Final Summary Overview

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Project Title: Chemical Imaging of Latent Fingerprint for Forensic Evidence

PI: Young-Jin Lee
Professor
yjlee@iastate.edu
515-294-1235

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DUNS:

EIN:

Recipient Organization: Iowa State University of Science and Technology
1138 Pearson Hall Ames, IA 50011

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Digitally signed by Tammy Polaski
DN: cn=Tammy Polaski, o=Iowa State University, ou=Office of Sponsored Programs Administration, email=tra@iastate.edu, c=US
Date: 2020.07.10 09:38:23 -05'00'

Tammy Polaski, Assoc. Director
Office of Sponsored Programs Administration
Iowa State University

Purpose

Chemical information of the fingerprints could provide a new avenue in individual identification. It may reveal the details of criminal activities or additional information about the suspect such as gender, age, or medical conditions. However, current gas-chromatography or liquid-chromatography based techniques have many limitations including the loss of fingerprint evidence due to extraction procedure, low sensitivity to detect low abundance compounds, and contamination form surface chemicals.

Mass spectrometry imaging (MSI) is a state-of-the-art technique for surface chemical imaging due to its extremely high sensitivity, label-free detection, and unbiased molecular characterization. There have not been much efforts to use this technique for forensic fingerprint analysis. The propose of this research is to adopt our expertise in MSI of small molecules and evaluate mass spectrometry imaging technique for forensic fingerprint analysis.

Research Design and Methods.

We have many years of expertise in matrix-assisted laser desorption ionization (MALDI)-MSI of small molecules[1-12]. The proposed research project is designed to best utilize our skills and know-hows, and to overcome the current four major bottlenecks in chemical fingerprinting: 1) limited sensitivity of low abundance small molecules, 2) ambiguity of chemical identifications, 3) lack of robust protocols compatible with traditional forensic investigations, and 4) demonstration of unique applications. Below is the excerpt of the proposed research plan.

Specific Aim 1: Nanoparticles to Assist Chemical Fingerprinting.

We hypothesize that nanoparticles or a mixed use of nanoparticles with traditional organic matrixes could be a good alternative matrix for small molecule analysis. As we have

shown in our recent study in nanoparticle screening as a matrix for MALDI-MS, some NPs show very unique analyte dependent specificity, which is consistent in both positive and negative ion mode. We will screen and test various NPs as well as a binary mixture of matrixes, and test their efficiency for MALDI-MS of fingerprint compounds.

Specific Aim 2: High mass resolution and high spatial resolution mass spectrometry imaging (HR^2MSI) for advanced chemical imaging of fingerprint.

Instrumentation in the Lee group (MALDI-ion trap-Orbitrap; Thermo Finnigan, San Jose, CA) has the capability of high-resolution MSI in both spatial and mass dimensions. High-mass resolution is possible with Orbitrap high-mass resolution mass spectrometer and high-spatial resolution is possible with a home-built laser optics that can reduce the laser spot size down to 5 microns[1]. Combining these, we can obtain high quality information in both chemical and spatial dimensions using high-resolution MSI in both the dimensions, or HR^2MSI.

Multiplex MSI strategy that we have previously developed is very useful to identify unknown compounds directly on the surface by obtaining MS/MS at the same time with MSI data acquisition[9]. This strategy will be adopted and optimized for chemical fingerprinting. Each imaging pixel will be split into four spiral steps. The first step will be used for Orbitrap MS scan in the low mass range (m/z 50-400) followed by data-dependent MS/MS scan on the second step. The third spiral step will be used for Orbitrap MS scan in the high mass range (m/z 400-1,000) followed by data-dependent MS/MS scan on the forth step. With dynamic exclusion, the data-dependent MS/MS scans will allow us to obtain tens of thousands of MS/MS spectra, which can be utilized to identify unknown compounds combined with accurate mass information from the Orbitrap scan. If MS/MS of unknown compounds are not present in our MS/MS library, an MS/MS search can be performed against a public MS/MS library.

Specific Aim 3: Expanding Utility of Mass Spectrometry Imaging for Fingerprinting.

The three sub-aims of the goal are to 1) aging study of chemical fingerprint, 2) surface modification of amino acids, and 3) compatibility with traditional fingerprint development techniques.

For aging of chemical fingerprints, we study the diffusion of fingerprint chemicals on surface as suggested by a group of researchers in NIST using fatty acid diffusion[13], however also to include triacylglycerols (TGs) that have three times higher mass and supposed to have slow diffusion. Amino acids have a good chance to be used as individual identification markers. But as discussed in the literature survey, it is challenging to reliably detect amino acids on fingerprints. In order to dramatically improve sensitivity and minimize local environment effects on the LDI efficiency of amino acids, we will adopt on-surface derivatization of amino acids previously developed by others[14, 15]. For sub-aim 3, we will test multiple development techniques for their compatibility with MALDI-MSI, cyanoacrylate fuming, forensic carbon powders, ninhydrin development, iodine fuming, and TiO_2 development. They will be compared with and without the development, to understand what are the chemicals affected by the development and whether we can overcome the limitation.

Specific Aim 4: Individual differentiation based on chemical fingerprint.

While exogeneous compounds can provide critical information about the suspect, it may not be always present or necessarily connected to individuals. It would be desirable if endogeneous compounds can provide additional information for individual differentiation. We will test this hypothesis with free fatty acids, TAGs and AAs that are commonly present in fingerprints as potential individual markers.

Findings and Scholarly Products

In total, we published seven peer-reviewed papers in scientific journals, and eight oral and nine poster presentations in scientific conferences. Here is the excerpt of the findings categorized to each specific aim along with full citations. It should be noted some of the outcome is done by Kelly O'Neill under her NIJ Graduate Fellowship as marked by #.

Specific Aim 1: Nanoparticles to Assist Chemical Fingerprinting: We found several nanoparticles are useful as a matrix for MALDI-MSI of fingerprints. Most notably, gold thin film sputter coating combined with sodium ion spray is very useful to improve the detection of TGs, and silver sputtering is useful for negative ion mode. These findings are not published separately but as a method part of other studies in application-oriented studies below.

Specific Aim 2: High mass resolution and high spatial resolution mass spectrometry imaging (HR^2MSI) for advanced chemical imaging of fingerprint: We have successfully adopted multiple MS imaging strategy combining MS/MS and high-mass resolution MS, to improve identification of unknown compounds directly from fingerprints. It has been demonstrated to characterize unknown exogeneous compounds from fingerprint that can be used to reveal individual lifestyle, as published in Scientific Reports. Demonstrated application includes the detection of marker compounds in bug spray, sun spray, food oils, citrus fruits, and wines.

1. "Revealing Individual Lifestyles through Mass Spectrometry Imaging of Chemical Compounds in Fingerprints", Paige Hinners, Kelly C. O'Neill, and **Young Jin Lee***, Scientific Reports, **2018,** 8:5149, DOI:10.1038/s41598-018-23544-7.

Specific Aim 3: Expanding Utility of Mass Spectrometry Imaging for Fingerprinting: In sub-aim 1) aging study of chemical fingerprint, we studied the diffusion of TGs and fatty acids

on various surface types, and found the surface interaction is more important that the molecular weight of the compounds as proposed by NIST scientist previously. As a result, the use of diffusion for fingerprint aging seems to be much more complicate and difficult.

2#. "Effect of Aging and Surface Interactions on the Diffusion of Endogenous Compounds in Latent Fingerprints", Kelly C. O'Neill and **Young-Jin Lee***, J. Forensic Science, **2018**. 63, 708-713, doi: 10.1111/1556-4029.13591.

Instead, we found ambient ozonolysis of unsaturated TGs is much more effective to determine the fingerprint aging as recently published in Analytical Chemistry. Namely, carbon-carbon double bond in the side chain of TGs reacts with ambient ozone and slowly degrades over time. We thoroughly studied the mechanism and demonstrated its applicability in a proof-of-concept result. This approach is much more reliable and has a good potential to be eventually applicable to forensic field. The expanded research plan is proposed to NIJ for further study and recently funded for the continuation of this work (2019-DU-BX-0134).

3. "Determining fingerprint age with mass spectrometry imaging via ozonolysis of triacylglycerols", Paige Hinners, Madison Thomas, **Young Jin Lee*,** Analytical Chemistry, 2020, **92(4),** 3125-3132 (selected as a supplementary journal cover), https://doi.org/10.1021/acs.analchem.9b04765.

In aim 2) surface modification of amino acids, we have successfully developed a protocol to enhance amino acids on surface; however, we could not find an appropriate method for quantification, especially how to adequately normalize the difference in initial deposited materials. As such, we did not further pursue the applicability of this method.

In aim 3) compatibility with traditional fingerprint development techniques, we have successfully published three papers, one submitted, and one more to be submitted. In chemical imaging of cyanoacrylate fumed fingerprints, we demonstrated MALDI-MSI is compatible with this development technique, except for quaternary ammonium ions, which is mostly from

hygiene products such as shampoo and soap and usually not so useful due to ubiqutous nature of this chemicals. We also studied the mechanism of cyanoacrylate fuming using mass spectrometry.

4#. "Chemical Imaging of Cyanoacrylate Fumed Fingerprints by Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging", Kelly C. O'Neill, Paige Hinners, **Young Jin Lee***, J. Forensic Science, **2018**, 63(6):1854-1857, doi: 10.1111/1556-4029.13773.

5#. "Study of the Cyanoacrylate Fuming Mechanism by Matrix Assister Laser Desorption/ Ionization Mass Spectrometry", Kelly C. O'Neill, **Young Jin Lee***, J. Mass Spectrom. **2019**, 54(3), 222-226. DOI: 10.1002/jms.4325.

We also tested the compatibility of forensic carbon powders. As reported by others, contamination by carbon cluster peaks is seriously limitation of adopting this development technique for MALDI-MSI. But, we could still distinguish the carbon cluster peaks from fingerprint chemicals by adopting high-resolution Orbitrap mass spectrometry because of their exact mass difference. Furthermore, carbon powders have inherent laser absorption and could be used without additional matrix. We also demonstrated that this approach is so gentle that there is no apparent damage in optical fingerprint images after MALDI-MSI analysis.

6. "Carbon-Based Fingerprint Powder as a One-Step Development and Matrix Application for High-Resolution Mass Spectrometry Imaging of Latent Fingerprints", Paige Hinners, **Young Jin Lee***, J. Forensic Science, **2019**, 64(4):1048-1056. **DOI: 10.1111/1556-4029.13981.**

Other development techniques, ninhydrin and iodine fuming commonly used for porous surface and TiO_2 nanoparticles used on dark surface, are also tested and the results are either just submitted or intend to submit soon.

* "Chemical Imaging of Latent Fingerprints Deposited on Porous Surfaces Developed by Ninhydrin and Iodine Fuming", Emily C. King, Paige Hinners and Young Jin Lee*, to be submitted to J. Forensic Science.

* "Mass Spectrometry Imaging of Fingerprints Using Titanium Oxide Development Powder as an Existing Matrix", Paige Hinners and Young Jin Lee*, revision submitted to J. Mass Spectrometry on Jun 1, 2020.

Specific Aim 4: Individual differentiation based on chemical fingerprint: We hypothesized TGs might be influenced by exercise, diet, and health conditions, which might be useful to get information about the individuals from the fingerprints. A proof-of-concept was successfully demonstrated for a small group of people as published in Analytical Methods. The continuous work this research, especially for diabetes patients, is a part of the newly funded NIJ project.

7#. "Potential of triacylglycerol profiles in latent fingerprints to reveal individual diet, exercise, or health information for forensic evidence" Kelly C. O'Neill, Paige Hinners, and **Young Jin Lee***, Analytical Methods, 2020, **12**, 792, **https://doi.org/10.1039/C9AY02652E**.

We made three invited oral presentations for the work supported by this work:

1. Pittcon, National Institute of Justice-Forensic Science Symposium for Emerging Technologies, Mar 1-5, 2020, Chicago, IL.
2. "Mass spectrometry imaging of latent fingerprints for forensic evidence", Department of Chemistry, Indiana University, Apr 9, 2019.
3. Pittcon, National Institute of Justice-Forensic Science Symposium for Emerging Technologies, Mar 17-21, 2019, Pittsburgh, PA.

Additionally, fiver oral presentations are made by students in various conferences:

4. "Large-Scale Study for the Differentiation of Individuals Based on Triacylglycerols in Latent Fingerprints", Kelly O'Neil, Young Jin Lee, American Academy of Forensic Sciences, Baltimore, MD, Feb 18-23, 2019.
5. "Chemical Imaging of Latent Fingerprints Deposited on Porous Surfaces Developed by Ninhydrin and Iodine Fuming", Emily King, Paige Hinners, and Young-Jin Lee, ACS Midwest Regional Meeting, Ames, IA, Oct 21-23, 2018.

6. "Revealing individual lifestyles through mass spectrometry imaging of chemical compounds in fingerprints". P. Hinners, Young Jin Lee, ACS Midwest Regional Meeting, Ames, IA, Oct 21-23, 2018.
7. "Lifestyle Determination From Chemical Identification in Fingerprints", Paige Hinners, Kelly O'Neil, Young Jin Lee, American Academy of Forensic Sciences, Seattle, WA, Feb 19-24, 2018.
8. "Chemical Imaging of Cyanoacrylate Fumed Fingerprints Using Mass Spectrometry", Kelly O'Neil, Paige Hinners, Young Jin Lee, American Academy of Forensic Sciences, Seattle, WA, Feb 19-24, 2018.

Additionally, eight poster presentations were made in various conferences.

1. “Triacylglycerol Profiles in Latent Fingerprints Reveal Diet, Exercise, and Health Information for Forensic Evidence”, Kelly C. O’Neill, Young Jin Lee, Merck Symposium, Rising Stars in Analytical Chemistry & Materials Science, Rahway, NJ, Nov 21-22, 2019.
2. "Use of Image Quality Scores to Determine Fingerprint Age in MALDI imaging", Madison Thomas, Paige Hinners, Young-Jin Lee, ASMS Conference on Mass Spectrometry and Allied topics, Atlanta, GA, Jun 2-6, 2019.
3. "Identifying Suspect Relevance to a Crime Scene Based on Fingerprint Age Biomarkers Using MALDI Imaging", Paige Hinners, Young-Jin Lee, ASMS Conference on Mass Spectrometry and Allied topics, Atlanta, GA, Jun 2-6, 2019.
4. "Determination of Health Status by MALDI-MSI of Latent Fingerprints", Kelly O'Neill, Young-Jin Lee, ASMS Conference on Mass Spectrometry and Allied topics, Atlanta, GA, Jun 2-6, 2019.
5. "Feasibility of Carbon Fingerprint Development Powder As a Matrix for MALDI-MSI of Latent Fingerprints", Paige Hinners, Young-Jin Lee, ASMS Conference on Mass Spectrometry and Allied topics, Jun 3-7, 2018, San Diego, CA.!
6. "Chemical Imaging of Latent Fingerprints Deposited on Porous Surfaces Developed by Ninhydrin and Iodine Fuming", Emily King, Paige Hinners, Young-Jin Lee, ASMS Conference on Mass Spectrometry and Allied topics, Jun 3-7, 2018, San Diego, CA.!

7. "Chemical Imaging of Cyanoacrylate Fumed Fingerprints Using MALDI-Orbitrap", Kelly O'Neill, Paige Hinners, Young Jin Lee, ASMS Conference on Mass Spectrometry and Allied topics, Jun 4-8, 2017, Indianapolis, IN.!
8. "Chemical Identification in Latent Fingerprints for Lifestyle Determination", Paige Hinners, Kelly O'Neill, Young Jin Lee, ASMS Conference on Mass Spectrometry and Allied topics, Jun 4-8, 2017, Indianapolis, IN.!
9. "Diffusion of Triacylglycerols to Determine Age of Latent Fingerprint", Kelly O'Neill, Young-Jin Lee, ASMS Conference on Mass Spectrometry and Allied topics, Jun 5-9, 2016, San Antonio, TX.!

Implications for criminal policy and practices in United States.

Under this NIJ supported project, we have successfully demonstrated the potential of MALDI-MSI as a state-of-the-art forensic tool that can overcome previous limitations in forensic labs. It is still early stage to apply for real forensic cases yet; but with additional demonstration to more complex and currently impossible forensic mock cases, this technique has a great potential to become a useful tool in forensic applications. As an example, our recent ambient ozonolysis fingerprint aging study has been highlighted by many news outlets:

* **ACS News Weekly PressPacs**, "Residues in fingerprints hold clues to their age", Jan 22, 2020. https://www.acs.org/content/acs/en/pressroom/presspacs/2020/acs-presspac-january-22-2020/residues-in-fingerprints-hold-clues-to-their-age.html
* **The Economics**, Science and technology column, " Fingerprints can now be dated to within a day of when they were made", Jan 30, 2020. https://www.economist.com/science-and-technology/2020/01/30/fingerprints-can-now-be-dated-to-within-a-day-of-when-they-were-made
* **Iowa State University, News Service**, "Chemists use mass spectrometry tools to determine age of fingerprints", Feb 18, 2020, https://www.news.iastate.edu/news/2020/02/18/fingerprints

* **Iowa Public Radio**, talk show by Ben Kiefer, River to River, Feb 21, 2020, our story starts at 31:50, https://www.iowapublicradio.org/post/declining-bald-eagle-population-isu-research-fingerprints-and-funnel-week-iowa-statehouse#stream/0
* **Channel 5, 6pm Local News**, Feb 21, 2020, "New forensic technology at Iowa State University could determine the age of fingerprints", https://www.weareiowa.com/article/news/local/fingerprint-age-technology-iowa-state-university-solve-crimes/524-5077a6ce-e0b7-46cd-acf7-eb14f2898bc4
* **Channel 8, 6pm Local News**, Feb 21, 2020, "Iowa State University researchers find breakthrough method to fight crime", https://www.kcci.com/article/iowa-state-university-researchers-find-breakthrough-method-to-fight-crime/31050430#
* **Channel 13, 6am Local News,** Feb 24, 2020, "ISU Students and Staff Make Groundbreaking Discovery That Could Impact Criminal Investigations", https://whotv.com/news/isu-students-and-staff-make-groundbreaking-discovery-that-could-impact-criminal-investigations/

Citations

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8. Horn, P.J., et al., *Spatial Mapping of Lipids at Cellular Resolution in Embryos of Cotton.* The Plant Cell, 2012. **24**(2): p. 622-636.
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14. Manier, M.L., et al., *A derivatization and validation strategy for determining the spatial localization of endogenous amine metabolites in tissues using MALDI imaging mass spectrometry.* Journal of Mass Spectrometry, 2014. **49**(8): p. 665-673.
15. Shariatgorji, M., et al., *Direct Targeted Quantitative Molecular Imaging of Neurotransmitters in Brain Tissue Sections.* Neuron, 2014. **84**(4): p. 697-707.