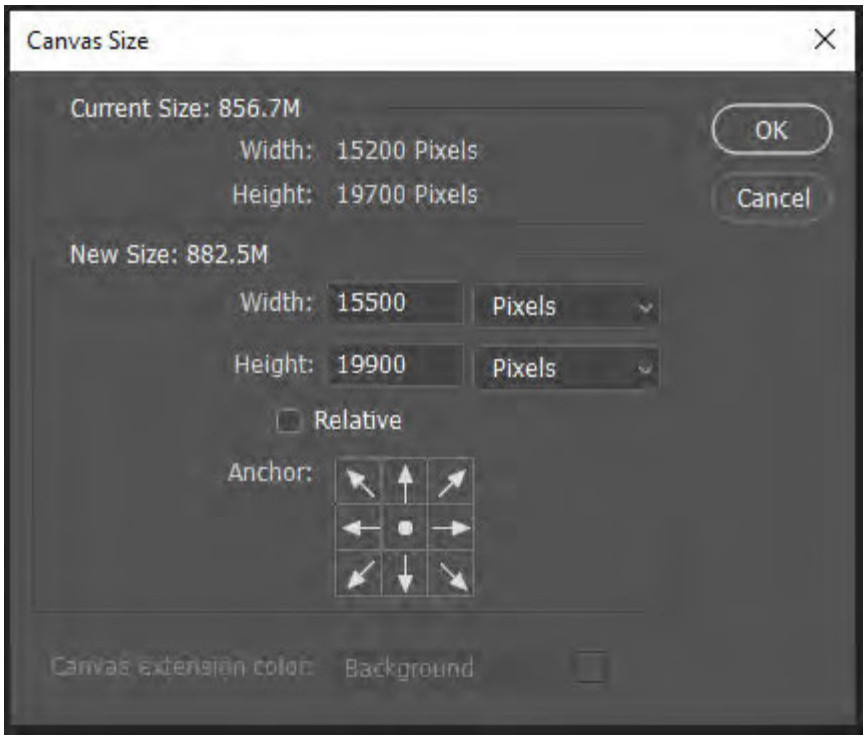
a. Switch to the histological image tab and use Edit → Paste (or Ctrl + V) to paste the enlarged ROI as a new layer
b. Grab and drag the ROI layer to the top of the layer stack if needed. The next layer down should be a solid histological image (either Pol or DIC)

11. Reduce opacity of the ROI to about 50%

12. Select one of the histological images and use Edit → Free Transform (or Ctrl + T)
    a. A blue box appears outside the image frame
    b. Move the mouse **outside** the blue box to be able to grab and **rotate** the histological image. Move the mouse **inside** the blue box to be able to grab and **drag** the histological image laterally.
    c. The histological image may not match the ROI exactly. Try to align the angle of the linea aspera (point at the bottom of the image)
    d. Press Enter to complete the transformation



13. If the histological image now reaches outside of the canvas, increase the canvas size using Image → Canvas Size (or Alt + Ctrl + C)
    a. Keep the Anchor in the default center
    b. Increase Width and Height by several hundred pixels to accommodate the widened picture. It's fine to overshoot – we will crop in the next step.
    c. Select OK
    d. Turn all layers off with the eye icon in the layer panel. Turn each histological layer on with the eye icon to check that it is fully inside the image Canvas.

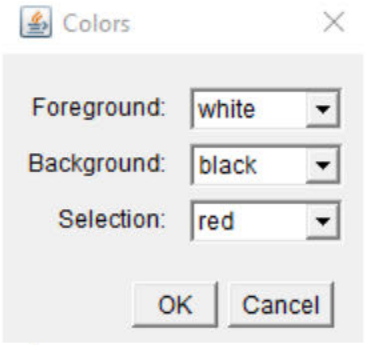


14. To crop to the image boundaries if you overshot the enlargement, select all three histological images in the layer panel.
    a. Image → Trim → Based on transparent pixels
15. Exporting Oriented Layers
    a. Turn on visibility **only** for the finalized DIC, Flo, and Pol layers
    b. File → Export → Layers to Files
        i. Make a new destination folder like "Anatomically Oriented"
        ii. Append Prefix with slide name (e.g. 18lf_91um_)
        iii. Check box for visible layers only
        iv. File Type: TIFF
        v. Image Compression: None
    c. Click Run

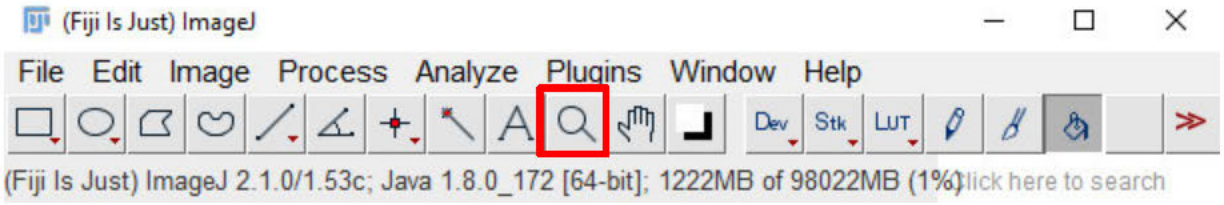
5. Clean Periosteal and Endosteal Boundaries in ImageJ / FIJI

Download the FIJI distribution of ImageJ if you do not have it installed:
https://imagej.net/Fiji/Downloads

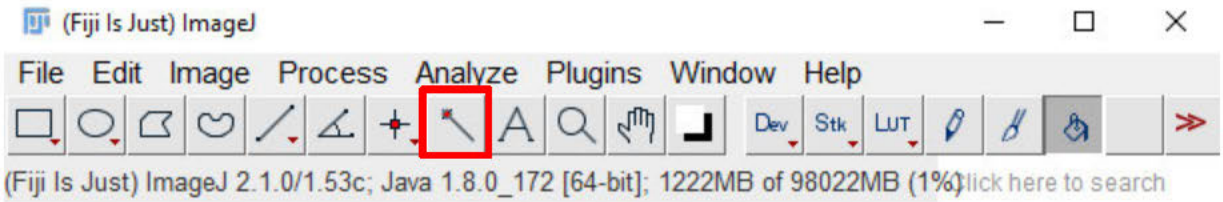
1. Open FIJI
2. Make sure that your colors are set to Foreground = White and Background = Black under Edit → Options → Colors



3. Open the DIC image with File → Open, or by dragging and dropping the image from its folder onto FIJI
4. Select the Zoom tool. Right-click to Zoom in and left-click to Zoom out. Zoom out until the full DIC image is visible in the window.
    a. You can also grab and drag the window edges to expand them on your desktop.



5. Select the Wand tool. Click in the off-white area outside of the bone (not the absolute white space outside the image itself). Most likely you will see nothing selected.

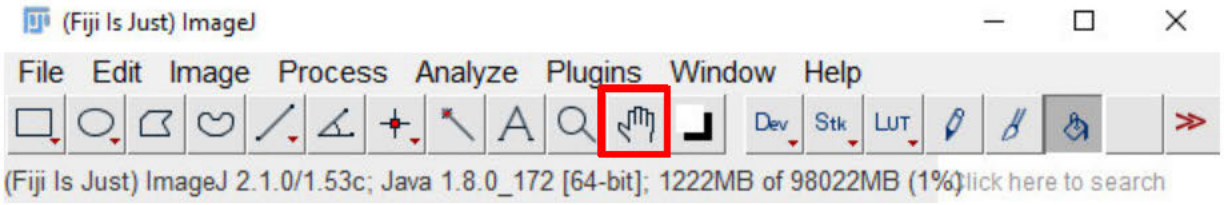


6. Double-click on the Wand tool. The Wand tool tolerance popup appears. Increase the tolerance until you can see a red line form around the outside (periosteum) of the DIC image
    a. Stop if the red line starts to penetrate into the bone itself. If you're not sure, if the red line is inside the bone, click "Ok" on the Wand tool tolerance popup and then zoom in to the region in question. You can always double-click to re-open and adjust the wand tool tolerance more or less.
    b. You may only be able to outline a portion of the bone from your starting position. It depends on the location of your original click.

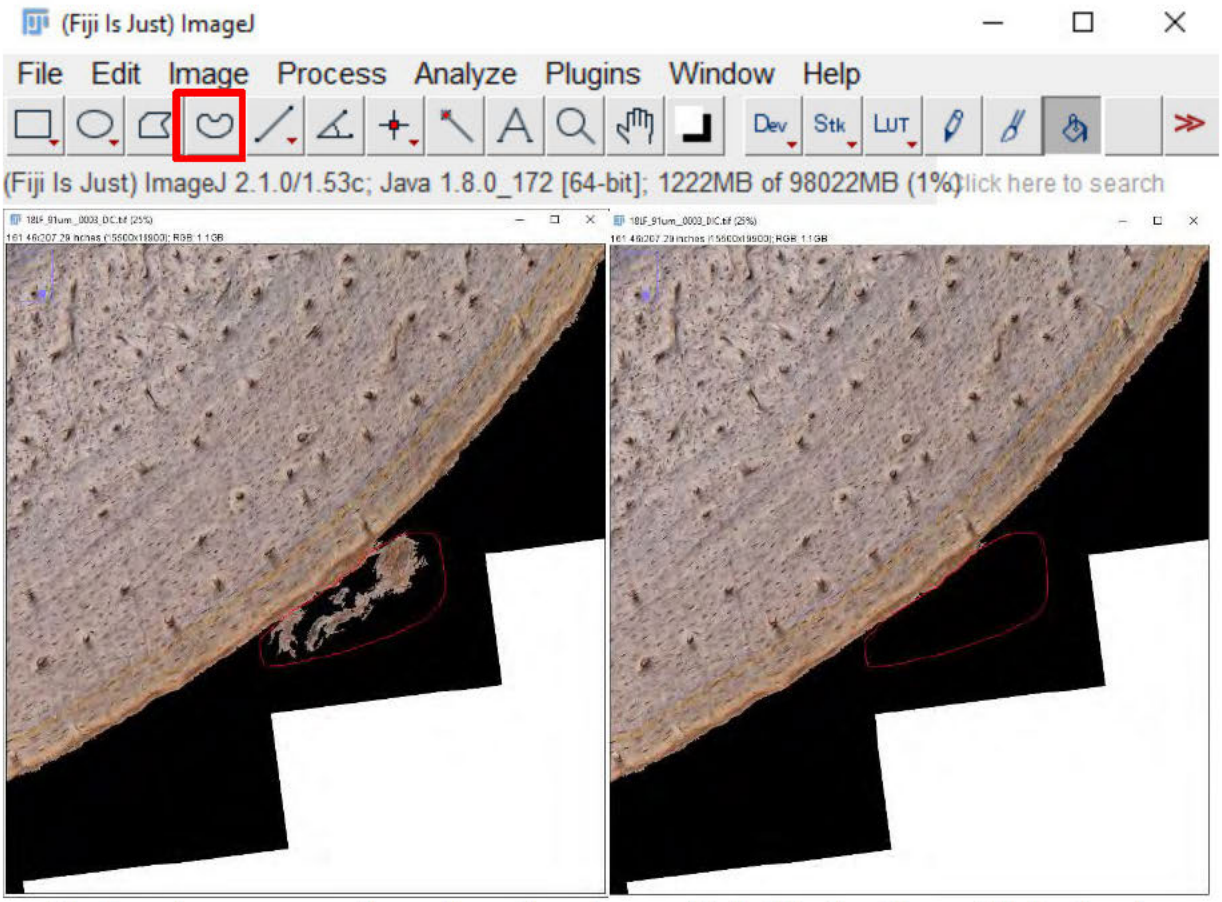
7. Click OK in the Wand tool tolerance popup to finalize the selection
8. In most cases, the selection forms a ring around the off-white region external to the periosteum. In this case, Select Edit → Clear or press Backspace to clear the selected region, turning it black.
    a. If this turned the bone black, then the selection formed a ring around the periosteum itself. In this case, select Edit → Undo to revert, and then Edit → Clear Outside to turn the region outside of the selection black.

9. If there are any remaining off-white regions external to the periosteum, click near those regions and repeat steps 5-8

10. There will be small pieces of membrane not selected by the Wand tool. Use the Zoom tool to zoom in on the border. Use the Scrolling tool to move around the border while zoomed, looking for this membrane.

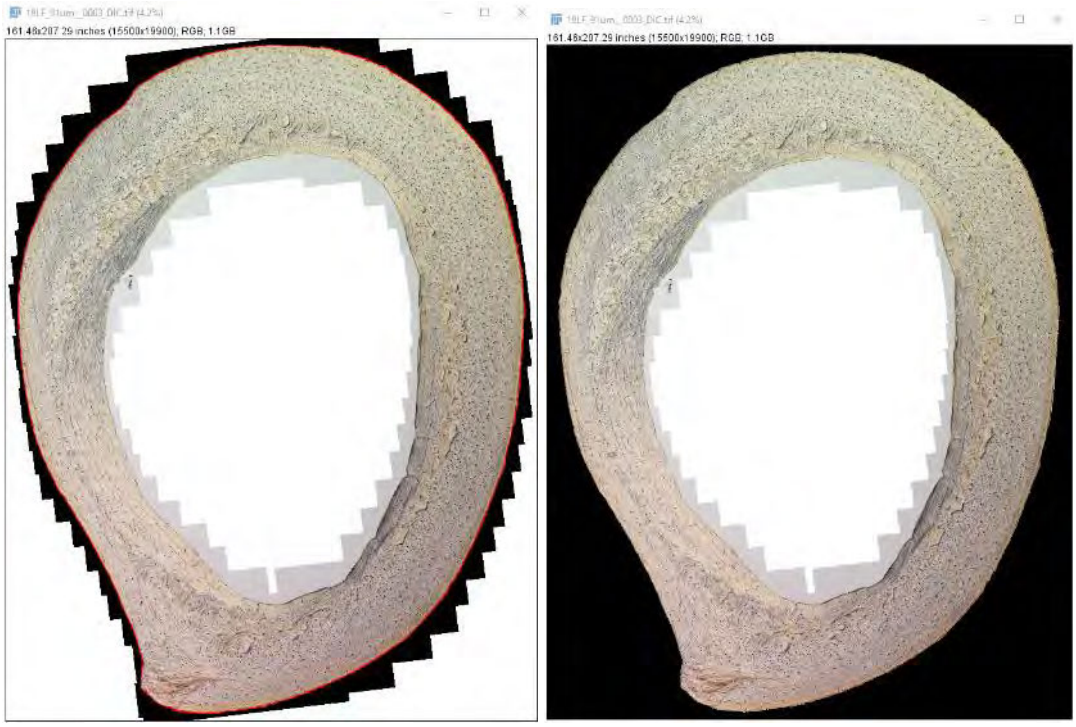


11. Use the Freehand selection tool to circle the membrane. Then use the Backspace key or Edit → Clear to delete the circled region.



12. Sometimes you can select regions of membrane with the Wand tool by modulating its tolerance, but be careful that they do not extend into the bone.

13. To clear absolute white panels external to this new black region, click in the new black border with the Wand tool. Double-click on the Wand tool and set tolerance to zero. This should outside the bone itself.



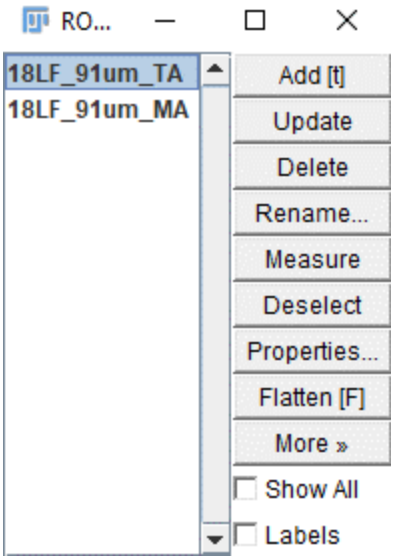
14. I recommend saving a copy of the DIC image (e.g. Cleared Outside) in case you mess up the marrow clearing and have to restart.
15. Repeat steps 5-12 for the endosteum (marrow cavity)

16. After the DIC image is cleared, save a copy (e.g. 18LF_91um_DIC_Clear) using File → Save As → TIFF

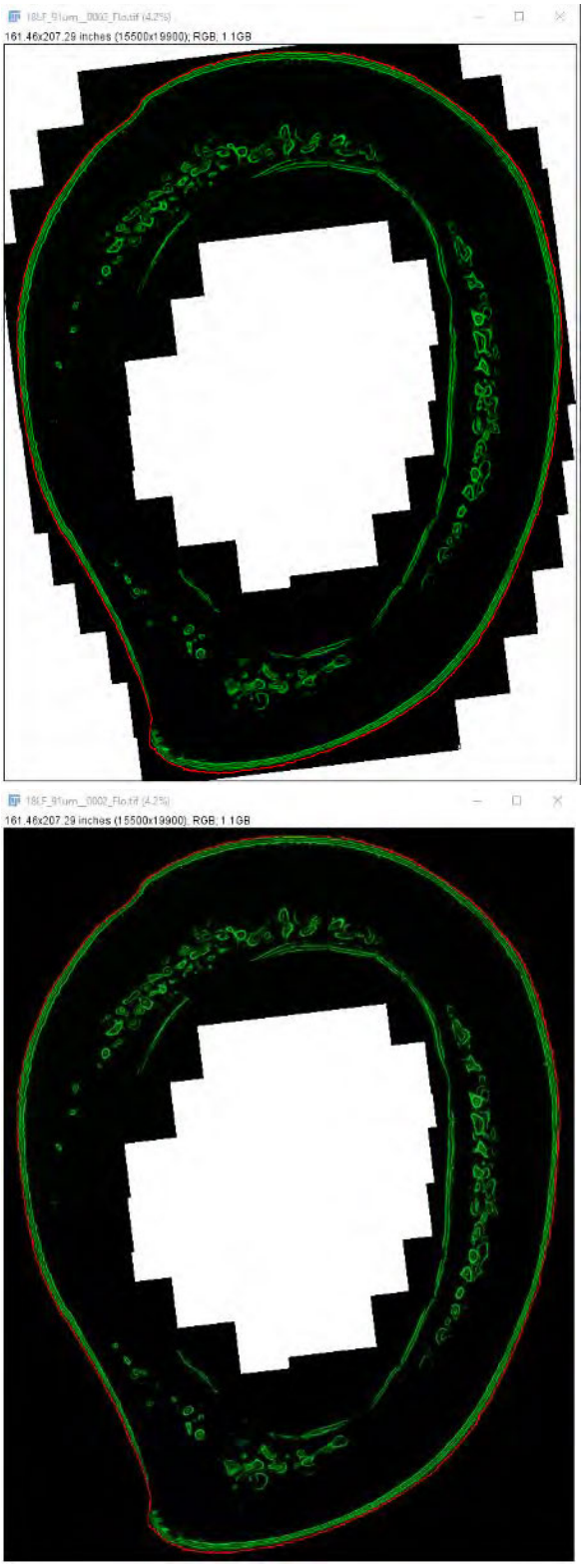
17. Keep the DIC image open in FIJI. Using the Wand tool with tolerance set to 0, click outside the bone to select the periosteum. Use hotkey “t” to add the periosteum to the ROI manager, which pops up automatically when “t” is pressed.
18. Use Edit → Selection → Select None to clear the periosteum selection, or just click outside the periosteum selection on the image. The ROI remains in the ROI manager.
19. Now click inside the bone to select the endosteum, and use “t” to add it to the ROI manager

Selection of Periosteum (Left) and Endosteum (Right)

20. Select the top ROI in the ROI manager. In the ROI Manager, select More Save… and save this ROI as “(Sample Number)_TA” (e.g. 18LF_91um_TA) in the same folder as the anatomically oriented histological images. The TA stands for “Total Area”
21. Select the bottom ROI in the ROI manager. In the ROI Manager, select More Save… and save this ROI as “(Sample Number)_MA” (e.g. 18LF_91um_MA) in the same folder as the anatomically oriented histological images. The TA stands for “Marrow Area”
22. Saving the ROIs will also change their names in the ROI manager

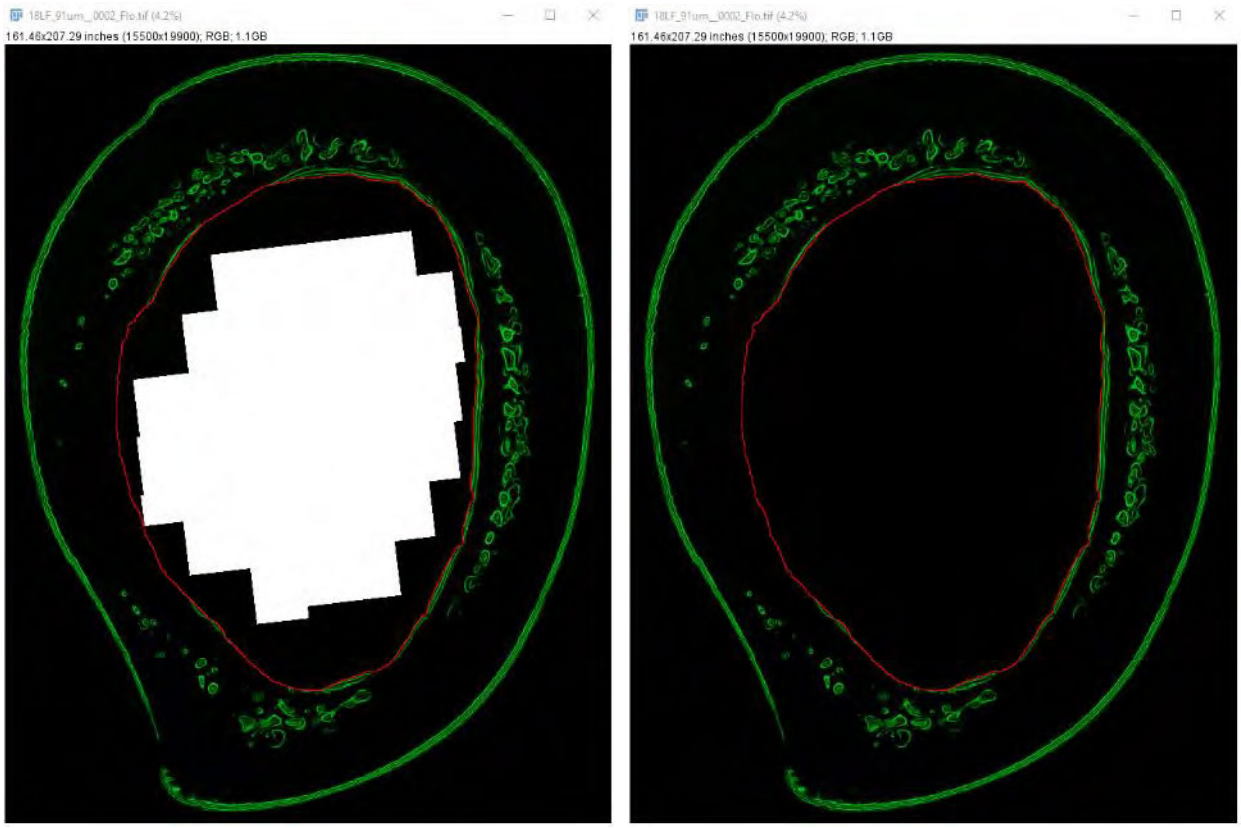


23. Close the DIC image. Keep the ROI manager open with the two loaded ROIs. You can always load the saved ROIs for a given image by dragging their saved .roi files from the folder onto ImageJ.
24. Open the Fluorescence image using File → Open, or by dragging and dropping the image from its folder onto FIJI.
25. Click on the new fluorescence image window to select it
26. Click on the top ROI in the ROI manager (the periosteum or TA). It should appear on the fluorescence image. Use Edit → Clear Outside to clear outside the periosteum



27. Click on the bottom ROI in the ROI manager (the endosteum or MA). It should appear on the fluorescence image.

a. Use Edit → Clear to clear inside the endosteum

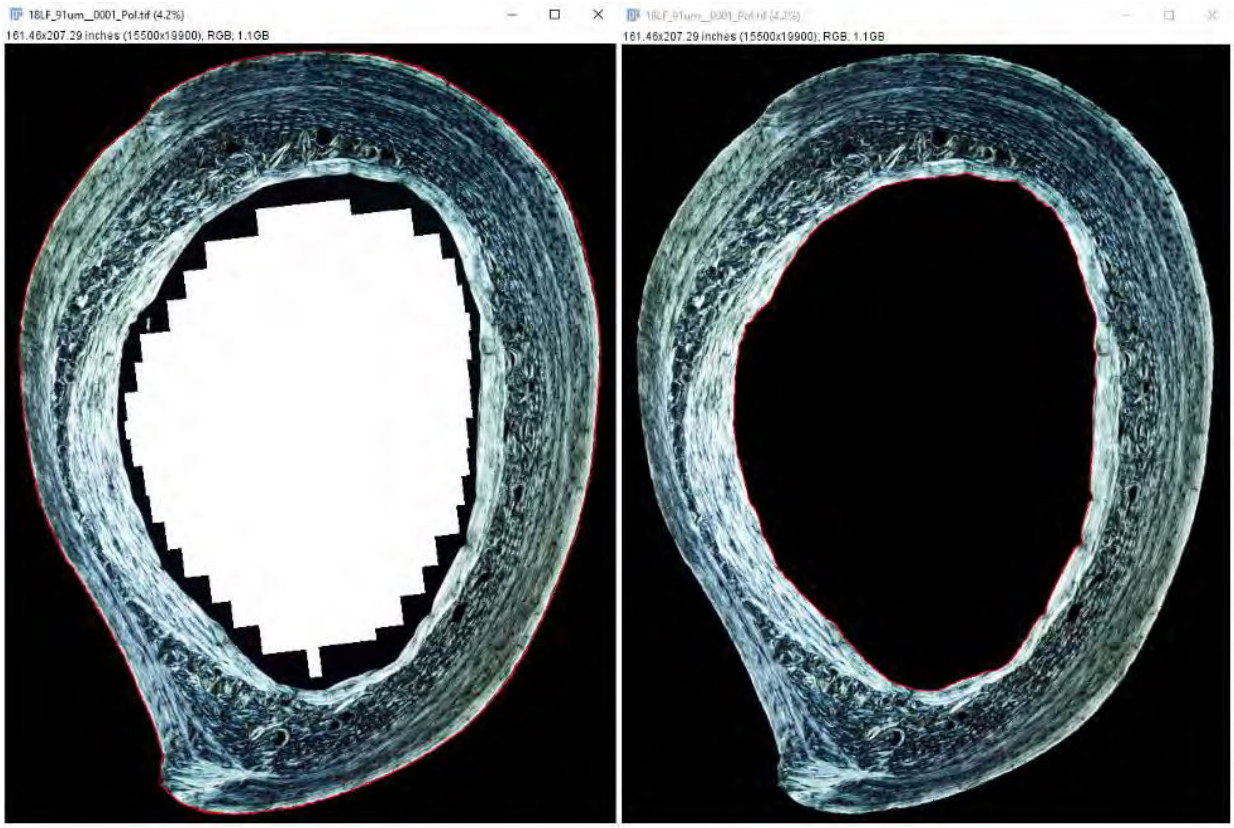


28. Use Edit → Selection → Select None to clear selections from the fluorescence image

After the fluorescence image is cleared, save a copy (e.g. 18LF_91um_Flo_Clear) using File → Save As → TIFF

Close the fluorescence image

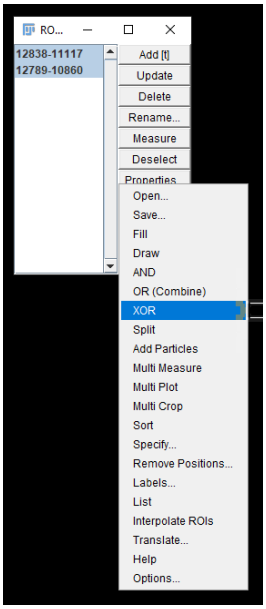
Open the polarized image using File → Open, or by dragging and dropping the image from its folder onto FIJI. Repeat steps 24 – 27 to clear inside and outside of the bone using the saved periosteum and endosteum ROIs.



Use Edit → Selection → Select None to clear selections from the polarized image
After the polarized image is cleared, save a copy (e.g. 18LF_91um_Pol_Clear) using File → Save As → TIFF

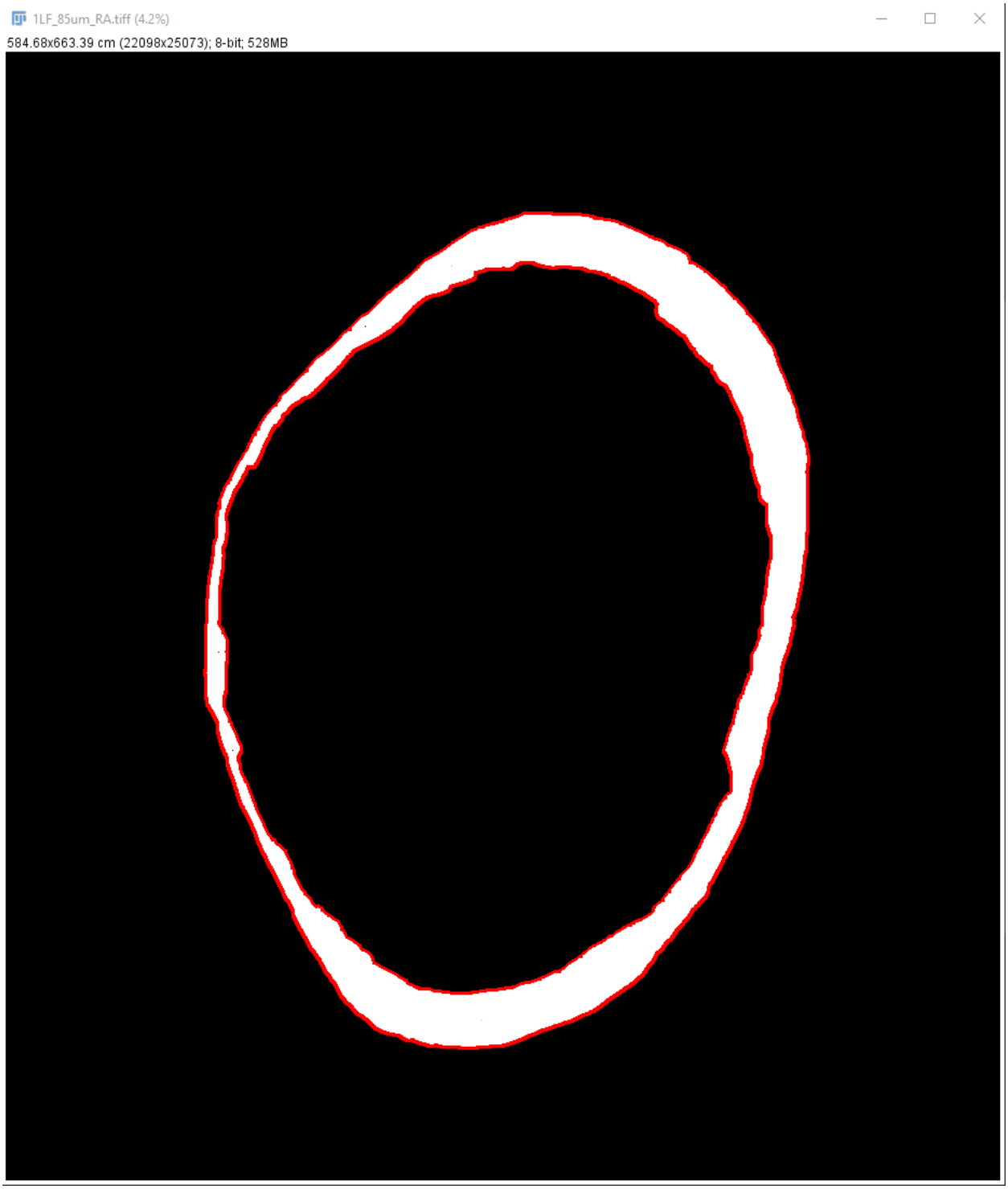
Appendix XXXII: Converting Dragonfly ROIs into ImageJ ROIs

1. In Dragonfly, right-click the ROI → Extract ROIs
2. Two new files will appear: one with the file name and an asterisk (e.g. Manual 1LF*) and one with the file name and ROI label 2 (e.g. Manual 1LF ROI label 2)
3. Right-click the asterisked file (e.g. Manual 1LF*) → Export → ROI as Binary
4. In the Save As popup, change Save as type to Tiff and give it an appropriate file name, such as "1LF_85um_RA", where RA stands for "Remodeling Area"
    a. The Tiff file should be the white ROI on a black background
5. Open ImageJ
6. Drag and drop the saved ROI into ImageJ, or use File → Open
7. Select the Wand tool: 
8. Click **outside** the white ROI. It will be selected in red, or whatever color is the default for ImageJ selections on your system
9. On the keyboard, press "t" to add the **outside** to the ROI manager, or use Edit → Selection → Add to Manager
    a. This will make the ROI Manager appear as a separate window
    b. Note that the ROI appears as a random number
10. Clear the selection using Ctrl + Shift + A, or use Edit → Selection → Select None
11. With the wand tool still selected, click **inside** the white ROI. It will be selected in red, or whatever color is the default for ImageJ selections on your system
12. On the keyboard, press "t" to add the **inside** to the ROI manager, or use Edit → Selection → Add to Manager
13. In the ROI Manager, click the first (outside) ROI, hold down Ctrl, and click the second (inside) ROI. This will cause them both to be highlighted.
14. In the ROI Manager, select More → **XOR**



15. Immediately press "t" on the keyboard. A new, combined third ROI will appear in the ROI manager. You can click on its numeric title to see it displayed on the image.

a. Note: In this image, line width has been increased to 75 pixels to make it visible for the screenshot



16. Click on the numeric title of this combined ROI. Select More → Save
17. In the Save Selection popup, choose an appropriate file name (e.g. 1LF_85um_RA)
    a. You can leave File Type as "All Files" – it will still save as a .roi file

18. Now the .roi file can be opened on top of any version (RA, DIC, Flo, Pol) of the oriented image for that sample by dragging and dropping it into ImageJ and then pressing “t” on the keyboard, or by using More → Open within ROI Manager

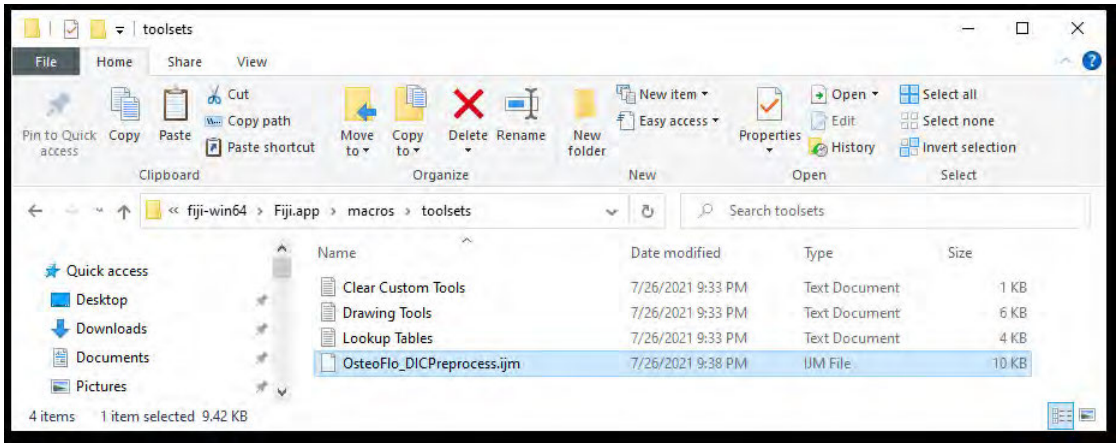
Appendix XXXIII: DIC Preprocess SOP

First-Time Installation

1. Make sure you have downloaded the FIJI installation of ImageJ: https://imagej.net/software/fiji/
    a. Move the fiji-win64.zip download to a logical place (can be on the Desktop or elsewhere)
    b. Unzip via right-click → Extract All
2. After unzipping, navigate to fiji-win64 → Fiji.app and click on the ImageJ-win64 application to open FIJI

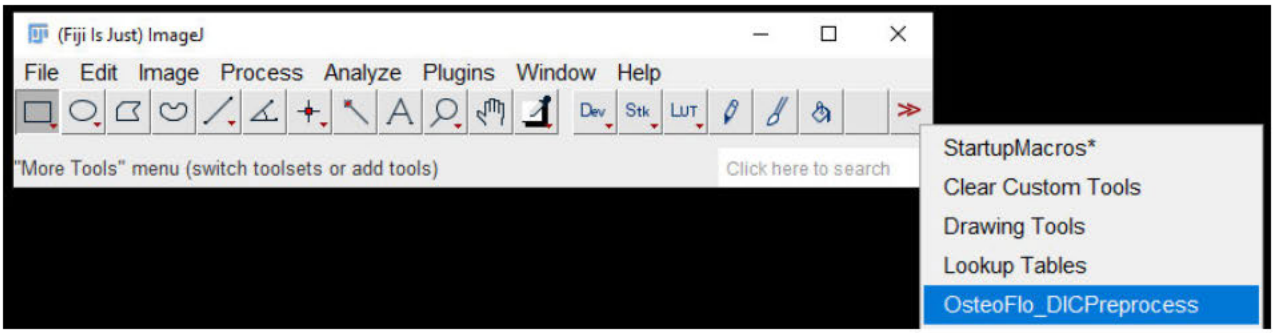
Start here if you already have FIJI downloaded:

3. In FIJI, go to Help → Update… (note this is different than "Update ImageJ")
    a. A progress bar will check for updates
    b. You may be asked to close and reopen ImageJ; if so call Help → Update… after you reopen ImageJ
4. The ImageJ Updater window will pop up with a list of updates
    a. In the lower left corner, select "Manage update sites"
    b. In the Manage update sites popup window, check the box for "**BoneJ**" and the box for "**BioVoxxel**" then click Close
    c. Back in the main ImageJ Updater Site, the BoneJ update site should have been added. Select "Apply changes"
    d. Close and reopen ImageJ as prompted
5. In FIJI, go to Help → Update ImageJ… and upgrade to the most recent version (v1.53k at the time of this SOP)
    a. The ImageJ-win64 application will close during upgrade
    b. Note that if you update ImageJ before applying package updates, it will revert to the original installation version
6. Select your downloaded OsteoFlo_DICPreprocess.ijm file. Cut and paste into the folder fiji-win64 → Fiji.app → macros → toolsets

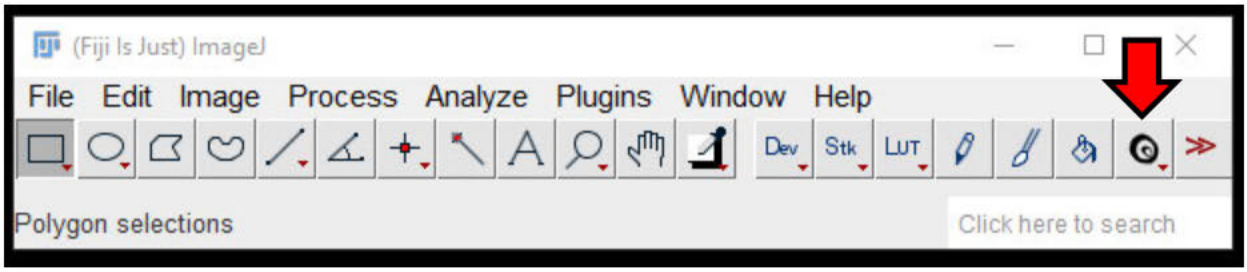


Every Time You Open a New Session of FIJI

1. Open the ImageJ-win64 application
2. In the FIJI toolbar, click on the >> arrow to the far right. Select OsteoFlo_DICPreprocess from the dropdown menu



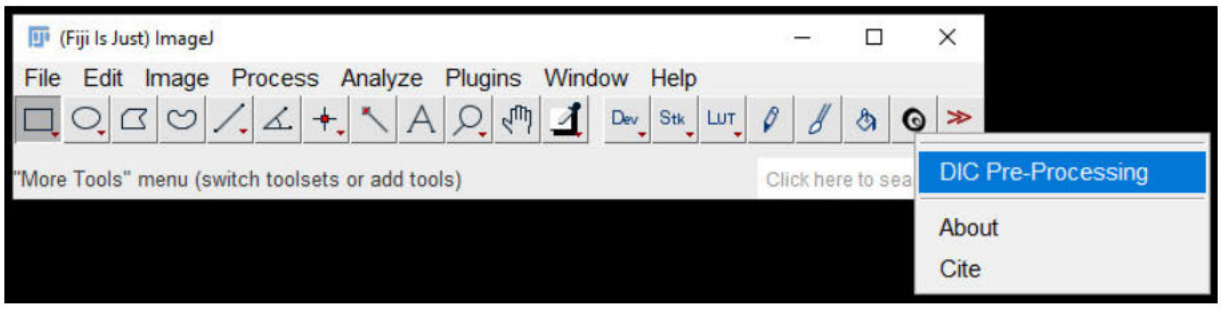
3. The OsteoFlo icon appears in the toolbar



To Preprocess a Given DIC Image

1. Download the cleared, oriented DIC Image from the Oriented Images subfolder "Cleared" for that sample on the drive: https://drive.google.com/drive/u/1/folders/1BeUI30l6p1AjaSR8_a1yixpbGdpmwW51

2. Click on the OsteoFlo icon and select the dropdown menu option "DIC Pre-processing"



3. You will be greeted by a popup that warns you that any active windows will be closed. Click OK.
4. In the popup window "Load the Cleared DIC Image", navigate to and click on the cleared, oriented DIC image, then click Open
5. In the popup window "Select Output Location", select the folder where the output image will be saved. It can be the same folder as the cleared, oriented DIC image if you like because this macro generates a subfolder.
    a. You will need to click into the folder where you want to save the output file and then click Select. Note that any individual files in that folder will not appear; the popup window will be blank unless it contains subfolders.
6. The macro will run on its own for a length of time dependent on your system capabilities.
    a. It took about four minutes on my system
7. You can see the progress statements for the High Pass filter in the ImageJ toolbar.
    a. You may see the Results window pop up periodically
    b. You will know the macro has finished when [Filename]_Preprocessing.tif is saved to the output subfolder "DIC Preprocessing"
8. After the macro finishes running, your selected output location will contain the subfolder "DIC Preprocessing." The contents of this folder should be:
    a. [Filename]_TA.roi
    b. [Filename]_CA.roi
    c. [Filename]_MA.roi
    d. [Filename]_Preprocessing.tif
9. Upload the DIC Preprocessing folder to the Oriented Images folder for that sample
10. Check the box for DIC Preprocessing for that sample on the Photomerge Tracker spreadsheet: https://docs.google.com/spreadsheets/d/1hiVpAoNIdxf6D_WLi3GdNCEw5_aIAdImGGwzCdxvWVw/edit#gid=0