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Compatibility of matrix-assisted laser desorption/ionization-mass spectrometry imaging with porous fingerprint development techniques

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Compatibility of matrix-assisted laser desorption/ionization- mass spectrometry imaging with porous fingerprint development techniques

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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NOMENCLATURE

MALDI	matrix assisted laser desorption/ionization
MSI	mass spectrometry imaging
MS	mass spectrometry
m/z	mass to charge ratio
FA	fatty acid
CHCA	alpha-cyano-4-hydroxycinnamic acid
DHB	2,5-dihydroxybenzoic acid
GC	gas chromatography
LC	liquid chromatography

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ABSTRACT

This thesis encompasses work done to aid in the incorporation of matrix-assisted laser desorption/ionization - mass spectrometry imaging (MALDI-MSI) into the forensic workflow. MALDI-MSI is a valuable technique in that chemical information, in addition to the location of the compounds, can be obtained. In the first chapter, an introduction to the technique and its applications and benefits for the field of forensics are presented. The following chapter details a set of experiments comparing MALDI-MSI with porous surface fingerprint development techniques, specifically ninhydrin and iodine-fuming. It was determined that MALDI-MSI is compatible with both techniques and able to reveal chemical information from a fingerprint. The MS image quality was also compared, and iodine-fumed fingerprints showed analogous images to the non-developed fingerprint, while ninhydrin-developed fingerprints seldom did. With iodine-fumed fingerprints maintaining ridge detail, further information can be determined, such as the deconvolution of the fingerprint and the surface surrounding the fingerprint. The final chapter concludes with an outlook on future directions of research related to this work.

CHAPTER 1. GENERAL INTRODUCTION

General Description of Mass Spectrometry

Mass spectrometry (MS) is a widely used analytical method in which analytes can be detected and identified. It has been implemented in a variety of fields to examine the chemical composition of a sample. MS operates on the principle of creating ions in the gas phase, which are then measured by their mass to charge ratios (*m/z*).[1] A mass spectrum is produced with the y-axis displaying the abundance and the *m/z* on the x-axis. Depending on the polarity (positive or negative) and ionization methods used, various adducts can be seen in the mass spectrum. Some of these variations include protonated, deprotonated, or in some instances sodiated, ion adducts. An assortment of mass analyzers and ionization techniques have been developed for the analysis of various sample types.

Time-of-flight, quadrupole, Fourier transform ion cyclotron resonance, and Orbitrap are a few of the commonly utilized mass analyzers.[1] For the research conducted in this thesis, an Orbitrap was used. An Orbitrap mass analyzer separates ions based on an electric field using a football-shaped outer electrode and a central electrode. It then detects the ions based on image current detection and uses Fourier transform to convert the image current into a frequency domain. High resolving power and high mass accuracy are some of the advantages of this mass analyzer.[1] To produce ions, methods such as electron/chemical ionization, fast atom bombardment, matrix-assisted laser desorption/ionization (MALDI), electrospray ionization and desorption electrospray ionization are frequently implemented.[1]

Mass Spectrometry Imaging

Overview

In contrast to traditional MS, mass spectrometry imaging (MSI) preserves the localization of analytes in a sample. To perform MSI, different types of sample introduction methods are used instead of the conventional extraction-based chromatography methods (i.e., liquid-chromatography or gas-chromatography). Different sample introduction/ionization methods such as secondary ion mass spectrometry (SIMS), desorption electrospray ionization (DESI), and MALDI preserve analyte localization.[1] SIMS is a hard ionization technique (i.e., compounds are readily fragmented), however, it has a very small spatial resolution (2-3 μm). DESI is a soft ionization technique (i.e., less fragmentation occurs), but it has a much larger spatial resolution (>150 μm).[2,3] MALDI is one of the most used imaging methods as it combines the advantages of the other techniques: soft ionization and high spatial resolution (5-300 μm). In addition to these benefits, MALDI has minimal sample preparation, is salt tolerant, and label-free. Furthermore, the ions are usually singly charged, which makes it easier to identify compounds. For the experiments discussed in this thesis, MALDI-MSI was used.

As described in the name of the technique, MALDI requires the sample to be covered by a matrix prior to the analysis. Organic compounds are customarily used to aid in the detection of a specific group of analytes, while other matrices such as carbon nanotubes and sputtered metals have also been investigated.[1,2,4,5] **Table 1** lists common matrix types and compounds with which they work well.[1,2,4,5] A matrix can be applied to the sample in a variety of ways including, but not limited to; sprayed, sputtered, spotted, and sublimated. With the different variations of matrix types, a wide variety of analytes can be detected.

Table 1. Common matrix types used in MALDI

Matrix Type	Matrix	Mode	Compound Types
Organic	α-cyano-4-hydroxycinnamic acid	Positive	Peptides
	2,5-dyhydroxybenzoic acid	Positive	Lipids
	1,5-diaminonaphthalene	Negative	Phospholipids
Metal	Silver	Positive/Negative	Olefins
	Gold	Positive/Negative	Triacylglycerols

Considerable research has gone into the mechanism in which ions form by MALDI, however, there is no definitive explanation. However, the importance of the matrix applied to the surface of the sample has been recognized. When the laser is fired on a sample it is absorbed with the help of the matrix if the analytes themselves are not able to. This, in turn, allows for the desorption and ionization of analytes, which permits MS detection.[1,2]

Forensics and MSI

There has been a great deal of work to test the capability of MSI for forensic use. This research was primarily started by the Francese group and continued by many others. The major advantage of MSI for fingerprint analysis is warranted because the technique enables the preservation of the fingerprint's evidentiary value. This is because an extraction process is not utilized. Chemical information such as the presence of endogenous and exogenous compounds can be identified. Endogenous compounds originate from the human body, whereas exogenous compounds do not. Research has been performed to determine the age, sex, ethnicity, and lifestyle of an individual with detection of these compounds.[6–8] Using endogenous compounds, it has been shown that the age of the fingerprint (how long since deposition) can be determined.[9] MSI analysis allows for a wealth of information to be gained from a single fingerprint.

Advances in the use of MALDI-MSI for the detection of forensically relevant compounds are important. However, in a realistic setting, a development technique will have been applied to the fingerprint prior to MALDI-MSI analysis. Fingerprints are mostly latent (invisible to the naked eye) at a crime scene and the development technique is needed for visualization. Investigation into the interference between development and analyte detection is critical for the incorporation of MALDI-MSI to the forensic workflow. Considerable work has gone into examining fingerprints that have been deposited on non-porous surfaces.[10–14] In contrast, work to investigate the effects of porous surface development techniques is lacking.[13,15] Work in this thesis aims to delve further into porous surface development techniques and determine their influence on MALDI-MSI analysis.

General MSI Workflow

All experiments were performed using a MALDI-linear ion trap-Orbitrap mass spectrometer. This instrument was modified in house to accommodate a 355 nm Nd:YAG laser. A schematic of this instrument can be seen in **Figure 1**. With this instrument, we have two mass analyzers. The first mass analyzer is a linear ion trap that is used mainly to perform tandem mass spectrometry to aid in the structural identification of compounds. The main analyzer used is an Orbitrap. This analyzer allows for the identification of compounds using the accurate mass as it has a high resolving power.

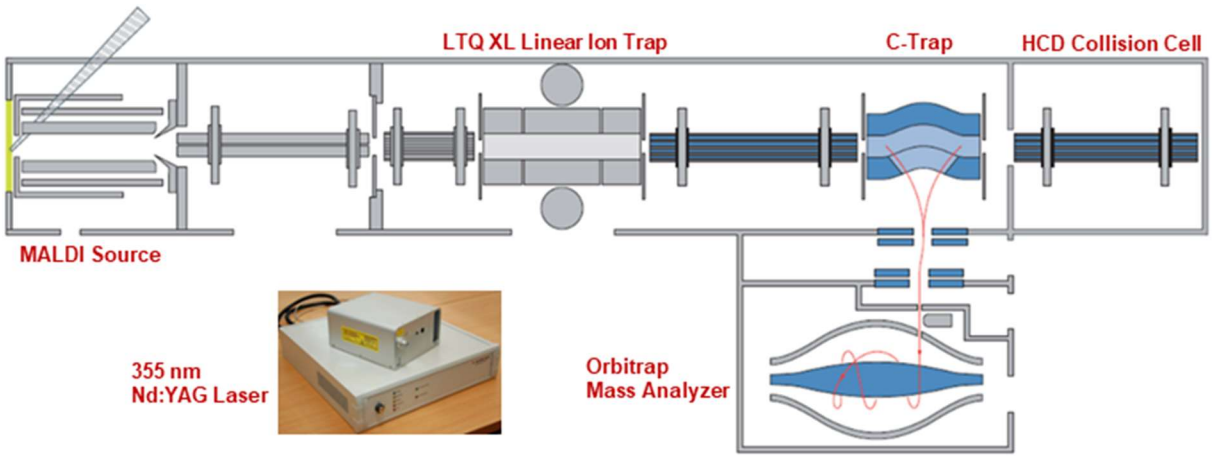

Figure 1. Schematic of MALDI-linear ion trap-Orbitrap mass spectrometer

Figure 2 shows the general workflow for an MSI experiment. The process can vary depending on what samples are being analyzed. In the first step, the fingerprint sample will be collected from a participant. For this thesis, all experiments were done on porous substrates (i.e. paper). An optional step is then performed in which the fingerprint is developed. Development techniques utilized include iodine-fuming and ninhydrin-development. Once the sample is prepared, a matrix is then added to aid in the ionization/desorption of the analytes. This can be done using a sprayer (i.e., TM-Sprayer) in the case of organic matrices or a sputter coater when applying metal matrices. The instrument experiment is then set up. In the work presented in this thesis, the samples were analyzed with a 100 µm raster step and a laser spot size of 15-20 µm. Once the data collection was complete the spectra are analyzed for any endogenous or exogenous compounds that may help identify more information about an individual of interest. To show the location and intensity of the compounds a pseudo heat map is compiled using software.

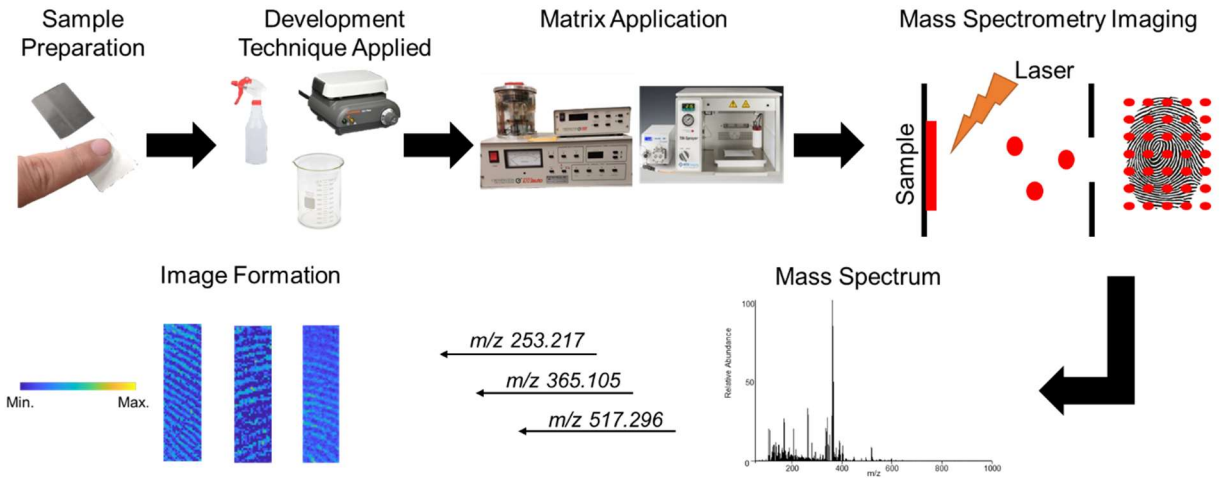

Figure 2. MALDI-MSI Workflow

Thesis Organization

This thesis is separated into three different chapters. In the first chapter, MS and MSI were discussed. An introduction to how the forensic community uses these techniques was reviewed. Chapter two is a submitted paper discussing the compatibility of MALDI-MSI with porous surface development techniques. Iodine-fuming and ninhydrin-development fingerprints are compared in parallel to a non-developed fingerprint to determine the impact of the development technique in the detection of forensically relevant compounds using MALDI-MSI. A summary of the work presented in this thesis is discussed in chapter three. Future directions and outlooks are also presented.

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CHAPTER 2. CHEMICAL IMAGING OF LATENT FINGERPRINTS DEPOSITED ON POROUS SURFACES DEVELOPED BY NINHYDRIN AND IODINE FUMING

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Abstract

RATIONAL: Latent fingerprints are invisible to the naked eye and require forensic development for visualization. Fingerprints can be analyzed using mass spectrometry imaging (MSI) which allows for chemical analysis of forensically relevant compounds to enhance evidentiary value, improving upon ridge detail identification. The compatibility of MSI with ninhydrin development and iodine-fuming was determined with an in-parallel comparison to a non-developed sample.

METHODS: A matrix-assisted laser desorption/ionization (MALDI)-linear ion trap-Orbitrap mass spectrometer (MS) was utilized to analyze fingerprint samples and chemical standards after development with ninhydrin or iodine-fuming. Signal intensities were directly compared to a non-developed sample to determine compatibility of MALDI-MSI with development techniques and forensic implementation.

RESULTS: MALDI-MSI is shown to be compatible with both development techniques, but iodine-fuming exhibited less signal suppression in positive mode and greater image quality. Ninhydrin provides much higher ion signals when used in negative mode.

CONCLUSION: MALDI-MSI is compatible with development techniques on porous surfaces, particularly with iodine-fuming in positive mode and ninhydrin in negative mode and can be implemented for the chemical analysis of fingerprints. The quality of MS images for iodine-

fumed fingerprints are analogous to non-developed fingerprint while ninhydrin-developed fingerprints appear to experience delocalization.

Introduction

In the field of forensics, fingerprints are commonly used to identify a person of interest with a comparison of ridge patterns in a database. Accompanying the ridge pattern evaluation, chemical analysis of fingerprints has been applied to provide additional information from fingerprints. There are two kinds of compounds that can be examined by chemical analysis; endogenous (i.e., naturally excreted from the body) and exogenous compounds (i.e., not naturally present in the body). Examples of endogenous compounds are amino acids, fatty acids, triacylglycerols, and cholesterol.[1–5] Forensically relevant exogenous compounds include drugs, explosives, and other compounds found in everyday objects such as food residue and sunscreens.[2,6–8] With this additional information, the lifestyle[6–9] or sex[10,11] of a person of interest can be narrowed down or confirmed/rejected.

Extraction-based chemical analysis methods have been most commonly used for the analysis of fingerprints. Some examples include, liquid chromatography-mass spectrometry (LC-MS),[12] and gas chromatography-mass spectrometry (GC-MS).[13–15] Although well-established and widely available, these techniques have many limitations including the loss of compound localization, contamination from background chemicals, and destruction of fingerprint evidence. Direct surface analysis with mass spectrometry imaging (MSI) is suggested as an attractive alternative as they allow for the spatial representation of the compound in the fingerprint. This proves useful when deconvoluting multiple fingerprints located on top of each other and distinguishing fingerprint compounds from surface contaminants.[16] Many ionization techniques have been used for this type of analysis including matrix-assisted laser desorption/ionization (MALDI),[2,3,6,15,17–21] desorption electrospray ionization (DESI),[22] secondary ion mass

spectrometry (SIMS),[15,23] and laser ablation inductively coupled plasma.[24] MALDI-MSI has become one of the most popular choices for the chemical analysis of fingerprints.[2,3,6,15,17–21]

Ridge patterns for latent fingerprints often require development to make them visible, as latent fingerprints are invisible to the naked eye. Most common fingerprint development methods include fuming, use of altered light sources, vacuum metal deposition, and powders,[25] which are readily applicable for non-porous surfaces (e.g., glass or plastic). Porous surfaces (e.g., paper or cardboard) require alternative development techniques such as 1,8-diazafluoren-9-one (DFO), physical developer, iodine-fuming, and ninhydrin.[25] Ninhydrin is one of the most commonly used porous surface development techniques. This solution reacts with the amino acids present in fingerprints and results in a purple (known as Ruhemann's Purple) developed print on paper.[25,26] A less commonly used technique is iodine-fuming. The fumes from the sublimation of iodine crystals interact with the sebaceous material in fingerprints,[27] resulting in a yellow/brown colored developed fingerprint.[25] This technique is simple to perform, but the development fades with time. However, techniques to increase the lifetime of a fingerprint developed by iodine-fuming have been studied previously.[27,28] Iodine-fuming is nondestructive, unlike the more commonly used ninhydrin technique. For example, Tsai et. al. demonstrated that there was no reduction in DNA quality after development with iodine-fuming, whereas ninhydrin-developed fingerprints demonstrated a significant decrease in DNA quality.[29,30]

We have previously determined the compatibility of MALDI-MSI with various development techniques such as cyanoacrylate fuming and carbon fingerprint powder.[2,3] Cyanoacrylate showed no signal suppression for most compounds except quaternary ammonium derivatives, and carbon fingerprint powder does not need additional MALDI matrix as it absorbs laser energy itself. Here, we report a thorough study on the compatibility of the two porous

development techniques, ninhydrin and iodine-fuming, with MALDI-MSI. Other groups have noted the compatibility of ninhydrin[18–20] with MALDI-MSI for the detection of endogenous and exogenous compounds, but have not performed a direct comparison to a non-developed fingerprint to show the impact of the development technique on compound signal intensities and MS image quality. Iodine-fuming however, to our knowledge, has not previously been studied for its compatibility with MSI.

Methods

Materials

Oleic acid (FA 18:1, ≥99.0%), cholesterol (≥99%), pseudoephedrine hydrochloride, lidocaine, cetrimonium bromide, 2,5-dihydroxybenzoic acid (DHB, 98%), α-cyano-4-hydroxycinnimaic acid (CHCA, ≥98%) and iodine crystals (≥99.8%) were all purchased from Sigma-Aldrich (St. Louis, MO). Ninhydrin (≥98%) was purchased from Tokyo Chemical Industry Co., Ltd. (Portland, OR). A silver target (99.99%, 57mm × 0.33mm) was purchased from Ted Pella Inc. (Redding, CA). Avobenzone, oxybenzone, homosalate, octisalate and octocrylene were ingredients in a sunscreen lotion that was purchased from a local retailer. Picaridin is an active ingredient in bug spray that was purchased from a local retailer.

Sample Preparation

The Iowa State University Institutional Review Board approved all experiments performed on human subjects. Standards were prepared at 10 mM concentrations in methanol or chloroform. Using an HTX TM-Sprayer (HTX Technologies, Chapel Hill, NC), standards were sprayed onto paper sections. The solutions were sprayed at a rate of 0.03 mL per minute for eight passes with a velocity of 1200 mm per minute and a temperature of 30 °C. After application of standard, the paper was then split into three sections and prepared with different techniques; one

was developed with ninhydrin, another with iodine-fuming, and the last section was left with no development to serve as the control. The development techniques are described below.

To prepare fingerprint samples, fingers were rubbed in the crease of the nose and then pressed onto paper for several seconds. Fresh fingerprints were prepared in triplicate for each analysis and comparison. A split fingerprint design (i.e., the fingerprint was cut in half lengthwise) was used to compare a developed section and a non-developed section of the same fingerprint, just as with the standards. For mock exogenous fingerprints, the sunscreen or bug spray was applied as directed on the product prior to depositing a fingerprint onto the paper surface. Powders were handled by the participant and then a fingerprint was deposited. The majority of this work was performed on printer paper. Notebook and receipt paper were used in the last experiment for paper type comparison.

Development

Development procedures were taken from the FBI processing handbook for developing latent fingerprints.[25] Iodine crystals were placed into a glass container. Paper sections containing fingerprints or standards were taped to the side of the container, and it was left to sit at room temperature. A paper towel was placed on top of the container to contain the iodine vapors. Once development was complete (approximately two minutes), samples were removed.

Ninhydrin solution was prepared by mixing five grams of ninhydrin with 1L of a methanol, isopropanol and petroleum ether mixture (30:40:930 V:V:V).[25] Paper sections with a fingerprint or standards were sprayed with the ninhydrin solution using a spray bottle. After samples were completely dry, they were placed on a heating block with a damp paper towel until developed (approximately five minutes).

Matrix Application

Silver was sputter coated on the samples using a 108 Auto sputter coater (Ted Pella Inc., Redding, CA) for five seconds at 40 mA. Solutions of 2,5-dihydroxybenzoic acid (DHB, 40 mg mL^{-1} in MeOH) and α-cyano-4-hydroxycinnamic acid (CHCA, 5 mg mL^{-1} in 70:30 acetonitrile: 0.1% aqueous trifluoroacetic acid) were sprayed on the samples using a TM Sprayer (HTX Technologies, Chapel Hill, NC). CHCA was applied with a flow rate of 0.03 mL per minute for eight total passes with a velocity of 1200 mm per minute. A 0.1 mL per minute flow rate was used for DHB with a total of eight passes with a velocity of 1200 mm per minute. CHCA was applied at a temperature of 30 °C and DHB was applied at 75 ° C.

Matrix Selection

The matrices tested were sputtered silver , CHCA, and DHB, as they have all been utilized for the analysis of fingerprints.[3,6,15,17,19–21] Selection of a suitable matrix is essential to ensure the detection of fingerprint species or forensically relevant compounds. A comparison of standard intensities using the three different matrices was conducted. Standards such as FA 18:1 and cholesterol represent compounds that would naturally be present in the fingerprint (endogenous compounds). As compounds not naturally present in the fingerprint (exogenous compounds), lidocaine, cetrimonium, and pseudoephedrine were used. Apart from cholesterol and cetrimonium, the sputtered silver matrix produced the highest overall signal intensity. Sputtered silver matrix was used for the rest of this study, as it worked well across a variety of compounds and maintained ridge detail. The sputtered silver matrix can also be used in both positive and negative mode allowing for a more complete analysis of compounds.

Mass Spectrometry Imaging

Samples were analyzed using a MALDI-linear ion trap-Orbitrap mass spectrometer (MALDI-LTQ-Orbitrap Discovery; Thermo Finnigan, San Jose, CA) coupled to a 355 nm

Nd:YAG laser (UVFQ; Elforlight, LT., Daventry, UK). A 100 µm raster step, 15 µm laser spot size, and 10 laser shots were used for all MSI experiments. The *m/z* range of 50-1000 was observed using the Orbitrap mass analyzer (resolution of 30,000 at *m/z* 400). Samples were analyzed in both positive and negative modes.

Data Analysis

Mass spectra were averaged and exported using QualBrowser software (Thermo Scientific). ImageQuest (Thermo Scientific) was used to export files to imzML format. Mass spectrometry images were produced using MSiReader[31,32] (North Carolina State University). All images of the same compound were exported with the same intensity scale, which is indicated on the figures. The effect of the development technique on the different compounds was measured by normalizing the signal intensity of the developed sample to its corresponding non-developed fingerprint.

Results and Discussion

Technique Compatibility

Figure 1 compares the optical images of fingerprints after development with iodine-fuming or ninhydrin, matrix application, and MALDI-MS analysis. As it is difficult to quantitatively compare fingerprint chemicals before and after the development, because of varying amounts of chemical residue, fingerprints deposited on paper were split in half lengthwise. One side is undeveloped, to be used as a control, and the other side is developed using iodine-fuming or ninhydrin-development, as shown in **Figure 1A**. For both non-developed sections, the fingerprint is invisible to the naked eye with no apparent ridge detail. Ridge detail is clearly visible in both developed fingerprints with iodine-fumed fingerprints as brown and ninhydrin-developed fingerprints as purple. After the application of silver nanoparticle matrix, the background color changes to purple in iodine fumed and yellow in ninhydrin developed

printer paper as shown in **Figure 1B**. The fingerprints after analysis by MALDI-MSI are displayed in **Figure 1C**, with black boxes indicating the region analyzed with MALDI-MSI. Mass spectrometry is generally a destructive technique, but MALDI-MSI is a very gentle technique with minimal sample damage. Even after silver sputtering and MALDI-MSI analysis, the ridge detail on the fingerprints is still clearly visible. In fact, there is no apparent difference in the image quality between the analyzed and unanalyzed fingerprint sections. This suggests that the fingerprints can be kept for court evidence after MALDI-MSI data acquisition.

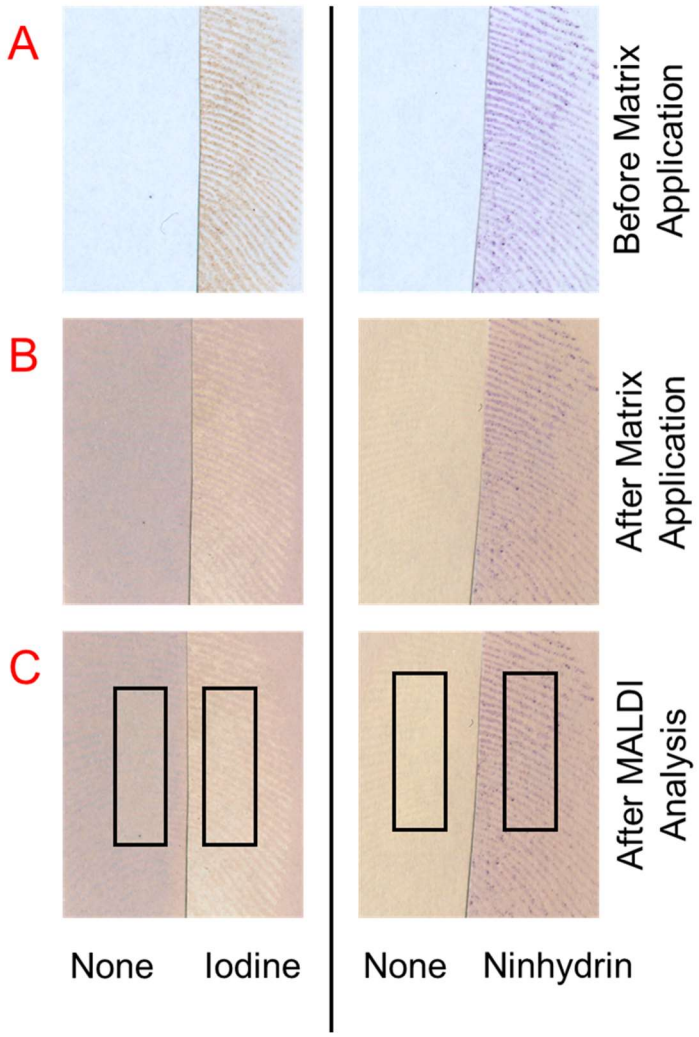

Figure 1. Optical images of latent fingerprints on printer paper **(A)** with iodine-fuming and ninhydrin-development compared with non-development, **(B)** after silver matrix application, and **(C)** after MALDI-MSI analysis. Black boxes indicate the area where the MALDI-MSI analysis was performed. 'None' indicates no development, and 'Iodine' and 'Ninhydrin' indicate iodine fumed and ninhydrin developed, respectively.

Selection of a suitable matrix is essential to ensure the detection of fingerprint or forensically relevant compounds. The matrices tested were silver, CHCA, and DHB, all of which worked as matrices for latent fingerprints on porous surfaces for both standards as well as the fingerprints (data not shown). Silver nanoparticles were used for the rest of this study as it worked well across a variety of compounds and maintained ridge detail. It can also be used in both positive and negative mode allowing for analysis of more compounds.

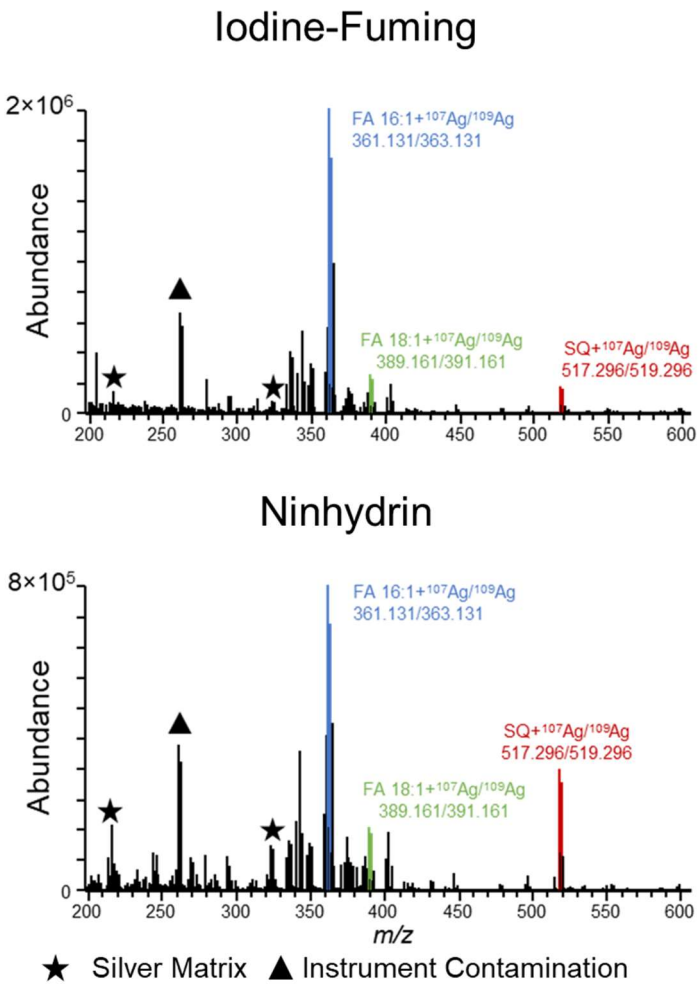

Figure 2. Mass spectra of fingerprints in positive mode after development with (**Top**) iodine-fuming and (**Bottom**) ninhydrin.

A representative positive ion mode mass spectrum using silver as a matrix from each development type is shown in **Figure 2** for the m/z range of 200 to 600. Compounds, such as fatty acids and squalene, are observed as a silver ion adduct after both iodine-fuming and ninhydrin-development. A characteristic doublet pattern is seen due to silver isotopes at 107 u and 109 u with a natural abundance of 1 to 0.93. The signal is higher after iodine-fuming for fatty acids (see difference in y-axis scale) but smaller for squalene.

Fingerprint Analysis

MSI was performed to examine the effect of the fingerprint development techniques on MALDI image quality and fingerprint compound intensity. The developed fingerprint signal intensity was normalized to the non-developed fingerprint signal intensity from the split fingerprints as shown in **Figure 1**. **Figure 3A** shows the normalized intensities of endogenous compounds comparing iodine and ninhydrin development for both positive and negative mode. A normalized intensity of one indicates that there is no signal suppression by the development procedure and is completely compatible with MALDI-MSI. Both ninhydrin-development and iodine-fuming are compatible with MALDI-MSI for fatty acids in positive mode with a normalized intensity of around one. However, for dihexose and squalene, there was a decrease in the normalized intensity for both development types, with a less significant decrease for ninhydrin-developed fingerprints. In negative ion mode, fatty acid signals are decreased for both development procedures but much more significantly for iodine-fumed fingerprints, whereas ninhydrin suffers only half the signal loss.

In **Figure 3B**, the MS images of endogenous fingerprint compounds are displayed. Note that the same maximum intensity scale is used for each compound across all development types to display intensity comparison. When comparing the image quality of all compounds, ninhydrin shows a lack of ridge detail. Ninhydrin-developed fingerprints appear to display delocalization of

the analytes. Iodine-fumed fingerprints exhibit ridge detail comparable to the non-developed fingerprint in positive mode, but almost imperceptible in negative mode. Based on MALDI-MS image quality, iodine fuming is compatible with MALDI imaging in positive mode and can therefore be incorporated into the forensic workflow.

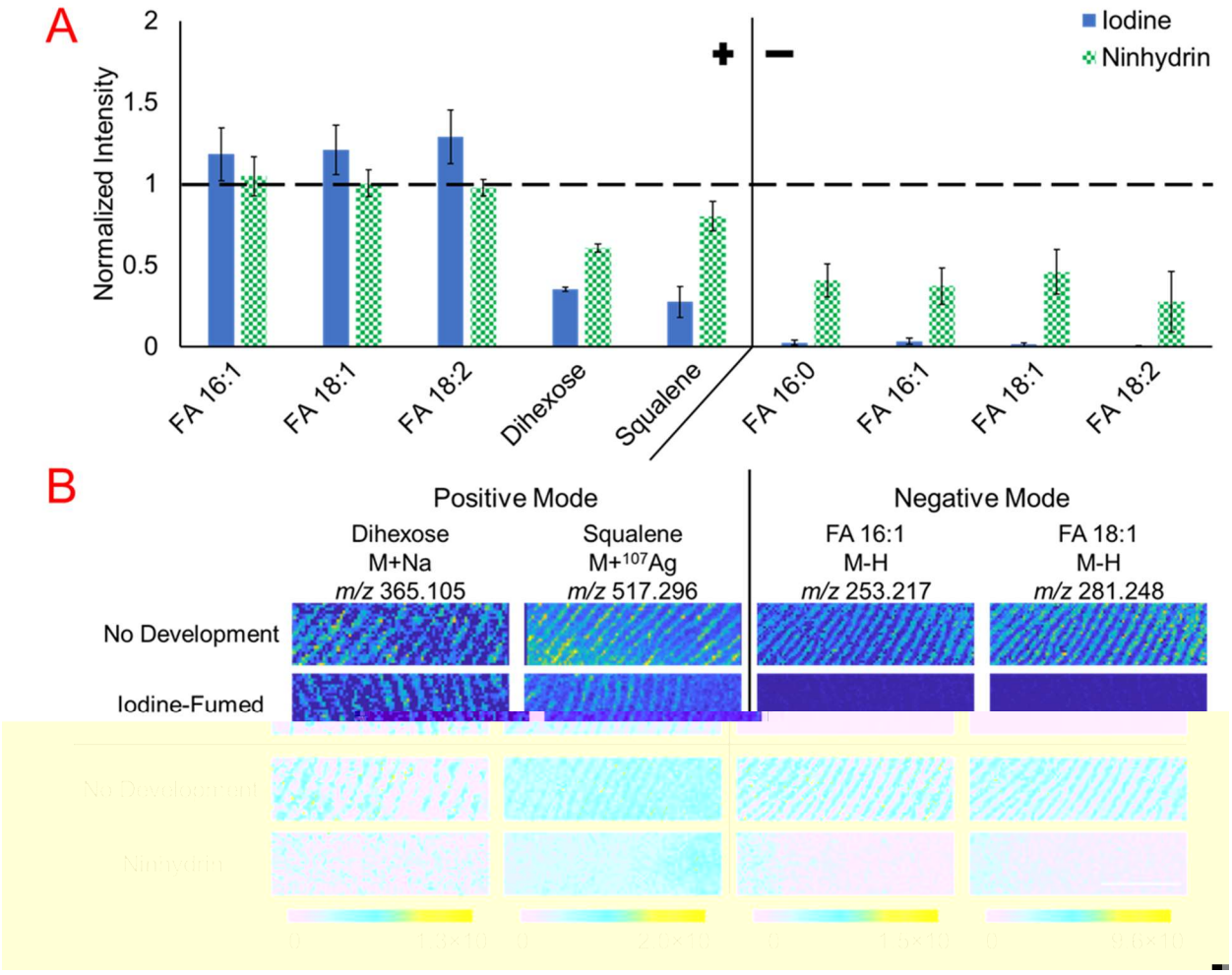

Figure 3. Comparison of selected endogenous compounds after ninhydrin and iodine fuming development: **(A)** Ion signal intensities normalized to those of no development, and **(B)** MALDI-MS images. Scale bar: 5 mm

A comparison of mock exogenous compounds that were applied to the finger is shown in **Figure 4A**. In positive mode, a decrease in intensity was observed for some compounds in

ninhydrin-developed fingerprints, while oxybenzone, lidocaine and picaridin did not. Pseudoephedrine is a secondary amine which will react with ninhydrin.[33] Both picaridin and lidocaine have tertiary amine groups which will not react with ninhydrin.[33] Avobenzone is not an amine, but possesses a beta-dicarbonyl known to react with ninhydrin.[34] We believe that this is the difference in reactivity and in turn signal intensity. By comparison, the iodine-fumed fingerprint did not exhibit a decrease in signal intensity for any exogenous compound analyzed in positive mode. In negative mode, iodine-fumed fingerprint samples displayed lower intensity values for each compound in comparison to the non-developed fingerprint. There is a slight signal decrease for avobenzone, but the rest of the compounds produced signals comparable to the non-developed in ninhydrin-developed fingerprints. Based on overall normalized intensity, iodine-fuming is more compatible than ninhydrin-developed samples when analyzed in positive mode, however ninhydrin is more compatible in negative mode.

MS images of the exogenous compounds in **Figure 4B** provide similar conclusions as in the endogenous compound images (**Figure 3B**) and the ion signals of exogenous compounds in **Figure 4A**. Iodine-fuming maintains high quality images in positive mode but suffers from lack of signal in negative mode. Ninhydrin has high signals for some ions in positive mode and almost all in negative mode, but show little ridge detail, except with picaridin. Diffusion of fingerprint compounds is not surprising as the paper gets wet during ninhydrin development. This is in contrast to the fine ridge detail of visual image, coming from ninhydrin-reacted amino acids. It might be due to the high affinity of amino acids for cellulose[26,35] which may allow them to retain fingerprint ridges after development. There are many different reasons why having clean fingerprint images from the MS imaging is important. One reason is that MS images can determine whether a substance originated from the surface or the finger. As shown in previous

research,[6,16,21] the MS images can also be useful for distinguishing two overlapping fingerprints. High quality MS images of iodine-fumed fingerprints suggest iodine-fuming is more compatible with MS imaging than ninhydrin development.

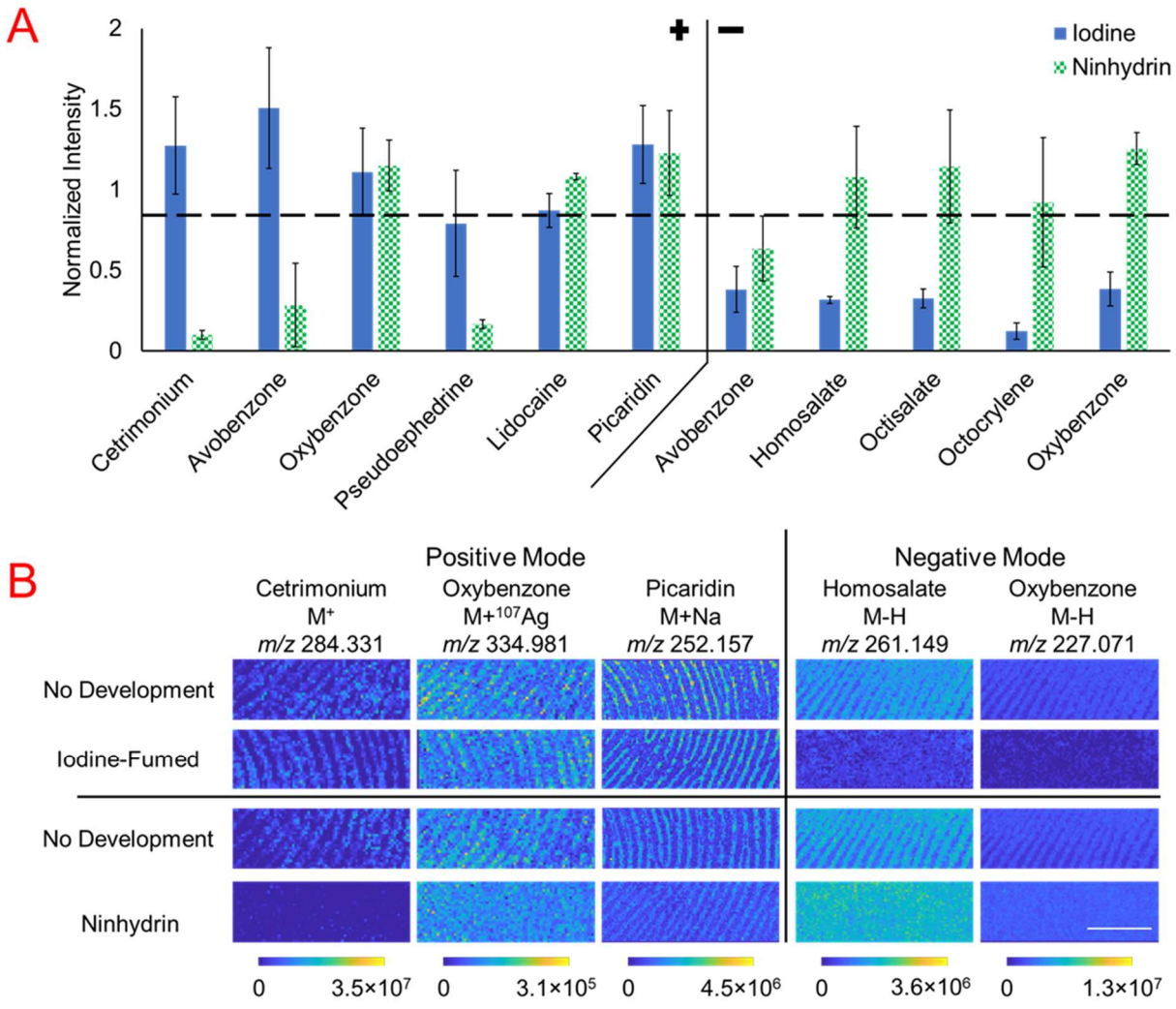

Figure 4. Comparison of selected exogenous compounds after ninhydrin and iodine fuming development: **(A)** Ion signal intensities normalized to those of no development, and **(B)** MALDI-MS images. Scale bar: 5 mm

Multi-Surface Analysis

In a real-world setting, there are many different types of porous surfaces present at a crime scene that need to be processed. A brief comparison of porous surface types was

conducted in this study to determine whether there is any difference in image quality or signal intensity related to the porous surface itself rather than the development. Printer paper, which had been used for the previous experiments, was used as a control. The other two types of porous surfaces tested were notebook paper and receipt paper as they are common in everyday use. Notebook paper is thinner than printer paper and receipt paper has a glossy coating. Heat map images of picaridin (exogenous compound) set to the same maximum signal intensity can be seen for each porous surface type in **Figure 5**. Different surfaces displayed slight changes in overall intensity and image quality. Overall, similar results were obtained with the previous analysis; iodine-fumed fingerprints provide comparable signals with non-developed while maintaining ridge detail, but not as distinctly in the ninhydrin-developed fingerprints. By showing that this technique can be utilized with different porous surface types, further integration into the forensic workflow is possible.

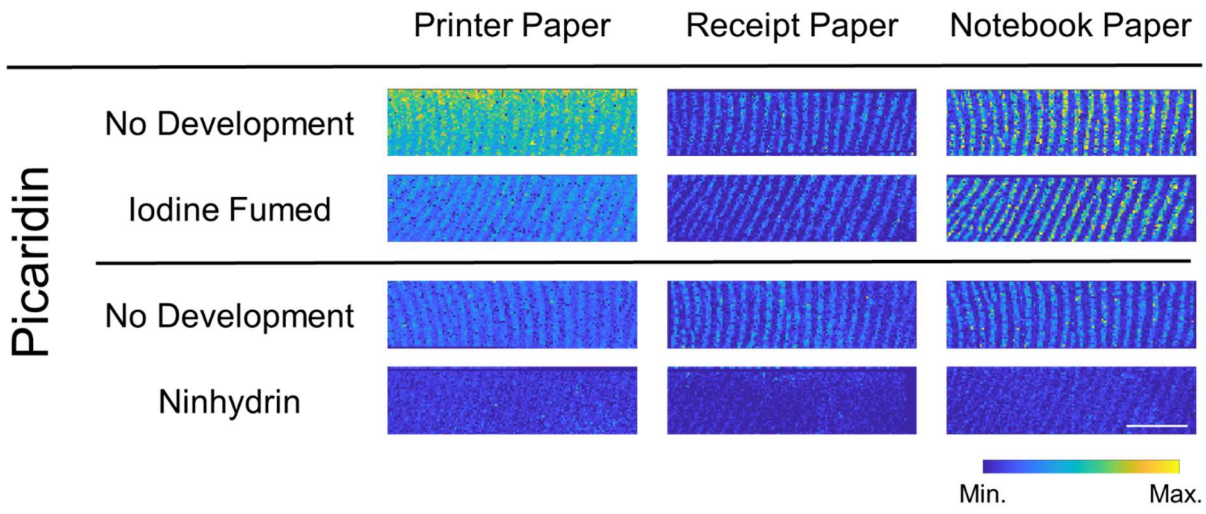

Figure 5. Comparison of MALDI-MS images of picaridin on three different porous surfaces after iodine- fuming and ninhydrin-development. Picaridin, [M+Na]+ at m/z 252.157, is used for MS images. Scale Bar: 5 mm

Conclusion

Overall, both the development techniques for porous surfaces are compatible with MALDI-MSI but with varying degree of success. In comparison to the non-developed fingerprint, signal intensities were similar with iodine-fumed fingerprints in positive mode and ninhydrin-developed fingerprints in negative mode. However, ridge details are well maintained in iodine-fuming in positive mode, but rarely in ninhydrin.

The compatibility of MALDI-MSI with both development techniques allows for the integration of MALDI-MSI into the forensic laboratory for chemical profiling of fingerprints. Although ninhydrin-development is more widely used by forensic personnel, MALDI-MSI is overall more attuned with non-destructive iodine-fuming. In future studies, it may prove valuable to consider the compatibility of MALDI-MSI with techniques that improve the permanence of iodine-fumed fingerprints.

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CHAPTER 3. GENERAL CONCLUSIONS

Summary

Evidence permissible in court must be backed by analytical techniques that are heavily validated and approved by the forensics community. This thesis focused on the incorporation of MALDI-MSI into the forensics workflow. This technique can provide more in-depth chemical information from a fingerprint left at a crime scene by examining both endogenous and exogenous compounds. To determine the compatibility of MALDI-MSI in the workflow, it is necessary to understand the influences current fingerprint development techniques on the detection of the analytes.

Chapter two dives into the compatibility of MALDI-MSI when a fingerprint on a porous surface has been developed by ninhydrin or iodine-fuming. From this work, it was determined that when a fingerprint is developed by iodine-fuming, there is little signal suppression for analytes detected in positive mode MALDI-MSI. However, the same cannot be said about negative mode where a significant decrease was seen in comparison to the non-developed fingerprint. Conversely, ninhydrin-developed fingerprints showed signal suppression in positive mode for some compounds but minimal suppression in negative mode. By determining the difference in detectability of these compounds in comparison to the non-developed fingerprint, it allows for further compatibility determination.

The critical aspect of wanting to perform MALDI-MSI on these samples is the addition of the chemical image of a given compound. As stated previously, these images can be important in determining where the compound is located (on the fingerprint ridge or surrounding the fingerprint). From this chemical image, you can also potentially piece together a fingerprint image or differentiate overlapping fingerprints.[7,35] With the data shown in chapter 2, iodine-

fumed-fingerprints would be able to take advantage of these benefits as they showed ridge detail comparable to the non-developed fingerprint. Ninhydrin-developed fingerprints, on the other hand, would not be able to as they appear to exhibit a delocalization of the compound.

Future Work

There has been a large amount of work that has gone into the determination on whether MALDI-MSI is a reliable technique for the analysis of fingerprints. Although work done by those in the Francese group, our own, and many others has shed light, there is still more validation necessary before MALDI-MSI is accepted into the forensic workflow. While work presented in chapter two is a conduit to the integration of MALDI-MSI into the forensics workflow, there is still much to be done. As MALDI-MSI was found to be more compatible with iodine-fuming, it would be beneficial to investigate ways to improve the permanence of the development technique. Different methods have been presented in which the iodine-fumed fingerprint is either dipped or sprayed with a solution to promote long term retention of the fingerprint development.[27,28] The permanency of the ninhydrin reaction is one of its main benefits as the fingerprint can then be kept for long periods of time as evident. By applying these fixation methods to the iodine-fumed fingerprint this may be achieved. However, the impact of the fixation technique on the chemical localization and subsequent detection with MALDI-MSI will need to be explored.

As ninhydrin is one of the most common development techniques used by investigators, it could be beneficial to improve the detectability of chemical compounds and MS image quality. There are many methods in which to prepare the ninhydrin solution (i.e., petroleum ether, acetone).[25] Petroleum ether based solution was used for the work presented in chapter two as it is standard in the USA. A comparison of not only the two methods, presented in the FBI processing handbook, should be investigated but also different variations that can be found in other parts of

the world. Also, it would be beneficial to evaluate the different application methods (i.e., spray, dip, or paint).

Work presented in chapter two dealt with groomed fingerprints that were intentionally prepared. For MALDI-MSI to be employed in the forensic workflow, this same analysis should be performed using ungroomed fingerprints. It should also be evaluated using fingerprints that are not fresh. By performing the same analysis in the ways mentioned above, it would create a more realistic setting. Eventually, work will need to be performed on real samples to determine the compatibility of MALDI-MSI in the forensic workflow.

MALDI-MSI has the potential to be a great addition to the forensic workflow as it provides chemical information about a fingerprint. The work done in this thesis demonstrates the compatibility of MALDI-MSI with the already existing porous surface development techniques. Further research will still need to be performed to further determine the existence of it in the forensic workflow.

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APPENDIX. INSTITUTIONAL REVIEW BOARD APPROVAL

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Institutional Review Board
Office for Responsible Research
Vice President for Research
2420 Lincoln Way, Suite 202
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515 294-4566

Date: 2/22/2017

To: Dr. Youngjin Lee
0035 Carver Co Laboratory

From: Office for Responsible Research

Title: Chemical Imaging of Latent Fingerprint for Forensic Evidence

IRB ID: 16-600

Approval Date:	2/22/2017	**Date for Continuing Review:**	2/9/2018
Submission Type:	Modification	**Review Type:**	Expedited

The project referenced above has received approval from the Institutional Review Board (IRB) at Iowa State University according to the dates shown above. Please refer to the IRB ID number shown above in all correspondence regarding this study.

To ensure compliance with federal regulations (45 CFR 46 & 21 CFR 56), please be sure to:

- **Use only the approved study materials** in your research, **including the recruitment materials and informed consent documents that have the IRB approval stamp.**
- **Retain signed informed consent documents for 3 years after the close of the study,** when documented consent is required.
- **Obtain IRB approval prior to implementing any changes** to the study by submitting a Modification Form for Non-Exempt Research or Amendment for Personnel Changes form, as necessary.
- **Immediately inform the IRB of** (1) **all serious and/or unexpected adverse experiences** involving risks to subjects or others; and (2) **any other unanticipated problems involving risks** to subjects or others.
- **Stop all research activity if IRB approval lapses,** unless continuation is necessary to prevent harm to research participants. Research activity can resume once IRB approval is reestablished.
- **Complete a new continuing review form** at least three to four weeks prior to the **date for continuing review** as noted above to provide sufficient time for the IRB to review and approve continuation of the study. We will send a courtesy reminder as this date approaches.

Please be aware that IRB approval means that you have met the requirements of federal regulations and ISU policies governing human subjects research. **Approval from other entities may also be needed.** For example, access to data from private records (e.g. student, medical, or employment records, etc.) that are protected by FERPA, HIPAA, or other confidentiality policies requires permission from the holders of those records. Similarly, for research conducted in institutions other than ISU (e.g., schools, other colleges or universities, medical facilities, companies, etc.), investigators must obtain permission from the institution(s) as required by their policies. **IRB approval in no way implies or guarantees that permission from these other entities will be granted.**

Upon completion of the project, please submit a Project Closure Form to the Office for Responsible Research, 202 Kingland, to officially close the project.

Please don't hesitate to contact us if you have questions or concerns at 515-294-4566 or IRB@iastate.edu.