STRONG-FIELD IONIZATION APPLIED TO SPUTTERED NEUTRAL MOLECULES FROM VARIED CHEMICAL ENVIRONMENTS

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by
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ABSTRACT

The advent of cluster ion sources and improved function of time-of-flight mass spectrometers has helped usher in the use of secondary ion mass spectrometry (SIMS) for exceedingly complex biological samples, however further advancements are needed to enable viable quantitative analysis of in situ biomolecules. Specifically, ionization matrix effects due to the local chemical environment makes quantitation with SIMS very difficult. Similarly, even qualitative measurements may be hindered due to ionization enhancement or suppression as an effect of the local chemical environment. The use of laser post-ionization (LPI) for the ionization of sputtered neutrals enables SIMS ionization mechanisms to be decoupled from the sputtering event.

The LPI scheme used in this research utilizes an ultra-short pulse of high power laser radiation in the infra-red region to photoionize sputtered neutral molecules which have been released from the sample’s surface following primary ion bombardment. In this process, the SIMS sputtering event is decoupled from the SIMS ionization event. For samples where matrix effects are present, observing neutral species via photoionization may provide more quantifiable information. The ionization process during LPI analysis creates ions which are not determined by the local chemical environment.

Employing LPI concurrently with SIMS experimentation affords researchers the ability to negate matrix effects and acquire data which more accurately depicts the chemical environment, particularly for samples whose SIMS ionization mechanisms are affected by matrix effects. Research is presented which utilizes various approaches to 1) understand the effect of local chemical environment for the formation of SIMS molecular ions, 2) how LPI can be used in conjunction with SIMS to increase sensitivity and overcome matrix effects associated with SIMS.
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Chapter 1 Introduction

1.1 Research Introduction

Mass spectrometry (MS) is an invaluable analytical tool that is utilized across nearly every branch of the sciences. The underlying principle behind all MS techniques is the ionization of some component of a sample and discrimination of those ions based on their mass to charge ratios (m/z). While a wide variety of MS techniques exist, secondary ion mass spectrometry (SIMS) provides unique and distinct advantages over others. SIMS has the benefit of extreme analytical sensitivity (reported to attomolar concentrations\(^1\)) and the ability to create 2D and 3D images with primary ion beams achieving sub 5 nm sampling depth\(^2\) and sub 100 nm lateral resolution\(^3-4\). In addition to SIMS, complementary laser post-ionization (LPI) analysis enables researchers to sample the enormous amount of sputtered neutral material that remains un-ionized by the SIMS process. LPI is able to monitor this additional chemical information via photoionization of sputtered neutral molecules with a high powered laser\(^5-7\). LPI enhances sensitivity due to the additional molecular ion signal it obtains. Also, LPI photoions are not affected by the local chemical environment to the extent at which SIMS ions are and yields data that is free of matrix effects\(^5,8\).

The versatility of SIMS and LPI is becoming increasingly apparent to a growing body of researchers across many fields. Recent exciting SIMS studies are branching out from the common areas and into others including studies of Renaissance art\(^9-10\) and the color of dinosaur skin\(^11\). Research in the field of LPI is equally exciting with researchers applying methodologies to analyze increasingly complex systems including tissues\(^12-13\) and cosmic materials\(^14-15\).
Additionally, successive advancements in primary ion technology\textsuperscript{16-17}, instrumental capabilities\textsuperscript{18} and ionizing lasers\textsuperscript{5} are making these advancements possible.

To aid in this continuously advancing technique, the research from this thesis body is presented with two main goals in mind. The primary goal is to show that LPI analysis provides a means to mitigate the matrix effects so often observed with SIMS analyses of complex molecular samples and to also increase the technique’s sensitivity for organic molecules when compared to SIMS. Additionally, during the course of these studies it will be shown that recent advancements in SIMS primary ion sources, namely the C\textsubscript{60}\textsuperscript{+} primary ion, lends itself towards incredible versatility in analysis of a wide variety of systems.

\subsection*{1.2 Introduction to SIMS and LPI}

The SIMS process utilizes a high energy primary ion beam which is accelerated toward a sample surface with the purpose of desorbing and ionizing surface-bound materials. The high energy collision between the primary ion and sample surface liberates material in the form of molecules, electrons, atomic species and fragments. Due to charge migration and charge imbalances a portion of the sputtered materials have the possibility of becoming either positively or negatively charged. These ions can be separated by their m/z ratio and their relative intensities recorded to yield a mass spectrum.

SIMS has been shown to be very effective at a vast array of analyses, however the analyses performed are not without issues. Mainly, a low abundance of secondary ions are generated (typical efficiencies for organic molecules range from $10^{-4}$ to $10^{-6}$) and influence of ionization behavior due to the local chemical environment can affect ion signal\textsuperscript{19}. These ionization effects become extremely apparent when analyzing complex biological samples where
an added decrease in sensitivity due to a matrix effect of an already low concentration will likely result in analysis beyond the limit of detection.

Historically, methods to enhance sensitivity have been investigated and include sample modification through the addition of thin-films of metals\textsuperscript{20}, adsorbed chemicals\textsuperscript{21} and ionic liquids added to the surface\textsuperscript{22} among other methodologies. These results have proven fruitful in enhancing specific ion signals, however these modifications will almost assuredly modify the sample surface. For studies of biological systems and other systems where the native state is to be analyzed, modifications must be kept to a minimum. Additionally, these surface modifications are often targeted for specific molecules or classes of molecules, making their application case-specific. A method to universally increase sensitivity and mitigate SIMS matrix effects is sought and a promising candidate, which requires no sample modification, is LPI.

The technique of analyzing sputtered neutrals in a mass spectrometer by subsequent ionization is entitled secondary neutral mass spectrometry (SNMS). Utilization of a laser to photoionize the neutral species has been given the name LPI, to indicate that sputtered neutral species are photoionized following primary ion bombardment. This technique has indeed existed for some time and currently exists with a variety of iterations which vary primarily based on the incident photon’s energy. Regardless of the differing approaches, the underlying methodology remains the same: to photoionize sputtered neutral species with a laser.

The fundamental principle of adding photoionization to a SIMS instrument is relatively straight-forward (though the actual application of it is anything but). A laser beam is set to intersect the region directly above the sample surface where, after primary ion bombardment, liberated neutral species will encounter the intersecting laser field.

With LPI it is known that the ionization process and the sputtering process are de-coupled from each other\textsuperscript{5,23-24}. Thus, information from the neutral species which become photoionized in the laser field give more accurate depictions of sample concentration\textsuperscript{8}, particularly when matrix
effects dominate the sample. Additionally, recent research has shown marked increases in LPI molecular ion signal when compared to the analogous SIMS signal\textsuperscript{5}. Together, the beneficial increase in sensitivity and negation of matrix effects afforded by LPI can be universally applied. Advancements such as these shall aid in the identification and quantitation of low-concentration analytes in complicated samples.

The field of LPI research is still making strides which allows its benefits to be applied universally. Recent research and research presented in this thesis shows that long wavelength (near-infrared) ultra-short pulse width lasers can ionize a variety of organic molecules and increase sensitivity over SIMS. This universality of LPI sensitivity enhancement provides an exciting time for SIMS and LPI research.

1.3 ToF-SIMS Instrument Introduction and Details

The ToF-SIMS instrument has been designed for exceptional surface sensitivity and high mass resolution. The specific instrument utilized in this body is described elsewhere\textsuperscript{25}, however, a generalized description of ToF-SIMS is provided. For a better understanding of the instrument it can be broken down into three parts: 1) the primary ion source 2) the mass analyzer and 3) the detector. Other ancillary equipment, such as vacuum pumps, gas analyzers and other peripherals are indispensable, but at the core of the mass spectrometer are these three main components.

An ion gun provides primary ions for sample interrogation. A variety of sources and generation techniques are available. Liquid metal ion guns (LMIGs) generate highly focused beams of atomic ions or small clusters \((n = 2,3)\) of ions\textsuperscript{3}. Sublimation based ion guns such as \(\text{C}_{60}^+\) sources generate gaseous molecules which are then ionized with electron impact\textsuperscript{26}. The most recent advancements in primary ion technology include massive cluster beams\textsuperscript{16-17}. This new generations of gas-cluster ion beams (GCIBs) are created through supersonic expansion of gases
through a nozzle facilitated by differential pumping. Alternative ion sources, such as those with MeV particles have been utilized, though most commercially available instruments incorporate the more traditional ion sources mentioned above. Regardless of identity, the ions are accelerated through the vacuum system at energies typically on the order of 10’s of keV towards the sample surface. After impacting the sample surface they liberate atoms and clusters of atoms when inorganic samples are analyzed, and intact and fragmented molecular species for molecular samples. The ejected species are neutrals, electrons and, albeit at lower abundance compared to the neutrals, positive and negative ions.

An axial ToF-SIMS instrument must operate in pulsed mode due to the nature of its ion extraction, therefore the operation of the ToF-SIMS instrument must occur at some frequency. The instruments utilized in these experiments can operate at rates up to 3 kHz, equating to 3,000 analysis cycles per second during normal SIMS operation. In order to remain sensitive to the surface alone and to avoid multiple samplings of the same region, less than 1% of the sample surface is to be analyzed. This limit, the so called static limit, is achieved by maintaining the dosage under the threshold of $1 \times 10^{12}$ primary ions/cm$^2$. For reference, an instrument operating at 3 kHz, with a 200 pA beam rastered over an area of $100 \times 100 \mu$m$^2$ the static limit is reached in less than a second of analysis.

Mass spectrometers with electrostatic and magnetic analyzers rely on the interaction of their fields with the ion to deflect its trajectory and effectively separating ions with differing m/z ratios. These analyzers have been proven very effective, particularly for instruments which analyze with a continuous primary ion beam. However, parallel detection of ions with varying m/z with electrostatic or magnetic analyzers requires multiple detectors which yields a limited detection range due to practical size constraints. Non-parallel detection is possible by scanning the strength of the electric or magnetic field, but comes at the cost of reduced sensitivity due to wasted signal during the duty cycle. The ToF analyzer, however, allows for parallel detection of
all ions generated in each instrument cycle. In theory, unlimited mass separation is possible with an infinitely long flight path, however practical size considerations limit flight tube length.

Beyond excellent mass resolution, ToF analysis presents other distinct advantages for SIMS. During operation ions are not always immediately extracted directly from the sample surface in SIMS and often they are allowed to drift in a field-free region during delayed extraction methodologies. Once the extraction field is applied, the ions are present with varied height from the origin of the extraction field and are acted upon by varied potential. Additionally, molecular ions are typically not sputtered in the direction of the flight tube. This will result in a broadening of the ion packet in space as it travels along the flight tube over sufficiently long distances. A way to counteract these effects is to utilize a reflectron-type ToF analyzer. The reflectron-type analyzer acts as an ion mirror which reflects ions back upon their initial trajectory (though a slight angle is utilized so the ions are not reflected back upon their initial starting point). A key benefit of the reflectron is the ability to refocus the ions in space, which is particularly advantageous for ions extracted from varied regions above the sample surface. For a given m/z with varying initial energies, an ion with higher kinetic energy (KE) pushes deeper into the reflectron than a similar ion with lower KE, thus sending it on a longer path-length. An ion of the same mass, yet with a smaller KE will have a shorter path through the reflectron. The geometry of the flight tube and potentials utilized for the reflectron allow the ions to be refocused in space to a terminal point at the detector. This leads to heightened mass resolution and peak widths which are less than the primary ion pulse width (when used with delayed extraction). And, as an added benefit of increasing the path-length, the reflectron is able to double the distance traveled while remaining roughly the same size as a linear flight tube of half the length.

At the terminus of the flight path is a dual channel micro-channel plate (MCP) detector. A record of flight times is recorded via the MCP detector and a computer controlled digitizer. Chevron-type MCP’s are utilized to create an electron cascade after ion impingement. In the
following research the electrical response is recorded with either of two digitizers. For SIMS experiments a time-to-digital converter (TDC) is utilized which monitors the current from the detector and records events at a given time. The TDC bins ion events in a binary fashion at ns intervals. Due to the high sampling frequency of SIMS analysis, the TDC is chosen and a mass spectrum is made from a histogram of the additive mass spectra generated from the individual binned data.

For LPI studies a transient digitizer (TD) is utilized. The lower sampling frequency (governed by the laser frequency - 1 kHz in these studies) requires either 1) more analysis cycles or 2) better ion counting. Option 2 is explored in these studies where a TD is utilized. The TD records timed events and an associated signal (rather than the binary encoding performed by the TDC). The TD can record 256 bits/bin and, if sufficient ion signature is present, an entire mass spectrum can be generated from one cycle. Though in practice multiple analysis cycles are added to provide ample signal.

1.4 The C_{60} Primary Ion Source and Further Advancements

Recent advancements in primary ion technology have yielded improvements in ionization efficiency for organic species. Early primary ion sources utilized atomic ions generated from liquid metal ion guns. While these sources are still very popular for inorganic analyses they have been shown to be less effective than their clustered counterparts for molecular ionization. Even utilizing clusters of metals such as Au\textsuperscript{n+} or Bi\textsuperscript{n+} has been shown to greatly enhance the molecular ion signature from organic molecules\textsuperscript{4}. These improvements in ionization efficiency have spurred researchers on to create varying types of cluster-based primary ion sources such as SF\textsubscript{5}\textsuperscript{+32}. These advancements are indeed very important, particularly in the analysis of molecules and
polymers, however the most remarkable advancements in the SIMS field in recent years comes from the utilization of the Buckminster Fullerene primary ion source.

The innovative use of the $C_{60}^+$ cluster ion source and advancements in $C_{60}^+$ ion gun technology helped to usher in a new era of SIMS analysis. The $C_{60}^+$ primary ion source possesses many advantages over its smaller-cluster counterparts. These benefits, for organic molecules, are primarily; increased ionization efficiency, decreased fragmentation of molecular species and exceptional damage removal$^{33-34}$. The $C_{60}^+$ primary ion source yields high molecular ion signal on samples where molecular ions were not observed during analysis with smaller or atomic sources. This increase in ionization efficiency is due mostly to the soft ionization of the $C_{60}^+$ primary ion. When considering the effect of a $C_{60}^+$ ion impinging upon a sample surface, its total KE is divided amongst its 60 individual atomic components. For comparison consider both $C_{60}^+$ and $Au_3^+$ primary ions, each accelerated at 20 keV. The total KE of the $C_{60}^+$ ion is spread amongst 60 individual atoms, or 333 eV/atom. For the $Au_3^+$ primary ion, the KE is dispersed over only three atoms or 6.66 keV/atom, a value much higher than that of the fullerene source. This reduction in energy per nucleon allows the primary ion to spread its energy out over the surface of the target material thereby creating less damage and disruption, and also sputtering away more material from the topmost layers$^{35}$.

These findings are emphasized in recent molecular dynamics (MD) simulations$^{35-37}$. In these simulations it is shown that the $C_{60}^+$ primary ion more readily disperses its energy at the surface of an analyte, in fact much more-so than atomic species. In some simulations only the top 5 nm of material is disturbed during interrogation with $C_{60}^+$ whereas atomic species tend to cause disruption and chemical damage much deeper within a sample surface$^{35}$.

As previously mentioned, the $C_{60}^+$ primary ion source also causes much less chemical damage than its smaller counterparts. This ability has enabled researchers to accurately depth profile a multitude of organic species with a versatile primary ion source for the first time. With
other primary ion sources, a decreasing steady states was are observed due to too much chemical damage being formed by the primary ion bombardment process\textsuperscript{38}. The C\textsubscript{60}\textsuperscript{+} primary ion source does not often suffer from this drawback as it is often capable of removing the same amount of damage it creates for organic species. Therefore accurate depth profile analysis of many organic systems is readily attainable with C\textsubscript{60}\textsuperscript{+} primary ions.

The C\textsubscript{60} primary ion source has greatly enhanced the field of SIMS, but primary ion technology is still being advanced to provide yet softer ionization. Most recently it appears that massive GCIB’s in the form of either Ar\textsubscript{n}\textsuperscript{+} and (H\textsubscript{2}O)\textsubscript{n}\textsuperscript{+} are gaining much attention for even further enhanced ionization while providing minimal surface disruption\textsuperscript{16}. These types of advancements will ensure that the field of SIMS continues to prosper in even more fields of research.

1.5 SIMS Ionization and Organic Molecules the [M+H]\textsuperscript{+} Molecular Ion

The C\textsubscript{60}\textsuperscript{+} primary ion source has proven to be a very viable source for interrogation of mixed organic and biological samples. It shows enhanced molecular ion formation and decreased fragmentation when compared to atomic and small cluster sources. Commonly with the ionization of organic species a protonated quasi-molecular ion (QMI) is formed. This [M+H]\textsuperscript{+} ion relies on the local chemical environment for an abstractable proton. The primary ion bombardment process is known to produce significant amounts of damage to molecular species and readily creates free protons. Additionally, studies have shown that molecules in the presence of H\textsubscript{2}O and D\textsubscript{2}O also release free protons or, analogously, deuterons which can generate [M+H]\textsuperscript{+} ions with nearby molecules\textsuperscript{39-40}. The protonation mechanism is believed to occur as either 1) pre-formed ions which are created in the damage selvedge following primary ion bombardment or 2) in the sputtered plume following primary ion bombardment.
Neat molecules (without the presence of any significant contaminants) which create 
[M+H]+ molecular ions will do so by obtaining a liberated proton from the local environment.
The ionization efficiency provided by the neat material, however, may be different when present
in a mixture. As stated previously, it has been shown that adsorbed water is known to be a source
of protons for molecular ions. In fact, the presence of water has been shown to increase
molecular ion signal\textsuperscript{1} for some molecules. Indeed, the local chemical environment plays a very
important role in the ionization of, particularly, [M+H]+ ions.

A defining factor in the ionization behavior is governed by the relative gas phase basicity
(GPB) of the involved molecules\textsuperscript{19,41}. Generally speaking, molecules with lower GPB will see
diminished ionization, whereas molecules with higher GPB will show increases in ionization.
Investigations into these implications and ways to circumvent the deleterious effects, such as with
LPI analyses, are crucial.

\section*{1.6 Strong Field Ionization}

A variety of wavelengths have been utilized for ionization from vacuum ultraviolet
(VUV) radiation\textsuperscript{42}, ultraviolet (UV)\textsuperscript{30,43}, visible infrared (IR) region\textsuperscript{44-45} as well. Each respective
wavelength range proceeds with a certain ionization mechanism which is governed by the energy
of the incident photon. Wavelength withstanding, the benefit of LPI analysis remains the same:
to create abundant ions from residual neutral species.

High energy, short wavelength (VUV/UV) photons fall under the single photon
ionization (SPI) regime. Under this mechanism, the energy of the incident photon meets or
exceeds the energy required to remove an electron from an atom or molecule. Exceeding the
ionization energy for atomic species is relatively inconsequential due to the inability of the atomic
species to dissociate, however molecular species tend to adjust to the excess energy imparted onto
them by other means such as fragmentation. Commercially available UV lasers such as F₂ excimer lasers have only a limited photon energy. For example, the F₂ excimer produces 157 nm photons with energies of 7.9 eV. Higher energies necessitate even shorter wavelengths that are typically achieved from synchrotron sources, limiting the practicality of such experiments. Due to this efforts to minimize molecular fragmentation while still creating appreciable ionization of molecular species is still sought.

In contrast to SPI with an excess of energy, resonant single photon ionization occurs when the incident photon energy is equivalent to the ionization potential of an atom or molecule. In these cases extremely high efficiencies have been observed, however this powerful technique may only be utilized for cases where information about specific analyte, with known transitions and accordingly coincidently resonant radiation, are possible. As exceptional as this technique may be it is limited in its application of universality.
Figure 1.1: Photoionization schemes. Single photon ionization proceeds when the energy of the incident photon is greater than or equal to the ionization potential. Multiphoton events occur when the energy of the photon is less than the ionization potential. Multiphoton events that do not proceed through transition states are non-resonantly enhanced. Procession through excited states where the photon energy equals an excited state transition is resonance enhanced multiphoton ionization. Longer wavelengths require increasingly higher orders of photons for ionization.

Longer wavelength light is used to alleviate some of the fragmentation issues associated with SPI for systems with too much energy per incident photon. In these latter cases multiple photons are used for ionization in a process termed multiphoton ionization (MPI). These ionization processes may be resonant or non-resonant. Resonance enhanced multiphoton ionization (REMPI) proceeds through one or more known excited state transitions by utilizing a photon whose energy is resonant with the transition\textsuperscript{46-47}. More photons are then used to excite an electron beyond the ionization threshold. REMPI has been shown to drastically enhance sensitivity, but again suffers from a lack of universality in its application. A more broad application of SNMS can proceed through non-resonant multiphoton ionization (NRMPI). With NRMPI, a very high flux of incident photons, at energies less than the ionization threshold value,
coincide with species to be ionized and often proceed through non-resonant virtual excited states as a result of multiple photon events\textsuperscript{24,48}. High photon densities are necessary in the NRMP1 regime to increase the probability of multiple photon incurring a target atom or molecule.

Recent work with high intensity IR wavelengths have shown an increase in ionization of the molecular ion and decreased fragmentation\textsuperscript{44}. The intensities utilized in these studies are in excess of $10^{13}$ W/cm\textsuperscript{2} and a different ionization mechanism, other than MPI, is believed to be dominant at longer wavelengths and such intensities. The ionization in these fields are facilitated by strong field ionization (SFI).

During adiabatic ionization at such high laser intensities the electric field amplitude nears or exceeds the values of the Coulombic field which retains an electron in an atom or molecule\textsuperscript{24}. Interactions between the electric field of the laser and the atom or molecule become very prominent at SFI intensities. At these intensities ionization proceeds through differing mechanisms when compared to photon absorptive processes. Two such interactions are shown in Figure 1.2. In this figure, the potential well with a captive electron is distorted at half-frequency intervals of the laser period. The potential well is reduced due to the electric field interaction which gives the electron increasingly higher probabilities of tunneling out and, in some cases, the barrier is reduced so much that an electron can freely migrate out\textsuperscript{49}. 


Figure 1.2: Behavior of an electron in a very strong laser field. Interactions between the electric field of the laser and the electron’s Coulomb barrier enable the electron to tunnel out or even freely exit during complete barrier suppression.

The relative probability of this type of adiabatic ionization can be described for a one-body system by the Keldysh equation\textsuperscript{50}. For elemental species this relationship describes which dominant adiabatic ionization scheme occurs at high laser intensities. As it appears below, the equation takes into account the ionization potential ($IP$ in terms of eV) of the atom in question, the intensity of the laser ($I$ in terms of W/cm$^2$) and the wavelength of the incident photon ($\lambda$ in $\mu$m). The unitless term $\gamma$ is known as the Keldysh adiabaticity parameter. When $\gamma << 1$ SFI is the dominant mechanism. For values of $\gamma > 5$, MPI is the dominant mechanism. For values $1 < \gamma < 5$ both mechanisms are present, yet remain indistinguishable from one another.

$$
\gamma = \sqrt{\frac{IP}{1.87 \times 10^{-13} I \lambda^2}}
$$

(1.1)

In consideration of the adiabaticity parameter for a given specimen with a fixed $IP$, two values are able to be modified in order to bias the ionization towards SFI. An increase in either laser field intensity or wavelength (or a combination of both) will yield decreased values of $\gamma$ and
will bias the dominant ionization mechanism towards SFI. The Keldysh equation was formulated to describe atomic species where fragmentation will not occur, though the trend has been shown empirically to be applicable to molecular species as well. Though molecular species present a much more complicated system, descriptions of their ionization behavior have been visited, particularly for non-adiabatic multi-electron dynamics (NME)\textsuperscript{51-52}.

Consider the interactions of a strong laser field on the closely-spaced molecular orbitals (MO) of a molecule. These MO’s will Stark shift in the presence of a strong electric field and their energy levels will become convoluted with each other. This overlap of MO’s produces a near continuum of excited states. Entry into this continuum occurs through a low-lying doorway state. After entry into the continuum further excitation is rapidly facilitated and the electron can be ejected from the molecule with ease. The probability of entry into the doorway state is provided by Equation 1.2 below.

\[
P_{DS} = \exp \left( -\frac{\pi \Delta_0^2}{4 \hbar \omega \varepsilon_0} \sqrt{\mu^2 + \frac{\alpha g \Delta_0}{2}} \right)
\]

The above equation describes the probability of an electron being excited into a doorway state \((P_{DS})\). The term \(\Delta_0\) is the energy difference between the ground state and the doorway state without the presence of the laser’s electric field. The \(\omega\) and \(\varepsilon_0\) terms are the angular frequency of the laser and the field amplitude of the laser respectively. The dipole moment of the transition is represented by \(\mu\) and the dynamic polarizability of the ground state as \(\alpha_g\).

These two descriptions of ion behavior at high intensities both show the trend that longer wavelengths will bias the ionization mechanism away from absorptive events to strong-field effects. It is with this information and with a growing body of empirically derived support that SFI mechanisms are applied to LPI SNMS.
1.7 Conclusions

This preceding sections have shown that advancements in SIMS technology has allowed researchers to investigate new and complex systems, with particular interest on biological systems. The extreme sensitivity and 2D and 3D SIMS capabilities has enabled research in fields that were not accessible before. Now, with the advent of commercially available high-powered femtosecond lasers with tunable-wavelength output researchers are able to push the boundaries of LPI even further. Coupling SFI regimes to LPI analysis provides a new prospect of broad ionization capabilities for a wide range of organic molecules that may not be observed with other photoionization mechanisms. SFI LPI analysis can afford researchers better sensitivity over SIMS and also enable complementary analyses which are matrix effect free.

1.8 References

1. Passarelli, M. K.; Winograd, N., Lipid imaging with time-of-flight secondary ion mass spectrometry (ToF-SIMS). *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 2011, 1811 (11), 976-990.


Chapter 2
Experimental Detail

2.1 Abstract

The information provided in this chapter describes important experimental details for secondary ion mass spectrometry (SIMS) and laser post ionization (LPI) analyses. Sublimation and solution-based sample preparation methodologies are described in detail. Experimental analysis conditions and the rationale for such conditions utilized in SIMS surface and depth profile analyses are given. Additionally, a discussion which describes optimized laser conditions for LPI experiments are detailed. The reasoning behind defocusing the laser beam for optimal ionization of molecular ions is also described.

2.2 Sample Preparation

2.2.1 Physical Vapor Desorption

A unique benefit of the specific PVD apparatus used to create glassy and contaminant free films is that it resides on the ToF-SIMS instrument. This provides a means to create samples under ultra-high vacuum (UHV) conditions without having to expose them to atmosphere prior to analysis. This method of preparation ensures contaminant free sample preparation. Additionally, the PVD chamber has provisions to deposit two unique materials independently, enabling creation of multi-layer bi-component films.
The deposition chamber was designed and built from existing apparatuses by David Willingham, a former graduate student in the Winograd research lab. The capabilities of the chamber have been previously described\(^1\) but are briefly explored here. PVD sample preparation yields incredibly neat films \textit{via} sublimation of a material under UHV conditions onto a target substrate. Interestingly, sublimation can be used as a method of purification since different materials can be separated by their respective sublimation temperatures\(^2\). Utilizing sublimation to prepare films ensures contaminant-free homogenous samples. In the employment of PVD sample preparation a commercially available organic compound (typically > 99% purity) is placed into a non-reactive alumina crucible. The crucible is then placed into a coil of tungsten wire which acts as both a crucible holder and heating filament. The filament is connected to an external power supply (a re-purposed power supply from a Ti-sublimation pump) \textit{via} electrically isolated ceramic and copper UHV feedthroughs. The filament is resistively heated with 8-13 A which provides enough heat to sublime the material in the crucible. A metal grid acts to diffuse the sublimate material and enhances the film quality.

Above the sublimate, the sample substrate is suspended facing downwards on a translation arm. This orientation places the target substrate directly in the path of the sublimate. Additionally, a quartz crystal microbalance (QCM) is also present above the crucible for thickness measurements. Utilizing the QCM, thicknesses of the material can be monitored in real-time. It has been found that deposition rates of 2-6 Å/s provide smooth films in a timely manner. Since no direct measurement of temperature is available in the PVD chamber, the deposition rate is used as a proxy for sublimation rate.

The PVD chamber may also be used to deposit volatile liquids with a slight modification. The modification involves removing one of the electrical feedthroughs and associated filament/sample holder and attaching a leak valve in its stead. This leak valve can be used to deposit controlled amounts of a volatile liquid onto a cooled sample in a similar manner as PVD.
adsorption. Measurements show that the sample should either be pre-cooled in the sample stage
to 93 K or the sample block should be placed in direct contact with the pre-cooled QCM to ensure
low enough temperatures are reached.

2.2.2 Spin-Coating and Drop Drying

Spin-coating and drop drying have been employed in a multitude of studies of organic
materials. Neat films and mixed films can be created by these methods with precise control of
stoichiometric ratios due to their solubilized nature\textsuperscript{3-4}. Preparation of a solution enables the
analytes in the mixture to be homogenously distributed as they should remain so upon drying.
Typically, small volumes of solutions between 5-10 µL are utilized for preparation on small Si
substrates (5 x 5 mm\textsuperscript{2}). For larger substrates, such as the 4 inch Si wafer, a much larger (~10 mL)
aliquot is necessary. Multiple additive layers can be sequentially deposited to form thick films,
however in the research presented here 100-200 nm thick films are created with one application
of samples from mM solutions. Spin-coating is a complicated interaction between adhesion,
rotational speed, solvent volatility and viscosity, and multiple attempts to adjust spin speed and
concentration were typically made before ideal conditions were met.

2.3 ToF SIMS and Depth Profile Analyses

Singly charged C\textsubscript{60}\textsuperscript{+} primary ions are utilized for all studies presented in this thesis. The
accelerating potential of the C\textsubscript{60}\textsuperscript{+} ions varies depending on instrumental setup and is either 20 keV
or 40 keV. Typical ion currents range from 20-400 pA. Analysis, however, takes place with 50-300 ns pulsed packets of C\textsubscript{60}\textsuperscript{+}. Short pulse widths such as these provide appreciable secondary
ion signal and good mass resolution while still maintaining analysis under the static limit of 10\textsuperscript{12}.
primary ions/cm². Analysis below this threshold ensures that less than 1% of the sample surface is investigated which provides data from pristine surface regions which have not had previous bombardment. SIMS analyses were typically collected with delayed extraction from 10-50 ns after the finish of the C₆₀⁺ pulse train. This provides an ideal balance between mass resolution and loss of low-mass species.

Typical voltage settings for the SIMS instrument are (+) 2500 V bias applied to the stage as an extracting voltage. A (-) 4300 V bias to the extraction lens 1 and extraction lens 2 remains grounded. Reflectron values varied from (+) 1100-1200 V retarding voltage and (+) 2450-2550 V reflecting voltage. These values are modified as necessary to ensure ideal peak profiles.

2.4 LPI Conditions

2.4.1 Mass Spectrometer Conditions for LPI Analysis

The laser system utilized for this experimentation is a commercially available Coherent (Santa Clara, CA) brand Mantis Oscillator with a Legend Elite Duo amplification system. The output of the Legend Elite Duo is used to pump a TOPAS-HE (Light Conversion, Lithuania) optical parametric amplifier (OPA) which is used to create tunable wavelength radiation.

The Mantis oscillator produces an 800 nm, 40 fs seed beam. The Ti:Sapphire gain media itself is pumped by the 532 nm output (doubled from 1064 nm with a lithium triborate (LBO) crystal) of a diode pumped quantum well arrangement. The round trip time through the cavity is 12.5 ns at a frequency of 80 Mhz. The cavity is designed to sustain modelocking passively. A mirror mounted on a piezo-electric device in the cavity is temporarily disturbed at some controlled frequency which enables modelocking⁵. This disturbance induces constructive and destructive interference of the phases within the cavity at extremely short durations. The
modelocking is maintained passively by the Kerr Lensing Effect in the gain media. Within the gain media the high intensity modelocked beam has higher gain at the center of the media where the smaller modelocked beam is amplified, rather than at the larger volume where the continuous wave mode is amplified. Therefore the high intensity modelocked beam is pumped preferentially and maintained without further intervention.

The 40 fs, 800 nm output of the oscillator is amplified in the Legend Elite Duo through the process of chirped pulse amplification (CPA). The CPA method must be used to avoid damaging optics and to avoid self-focusing through gain media. The chirp namesake refers to separation of the higher frequency components from the lower frequencies. The higher frequency components of the pulse are sent over a longer path-length than the shorter frequency components after separation by a series of gratings in the stretcher cavity. Chirping stretches the beam temporally and reduces its peak power in order to avoid damage to the optics during further amplification. After the beam’s pulse width is stretched, it is passed multiple times through Ti:Sapphire gain media. The multiple passes are controlled through Pockel’s cells and are dumped out of the regenerative amplifier and into the second amplification stage before diminishing returns from the gain media are observed. The seed beam only passes through the Ti:Sapphire gain media of the single pass amplifier once and is then expanded with a set of telescopic lenses and sent into the compressor. The compressor acts as the reverse of the stretcher and sends the lower frequency portion of the pulse over a longer path length and compresses the beam temporally back to the initial 40 fs pulse width. The output is now 10 mJ at 800 nm. This beam can be used for analysis directly, however longer wavelengths are provided by the OPA.
Figure 2.1: Diagram of individual laser components. The final output of the system is tunable wavelength radiation from 1200-2500 nm with energies over 3 mJ at 1 kHz.

A CaF$_2$ window mounted onto a specially designed recessed vacuum flange allows the laser to be transmitted from atmosphere into the vacuum chamber. The flange is designed such that a focusing lens is in close enough proximity to the ionization volume as is practically possible. The lens itself is made of Bk7 material with a focal length of 150 mm. Minimization of focal length is necessary to produce the smallest beam waist possible which ultimately determines the power density of the focused laser beam (assuming consistent maximum output from the laser). The lens is mounted on an x,y,z translation stage which allows for precise overlap with the sputtered plume, and also gives the ability to adjust the power density of the overlap region by moving the beam along its axis of propagation. The beam focus is governed by the equation listed below (Equation 2.1) where $w_0$ is the calculated beam waist. The $M^2$ term is the beam quality factor which is 1 for a Gaussian beam and is 1.5 for the described system (as provided by
Coherent during installation). The wavelength of the laser is \( \lambda \), the focal length of the beam is \( f \) whereas \( w_D \) is the initial beam radius. As the focal length decreases, a smaller beam waist is achievable. The setup described allows for power densities of \( 10^{15} \) W/cm\(^2\).

\[
    w_0 = \frac{\lambda f M^2}{\pi w_D}
\]

(2.1)

Figure 2.2 shows a plot of laser the volume of the laser along the axis of propagation after the focusing lens. This plot is shown to provide a visual representation of why analyses are often taken at reduced focus where the power density is less than the maximum achievable. As Adnrew Kucher has shown for some organic molecules\(^7\), saturation intensity occurs for some organic molecules at around \( 10^{13} \) W/cm\(^2\). Analysis at \( 10^{15} \) W/cm\(^2\) will still be saturated however at this focus significantly less volume of the plume is sampled. Whereas at a defocused intensities of \( 10^{13} \) W/cm\(^2\) provides higher plume overlap. The saturation intensity is still achieved and the sampled area is increased which leads to enhanced sensitivity.
Figure 2.2: Plot of the focused laser beam along the axis of propagation near the point of maximum intensity. The area of peak power density at $z = 0$ has a typical value near $10^{15}$ W/cm$^2$. As the value of $z$ increases, decreasing power densities are observed. Additionally, the area of the laser cross-section increases with increasing $z$ values.

2.4.2 LPI Experimental Conditions – Excessive Ion Signal

This SIMS instrument is designed to account for single ion events at the detector due to the relatively low ionization efficiencies observed with SIMS. Application of the same instrumentation to LPI analyses, however requires methods of dealing with the excessive signal present from 1) the overabundant low-mass fragment signal and 2) SIMS signal in the LPI spectra.
One method for removal of the positive SIMS ions from the LPI spectrum is to generate a negative bias on the stage directly following the impact of the primary ion pulse. A timings schematic of this process is depicted in Figure 2.3. The negative stage bias will attract the positive ions instead of accelerating them into the mass analyzer. Conversely, any negative ions will be accelerated into the mass analyzer, however their paths will not terminate at the detector since the reflectron is set to a positive voltage. The negative bias is applied for ~100 ns before being quickly switched to a positive accelerating bias. Shortly after the positive bias is set, the laser beam intersects the plume and creates positive photoions which are immediately accelerated into the mass analyzer. This yields an LPI spectrum that is generally free of SIMS ions. This method is particularly useful, though when comparing SIMS and LPI signals it requires multiple spectra to be obtained with and without suppression for comparison. A different approach for the differentiation of SIMS and LPI ions is to offset their entry into the mass analyzer in time.

Figure 2.3: Timing diagram for LPI analysis with suppression of SIMS ions. A negative stage bias is applied at the terminus of the primary ion pulse to prevent positive SIMS ions from entering into the mass analyzer. Shortly after the end of the primary pulse, the stage is set to a positive accelerating voltage and the laser beam intersects the neutral plume. The photoions generated in the process are immediately accelerated into the mass analyzer by the positive accelerating bias.
Overlap of the SIMS and LPI ions will be present in the mass spectrum if the photoions and SIMS ions are extracted simultaneously. However, if a delay is present between the extraction of SIMS ions and the generation/extraction of the photoions (which are generated nearly instantaneously after interaction with the laser) the differently generated ions will be delayed in time entering the mass analyzer. This temporal separation will allow for differentiation between the two types of ions in the mass spectrum. A schematic diagram is presented to depict the timings of these events in Figure 2.4. The 40 fs packet of photons intersects the sputtered plume after extraction of SIMS ions has already occurred. The positive 2500 V stage is applied directly after the termination of the primary ion pulse packet and remains on for 2 µs. This enables the photoions to be immediately extracted after they are generated. These carefully set timings can allow for offset of the SIMS and LPI ions by ~0.5 amu in the mass spectra and allows for easy differentiation of SIMS and LPI ions.

Figure 2.4: Timing diagram for concurrent SIMS and LPI analyses. The positive stage bias is applied immediately at the terminus of the primary ion pulse. A temporal offset between stage bias application and the intersection of the laser in the plume allows for separation of SIMS and LPI ions in the mass spectrum.

The abundance of LPI ions that reach the detector are incredibly high, particularly for fragmented species since detector saturation effects become prevalent at such high signal levels. The multi-channel plate detector relies upon a cascade of electrons to amplify signal and with such a great abundance of low-mass ions hitting the detector it is unable to recover enough
electrons in time. A capacitor is present at the power input of the detector to counter this action, however it is insufficient at supplying the needed current to replenish the electron supply. Lowering the detector gain can be effective at diminishing these effects, however this greatly reduces the sensitivity of the instrument and severe diminishment of high mass ions is observed. Another method of removal of low mass ions from hitting the detector is required.

The original detector design incorporated a grid above the MCP plates which had previously been grounded during routine operation. This grid provides a means to apply a potential which can selectively steer ions away from the detector. A high-voltage switch from a pre-existing instrument was utilized in conjunction with a power supply to apply the potential at the detector. The switch is controlled by a Berkeley Nucleonics BNC-575 8-channel delay generator which is already incorporated into the ToF-SIMS instrument. The timing is set to selectively diminish low mass signals by applying a voltage of (+) 2600 V to the grid for a selected duration. The nature of the grid provides an imperfect system which still allows some ions to pass, however it is extremely successful at removing the majority of low-mass signal. The effect of the grid is no observable detector saturation under normal operating conditions. A recent upgrade to the system was produced by relocating the high-voltage switch. A long cable was previously used to connect the switch to the detector, and due to the increased capacitance, long rise times were observed. Now a very short cable (< 0.3 m) connects the grid to the associated high voltage switch which provides a sharp cutoff of removed ions from the mass spectrum.
2.5 References

Chapter 3

Evidence for the Formation of Dynamically Created Pre-Formed Ions at the Interface of Isotopically Enriched Thin Films

This chapter has been adapted from Lerach, J. O.; Winograd, N., Evidence for the formation of dynamically created pre-formed ions at the interface of isotopically enriched thin films. *Surface and Interface Analysis* 2013, 45 (1), 54-56

3.1 Abstract

A novel approach to elucidate the SIMS ionization mechanism of the commonly formed \([M+H]^+\) molecular ion is investigated. Molecular depth profiling of isotopically enriched thin films provides insight into the ionization mechanism. Using a model bi-layer film of phenylalanine and phenylalanine-D$_8$, the depth profile results show evidence of formation of an \([M+D]^+\) molecular ion generated from the non-enriched phenylalanine parent molecule. This unique ion formation is attributed to the mixing of chemical damage with intact molecules due to successive primary ion impacts. The \([M+D]^+\) has an observed thickness of 19.9 nm for the enriched-on-top system, and 9.9 nm for the enriched-on-bottom system. This ion formation is direct evidence for dynamically created pre-formed ions as a result of chemical damage rearrangement which is induced by successive primary ion bombardment events.

3.2 Introduction

The versatility of secondary ion mass spectrometry (SIMS) enables researchers to investigate increasingly complicated systems. Due to the advances in recent research in organic and biological systems it is imperative to understand the fundamental ionization processes...
involved during the layer-by-layer analysis of molecular depth profiling. Various groups have already reported methodology to investigate the interfaces of two differentiable materials. For example, water-ice overlayers on the surface of some organics have been employed to improve ionization\(^8\). Additionally, delta layers of differing Irganox materials have been utilized to achieve precise control over film thickness and roughness\(^9\). In these systems, the Irganox molecules used possess the same sputter yield which ensures consistent sputtering rates during depth profile analysis, regardless of the layer’s identity. However in these studies the ionization efficiency of the individual components is still not under direct control due to the involvement of structurally different molecules. It follows that a new method is necessary to generate a controlled interface between two thin films which are as chemically similar as possible, yet remain differentiable in a mass spectrometer.

Deuterium labeled molecules have been proven to be successful in the isolation of the identity of the proton or deuteron donor responsible for ionization. Previous studies involving a complex system of fatty acids in a matrix with varying water or D\(_2\)O concentrations were used to elucidate the proton transfer mechanism\(^10\). Tracing the origin of a proton donor in a film of only one material is impossible to determine, however it is possible to utilize isotopic labeling to monitor the ion formation. The methodology used in this study requires the preparation of a bi-layer film of a molecule and an its deuterated, isotopically enriched analog. Molecular depth profiling of this bi-layer system, with the focus being on the interface, should provide unequivocal evidence for the origin of the proton responsible for ionization.

This study utilizes an enriched form of phenylalanine (PHE, Figure 3.1) to provide large amounts of deuterium which will become available upon fragmentation following primary ion impacts. Phenylalanine-D\(_8\) (PHE-D\(_8\), Figure 3.1) was chosen due to its high level of enrichment at very stable covalently bound C-D sites along the phenyl ring (five deuterons), on the β-carbon (two deuterons) and on the α-carbon (one deuteron). In contrast, the amino and carboxyl groups
remain protonated as these sites may be able to exchange protons with other molecules with other available or weakly bound protons/deuterons. Thus, the possibility of deuteron exchange which is not facilitated by primary ion impact is negligible.

![Phenylalanine molecule and isotopically enriched Phenylalanine-D₈ molecule.](image)

**Figure 3.1:** a) Phenylalanine molecule and b) isotopically enriched Phenylalanine-D₈ molecule.

Molecular depth profiling of these bi-layer films is performed and the subsequently ionized species are monitored. The exchange of a deuteron from fragmented PHE-D₈ molecules is observed in the form of the [MₐPHE+D]⁺ molecule, yielding strong evidence for dynamically created pre-formed ions (DCPI)¹¹⁻¹³.

### 3.3 Materials and Methods

#### 3.3.1 Sample preparation

PHE-D₈ was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada) and PHE was obtained commercially from Acros Organics (Geer, Belgium). Both samples were used without further purification. Bi-layer samples were created in a previously described, laboratory-built physical vapor deposition (PVD) chamber¹ which is attached to the ToF-SIMS system. Each layer was 100 nm to 300 nm in thickness as determined by a quartz crystal microbalance (QCM). Briefly, an alumina crucible containing 0.2 g of either analyte was brought to
sublimation temperature by resistive heating of a tungsten filament. The resulting sublimate is
diffused through a uniform grid which is suspended above the crucible. The sublimate is
adsorbed onto a pre-cleaned 5 x 5 mm² clean Si wafer (Ted Pella, Redding, CA). The sample
wafer is attached to a Cu sample block via double-sided copper tape (3M, Saint Paul, MN). The
block holding the wafer is placed in contact with a liquid N₂ cooled QCM, from which thickness
was monitored. The deposition chamber, which is also connected to the SIMS instrument, is able
to deposit from either of two unique crucibles thus enabling a multi-layer film with up to two
components to be created and analyzed without having to expose the sample to atmosphere.

### 3.3.2 Depth profile analysis

Analyses were performed on a Bio-ToF mass spectrometer which has been previously
described\(^\text{14}\). Mass spectra were collected at 3 KHz with a 100 ns pulsed primary ion beam of 20
keV C\(_{60}^+\) (IOG-C60-20, Ionoptika, Warriors Mark, UK) with intermittent erosion cycles of seven
seconds. A primary ion beam with current between 100 pA to 140 pA was rastered across an area
of 240 x 300 μm\(^2\) as measured by atomic force microscopy (AFM) profilometry. AFM
profilometry measurements were taken on a KLA Tencor Nanopics 2100 (Milpitas, CA) in
contact mode providing the researchers with accurate depth information of the sputtered film\(^\text{15}\).

During analysis, the sample stage was cooled to 93 K to minimize damage accumulation
during erosion. Earlier results also indicate that PHE does not achieve a steady state for depth
profiles taken at ambient temperature. In contrast, analysis performed at 93 K alleviates this issue
and a consistent steady state is achieved. In addition to increased depth profile performance at
such temperatures, molecular motion is not expected between the two analytes in the bi-layer
system at reduced temperatures and should provide an ideally abrupt interface during analysis.
3.4 Results and Discussion

Complementary depth profile analyses were performed on two separate bi-layer samples, where the enriched layer was either on top or bottom relative to the non-enriched layer. The respective depth profiles through each type of film are shown in Figure 3.2. In each depth profile a small increase in molecular ion (MI) intensity of the top layer is observed and followed by a rapid fall of the signal into the steady state. At the interface of each film the MI signal for the top layer approaches zero, whereas the lower layer’s MI rapidly increases and reaches its steady state value. An obvious difference can be observed between the steady state values for the respective MI's as the PHE-D₈ MI has lower intensity when compared to the PHE MI. This difference is due to the lower abundance of protons within the enriched sample. This observation directly suggests that diminishing proton sources by replacement with deuterons diminishes the formation of [M+H]⁺ and favors the formation of [M+D]⁺.
Figure 3.2: Depth profiles of PHE and PHE-D₈ bi-layer films. Molecular ion signal is plotted for each species as a function of depth. a) PHE as the top layer b) PHE-D₈ as the top layer

The focus of this study is at the interface of these bi-layer systems. First, consider the system with PHE-D₈ film on top of the PHE film. As the depth profile proceeds, a layer of chemical damage is built up and subsequently removed as the steady state is achieved. In this region damage is accumulated and removed at the same rate, yielding a static identity to the fragments which remain near the surface of freshly exposed film. Since the identity of the top film is PHE-D₈, the fragments are rich in deuterium. These existing fragments are subject to beam-induced mixing from subsequent primary ion bombardment, which may allow them to interact with the lower levels of the film. As this deuterium-rich damage layer is brought closer to the PHE layer, perturbation of the two films occurs as fragments from the PHE-D₈ layer migrate into the PHE film. This mixing allows deuteron rich damage to associate to PHE, which
are observable in the form of deuterium-associated ions. The ions are differentiable from non-enriched material by \( n+1 \) amu in a mass spectra (\( n \) = number of associated deuterons). By monitoring the \([\text{M}_{\text{PHE}}+D]^+\) ion, the source of deuterium can be definitively determined to be from the upper PHE-D\(_8\) layers. Since a finite amount of deuterium rich damage is present, the existence of the \([\text{M}_{\text{PHE}}+D]^+\) ions is likely to be short-lived.

Figure 3.3 shows the anomalous ion signal at m/z 167 relative to the common trends of ions from respective layers of the film. This plot was created after normalizing the steady state values of common ions of each layer to unity and determining the mean and standard deviation of those values using OriginPro 8.1 (OriginLab, Northampton, MA). Ions chosen for the enriched sample were at m/z 81, 98, 128, 173, 174 and 175 while analogous ions for the non-enriched sample had m/z ratios of 77, 91, 120, 128, 165, and 166. The signal at m/z 167 was plotted separately given its disparate behavior resultant from the formation of \([\text{M}_{\text{PHE}}+D]^+\) at the interface. Beyond the interface, the formation of \([\text{M}_{\text{PHE}}+D]^+\) decays and the signal which remains is due to the steady state value of the naturally occurring \([^{13}\text{C}\text{M}_{\text{PHE}}+\text{H}]^+\) isotope.
Figure 3.3: Normalized depth profile data for PHE-D₈ top-layer sample. The averaged signals for the respective layers are shown in green for PHE-D₈ and blue for PHE. The anomalous signal at m/z 167 is also plotted in red with its steady state value normalized to the average steady state value of PHE ions. The peak at the interface of the m/z 167 ion is due to \([M \text{PHE} + D]^+\) formation.

In order to extract the signal for \([M \text{PHE} + D]^+\) from the \([^{13}\text{C} M \text{PHE} + H]^+\) isobar, a sigmoidal trend was fit to the average value of the non-enriched ions using OriginPro 8.1 and subtracted from the normalized m/z 167 signal. Since the data points are normalized to the same y-value, the representative subtraction spectrum will yield depth information which is pertinent to the x-axis. Therefore, the subsequent subtraction spectrum yields a peak which represents the abundance of the \([M \text{PHE} + D]^+\) ion as a function of depth as evident in Figure 3.4. After fitting a
Gaussian peak to the subtraction spectrum it was determined that the full width at half-maximum (FWHM) intensity is 19.9 nm.

For comparison, a separate experiment was performed by depth profiling through a bi-layer film where the non-enriched sample resided on top of the enriched sample. The same data treatment method was used to examine these separate systems. Considering again the signal due to only $[\text{MPHE}+\text{D}]^+$ for each system at the interface, it can be seen that the width of the peak for the enriched-on-top depth profile is significantly larger than the enriched-on-bottom depth profile at 19.9 nm and 9.9 nm respectively. The differences in peak widths describe what mechanisms are causing DCPI formation in each system.

Figure 3.4: Subtraction spectra obtained for the $[\text{MPHE}+\text{D}]^+$ ion plotted with a fitted Gaussian curve. A value of 19.9 nm is calculated for FWHM.
In order for $[\text{M}_{\text{PHE}} + \text{D}]^+$ to form, a free $\text{D}^+$ must reside near a PHE molecule. Considering the case with the enriched-on-top sample, chemical damage due to the primary ion impact will yield a surplus of deuterium-rich fragments. These fragments are known to occur below the surface and, in the case of H or D, have been known to migrate within the sample. This allows deuterium-rich fragments from the enriched layer to come into contact with PHE molecules in lower layers. This is a process in support of DCPI proposed recently. Alternatively, in the case of the enriched-on-bottom film, the width of the $[\text{M}_{\text{PHE}} + \text{D}]^+$ peak is half the size of the preceding case. This narrowing is due to the low probability of intact PHE molecules being forced to lower depths within the sample and coming in contact with deuterium-rich fragments from the PHE-D$_8$ molecules. It is known that the layers of a film below the surface do receive some chemical damage due to primary ion impact, however, the forces at the lower levels are greatly diminished compared to those nearer the surface, leading to less damage. The lower mobility of an intact PHE molecule relative to fragmented molecules or free deuterons limits mobility within the sample, hence the narrower width of the $[\text{M}_{\text{PHE}} + \text{D}]^+$ signal.

The formation of DCPI is evident from the formation of the $[\text{M}_{\text{PHE}} + \text{D}]^+$ ions. The identity of the ionizing atom is easily traced to the PHE-D$_8$ layer. Conclusions made about the $[\text{M} + \text{D}]^+$ ion formation can be analogously applied to $[\text{M} + \text{H}]^+$ formation due to their chemical similarity. In the two cases outlined in this study, primary ion impact is responsible for the formation of deuteron-rich fragments and mobility of the fragments within the top few layers of the film. If excess deuteron-rich material comes into near enough contact with a PHE molecule, the basic R-NH$_2$ (pKa = 9.31) site may abstract that molecule to form a DCPI. Upon successive primary ion impact the DCPI is liberated from the confines of the film and enters into the vacuum of the ToF-SIMS instrument where it is subsequently analyzed.
3.5 Conclusions

A unique method for interfacial SIMS studies has been reported. Utilization of this system makes it possible to directly observe DCPI formation by monitoring the \([M_{\text{PHE}}+D]^+\) signal at the interface of an isotopically-enriched and non-enriched film. The mechanism of \([M_{\text{PHE}}+D]^+\) formation will be similar to \([M+H]^+\) ion formation in a film of either PHE or PHE-D$_8$ and possibly other similar organic films.

This method can also be extended to investigate the fundamental aspects of depth profiling due to the limited perturbation of a system by isotopic enrichment. Many common organic analytes can be obtained commercially or synthesized with varying degrees of enrichment. Inserting an isotopic marker at specific sights on a molecule can help tailor experiments to probe fundamental questions incurred for depth profiling studies. This protocol should also be useful for elucidating the details of ionization during gas cluster ion bombardment where the chemical damage is smaller, and the ion beam mixing effects are reduced further.

3.6 References


Chapter 4

Investigations into the Interactions of a MALDI Matrix with Organic Thin Films using C_{60}^+ SIMS Depth Profiling

This chapter has been adapted from Lerach, J. O.; Keskin, S.; Winograd, N., Investigations into the interactions of a MALDI Matrix with organic thin films using C60+ SIMS depth profiling. *Surface and Interface Analysis* 2014, 46 (S1), 67-69

4.1 Abstract

Molecular depth profiling of multilayer organic films is now an established protocol for cluster secondary ion mass spectrometry (SIMS). This unique capability is exploited in the following sections to study the ionization mechanism associated with matrix-enhanced SIMS, and possibly, matrix assisted laser desorption/ionization (MALDI). Successful depth profiling experiments are performed on model bi-layer systems using 2,5-dihydroxybenzoic acid (DHB) as a matrix with dipalmitoylphosphatidylcholine (DPPC) or phenylalanine (PHE) as substrate. The interaction between the matrix and organic analyte is monitored at the interface of the films. Tri-layer films with D_{2}O as a thin-film sandwiched between the matrix and organic layers are also investigated to determine what role, if any, water plays during ionization. A temperature of 93 K was required for successful depth profiles. Mixing is observed at the interfaces of the films due to primary ion bombardment, however the mixing does not recreate the conditions necessary for ionization enhancement.
4.2 Introduction

Previous research has shown that the addition of certain molecules to the local chemical environment may enhance molecular ion signal during SIMS analysis\textsuperscript{1-3}. This is often termed matrix-enhanced SIMS (MeSIMS). Two common benefits of MeSIMS are increased ion signal and decreased fragmentation. In a variety of previous studies multiple matrices including common MALDI matrices\textsuperscript{4}, metals\textsuperscript{5}, metal nanoparticles\textsuperscript{6} and ionic liquids\textsuperscript{7} among others, have been shown to increase the molecular ion signal of various analytes. In addition to the identity of the matrix, the application of such matrices in SIMS is also under investigation\textsuperscript{8-10}. The mechanisms governing the observed enhancements are still being scrutinized\textsuperscript{11} and more mechanistic information is necessary to fully exploit the use of such matrices and to better tune them for SIMS experiments. This study aims to utilize precisely controlled interfaces of a simplified matrix-analyte mixture in an attempt to recreate enhancement conditions and to investigate the ionization enhancement mechanism. In the context of the research presented in this chapter, the commonly used MALDI matrix DHB is utilized to investigate ionization effects, if any, on thin films of the organic molecules DPPC and PHE. DHB is utilized as the matrix due to its broad SIMS ion enhancement abilities.

The model system utilized for this experimentation employs a bi-layer of matrix deposited on top of an organic analyte. Additionally a tri-layer system which incorporates a thin layer of D\textsubscript{2}O between the matrix and organic layers is also utilized and will help monitor the effects of excess water in the system. This experiment utilizes depth profiling analysis which for two purposes: the first of which is to monitor the formation of ions during the experiment \textit{via} SIMS and the second is to cause mixing of the matrix layers with the subsequent organic layers underneath through successive primary ion bombardment. The intentional primary ion
bombardment-induced mixing of unique layers in depth profiling has been exploited previously\textsuperscript{12} and is showcased in the preceding chapter.

It is known that the matrix/analyte ratio is crucial to the ionization behavior in MeSIMS experiments and utilizing primary-ion induced mixing of the matrix/analyte system may produce ideal stoichiometric conditions for ionization enhancement in the depth profile analysis. Should the ideal conditions be met, an increase in ion signal from the organic molecule may be observable in the plotted depth profile.

4.3 Materials and Methods

4.3.1 Thin film Preparation

DPPC was obtained from Avanti Polar Lipids (Alabaster, AL), PHE from Sigma Aldrich (St. Louis, MO) and D\textsubscript{2}O from Acros Organics (Geer, Belgium). DPPC films were prepared by spin-coating a 5 µL aliquot of 20 mg/mL DPPC in chloroform (EMD Performance Chemicals, Philadelphia, PA) onto a 5 x 5 mm\textsuperscript{2} Si wafer (Ted Pella, Redding, CA) at 3500 rpm for 30 s yielding 100 nm thick films. PHE samples were created by physical vapor desorption (PVD) onto a Si wafer. The PVD is the same setup used in the preceding chapter for PHE thin-film preparation\textsuperscript{12-13}. Samples were stored in a desiccator until use.

The prepared thin films were mounted onto a sample holder and inserted into the mass spectrometer which contains the PVD chamber. The sample block/wafer was cooled in the sample stage to 93 K then inserted into PVD chamber where a preheated crucible of DHB is subliming at 2-8 Å/s. Approximately 200 nm film of DHB was deposited onto the wafer/organic film and the samples were immediately inserted back into the cooled stage in the analysis chamber.
4.3.2 Me-SIMS Comparison Spectra

A comparison of DPPC with and without DHB matrix are compared for baseline measurements. Analytes were dissolved in solutions of 1:1 acetonitrile:water (ACN:H$_2$O), where either pure water or water containing trifluoracetic acid (TFA) was used. Solutions of 0.5 M DHB and 0.001 M DPPC in ACN:H$_2$O and ACN:(0.1%TFA)H$_2$O were prepared according to literature$^4$. A 5 µL droplet of DPPC solution from the ACN:H$_2$O solution was drop died on a pre-cleaned Si wafer for use a DPPC reference. 2.5 µL aliquots of DPPC solutions were mixed with 5 µL of corresponding DHB or DHB with TFA solutions then drop dried on wafers. A total of three spectra from as many unique points across each sample were collected.

4.3.3 D$_2$O Films

D$_2$O was incorporated into the model system by means of a leak valve. The D$_2$O was purified by 5 freeze-pump-thaw cycles. The leak valve is mounted onto the preparatory chamber of the mass spectrometer which is connected to the analysis chamber by a butterfly valve. Base pressure in the chamber is typically 2x10$^{-9}$ torr. With the leak valve open the pressure is adjusted to 1x10$^{-7}$ torr. A sample, pre-cooled to 93 K, of either DPPC or PHE was exposed to D$_2$O while static SIMS spectra were constantly being obtained. Once the D$_2$O$^+$ signal reached the same intensity as the [M+H]$^+$ molecular ion from PHE or the [C$_3$H$_{15}$NPO$_4$]$^+$ DPPC headgroup peak (since molecular ion was not observable for DPPC) bother the leak valve and the valve between the chambers were closed and the vacuum system allowed to equilibrate.
4.3.4 Film Characterization

Film thicknesses were monitored with atomic-force microscopy (AFM) profilometry on a Nanopics 2100 AFM profilometer (KLA Tencor, Milpitas, CA). Crater thicknesses of DPPC and PHE films were recorded. Thicknesses of DHB films could not be accurately recorded since DHB was observed to sublime at ambient temperature under UHV conditions.

4.3.5 SIMS Characterization and Depth Profiles

All depth profile analyses were collected at 93 K. Static SIMS spectra and depth profiles were recorded on a Bio-ToF mass spectrometer previously described\textsuperscript{14}. A 20 keV C\textsubscript{60}\textsuperscript{+} source (Ionoptika IOG-C60-20, Warrior Park, England) was utilized for sputtering and analysis. Depth profile spectra were obtained from a 200x200 \(\mu\text{m}^2\) analysis area in a 350x350 \(\mu\text{m}^2\) etch area. The primary ion beam typically measured 100-300 pA at direct current. For analysis a 60 ns pulse width beam with a repetition rate of 3 kHz was used to collect the 100,000 summed spectra per cycle.

4.4 Results and discussion

4.4.1 Reference Samples

In the reference spectra comparing DPPC signal both with and without applied matrix only a weak DPPC molecular ion was observed for any sample, however the common m/z 184 head-group ion was observed as a high-intensity peak in all spectra. It is known that the formation of the m/z 184 ion requires at least one proton from its surroundings, so DHB’s role as a possible proton donor may be exploited\textsuperscript{15}. An increase in signal of \(~ 60\%\) was observed for the
m/z 184 ion in both DHB/DPPC and DHB(0.1%TFA)/DPPC. These findings prove that the ion signature for the m/z 184 ion can be increased when DPPC and DHB are interrogated as a homogenously mixed sample.

4.4.2 Bi-layer films

Successful depth profiles were obtained for the DHB/DPPC samples at 93 K (Figure 4.1). A decreasing steady state and lateral mixing were observed when the samples were prepared and analyzed at ambient temperatures. These effects were overcome by cooling the samples during DHB sublimation and analysis as evident by consistent steady state values and distinct interfaces in the depth profile data. This observation is consistent with recent C$_{60}^+$ depth profiling experiments using amino acids where successful depth profiles at room temperature could not be obtained$^{16}$. Unique ions at m/z 155 for [MDHB+H]$^+$ and at m/z 184 for the [C$_{5}$H$_{15}$NPO$_{4}$]$^+$ DPPC headgroup ion are observed. Both ions are present for a time at the interface of the films, however, no significant or reproducible enhancement of the m/z 184 ion is observed.
Figure 4.1: Depth profile of DHB deposited onto a thin film of DPPC. No increase in ion signal at the interface is observed for the DPPC headgroup.

4.4.3 Tri-layer films

Water has the ability to lend protons during SIMS ionization and, whether considered beneficial or detrimental in SIMS analysis, is ubiquitous during sample preparation and inside of UHV systems. In this study water, in the form of D$_2$O, was intentionally added to investigate its role in ionization enhancement in this MeSIMS system. Deuterium’s additional neutron will make it differentiable from the naturally abundant hydrogen atoms in the mass spectra, thus the genesis of deuterated ions can be directly attributed to the added D$_2$O.
Samples of both DHB/D$_2$O/DPPC and DHB/D$_2$O/PHE were analyzed successfully with SIMS depth profiling. Ions from all three components of the respective films are present at the interface of the depth profile in Figure 4.2. The DPPC headgroup ion with an abstracted deuteron at m/z 185 is observed, however abundance is very low. This is not believed to be due to enhancement in the sense of matrix-enhancement, but rather due to the abundance of free deuterons which are able to act in non-enhanced ionization pathways. In cases with thick D$_2$O layers (thick enough that no mixing is observed between DHB and DPPC during depth profiling) deuterated molecules are observed at higher intensities (2x the $^{13}$C steady state) showing a relationship between D$_2$O and DPPC, yet by comparison, no matrix enhancement on behalf of DHB or a combination of D$_2$O and DHB (Figure 4.2). This type of behavior has been exploited in the previous study which is outlined in the preceding chapter, and leads the researchers to believe that the presence of water or D$_2$O in conjunction with DHB does not yield ionization enhancement in the context of this study.

Figure 4.2: Depth profiles of DHB/D$_2$O/DPPC (left) and DHB/D$_2$O/PHE (right). The addition of D$_2$O plays no noticeable role in the ionization behavior of the organic layer.
4.5 Conclusions & future prospects

In this work, a multilayer organic system aimed to mimic the MeSIMS environment is constructed and successful depth profiles with such systems have been acquired. The motivation for this experiment is to create an ion-beam induced mixed interface where ionization enhancement may be observed. However, no enhancement effects are evident. Perhaps the DPPC molecular ion behaves differently than the m/z 184 headgroup, but with this current instrumental setup the DPPC molecular ion is not observable. With this information it would appear that the correct environment to promote ionization enhancement has not been replicated. Moreover, even the addition of a thin D$_2$O layer to the interface does not appear to influence the ionization probability in any measurable fashion. In a conversation with Ron Heeren it was suggested that, much like the action of the MALDI matrix, the MeSIMS matrix necessitates solvent extraction, or more abundant localization of matrix molecules around the analyte. The ion-beam induced mixing may not cause enough distortion between the two unique layers to facilitate these types of interactions.

This very well-defined layered structure would seem to be a model which allows conditions to be controlled in a systematic way. Perhaps the enhancement effects sought may be found by choosing molecules that exhibit a larger MeSIMS enhancement effect, or, as additionally suggested by Ron Heeren, by using a molecule with a higher gas-phase basicity to induce more dramatic ionization effects. Indeed, this approach was pursued and is summarized in the following chapter.
4.6 References

12. Lerach, J. O.; Winograd, N., Evidence for the formation of dynamically created pre-formed ions at the interface of isotopically enriched thin films. *Surface and Interface Analysis* 2013, 45 (1), 54-56.
Chapter 5
Strong Field Laser Post-Ionization of Organic and Biomolecules
Monitoring the Effects of Gas-Phase Basicity on Bi-layer systems with SIMS
and LPI Depth Profiling

5.1 Abstract

Multiple experiments are presented which monitor varying degrees of ionization effects
with both secondary ion mass spectrometry (SIMS) and laser post-ionization (LPI). LPI analyses
are shown to be able to better characterize systems where suppressive ionization effects dominate
typical SIMS spectra. These findings help diminish the possibility of false negatives in data with
complementary SIMS and LPI analyses. In addition, explorative research of systems designed to
study ionization effects are also presented. Homogenously mixed films and bi-layer films with
distinct interfaces are utilized as controlled environments for the study of ionization effects.
Three distinct series of experiments are presented, the first two exploit behavior of molecules
with varying gas-phase basicity (GPB) of bi-layer and homogeneously mixed films. The last
series of experiments utilizes homogenously mixed and chemically-similar films to study
variations in molecular ion behavior for SIMS and LPI.

5.2 Introduction

The depth of information obtained with LPI is greatly beneficial to SIMS analyses since
it provides secondary information pertaining to sample composition due to the decoupling of
ionization from sputtering. For example, many organic molecules ionize in the SIMS process as
\([M_{\text{SIMS}}+H]^+\) ions whereas the same molecules will form \(M_{\text{LPI}}^+\) photoions in the presence of an
ionizing laser\textsuperscript{1,2}. Since sputtering events are independent of matrix effects the M\textsubscript{LPI}\textsuperscript{+} photoion may be a more accurate representation of abundance in a sample, as opposed to [M\textsubscript{SIMS}+H]\textsuperscript{+} ions which are known to be influenced by local chemistry. Additionally, a large number of biologically relevant molecules do not ionize as M\textsubscript{SIMS}\textsuperscript{+} SIMS ions, rather they form [M\textsubscript{SIMS}+H]\textsuperscript{+}. Furthermore, some complex biological molecules such as trehalose, cholesterol and other molecules ionize through proton loss or [-OH\textsubscript{n}] (n = 1,2) loss mechanisms to form positive ions in SIMS. These organic molecules are readily observed as M\textsubscript{LPI}\textsuperscript{+} photoions generated by the strong field LPI\textsuperscript{1}.

One of the factors which has been shown to have an influence on SIMS ionization is GPB of molecules in a heterogeneous mixture. This measure of basicity has been shown to greatly affect the ionization of various molecules. Recent findings with SIMS have shown that molecules with disparate GPB values will favor ionization of the molecule with higher relative GPB. In some cases the molecule with lower GPB can be completely quenched from a SIMS spectra even though it is known to be present in the sample.

In this section, a series of depth profile analyses of multiple bi-layer systems are examined using SIMS and LPI. The behavior of the individual analytes at the interfacial mixing region is monitored with complementary SIMS and LPI analyses to investigate the difference in ionization between the differing methodologies. Strong field ionization (SFI) is utilized due to its broad ionizing capabilities for organic molecules. The effects shown in these studies show that LPI is quite capable of observing photoions where SIMS ions of the same species are not observed. In addition, the quantitative effects of SIMS and LPI are compared.
5.2 LPI Analysis of Bi-Layer Films

5.2.1 Introduction – LPI Analysis of Bi-Layer Films

The construct of these model systems utilizes methodology applied previously in the study of \([M_{\text{SIMS}}+H]^+\) ionization (Chapters 3 and 4)\(^{3-4}\). SIMS depth profiling with the \(C_{60}^+\) primary ion excels at both the ionization of organic species and the sputtering organic material with minimized damage\(^5\). In addition, depth profiling of the model bi-layer films presents two unique experimental advantages: 1) For sufficiently thick films, where a steady state signal is achieved, an internal reference for the steady state signal of the pure material is afforded and 2) mixing of the subsequent layers of the sample, particularly at the interface of the two unique layers, is facilitated by primary ion bombardment. This mixing effect provides a region where the two unique species are no longer homogenously distributed about a finite interface, rather they become intimately mixed with each other for some duration\(^3\).

This controlled environment of chemical mixing may generate unique ions due to the local chemical environment. In the previous example in Chapter 3 it is shown that a non-enriched phenylalanine (PHE) became associated to a non-native deuteron owed to primary ion mixing from a separate enriched PHE-D\(_8\) layer. Deuteron mixing in this region was reported to exist for 19.9 nm in a previous publication\(^3\). Such is the rationale for using this type of bi-layer system to investigate matrix effects of two-component systems.

Coupling SIMS and LPI can afford much more information regarding the chemical consistency of the mixing layers with respect to species formed during the bombardment process. SIMS alone can provide information regarding only ionic species, whether pre-formed during the bombardment process or formed from interactions during/after liberation from the surface. This information is central to the SIMS process, however the observable data from SIMS-generated
ions remains inherently tied to ionization effects. Alternatively, the use of LPI gives researchers access to information from un-ionized species. During sputtering events neutral species are freed from the sample surface and can be photoionized in a laser field. Strong-field ionization is utilized in these studies since it is shown to increase molecular ion yield and decrease fragmentation when compared to other LPI regimes. Since LPI analysis is designed to only interrogate neutral species, the signal observed from LPI remains independent of SIMS ionization effects. This leads to a more accurate depiction of chemical makeup.

For the case of bi-layer films the formation of $[M_{\text{SIMS}}+H]^+$ SIMS molecular ions can be monitored and compared to $M_{\text{LPI}}^+$ LPI molecular ions (as well as fragments of the molecular ions) to yield information on the behavior of the formation of molecular ion which can be used as an indicator of any disparity between ionization and sputtering.

This particular project is framed insomuch that the relative GPB character between the unique molecules which comprise the respective layers may cause a change in SIMS ionization as primary ion bombardment facilitates churning of the surface and sub-surface layers. In terms of the effect of GPB on SIMS ionization, previous work has shown the trend that molecules with more positive GPB values will have a higher propensity to abstract a proton in the gas phase when compared to molecules with a relatively smaller value. Thus, a molecule with higher GPB is more likely to form an $[M_{\text{SIMS}}+H]^+$ than its lower GPB counterpart. This enhancement/suppression effect has been shown previously, with results even showing that even in a 1:1 mixture the lower GPB molecule is nigh unobservable.

Since the relative GPB character of the molecules has an effect on SIMS ionization, another method such as LPI analysis, is used for more accurate depiction of chemical makeup of the sample. The signal obtained from LPI analysis is from sputtered neutrals that are subsequently photoionized, as opposed to sputtered ions as with SIMS. Thus LPI analysis yields a depiction of neutrals within a sample which are not as greatly influenced by GPB character as
SIMS. In fact, in some cases where almost complete SIMS suppression was observed, LPI analysis shows ion signatures from the suppressed species.

A series of four bi-layer films were created to test the response of varied GPB character at the interface of two unique materials. The top-most layer’s identity, 2,5-dihydroxybenzoic acid (DHB) is kept constant, whereas the layer on which it resides can be any of four distinct materials, each with varied GPB character. Table 5.1 summarizes the GPB character of each of the molecules. Relative to DHB, arginine (ARG) and histidine (HIS) have very high GPB values. The phenylalanine (PHE) molecule has similar GPB character, albeit a slightly higher value, and barbituric acid (BA) is slightly lower. These materials provide a wide range of disparity between GPB values relative to DHB and should provide enough information to monitor trends if they are present and observable.
<table>
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<th>Molecule</th>
<th>Reported Gas Phase Basicity (kJ/mol)</th>
<th>Structure</th>
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<td><img src="image" alt="Barbituric acid" /></td>
</tr>
</tbody>
</table>

Table 5.1: Reported gas phase basicity values for the five molecules utilized for depth profile and homogenously mixed film studies.

### 5.2.2 Materials & Methods - LPI Analysis of Bi-Layer Films

A series of bi-layer systems were created using various combinations of spin-coating organic films from a solvent and/or physical vapor desorption (PVD). Molecules for the model system were based on variety of GPB character and ease of preparation. All samples created using 5 x 5 mm<sup>2</sup> Si wafer substrates (Ted Pella, Redding, CA). Thin films of all samples were created via PVD with the exception of ARG, which was drop-dried from a solution of 0.05 M 1:1 acetonitrile:water. PVD has previously been utilized to generate low roughness glassy films<sup>10</sup> of various organic molecules and the dried droplet technique has also shown success at creating
films for these studies11. PVD films were made by sublimation of the neat material on pre-
cleaned Si substrates and monitored by quartz crystal microbalance (QCM). All samples were
stored in a desiccator until further use.

The addition of DHB top-layer to existing films was made by depositing a layer of DHB
onto the previously prepared bottom-layer samples in the PVD chamber which resides on the
ToF-SIMS instrument. The sample was cooled in the sample stage to 93 K and inserted into the
PVD chamber where DHB was already subliming at a rate of about 3-8 Å/s as monitored by
QCM. Bi-layer films with the top PVD layer of up to 300 nm are created in a very short time
using this methodology. Samples were then inserted back into the cooled analysis stage and
analyzed.

LPI depth profile analyses were taken on a Bio-ToF reflectron-type time-of-flight
secondary ion mass spectrometer. Briefly, the instrument was run in positive-ion mode with a
pulsed stage bias of (+) 2500 V. When needed, to avoid saturation of the detector, a pulsed bias
of (+) 2600 V was applied to a grid just prior to the MCP to deflect the over-abundant low-mass
ions. An analysis area with a 50 µm field of view was established for sample interrogation,
whereas the sputtered area was 300 µm. The small analysis field of view ensures that the analysis
area does not overlap with the crater edges, so no edge effects are observed. Typical ion gun
current was kept under 121 pA in direct current mode, and sample analysis was performed at 1
kHz with a 2,000 ns primary ion pulse width. These parameters ensure that the primary ion
current stays below the static limit during analysis cycles.

A 50 fs ultra-fast pulse of 1333 nm laser radiation was utilized for photoionization. In
the optical parametric amplifier (OPA) system used, 1333 nm shows good conversion efficiency
and has been shown to ionize a variety of organic molecules quite well which is was selected out
of possible wavelengths. Laser power outside of the OPA was typically 2.1-2.4 W for analysis.
DHB ionizes remarkably well at most laser intensities so the laser focus was adjusted to enable
the ideal power densities for analysis of the bottom layer materials. ARG, BA and HIS utilized power densities from 1-2x10^{13} \text{ W/cm}^2 and PHE was ionized at narrower focus and a power density of 5x10^{14} \text{ W/cm}^2.

5.2.3 Results and Discussion pt. I: Depth Profile Analyses – LPI Analysis of Bi-Layer Films

Preliminary depth profile data of bi-layer films of PHE on ARG and BA on ARG possess an increase in ARG [M_{\text{SIMS}}+H]^+ signal relative to its steady state value at the interface of the two films during depth profile analysis. An increase in signal of the molecule with higher GPB could be indicative of ionization enhancement based on GPB character. With this information, four new bi-layer films were created and had the identities: DHB/ARG, DHB/HIS, DHB/PHE and DHB/BA. These systems exploit a wide range of variation in GPB and were analyzed concurrently with SIMS and LPI analyses.

Of the four systems, clear evidence of a varied ionization effect is only observable for ions from ARG in the DHB/ARG system (Figure 5.1). The enhancement effect at the interface is manifested as an increased in intensity at the interface relative to the steady state value. The three other systems show no clear evidence of ionization enhancement or suppression, for either the DHB layer, or the varied bottom layer. (Additional depth profile data is presented in Appendix B). The only other molecule with a higher GPB than DHB was HIS and no noticeable enhancement was observed. Additionally, DHB, which has a higher GPB than PHE or BA was also not observed to be enhanced in those respective films either.
Figure 5.1: Depth profiles obtained from SIMS (left) and LPI (right) data. ARG [M+H]^+ enhancement is observed at the film interface for both SIMS and LPI depth profiles.

For the SIMS and LPI ions plotted in the DHB/ARG depth profile Figure 5.1, an enhancement for the molecular ion of ARG is present for both techniques. However, it is important to note that the ARG molecular ion for LPI is observed as an [M_{LPI}+H]^+ ion (speculation of [M_{LPI}+H]^+ ion formation provided in Section 5.2.4). Contrarily, LPI molecular ions typically take the common form of M_{LPI}^+ ions, and in addition SIMS molecular ions are generally observed as [M_{SIMS}+H]^+. This ionization behavior makes the ARG [M_{ARG}+H]^+ a unique case. In addition to its unique structure, its behavior at the interface is also anomalous since it too shows an unexpected ionization enhancement at the interface. Much of the research shown in this body and elsewhere have shown decoupled sputtering from ionization and, in this case, it appears that there must be some relation for this unique case.

5.2.4 Results and Discussion pt. II: The Arginine [M_{SIMS}+H]^+ and [M_{LPI}+H]^+ Molecular Ion – LPI Analysis of Bi-Layer Films

An intriguing outcome of the analysis of ARG with strong field LPI shows evidence for [M_{LPI}+H]^+ ion formation as opposed to the expected M_{LPI}^+ formation observed by all other
molecules under the same type of ionization conditions. The formation of $[\text{MLPI}+\text{H}]^+$ ion necessitates at minimum an intact arginine molecule and a proton. The following equations provide possible means by which this ion may form. The process of adding a proton and electron to balance charge of an ARG molecule (Equation 5.1) followed by subsequent removal of an electron facilitated by a strong laser field (Equation 5.2) is one method by which $\text{MH}^0$ formation may occur. Additionally unimolecular dissociation of a dimeric cluster may also occur as illustrated in Equation 5.3.

\[
e^- + M + H^+ \rightarrow \text{MH}^0
\]  

(5.1)

\[
\text{MH}^0 \xrightarrow{hv} \text{MH}^+ + e^-
\]  

(5.2)

\[
M_2 \xrightarrow{hv} \text{MH}^+ + [M - H]^-
\]  

(5.3)

It is unknown how to determine if the ionization follows the path outlined by equations 5.1 and 5.2 or 5.3, however investigations into dimer formation are possible. A separate series of depth profiles were run to investigate the effects of temperature on dimer formation during SIMS analysis in the steady state region. Analyses at both ambient temperature and at 93 K were collected for the same sample of ARG. Data were collected on the same day under the same conditions with the exception of varied temperature. The SIMS signal for the $[\text{ARG}_2+\text{H}]^+$ SIMS dimer at m/z 349.2 is evident at both temperatures, however the ions shows very low abundance when collected at room temperature, yet increases in intensity by 6.8 times when observed at 93 K. In addition to the dimer data, the signal for the $[\text{ARG}+\text{H}]^+$ molecular ion intensity increases
by 2.8 times at 93 K. These results are similar to findings by Piwowar et al where they report a 6.3-fold increase in dimer formation of ARG at cryogenic temperatures\textsuperscript{12}.

The dimer, a weakly bound state, can be observed to have higher abundance when analyzed at lower temperatures with SIMS as noted here and elsewhere\textsuperscript{12}. Likewise an increase in LPI signal should also be observed assuming its ionization efficiency does not change by much as a function of temperature. Though recent studies have shown that cryogenic temperatures do not necessarily yield higher molecular ion signal for some organic species with LPI for $M_{LPi}^+$ ions\textsuperscript{13}. The $M_{LPi}^+$ and $[M_{LPi}+H]^+$ ions differ in their formation and, while the data shown is not a direct measurement of how the $[M_{LPi}+H]^+$ ion forms, it may lead to insight into whether unimolecular dissociation of dimeric ARG is a viable route by comparing the relative SIMS and LPI signals.

5.2.5 Conclusions – LPI Analysis of Bi-Layer Films

The established bi-layer protocol has been successful at investigating ionization effects at the interface of two materials as exhibited in the previous two chapters. Ionization effects such as enhancement and suppression are readily observed in various studies. In the studies shown in this chapter, successful depth profiles for all materials were obtained, however with the results presented it is unclear whether the bi-layer approach was successful. The SFI ionization mechanisms for ARG molecular ion is different than all other molecular ions reported, and while it shows an increase in molecular ion for SIMS, it also shows an increase in the LPI molecular ion as well. These results are intriguing since the SIMS ionization and neutral sputtering event are expectedly decoupled from each other. Interestingly, the LPI molecular ion for ARG being anomalously protonated does not necessarily add or detract from the decoupling of ionization and sputtering since its behavior is not fully understood.
The rest of the molecules analyzed show little to no variation in their depth profiles suggesting that the ideal stoichiometric ratios were not met, or that the change in ionization was not significant enough to be observable on the scales of the depth profile.

Though the bi-layer depth profile system has been successful at other analyses the mixed results proves it to be less than successful at creating conditions by which ionization suppression/enhancement can be studied in these cases. Pursuant to the goal of determining the efficacy of SFI LPI to ameliorate SIMS ionization effects further research is warranted under differing conditions. These goals are achieved using non-varying mixtures of materials prepared with other established methods such as spin-coating and drop drying of mixtures of materials. These types of studies which analyze the variances between SIMS ionization and the sputtering event, and other LPI studies, are investigated in the following section.

5.2 Recovery of Suppressed SIMS Data with SFI LPI

5.2.1 Introduction - Recovery of Suppressed SIMS Data with SFI LPI

The matrix effect in SIMS is a broad term which is used to describe either suppression or enhancement of an analyte due to the local chemical environment. These effects are caused by a multitude of conditions in varied analyses. Perhaps one of more thoroughly studied systems of matrix effects exist with respect to diversity in GPB within a heterogeneous mixture. Recent SIMS studies by Heeren and Lockyer’s groups bolster the postulation that a molecule with higher GPB shall suppress the ionization of a molecule with lower GPB. In some cases, the higher GPB molecule will even have an enhanced effect, likely due to its increased affinity for protons from the local chemical environment. In this study, two-component mixtures of equimolar concentrations are investigated with SIMS and LPI to further investigate the role which GPB
character plays with enhancement/suppression and determine the efficacy of LPI analysis to mitigate these effects.

5.2.2 Materials & Methods - Recovery of Suppressed SIMS Data with SFI LPI

Neat samples of PHE, ARG, DHB and BA are analyzed with SIMS as reference materials. SIMS spectra of equimolar mixtures of ARG with each of the three other materials are collected for reference. For comparison, LPI spectra of equimolar mixtures are obtained. In addition to the equimolar mixtures, mixtures of ARG:PHE at 1:1, 10:1, 100:1 and 1000:1 are investigated with SIMS and LPI to compare variations over a wide range of concentrations.

Four of the molecules utilized in this study are listed according to their GPB values in Table 5.1. Neat solutions of PHE, ARG and DHB were prepared at a concentration of 0.05 M with 1:1 acetonitrile:water whereas BA was prepared in a solution of 1:1 methanol:water. Mixed samples with equimolar concentrations were prepared by combining equal volumes of two analyte solutions. All samples, both reference and mixed, were created by drop drying a 5 µL aliquot of the desired material or mixture of materials onto a pre-cleaned 5 x 5 mm² Si wafer. Varied concentration samples were similarly created by mixing appropriate amounts of 0.05 M solutions to yield the specified ratio, then drop drying by the above methods. Lastly, the PHE reference material was made via PVD.

All analyses were taken with 20 keV C₆₀⁺ primary ion beam current between 220-300 pA. Initial SIMS measurements used a primary pulse width of 100 ns whereas LPI measurements were taken with 2,000 ns primary ion pulse width. A total of 100,000 analysis cycles were collected per data point excepting the case of PHE and ARG which consisted of 50,000 cycles. All LPI measurements were collected with SIMS suppression via a (+) 2450 V stage bias for 100 ns prior to extraction of positive photoions. Additionally all LPI measurements were collected
with a pulsed (+) 2600 V bias applied to the deflection grid just before the detector to deflect low-mass ions. LPI analyses were carried out with 50 fs, 1350 nm radiation and power densities ranging from $1-3 \times 10^{13}$ W/cm$^2$.

5.2.3 Results & Discussion - Recovery of Suppressed SIMS Data with SFI LPI

A summary of the SIMS data collected for the equimolar mixtures is presented in Table 5.2. The molecular ion signal obtained from the 1:1 mixtures is reported is a % change from the reference material. The signal obtained was multiplied by a factor of two so it is comparable to the reference material. In each mixture the ARG component was kept constant whereas the second part of the binary mixture was adjusted to monitor effects due to disparity in GPB. In each case, the secondary molecule exhibited a decrease in molecular ion signal in the presence of the proton hungry ARG. Nearly complete suppression was observed for both DHB and BA, each having a difference in GPB from ARG of 147 kJ/mol and 223 kJ/mol respectively. Phenylalanine, which has a 118 kJ/mol difference from ARG, was only decreased by 29%. For ARG:PHE and ARG:BA the signal of the ARG molecular ion increases, and the ARG signal magnitude increases with increasing GPB disparity. Interestingly, the ARG signal obtained for ARG:DHB shows a decrease. These findings are in line with what other members of the SIMS community have reported, with the exception of ARG’s behavior in ARG:DHB. Perhaps the drop-drying methodology did not create and ideally homogenous mixture for ARG:DHB with pockets of DHB, though it is unlikely as an isolated pocket of DHB molecules should show some signal for DHB with the lack of ARG.
Table 5.2: Summary of mixed film SIMS data. The table reports the percent change of the molecular ion’s integrated intensity from 2x the individual component to its reference material. Matrix suppression effects are clearly observed for all molecules with lower GPB.

These results did indeed show that ionization behavior is being greatly affected by GPB character, and that the simplified system of mixed equimolar films provides a controlled method to study ionization behavior. Particularly in the cases of DHB and BA further research to determine the ability of LPI analysis to observe signal from those with such drastic SIMS suppression is investigated.

Table 5.3: Summary of SIMS and LPI comparison for mixed films with observable matrix effects. The table reports total integrated counts from the molecular ion of the individual component of the mixed film. SIMS and LPI data were collected under the same instrumental conditions. SIMS molecular ions and ARG LPI molecular ion are observed as [M+H]^+ ions whereas all other LPI molecular ions are observed as M^+ ions. Matrix suppression effects are most noticeable in SIMS for DHB and BA whereas these signals are recovered with LPI.

The data shown in Table 5.3 expresses variations in signal obtained during LPI experimentation compared to SIMS. First, consider the behavior of ARG. ARG was observable for each sample using SIMS and LPI. The resultant LPI signal is indeed lower than the SIMS signal, however this is not believed to be due to deleterious effects, rather ARG does not ionize as
well as some other molecules in the laser owing for its relatively low LPI intensity. Next, considering the molecules with lower gas phase basicity, all show appreciable LPI signal. With respect to the data shown in Table 5.2, PHE’s SIMS signal did not diminish by much compared to its reference and is still readily observable with both SIMS and LPI in Table 5.3. Excitingly, the SIMS signals for DHB and BA were almost completely suppressed by the ARG component and barely observable, yet with LPI analysis their intensities increase by multiple orders of magnitude! The SIMS signals for these molecules’ molecular ions were quenched by ARG, yet they are readily observable with the LPI.

![Figure 5.2: Comparison of response curves for LPI (left) and SIMS (right). Response curves are generated by monitoring the PHE molecular ion signal in multiple samples with varied amounts of PHE doped into ARG. The LPI response provides a useful analysis range to 1:100, whereas SIMS is only useful down to 1:10.](image)

Lastly, LPI and SIMS data are compared in Figure 5.2 for neat PHE and PHE doped at various concentrations in ARG from 1:1 to 1:1000. Results were run in triplicate and standard deviations are plotted. Noticeably, the variance in SIMS PHE reference signal is much larger.
than for its LPI counterpart. An $R^2$ value provided by OriginPro 8.5.1 for the LPI data is 0.9955, and for SIMS data is 0.9795 showing that the LPI data can provide a more accurate representation of the signal. Additionally, the LPI data is reliably observed down to 1:100, whereas after 1:10 for SIMS the intensity values become unreliable due to decreased sensitivity. Due to the complicated ARG [M+H]$^+$ LPI photoion behavior, the SIMS and LPI data for ARG ions is listed in Appendix C. Though, as was states earlier, the benefits of LPI analysis are greatly evidenced by its abilities to avoid false negatives, particularly when SIMS suppression effects are evident, and the recovery of DHB and BA signal in the presence of ARG exemplifies this.

5.2.4 Conclusions - Recovery of Suppressed SIMS Data with SFI LPI

The presence of ARG is shown to suppress the ion signal from molecules with lower GPB in SIMS. Complementary LPI analyses of like samples shows abundant signal from the SIMS suppressed species. This is increasingly important for complex systems where known suppression issues are present. In addition to providing secondary information to SIMS and increases in ion intensities, LPI analysis also affords researchers the capability of observing analytes which are suppressed by conventional SIMS studies.

5.3 - Irganox Mixed Film SIMS and LPI Comparison

5.3.1 Introduction - Irganox Mixed Film SIMS and LPI Comparison

Further comparisons of SIMS and LPI data collected on reliable systems are sought to show the broad value of LPI analysis. Sample construct must be easily controlled and co-analysis with SIMS and LPI must be attainable. A new system of Irganox 1330 and Irgnax 1010 is
utilized for such an analysis. This system was inspired by recent work by Shard et al\textsuperscript{14}. An intriguing method used in their analyses is the creation of intimately mixed films with various concentrations of two analytes. They have used a variety of materials including Irganox 1098 and Irganox 1010. These materials, of known concentrations, are analyzed with SIMS depth profiling and reported and variations in SIMS signal are observed. Similar analyses were sought by this lab to further investigate the role which LPI can play in providing matrix-effect free data.

A series of samples of including neat and mixed Irganox 1010 and Irganox 1330 were created for analyses (structures shown in Figure 5.3). Access to a precisely controlled PVD apparatus such as the one used by Shard et al is unavailable so spin-coating, which has been used in the past to form varied concentration samples, is utilized. Mixtures of the two materials at 20\% intervals were created and analyzed with both SIMS and LPI. The relative intensity of the ion signatures are compared and reported.

![Irganox 1330](image1)

![Irganox 1010](image2)

Figure 5.3: Chemical structures of Irganox 1330 and Irganox 1010.
5.3.2 Materials & Methods - Irganox Mixed Film SIMS and LPI Comparison

Reference and mixed films consisting of Irganox 1330 (>95%, TCI Chemicals, Portland, OR) and Irganox 1010 (Ciba Chemicals, White Plains, NY) were created from 0.025 M solutions in chloroform (EMD Performance Chemicals, Philadelphia, PA). Mixed solutions were prepared from reference solutions in ratios of 0.2, 0.4, 0.6 and 0.8. Thin-films of the materials were created by spin-coating from solution. A 5 µL aliquot of the desired material was applied to a pre-cleaned 5 x 5 mm² Si wafer and spun at 3000 rpm for 30 s. This methodology provided blue, glassy films for analysis.

A 300 ns pulse width beam of 20 keV C₆₀⁺ is used for analysis with a direct current reading of 196 pA. A pulsed bias of (+) 2600V was applied to a retarding grid just prior to the detector to remove low-mass ion signal. All analyses were collected at 93 K to diminish gas-phase photoion interference and to provide better depth profile data. A 2-step depth profile protocol was utilized to provide timely analysis of the surface and steady state for all six samples. The analysis area was 50 x 50 µm² in the center of a 300 x 300 µm² etched region. The etch time of 30 s (a sputter dose of 4x10¹³ C₆₀⁺/cm²) was determined by preliminary depth profile analysis to be sufficient in reaching the steady state of each film. Photoions were collected with 50 fs pulsed 1333 nm radiation at 1.67 W focused to a power density of 2x10¹³ W/cm² under the ionization optics. Molecular photoion signal for Irganox 1330 is observed at these values, however no molecular photoion is observed for Irganox 1010.

5.3.3 Results & Discussion - Irganox Mixed Film SIMS and LPI Comparison

The data collected during these experiments provide surface and steady state analysis of mixed films. Due to the overlap of the SIMS and LPI peaks at higher masses a large offset in the
laser delay was required to simultaneously observe SIMS and LPI peaks. An offset was provided which separated the SIMS molecular ion and LPI ions by about 4 amu. The regions at which distinguishable Irganox 1330 molecules reside are free from other ions, making simultaneous collection of SIMS and LPI data practical (and without the need for suppression of the SIMS ions). Due to the structural similarities between the two molecules only two distinguishable photoions, both from Irganox 1330, are observable. These ions are the molecular ion at m/z 774.6 and the fragment resulting in the loss of one dendrimer at m/z 565.4. SIMS ions above these masses were observed for Irganox 1010, however no appreciable LPI signal was observed.

Figure 5.4: Response curves for LPI (left) and SIMS (right) for films of Irganox 1330 doped into Irganox 1010. Irganox 1330 molecular ion (m/z 774.6) and a fragment (m/z 565.4) are reported at varied concentrations. Spectra were taken at the film surface (top) and in the steady-state region (bottom). Enhancement of the SIMS ions in the mixed films between 20% and 80% is observed. No such enhancement is observed for LPI data.
The integrated intensities for the Irganox 1330 ions with both SIMS and LPI and also at
the surface and in steady state region are shown in Figure 5.4. A large amount of variation in the
SIMS signal for Irganox 1330 at increasingly higher concentrations is observed. The SIMS
signals of 80% Irganox 1330 are equivalent or higher than the signal obtained for the Irganox
1330 reference material in both the surface and steady state regions. It appears that for the mixed
samples, the SIMS response is generally linear, however at the extremes of the graphs, with the
reference material, the reported values taper off from linearity. This behavior is especially
evident in the SIMS surface spectra. Considering the LPI response trends, only the value for 60%
Irganox 1330 is errant from a linear trend. The rest of the data from the LPI analysis shows a
relatively straight trend, particularly when compared to the SIMS data. Ideally, a quantitative
study would benefit more from the LPI analysis over the SIMS data. Regarding the errant LPI
peak at 60% Irganox 1330, it is speculated that this is due to the sampling methodology and a
possible variation in stage height.

5.3.4 Conclusions - Irganox Mixed Film SIMS and LPI Comparison

These studies show that the LPI analyses, for the majority of concentrations, provide a
better indication of sample concentration than the SIMS analyses do. This is particularly true
when comparing the mixed materials to the reference materials. Particularly in the case of SIMS
it appears that there is indeed some ionization effect occurring within the interactions of Irganox
1330 and Irganox 1010 to slightly enhance the ionization of the Irganox 1330 molecular ion and
fragment. LPI analysis, however, is less influenced by sample composition and may provide
more accuracy in quantitative measurements.
5.4 References

3. Lerach, J. O.; Winograd, N., Evidence for the formation of dynamically created pre-formed ions at the interface of isotopically enriched thin films. *Surface and Interface Analysis* 2013, 45 (1), 54-56.
Chapter 6

Behavior of Octadecane and Stearic Acid at Varying Power Densities and Wavelengths in the SFI Regime

6.1 Abstract

The behavior of stearic acid and octadecane under strong field ionization (SFI) conditions are presented. These classes of molecules, which have not been previously explored with SFI, are investigated with varied power densities at two distinct wavelengths. Molecular ion signal is monitored for each of the variables to determine the most efficient ionization conditions, which will aid in future studies. The behavior of the molecular ion is reported as a function of power density for each of two wavelengths. It is found that increasing the power density above $10^{14}$ W/cm$^2$ has little to no effect on relative fragmentation and that optimum ionization occurs near $10^{14}$ W/cm$^2$.

6.2 Introduction

The in situ SIMS analysis of biomolecules in a biological sample possesses many unique challenges. Low concentrations of analyte and matrix effects coupled with the sheer complexity of biological systems make precise sample preparation crucial since contamination can have serious detrimental effects on sensitivity$^{1-3}$. Methods for increasing the secondary ion signal with sample preparation protocols exist, however these may cause changes to local chemical environment and give an inaccurate depiction of the native construct$^{4-6}$. To avoid these negative interactions, a technique like LPI is utilized since it does not require modifications to the sample.
It has been shown that LPI is adept at increasing ion signal and is also resistant to matrix effects\(^7\)\(^8\). These benefits make it an ideal technique for mass spectrometry studies.

The specific biological systems of interest are the algal species \(B.\ braunii\), which are known to produce many different lipids and fatty acids which can be utilized for the formation of biofuels\(^9\)\(^10\). The methods by which the lipids are stored and ultimately secreted from the alga is still under investigation\(^11\)\(^12\). Recently, SIMS has been utilized to better aid in the understanding of these secretion methods, however more research is warranted. In the interest of furthering research in this field, the strong-field ionization behavior of two model molecules, stearic acid and octadecane (Figure 6.1), are studied in order to better understand the ideal laser ionization conditions. For the purposes of studying a sample as complex as lipids in \(B.\ braunii\) it is important to obtain as much signal from as possible from analytes which may only be present at very low concentrations.

Pursuant to these studies, two wavelengths were chosen (1200 nm and 2000 nm) which both provide high power densities in the laser system employed, thus allowing researchers to utilize two orders of magnitude change in power density. The molecular ion behavior of two model molecules are analyzed at these two wavelengths. These molecules will provide an insight into ionization behavior of pure aliphatic materials \(via\) octadecane and those with additional functionality such as the carboxylic acid headgroup of stearic acid.

In addition to the analysis of these specific biomolecules, this investigation aims to add to a growing pool of data which proposes ideal conditions to utilize SFI for LPI analysis\(^13\)\(^14\). LPI is a broad approach which has utilized photon energies ranging from the ultraviolet region to the near-infrared. Recently, LPI studies utilizing photons in the IR region (specifically in the strong-field regime at power densities greater than \(10^{12}\) W/cm\(^2\)) have shown decreased fragmentation and the ability to ionize a broad variety of organic molecules.
6.3 Power and Wavelength Dependence of Octadecane and stearic acid photoions

6.3.1 Gas Phase Power Studies

All materials were obtained from Alfa Aesar (Ward Hill, MA). Gas-phase photoionization measurements of stearic acid (> 99%) and octadecane (> 99.5%) were collected at room temperature as sublimation of material in the vacuum chamber of the instrument was utilized to generate a steady gaseous volume of the respective analyte. Each analyte was prepared as a powder pressed into In foil (> 99.99%). Gas-phase ionization studies were collected at two distinct wavelengths (1200 nm and 2000 nm) and over a variety of power densities (10^{12}-10^{15} W/cm²). Base pressure during analysis of the samples was ~10^{-8} Torr whereas typical base pressures in the instrument are 5-10x10^{-10} Torr.

Power density variations were achieved by translating the focusing lens which is attached to an (x,y,z) translation stage along the axis of laser propagation, effectively changing the focus under the ion extraction optics depending on the z-location. These methods have been expounded in Chapter 2. Extraction of the ions occurs only in a finite region about which the power density is known and previously calibrated using residual Xe^{n+} ions^{15}. For both reported wavelengths a total of 300,000 summed spectra were collected at each reported power density point by adjusting

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Figure 6.1: Structures of octadecane (top) and its carboxylic acid functionalized analog stearic acid (bottom).
the laser focus. Data are reported as molecular ion signal as a function of power density (Figure 6.2).

![Figure 6.2: Gas-phase molecular ion behavior for octadecane (left) and stearic acid (right) as a function of increasing power density. Data was collected with 1200 nm and 2000 nm radiation. Ideal analysis conditions are at the peak of the response curve where maximum signal is observed.](image)

6.3.3 SIMS-LPI Materials and Methods

For SIMS-LPI measurements the same In-pressed sample methodology was utilized, however measurements were taken at 93 K to prevent sublimation of the material thus negating gas-phase interference. A 2,000 ns pulsed 20 keV C\textsubscript{60}\textsuperscript{+} primary ion source were used for analysis with a dc current of 208-285 pA. A total of 100,000 spectra were collected per analysis. In an effort to simplify the spectra, SIMS ions were suppressed via a (-) 2450 V stage bias for 100 ns prior to extraction. The incident laser wavelength was 2,000 nm and power varied between 1.10-1.24 W. Plots of the LPI spectrum obtained for each molecule are shown in Figure 6.3 and 6.4.
Figure 6.3: An LPI spectrum with an inlay of the molecular ion signal obtained for stearic acid with 2000 nm radiation. Molecular ion is observed at m/z 284.2 and a loss of H₂O is evident at m/z 266.2.
6.4.1 Results and Discussion

Successful experimental data were collected for both molecules. SIMS LPI data (Figure 6.3 and 6.4) show molecular ion present from the sputtered materials, and gas phase power studies (Figure 6.2) show molecular ion behavior over a variety of power densities. The ionization of the octadecane molecule is an intriguing case since it is a completely aliphatic hydrocarbon system. Historically, SFI LPI has not dealt with these types of molecules. With regard to the stearic acid molecule, the question was initially raised as to where the positive character would reside; localized to the hydrocarbon tail or at the carboxyl group. The presence of octadecane molecular ion is indeed proof that the negative charge following SFI ionization...
does not necessarily localize to the carboxyl group and may indeed form on the hydrocarbon tail - an interesting prospect for possible studies with *B. braunii*.

The power scans at two distinct wavelengths show similar features for each molecule (Figures 6.2). At lower power densities, the molecular ion signal rises to an inflection in the mid to high $10^{14}$ W/cm$^2$. After the inflection, the molecular ion signal decreases in intensity to lower values where it begins to flatten out. These graphs show that the ideal conditions to ionize these molecules resides at the maxima of the curves where the most molecular ion signal is present due to increased ionization rates and increased laser and plume overlap. Indeed at these intensities the signal observed is more than two orders of magnitude higher than the lowest observed signal near $10^{13}$ W/cm$^2$. The molecular ion signal of the two molecules at the varied wavelengths for stearic acid favors ionization with 1200 nm light as opposed to octadecane whose molecular ion signal is slightly enhanced with 2000 nm light. The carboxylic acid headgroup likely plays a role in this behavior. Longer wavelengths should provide softer ionization according to the findings of David Willingham$^{14}$ and Andrew Kucher$^{13}$. If this is indeed the case, perhaps the electron delocalization capabilities of the carboxyl group of the stearic acid molecular ion act to stabilize the molecule, preventing some fragmentation. Alternatively, with octadecane the headgroup is not present and the molecule behaves similarly regardless of wavelength.

In addition to the plots of molecular ion as a function of power density, molecular ion-to-fragment ratios ($M^+/F_{tot}$) are also presented (Figure 6.5). These graphs present the data in a means that describes the relative amount of fragmentation occurring at such a power density. At lower power densities, there is relatively less fragmentation, however at power densities greater than $10^{14}$ W/cm$^2$ the trend flattens out yielding sustained fragmentation rates at these power densities. Above the $10^{14}$ W/cm$^2$ threshold it appears that the ionization becomes soft enough to not increase fragmentation relative to primary ion yield.
Figure 6.5: Mass-to-fragment ratios for octadecane (left) and stearic acid (right). The ratio is reported as molecular ion signal divided by the total fragment ion signal obtained at each respective power density. Above $10^{14}$ W/cm$^2$ the rate of fragmentation is constant.

6.4.2 Conclusions

Ideal values for the analysis of octadecane and stearic acid are presented in the previous sections. The ideal conditions for maximization of molecular ion signal for stearic acid stand near $8 \times 10^{13}$ W/cm$^2$ for either wavelength, though 1,200 nm yields more molecular ion. Octadecane ionizes best near $5 \times 10^{13}$ W/cm$^2$ and slightly better with 2,000 nm light. For both molecules and at each wavelength the molecular ion behavior above power density of $10^{14}$ W/cm$^2$ stabilization of the fragmentation rate is observed. At this point the molecular ion signal decreases, and the rate of fragmentation also decreases at an equal rate. This is an important finding since it provides further proof that longer wavelengths do indeed produce softer ionization.
6.5 References

Chapter 7

Fabrication of Highly Patterned Organic Thin Films for Ion Beam Characterization

7.1 Abstract

As chemical imaging of biological samples becomes more prevalent in the secondary ion mass spectrometry (SIMS) community new metrics are needed to establish baseline measurements of imaging performance. It follows that the primary ion beam needs to be monitored in order to precisely quantify its imaging capabilities. To suit these needs a highly ordered patterned organic film is sought for these purposes. One such method for the creation of a patterned thin film of the organic molecule rubrene is presented. Rubrene is a highly-conjugated molecule with a relatively high molecular weight of 532.2 amu. A thin film of rubrene is modified with various lithographic techniques to produce a highly ordered pattern with regular square features ranging from 5 µm to 500 µm. This patterned thin film will suit the characterization needs for a wide variety of primary ion sources.

7.2 Introduction

The overall process utilized for the generation of patterned rubrene films is outlined in Figure 1. A brief description of the process is provided here with a more thorough stepwise description outlined below. Rubrene is used due to its relatively high molecular weight and its capability as an organic semiconductor. It has been used previously to make field-effect transistors\textsuperscript{1-3} and light-emitting diodes\textsuperscript{4-5}. Rubrene is spin-coated from a solution in toluene onto a 4 inch Si wafer cut on the <111> face. A 50 nm sacrificial layer of Al is thermally deposited onto the rubrene layer to prevent deleterious interactions between rubrene and the photoresist.
Positive photoresist SPR 3012 is then spin-coated onto the Al layer. The resist is patterned in a contact aligner with exposure to high intensity ultraviolet (UV) light with an aqueous developing step to follow. The developing step reveals patterned photoresist on top of the Al and rubrene layers. A Cl₂/BCl₃ plasma is used to pattern the exposed Al regions, revealing the rubrene layer in select patterned regions. These select regions of rubrene are subsequently removed with an O₂ plasma to reveal the underlying Si in the patterned areas. A final aqueous develop step which removes the remaining photoresist as well as the Al layer ultimately reveals a patterned film of rubrene on Si. Characterization of the films is completed with atomic force microscopy (AFM) profilometry and scanning electron microscopy (SEM). Features sizes of 5 µm, 10 µm, 20 µm, 30 µm, 40 µm, 50 µm, 60 µm, 70 µm and 500 µm are produced.
7.3 Materials and Methods

Nearly all steps were performed inside the class 100 clean room facilities at The Pennsylvania State University’s Materials Research Institute Nanofabrication Laboratory. Spin-coat preparation of rubrene onto the Si substrate took place primarily in the clean room facilities, though initially it was performed in an external facility with a Laurell Scientific (North Wales, PA) WS-650-23B spin-coater. The use of the clean room for all other processes ensures minimal
contamination from dust or other foreign materials yielding a pristine final product. Rubrene samples were kept out of direct light whenever possible as photo-oxidation can occur.

7.3.1 Spin-Coating

A 4 inch Si wafer cut on the <111> face (Ted Pella, Redding, CA) is utilized as the sample substrate. Such a large wafer is pared after the initial patterning in order meet sample size requirements for many mass spectrometers.

A saturated solution of 99% purity rubrene (Acros Organics, Geel, Belgium) in ACS grade toluene (EMD Performance Materials, Philadelphia, PA) is prepared for spin-coating. The solution is sonicated for 5 minutes, then allowed to settle for at least 30 minutes before being decanted and vacuum filtered through a fine glass frit (Chemglass CG-1406-03, Vineland, NJ).

The spin-coating program proceeds through three different steps. An initial step of 2 s at 300 rpm is set to begin the rotation of the mounted wafer and is followed by a 5 s, 1000 rpm application step. During the application step about 5 mL of solution is applied in a continuous stream from a glass pipette. Lastly for 60 s a 5000 rpm drying step follows immediately after the application step. Following termination of the drying step the wafer is removed and baked for 60 s on a hotplate set to 100 °, then returned to an Al foil coated wafer carrier where it is stored so as not to be affected by ambient light. The thickness of the rubrene layer is typically 100 nm as measured by AFM profilometry.

7.3.2 Aluminum deposition

A 50 nm layer of Al is deposited using a Lab18 Thin Film Deposition System (Kurt J. Lesker, Pittsburgh, PA) as monitored by a quartz crystal microbalance. Deposition of Al is
controlled to 1 Å/s. The Al is sublimed under vacuum with heating from an electron beam. The wafer is continuously rotated during deposition to ensure even coating.

7.3.3 Photoresist application and patterning

A 1 µm thick layer of the positive photoresist SPR 3012 is applied to the Al layer. About 10 mL of SPR 3012 is applied during the first step of the dynamic spin-coating process which was provided by the MRI technical staff and was designed specifically for the selected photoresist. Initially, the wafer is spun at 900 rpm for 10 s while 10 mL of photoresist is applied. The application step is followed directly by a dynamic 4000 rpm, 45 s casting step. After spin-coating the wafer is immediately placed on a hotplate set to 95 ºC for 60 s, then cooled to room temperature on a cool plate for 30 s.

Following application of the photoresist the wafer is loaded into a SUSS MicroTec MA/BA6 contact aligner. A photomask is also loaded into the contact aligner and will provide the master pattern to be struck into the photoresist. The nature of the positive photoresist is to degrade upon exposure to intense UV light allowing for easy removal of exposed regions in the aqueous develop step. Selective areas are exposed through the photomask’s pattern which leave behind an identical copy of the master pattern in the remaining unexposed photoresist. Hard contact is made between the wafer and the photomask and a 4.5 s exposure to 8 mW/cm² UV (365 nm) light follows. It should be noted that typically 8 s is the ideal exposure time to properly pattern 1 µm of SPR 3012 photoresist at this power density (as per the information provided by the nanofabrication staff), however, due to the reflective nature of the Al layer the exposure time had to be scaled accordingly. A variable transmission mask was utilized to establish ideal exposure time.
Following exposure in the contact aligner the wafer is placed in a plastic vessel containing Microposit CD30 developer solution. The wafer is gently swirled for 60 s in the developer solution, then the wafer is removed and submerged in a bath of ultrapure H₂O to halt the developing process. The wafer is then gently rinsed with more ultrapure H₂O and dried under a gentle stream of N₂ gas. This solution removes the areas of photoresist that were exposed to UV light while leaving the unexposed areas intact. As of this step, the wafer consists of patterned photoresist on an unmolested layer of Al residing on top of rubrene. A comparison of the photomask used and a wafer after the patterning and develop steps is provided in Figure 7.2. Of note: Initially, CD26 develop solution was used, however it was found to etch away at the Al layer. This serendipitous result indeed works to the process’s advantage and is used in the final step of removing the Al layer from the rubrene as shown in section 7.3.5.

Figure 7.2: Images of the photomask (left) used for patterning and an image of a wafer with patterned photoresist (right). Successful transfer of the pattern from the photomask to the wafer is evident.
7.3.4 Plasma etching of Al and rubrene layers

A Cl₂/BCl₃ plasma reactive ion etching (RIE) step is utilized to selectively remove the Al layer, then an O₂ plasma is utilized to remove the newly exposed rubrene. The remaining photoresist acts as a barrier to the plasma and does not allow the underlying layers of Al and rubrene to be attacked by the plasma. The remaining photoresist ensures that only the areas where the resist was removed during the develop step are etched in the RIE systems.

A Plasma-Therm (St. Petersburg, FL) Versalock RIE system is used for the Cl₂/BCl₃ etching. The instrument is designed for use with 6 inch wafers, so the 4 inch wafer is mounted on a 6 inch carrier wafer with thermal release tape so it is compatible with the system. A 1:3 ratio of Cl₂/BCl₃ is utilized in favor of pure Cl₂ gas since the mixture does not yield much residual Cl⁻ at the film surface after the etching cycle. Pressure of the Cl₂:BCl₃ gas is adjusted to 5 mTorr. Chuck RF1 is set to 75.0 W and Coil RF2 is set to 800.0 W. The sample is cooled with He gas to prevent heat-related degradation. An etch time of 30 s was found to be sufficient for the removal of the 50 nm Al layer.

Oxygen plasma is supplied by a Plasma-Therm PT720 capacatively coupled RIE system. A 250 W plasma is applied with a DC bias of 492 V for 40 s with an O₂ pressure of 10 mTorr. These parameters are found to be sufficient in the removal of the rubrene layer. The etch rate of ~25 Å/s is empirically derived with ellipsometry measurements on a Gaertner Scientific (Skokie, IL) Ellipsometer with λ = 632.8.

7.3.5 Final developing step

After the two plasma etching steps, the rubrene layer is patterned yet still has a layer of Al and photoresist on top of it. In order to remove these layers, the wafer is put back into the
MA/BA6 contact aligner without the mask in place and the entire wafer is flood exposed to UV light for 10s. The wafer is then placed in CD26 developer solution bath and gently swirled. As mentioned before, CD26 fortuitously attacks the Al layer in addition to also removing the photoresist. After the Al is removed (4-5 min.) the wafer is removed and bathed in ultrapure H\textsubscript{2}O, then gently rinsed in ultrapure H\textsubscript{2}O and dried under a gentle stream of N\textsubscript{2} gas.

7.4 Results and Discussion

7.4.1 AFM Profilometry Data

AFM Profilometry data show successful creation of films with expected feature sizes. Of particular interest is the smallest feature size, 5 \( \mu \text{m} \), since the MA/BA6 contact aligner’s patterning capabilities begin to diminish near 3 \( \mu \text{m} \) feature sizes. Profilometry data presented in Figure 7 show excellent feature formation for the 5 \( \mu \text{m} \) features. In measuring the sheerness of the wall features, AFM profilometry data may not be entirely reliable due to the broadening of the AFM tip. When measuring a wall of 100 nm thickness errant readings may be obtained as the stylus body moves up the side of the wall. Scanning Electron Microscopy is utilized for these purposes and confirms a nearly shear wall with resolution of < 100nm. (Appendix D).
Figure 7.3: An image obtained from the AFM-profiling data of 5 µm features patterned in a rubrene thin film. The film’s thickness is 100 nm.

### 7.4.2 ToF-SIMS Analysis

Preliminary studies of spin-coated rubrene show molecular ion signal obtained with C\textsubscript{60}\textsuperscript{+} SIMS. Films were also characterized after the patterning process and are presented in Figure 7.4. These data show chemical images of successfully created patterned films. A variety of feature sizes, from 70 to 20 µm are presented. Data from SIMS images were collected and provided by Hua Tian of the Winograd research laboratory on the Ionoptika J105 Chemical Imager with 20 keV C\textsubscript{60}\textsuperscript{+} (Ionoptika, Warriors Mark, UK). The total ion image shows signatures from all ions collected, though signal comes mostly from the patterned rubrene areas. The molecular ion of rubrene (at m/z 532.219) is shown in the second columnn, whereas the potassium phoshpate salt
provides the relief image and is presented in the last column. The relief image of the salt is used due to the inability of the instrument to record ions at m/z < 100. Reliable Si₅⁺ clusters were not observed, however the residual salt from an aqueous develop step was observable. The patterned rubrene film are successfully created with intact molecular ions providing adequate signal for analysis. Consistent patterns are created which make analysis possible and repeatable.
Figure 7.4: ToF-SIMS images of patterned rubrene films. Data collected by Hua Tian using an Ionoptika J105 Chemical Imager. Total ion image is represented in grayscale on the left, signal from rubrene molecular ion is shown in the center and a residual salt ion is shown on the right as a relief image.
7.4.3 Other Comments

Rubrene’s intrinsic nature as a hydrocarbon allowed it to remain intact during the various steps of the presented process. Other molecules were investigated for such purposes, however they were unable to endure the process. The molecule of interest must be able to withstand heightened temperatures up to 95°C and also not dissolve or delaminate during exposure to an aqueous environment. Ease of preparation of the organic film is also a necessity and spin-coating of organic materials (particularly in such large dimensions as a 4 inch wafer) and has proven to be reliable, though other available methods exist for creating thin films of organic molecules such as rubrene such as physical vapor deposition\(^7\). Rubrene was found to match these criteria due to its high melting point (330-335°C) and its extensively pi-bound hydrocarbon structure which causes it to have very low solubility in water. Crystal violet was the initial molecule to undergo this study, however the residual Cl\(^-\) ion of the salt was found to deteriorate the Al layer if left unprocessed for more than 24-48 hours. Additionally, crystal violet was unable to survive the aqueous develop stages as it would dissolve into the CD30 develop solution.

7.5 Continued Work and Future Prospects: ITO glass as sample substrate

The Si substrate provides an incredibly flat, clean and well characterized surface from which to begin the process. It can be portioned into smaller, more useable pieces with ease too. However, its poorly-conductive nature does not allow for charge dissipation when an excess of charge is present (as can sometimes be the case with SIMS analysis). Also, is not optically transparent to UV light which is commonly used with MALDI, so one cannot use such a sample in a backside MALDI analysis. To this end another sample substrate is sought which is both
conductive and is somewhat optically transparent in the UV region. Glass with an indium tin oxide (ITO) coating possesses these properties.

The conductivity and optical transparency of ITO glass makes it popular in the mass spectrometry community as a sample substrate. Polished float glass coated in ITO has transmission properties that work in the UV range of MALDI laser wavelengths. As suggested by Paul Laibinis at Vanderbilt University, Delta Technologies (Loveland, CO) provides ITO glass that meets the needs specific to the MALDI community. A 4 inch wafer with 30-60 Ω surface resistivity provides an excellent substrate which should be able to be used as a direct replacement to a 4 inch Si wafer.

The use of a 4 inch ITO wafer was tested for compatibility into the existing patterning process. During the process delamination is believed to occur at the first aqueous develop step in the CD30 developer. The weak interaction forces that are present between the outermost oxide layer and the rubrene for the Si wafer must be stronger than the ITO-rubrene interactions. Some areas remain intact after processing and results show that it does indeed create some pattern of rubrene on ITO glass, however the delamination leads to poor results for rubrene-ITO which make the process impractical without modifications.

Due to the complexity of the process, rubrene (or another large hydrophobic hydrocarbon molecule) remains the ideal. Irganox 3114 and 1330 (mw of 784.08 g/mol and 775.22 g/mol respectively) are suggested molecules as they both possess extremely poor solubility in water, yet are able to be spin-coated on Si. In addition, their extensive use with the SIMS community in well controlled samples from the National Physical Laboratory in the UK make them ideal candidates as well. Since the method is shown to work with Si and the problem likely resides with the adhesion of rubrene to the substrate, chemical surface modification may enhance the interaction between the ITO surface and rubrene. Covalent attachment of a molecule which may
better interact with the extensively pi-bound structure of rubrene is a good candidate for surface modification 11-12.

In addition to other sample substrates, a more specific photomask should be created for to cover a more broad range of use. Ideally a 2 x 2 cm² repeating unit should be created which possesses multiple feature sizes on it, as opposed to large regions of a wafer being comprised of only one feature size.

Additionally, the need for bright and dark field features has been expressed. The current mask creates wells of silicon, surrounded by walls of rubrene. The negative of this pattern would be pillars of rubrene on top of Si. This may be accomplished using the same mask with a negative photoresist. Attempts at this methodology were made, however they proved to be unsuccessful. The negative photoresist JSR 105G was utilized, but during the developing process with Nanoremove PG, the rubrene delaminated from the Si substrate. Rather than further examining the negative resist route, one could simply design a complementary area of the proposed mask which creates pillars of rubrene on the same mask by reproducing the negative image of the existing structures.

As the limit of resolution for the contact aligner is just below 5 µm, the feature size is limited unless a different process is used. The nanofabrication facility has a GCA 8500 in-line stepper which is capable of producing sub µm features. These feature types would be particularly advantageous for atomic primary ion sources which reportedly generate 50 nm beams and also for the latest generation of C₆₀+ primary ion sources which are capable of being focused to 300 nm.

Regular features from 50 nm to 100 µm would do well for the SIMS imaging communities and cover a broad range of ion sources. Additionally, a secondary photomask which includes larger features from 100 µm to 1,000 µm for mass spectrometry equipment such as MALDI and other desorption/ionization techniques which have larger values of lateral resolution could be created with the same process while using a different photomask. The pre-existing
photomask worked wonderfully to create desired features, however a more purpose-driven photomask will create a more desirable product\textsuperscript{12}.

7.6 References


Chapter 8
Characterization of Hydroxyapatite on Synthetic Polymeric Bone-Graft Replacement Materials

This chapter has been adapted from Morozowich, N. L.; Lerach, J. O.; Modzelewski, T.; Jackson, L.; Winograd, N.; Allcock, H. R., Characterization of hydroxyapatite deposition on biomimetic polyphosphazenes by time-of-flight secondary ion mass spectrometry (ToF-SIMS). *RSC Advances* 2014, *4* (38), 19680-19689

8.1 Abstract

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is utilized to analyze mineralization of hydroxyapatite (HAP) on a series of polymeric structures that were created to function as synthetic bone-grafts. The polymers were designed to serve as a scaffold which promotes nucleation of HAP and whose structure diminishes over time while being replaced by the growth of the recipient’s natural bone. To achieve this, a series of polyphosphazene-based polymers were synthesized bearing either phosphoester or phosphonic acid moieties which serve as sites for HAP nucleation. Following exposure of the polymers to simulated body fluid, the amount of HAP formation is investigated. ToF-SIMS is utilized as the primary analytical tool since traditional spectroscopic methods cannot differentiate between the shared atomic species in the specific HAP phase of the apatite material and the similar chemical makeup of the polymer. ToF-SIMS provides unique and differentiable molecular information from polymer and HAP fragments which allows for identification of the respective phases. Two-dimensional analyses are also presented which clearly show localization of HAP signal to specific nucleation sites on the polymer. These SIMS chemical images correlate directly to additional images obtained using
environmental scanning micron microscopy (ESEMS) with energy dispersive spectroscopy (EDS).

8.2 Introduction

Though the synthesis is described elsewhere in detail\textsuperscript{1}, a brief synopsis of the polymer’s synthetic attributes is warranted. The context of this chapter is written from the perspective of the ToF-SIMS researcher. A more detailed description of the overall project can be found in the main body of the initial publication\textsuperscript{2}, and further details regarding the synthesis can be found in the preceding publication\textsuperscript{1}. The synthetic work in the creation of these compounds was performed in the Allcock research group at The Pennsylvania State University by Nicole Morozowich and Tomasz Modzelweski. Additionally, the relative quantitation scheme and all analyses on the QSTAR®XL were performed by Lauren Jackson, a graduate of the Winograd research group.

This design of this polymeric material was successfully created to meet three requirements which make it an excellent candidate for bone-graft replacements. Primarily, to act as a bone graft, it must be able to promote nucleation of HAP from within the human body. To accomplish these means the polymers were created with phosphonic acid and phosphoester groups which are capable of coordinating free Ca\textsuperscript{2+} ions and ultimately nucleating HAP. In this research a solution of simulated body fluid (SBF) is utilized to mimic physiological concentrations of human blood plasma as an analog for the determination of the polymer’s nucleation behavior in the human body. Secondly, for use in humans, the polymer must possess adequate bioerosion properties. If this property is not met, the HAP nucleation may encapsulate the outside, leaving the polymeric material trapped and unable to perform that task of nucleation. At proper bioerosion rates, HAP nucleation will occur while the polymer degrades into simpler...
biocompatible components which can easily be excreted, resulting in the complete degradation of the polymeric scaffold and full bone growth. Amino acid esters were incorporated into the structure to allow the polymers to undergo hydrolysis, thus breaking down to simpler biocompatible units. Lastly, the material must also be structurally compatible as it will be taking the place of the native bone until the new bone growth is fully incorporated. Aromatic units are incorporated into the structure to provide the needed structural rigidity.

The chemical structure of the resulting polymers yielded a new analytical challenge for previously utilized characterization techniques. The phosphonic acid and phosphoester units which are intrinsic to the polymer are also closely related to the phosphate groups associated with the specific apatite phase which was expected to be found: HAP. The chemical formula for the HAP extended structure, \( \text{Ca}_5(\text{PO}_4)_3\text{OH} \), also possesses the base phosphate group thus rendering differentiation between the phases exceedingly complex by traditional spectroscopic means. Signatures from Ca may be useful for determination of polymeric sites at which \( \text{Ca}^+ \) is coordinated, however this does not provide information regarding the phase of apatite which is being nucleated. Additionally Ca/P ratios cannot be used to determine phase since the polymer contains P also. These challenges rendered traditional spectroscopic methods unusable and a new analysis method was sought.

The information presented from ToF-SIMS data is uniquely poised to solve this problem. Though phosphate groups are present in both the polymer and apatite phases, enough chemical diversity between the two exist to differentiate between the polymer and apatite. In a SIMS mass spectrum unique ion signatures from reference polymer (before exposure to SBF) and HAP reference are characterized and identified. These characteristic ion signatures provide unique identifying markers which differentiation between the two materials is possible.

The primary ion beam used for this analysis is \( \text{C}_{60}^+ \) and the analyses presented herein showcase the exceptional range of analysis of this primary ion source. Previous fields of analysis
have included inorganic\textsuperscript{3-4}, polymers\textsuperscript{5-6} and successful differentiation of various apatites\textsuperscript{7}, however in this study both phases are identified simultaneously. The \textit{C}_{60}\textsuperscript{+} ion source is successful at these analysis whereas attempts at similar types of research have proven to be less successful with atomic primary ion sources\textsuperscript{8-9}. The data presented here show the analysis of polymeric materials and inorganic materials with excellent specificity, sensitivity and in the case of 2D analysis, imaging capabilities.

### 8.3 Materials and methods

The polymers are exposed to SBF (at 1.5x physiological concentrations) following a procedure initially prepared by Kokubo \textit{et al}\textsuperscript{10}. Mineralization of polymers after 2 and 4 weeks are analyzed and the amount of HAP nucleation is recorded \textit{via} ToF-SIMS and ESEM.

#### 8.3.1 Mass Spectrometry Data: Spectra and 2D Data

ToF-SIMS spectra and 2D images were collected on a Bio-ToF instrument with a 40 keV primary ion \textit{C}_{60}\textsuperscript{+} source (Ionoptika, Warrior’s Mark, UK). The instrument is described previously\textsuperscript{11}. The measured direct current of the analytical ion beam was 50-125 pA, though a 50 ns (at 3 kHz) primary ion pulse width was used for analysis. Images were taken over a 2008 x 2008 \textmu m\textsuperscript{2} area in a 256 x 256 pixel array, with each pixel representing a total of 30 unique mass spectra. A stage bias of (+) 2500 V was applied to aid in extraction. Analyses under such conditions are well below the static limit ensuring a true representation of the undamaged sample surface. This instrument is capable of high mass resolution (m/\Delta m 5,000)\textsuperscript{12}, however the large variations in sample height have diminished apparent mass resolution of the presented spectra.
Reference HAP Ca₅(PO₄)₃OH•H₂O material (> 85% Sigma Aldrich, St. Louis MO) was analyzed and compared to as-prepared polymer reference materials. Unique ions which represent the respective materials are identified. HAP ions have m/z 56, 96, 112, 159, 175, 231 and 287 and the respective identities of [CaO]⁺, [Ca₂O]⁺, [Ca₂O₂]⁺, [Ca₂PO₃]⁺, [Ca₂PO₄]⁺, [Ca₃PO₅]⁺, and [Ca₄PO₆]⁺. Polymer ions are reported as having m/z 77 and 91 ions which are both formed from the aryl benzene moieties. The ion at m/z 77 is the benzyl molecule [C₆H₅]⁺ and m/z 91 is identified as the tropylium ion [C₇H₇]⁺.

Large values of ion signature at m/z 73 and 147 are also observed in these samples. It is believed to be the common contaminant polydimethysiloxane (PDMS). Comparison of to PDMS reference spectra support this conclusion. The intensity of these peaks are diminished after hexane washes and the ion signature from the polymer benzyl units are observed to consequently increase. Spectral data for HAP reference material compared to polymer samples and polymeric peaks after hexane washes is shown in Figure 8.1. ToF-SIMS analysis on specific regions of samples which have previously been analyzed utilizing ESEM are presented. The regions of HAP determined by ToF-SIMS tend to directly correlate the suspected HAP regions in the ESEM images. Additionally, the ion signature from these specific regions are plotted and compared to HAP reference (Figure 8.2).
Figure 8.1: Mass spectra obtained from an SBF exposed polymer sample (top), a non-exposed reference (center) and a hexane washed sample (bottom). The ions $[C_6H_5]^+$ and $[C_7H_7]^+$ ions are present in all three samples and become more predominant following hexane washes.
Figure 8.2: Mass spectra of HAP reference material (top) and suspected HAP features on an SBF exposed sample (bottom). Spectral overlap of HAP reference ions is observed for the analyzed feature.

8.3.2 Mass Spectrometry Data: Relative Quanititation on the QSTAR® XL

A modified version\textsuperscript{13-14} of an AB/SCIEX QSTAR®XL with an incorporated 20 keV C\textsubscript{60}\textsuperscript{+} (Ionoptika, Warriors Mark, UK) primary ion source was utilized for high resolution and high sensitivity measurements. Peak assignments utilizing the QSTAR®XL possess < 10ppm accuracy. The instrument is operated in direct current mode with a 15 pA primary ion beam current. The relative quantitation methodology utilized here is described previously\textsuperscript{15}. During data collection the sample stage was constantly rastered by 10 µm steps to provide fresh analysis surface. A roughly 5 x 5 mm\textsuperscript{2} portion of each sample was analyzed for each of three unique analysis cycles per sample. A total of 180 scans are collected and summed per analysis cycle.
Data was collected in multi-channel acquisition with each scan lasting 0.33 s. Data are presented in Figure 8.3 which represent the respective ion signal form each respective data set.

Figure 8.3: Relative quantitation data which monitors the trends of some observed HAP ions with increasing exposure to SBF. An increase in HAP ion signature is observed for nearly all polymers with increasing SBF exposure.

8.3.3 Environmental SEM morphology measurements

A Philips FEI Quanta 200 ESEM (Hillsboro, OR) was utilized in vacuum mode for analysis. The electron source was operated at 20 keV with a working distance of 12 mm and pressure of 680 mTorr. Regions of nucleation were identified and analogously analyzed with ToF-SIMS.
8.4 Results and Discussion

ToF-SIMS analysis of the polymer samples were proven to be successful. Unique ionic signatures were identified for each respective species which aided in proper identification. Figure 8.1 shows prominent overlap between HAP reference data and suspected regions of HAP deposition on polymer samples. The identified ions were used in subsequent spectral and 2D analyses to determine location and relative abundance of HAP. Additionally, the benzyl fragments from the polymer are also visible when comparing polymer reference material (without exposure to SBF) to SBF exposed polymer. The PDMS contamination of the polymer material is observed to be mitigated following hexane washes of polymer reference material. The \([C_6H_5]^+\) and \([C_7H_7]^+\) ions increase in relative intensity after the washes, but are still visible without successive washing, making analysis successful without risking damage to deposited HAP. All of these unique ionic signatures from the respective materials bear no overlap with each other and make differentiation and identification with SIMS possible. Other ions from each species are possible to use, however they may have possesses isobaric overlap. The utilization of SIMS in this study is due to the availability of multiple ionic signatures which further aids in identification of the species.

Spectral analyses compared to reference material confirms the presence of HAP on the polymer surface for the materials exposed to SBF, and is also used to identify specific features as HAP. The images provided in Figure 8.4 depict an ESEM image (left) of a specific region of a polymer which was believed to have deposits of HAP present. This was confirmed with EDS which showed a high Ca concentration in the regions of interest. These samples were identified and transferred into the ToF-SIMS instrument for analysis. Once the features were located in the ToF-SIMS instrument, by using the attached microscope, 2D image spectra were obtained. The ion signature from the HAP ions (red) and the ion signature from the polymer (green) were used
to create a 2D image which shows excellent overlap of the features present in the ESEM work. Nearly all features are accounted for and those that are not present were likely physically dislodged from the sample during transit between the instruments. Individual spectra obtained from the regions of HAP deposition from the 2D analysis were compared to the HAP reference to confirm their identities (Figure 8.2).

Figure 8.4: Comparison of a sample region analyzed by both ESEM (left) and SIMS (right). Initial ESEM images provided a reference point which was aligned in the SIMS instrument with an optical microscope and SIMS images were collected on the correlative area. In the SIMS image green pixels indicate signal from the benzyl-fragment ions which are associated with the polymer and red pixels indicate ions associate with HAP.

In addition to the spectral and 2D images provided by the Bio-ToF instrument, high mass resolution and mass accuracy measurements were taken on the QSTAR®XL hybrid instrument. The relative quantitation data collected are presented in Figure 8.3. The high mass accuracy of the instrument allowed for further confirmation of the ions’ respective identities. The presented work shows ion signals as a function of time for four selected ions: 112.91, 158.88, 174.88, 286.9 respectively \([\text{Ca}_2\text{O}_2]^+, \text{[Ca}_2\text{PO}_3]^+, \text{[Ca}_3\text{PO}_4]^+\] and \([\text{Ca}_4\text{PO}_6]^+\). Ions below m/z 100 are generally not observed in the QSTAR®XL under normal operating parameters. All of the ions presented
show an increase in abundance after the second week exposure to SBF. Additionally, most ions are observed to continue increasing in abundance following the fourth week of exposure to SBF. These observations, coupled with weight gain data, support the increased presence of HAP with exposure to SBF.

8.5 Conclusions

The analysis presented in this chapter showcases the versatility of SIMS based on its ability to collect varied molecular data from exceedingly complex systems. The apatite phases which were nucleated on the films were positively identified with ToF-SIMS as HAP and the specific areas of deposition were provided and correlate with complementary ESEM data. The relative quantitation scheme further identifies the ions’ identities and also correlates increased HAP deposition to exposure to SBF, which is the anticipated result of this research.

8.6 References

5. Ren, X.; Weng, L.-T.; Chan, C.-M.; Ng, K.-M., Hollow Interior Structure of Spin-Coated Polymer Thin Films Revealed by ToF-SIMS Three-Dimensional Imaging. Analytical Chemistry 2012, 84 (20), 8497-8504.


Chapter 9
Concluding Statements and Future Prospects

9.1 Concluding Statements – SIMS Ionization Behavior

The ionization behavior of organic ions as monitored by SIMS and LPI is studied in chapters 3-6 in this thesis body. The local chemical environment is shown to have a great impact for the formation of [M+H]+ SIMS ions, specifically following the trends of gas-phase basicity (GPB) as outlined by others\textsuperscript{1-2} and exploited here. With isotopic enrichment it is shown that the ion beam induced mixing is responsible for generating free protons or deuterons from the sample itself. These free nuclei can associate with neighboring molecules to form [M+H]+ ions. Additionally, for mixed films of varying GPB suppression and enhancement effects are observed for SIMS ions. LPI analysis is used to study the photoions generated from the same systems and is shown to enable recovery of the lost SIMS signal. This is particularly useful for samples where the SIMS ions were not observed for species known to be present in the sample mixture. In addition, mixed samples over a variety of concentration ranges were analyzed and the results show that LPI outperformed SIMS in a quantitative sense. Not only is LPI able to recover lost signal and avoid false negatives, it is also able to provide better quantitation on the systems explored in the preceding chapters. This body of work adds to the growing pool of data which suggests that LPI can provide both quantitative and qualitative improvements over SIMS alone\textsuperscript{3-5}.

9.2 Concluding Statements – Application of Strong Field Laser Post-Ionization

The versatility of strong field ionization (SFI) is showcased in the multiple LPI experiments presented in this work. A multitude of organic samples ranging from completely aliphatic hydrocarbons to biomolecules and MALDI matrices were ionized with success in the
laser field. Each had signal intensity on par with or exceeding that of the SIMS signal. Defocusing of the laser beam to intensities near $10^{13}$ W/cm$^2$ was shown to provide ideal ionization conditions for most molecules. Additionally, since such laser power densities are achieved by selective positioning of the laser focus, an increase in overlap of the laser volume with the neutral plume is also observed which causes an increase in the amount of photoions generated. SFI of biological molecules is shown to provide a universal system to increase sensitivity and diminish matrix effects.

9.3 Future Prospects – Instrumental Modifications

Some suggested modifications to the existing instrument are provided which may increase its utility. Provisions for an electron flood gun currently exist in the instrument software, however the hardware for the flood gun is not present. The addition of a flood gun would enable research on samples such as polymers, or thick non-conductive organic films which are known to charge. For example, the National Physical Laboratory has created precisely controlled samples with various Irganox molecules in various in multi-layer samples$^{6-7}$. Analysis of these samples was attempted however, deleterious charging during depth profiling made complete analysis impossible. With a flood gun the precisely created samples could be analyzed with LPI and SIMS for and could yield interesting information regarding variations between LPI and SIMS ion generation.

The abundance of low-mass ions present in LPI spectra are problematic in terms of the replenishment of electrons at the detector. One method of alleviating this issue is to utilize a pulsed voltage at a grid just prior to the detector at the same frequency as the instrument cycle to selectively steer the trajectory of low-mass ions away from the detector. Removing these ions with the grid voltage minimizes detector saturation effects and does not sacrifice high-mass
sensitivity. Unfortunately, some noise due to the switching of the high-voltages is present at the detector and is observed as an increase in baseline noise counts. Due to the proximity of the deflecting optics to the detector, the switching is likely the cause. It is suggested to use another gating method to remove the low-mass contribution that may diminish some of the noise observed at the detector. Since the flight tube incorporates a reflectron into the ion’s path, either the retarding or reflecting voltage could be pulsed in a similar fashion to the grid to selectively to remove the low-mass ions.

Lastly, high mass sensitivity is required for analysis of larger molecules. As SIMS and LPI research progress more readily into biological communities, the necessity for high-mass sensitivity becomes crucial. Molecular ion of dipalmitoylphosphatidylcholine (DPPC) has been observed on an instrument very similar to the dedicated laser instrument, however it was not observed for SIMS or LPI with the laser instrument. The main difference between the two instrument lies in the post-acceleration apparatus just prior to the detector on the non-laser instrument. The addition of this can greatly increase the kinetic energy of high-mass ions and yield a greater electronic response in the detector. The post-acceleration apparatus could give the parent molecule at m/z 733.56 enough kinetic energy to be detected and add gains in sensitivity for high-mass ions.

9.4 Future Prospects – Experimental Approaches

Fragmentation of molecules is ubiquitous with SIMS and LPI. Excess energy imparted by the physical forces of the primary ion impact or the excitation due to the laser field is often removed by the molecule via fragmentation pathways. When considering LPI data, it is currently unknown if an observed fragment is the product of a dissociated photoion or of a photoionized
sputtered-neutral-fragment. An approach is suggested that may be useful in differentiating between the two.

For the incident laser volume that is fixed at some distance above the sample surface, a fixed amount of time is taken for a sputtered molecule to reach the volume. Likewise, a fragment molecule will also take some time to reach the volume, and its flight time will be a shorter than that of a larger molecule. If mass spectra are collected while varying the incident laser’s impact time from the time before the molecules enter the ionization volume (at some sampling interval) to well after the termination of the primary ion pulse, a histogram of intensity for each ion intensity at a specific overlap can be generated from the data. From this data, insight into the ionization pathway of the fragments can be investigated.

In this study, the molecular ion can be considered to be unique in the fact that it should solely originate from the photoionization of a neutral molecule. Its histogram should rise to a plateau that is equivalent to the duration of the primary ion pulse width then taper off. The nearest-mass fragment, however can be a product of either photoionization of a sputtered neutral, or a photo-dissociative process. This would lead to two possible distributions, which would be superimposed on each other in the histogram. Since a reference point for the photoionization of a sputtered neutral is given by the molecular ion, it is possible to de-convolute the two possible fragment distributions from each other by using the molecular ion histogram. After de-convolution the relative abundance of ions generated from photoionization and photo-dissociation may be compared.

This process would require small sampling intervals, likely controlled by the acquisition software. In addition, due to changing the timings of the laser beam, the mass spectra for each interval will be different. This type of analysis would likely require modifications to the acquisition software in order to collect the data in a timely manner.
The results observed from complementary SIMS and LPI experimentation have provided important results in the matters of quantitation in this type of mass spectrometry. They have also proposed intriguing questions as to formation of certain ions such as the \([\text{ARG}+\text{H}]^+\) photoion from LPI. Comparison of the ARG molecule’s kinetic energy distribution to its dimer or other molecules could give insight into its generation. Additionally, studying the kinetic energies of LPI-generated ions has been proven to be insightful in the past.

9.5 References

Appendix A – Additional Mass Spectra

Mass spectra which were obtained during the various experimentation are provided in this appendix section.
Positive SIMS spectrum of phenylalanine. Experimental description in Ch. 3.
Positive SIMS spectrum of phenylalanine-D₈. Experimental description in Ch. 3.
Positive SIMS spectrum of rubrene. Data taken with 40 keV C$_{60}^+$ on a BioToF Mass Spectrometer. Relevant data presented in Ch. 7
Negative SIMS spectra of rubrene. Data taken with 40 keV C_{60}^+ on a BioToF Mass Spectrometer. Relevant data presented in Ch. 7
LPI Spectrum of sputtered octadecane with 2,000 nm laser radiation. Experimental description in Ch.6
Gas-phase photoionization spectrum of octadecane with 2,000 nm radiation. Experimental description in Ch.6
Gas-phase photoionization spectrum of octadecane with 1,200 nm radiation. Experimental description in Ch.6
LPI spectrum of sputtered stearic acid with 2,000 nm laser radiation. Experimental description in Ch.6
Gas-phase photoionization spectrum of stearic acid with 2,000 nm radiation. Experimental description in Ch.6
Gas-phase photoionization spectrum of stearic acid with 1,200 nm radiation. Experimental description in Ch.6
Appendix B – Depth Profile for Mixed GPB Bi-Layer Films

Depth profile data for ions collected with LPI (left) and SIMS (right) for varied bilayer films.
Appendix C – ARG Ions for PHE-Doped ARG Films

ARG LPI (top) and SIMS (bottom) response as a function of concentration in mixed films with PHE are presented.
Appendix D – Patterned Thin Films of Rubrene Accompanying Information

An SEM image of the wall of a rubrene feature. The lighter areas are where material has been removed to yield Si, whereas the darker area is the rubrene film.
VITA

Jordan Oswald Lerach

Jordan was born in Pittsburgh, PA to Oswald and Linda Lerach. Jordan’s love of the sciences began at a young age and that love was nurtured during primary and secondary education in the Catholic Diocese of Pittsburgh school system. He continued his quest for scientific education and earned a B.S. in Chemistry from Duquesne University in Pittsburgh, PA. While there he began his research career by creating and characterizing novel materials with Dr. Jennifer Aitken. After graduation he entered into the terminal master’s program at Youngstown State University in Youngstown, OH. With the guidance of Dr. Brian D. Leskiw he earned an M.S. in Chemistry while monitoring gas-phase reactions with variety of mass spectrometers. After matriculation Jordan entered into and graduated from the Chemistry Ph.D. program at the Pennsylvania State University in University Park, PA. His Ph.D. work, with Dr. Nicholas Winograd, focused on secondary ion mass spectrometry and laser post-ionization under.