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Prospects for imaging TOF-SIMS: from fundamentals to biotechnology

N. Winograd*

Department of Chemistry, 184 MRI Building, The Pennsylvania State University, University Park, PA 16802, USA

Abstract

Molecular imaging with TOF-SIMS is considered with respect to existing technical challenges and potential applications. The major goal is to improve the sensitivity of measurement since desorption of molecules occurs from an area of much less than 1 μm in diameter and the number of molecules is limited. Improved knowledge of the fundamental aspects of the molecular collision cascade, bombardment with focused beams of cluster ions, laser postionization of neutral molecules and ion correlation techniques are suggested as areas where there may be considerable payoff. Potential applications to biomaterials, single biological cells, rapid screening in genomics and proteomics and molecular electronics are considered.

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1. Introduction

Molecular surface characterization of a variety of materials has been made possible in recent years by the emergence of static SIMS and related methodologies [1]. The characteristics of this approach are well-known to this community. Of special interest is the perfection of the TOF analyzer, where all secondary ions are detected in parallel fashion with nearly 100% efficiency, and the implementation of pulsed, liquid metal primary ion sources for imaging experiments. The combination of these components yields a technique with surface sensitivity, molecular specificity through mass spectrometry and submicron lateral resolution. With the introduction of ultra-high performance instrumentation over the last decade, hundreds of laboratories world-wide have begun to

explore the possible applications. As is evidenced from this conference, the community is reaching critical mass, producing a number of truly spectacular results.

Even with all of this progress, there is still many a difficulty remaining before molecule-specific imaging with TOF-SIMS reaches full potential. Probably the biggest issue is efficiency of formation of molecular secondary ions. As the size of the primary beam approaches zero, of course, the number of surface molecules approaches zero. In most cases, lateral resolution is limited not by the beam probe size, but by the number of counts acquired in each pixel. An equally important problem involves sample preparation. For many imaging TOF-SIMS applications, new ideas are needed to make sure the molecules of interest retain their spatial integrity and that contamination of monolayers does not obscure the desired information. These effects are particularly severe when examining objects with high vapor pressure like

* Tel.: +1-814-863-2619; fax: +1-814-863-0618.

E-mail address: nxw@psu.edu (N. Winograd).

single biological cells. Finally, there is still a real need for better fundamental understanding of both the sputtering and ionization of molecular systems. Improved theories might suggest novel experimental geometries and point to heretofore unknown strategies for enhancing the secondary ion yields.

There are hints coming from various laboratories that many of these issues will be resolved in the near future. If true, we have only begun to explore the possible scientific implications, and with each improvement, the possibilities seem to expand exponentially. In this report, the goal is to speculate about this future by extrapolating recent fundamental and applied advances forward a few years. Where is the field going? Can the difficulties be overcome? What will it take to entice new people with different perspectives into acquiring this instrumentation? How big of an impact will imaging TOF-SIMS bring to the scientific arena? It is always dangerous to predict such things since one is usually off track, but it is fun nonetheless.

2. Collision cascades in molecular solids

There has been considerable progress in acquiring a molecular level picture of the sputtering process associated with SIMS measurements. This understanding comes largely from molecular dynamics (MD) computer simulations of the ion bombardment event. Accurate interaction potentials for MD simulations first became available for metallic systems and were tested by comparing with experimental measurements of energy and angular distributions of sputtered neutral atoms [2]. More recently, modeling of organic compounds on metal surfaces has become feasible. For simple systems like a monolayer of benzene on Ag{111}, experiments have been carried out to measure desorbed neutral benzene molecules in specific quantum states by laser spectroscopic probing of the molecules after they leave the surface [3]. These results show that the translational energy of the molecules peaks at about 1 eV and that there is a rough correlation between translational energy and internal energy. Moreover, the kinetic energy distributions are well-reproduced with MD simulations which show that the molecules are desorbed by direct collisions with substrate silver atoms. Some illustrative results are given in Fig. 1. The calculations have also been

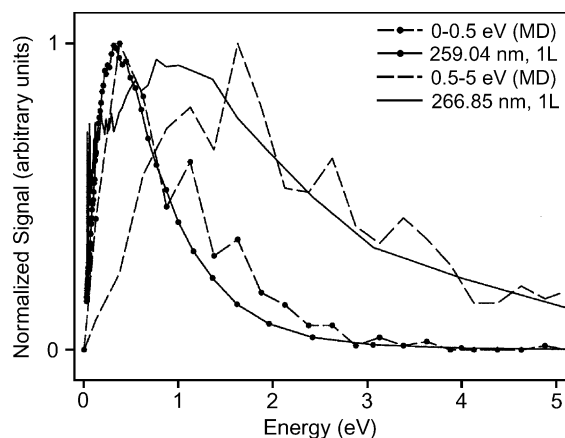


Fig. 1. Experimental and calculated kinetic energy distribution for ground state and excited state benzene molecules desorbed from Ag{111}. The experimental values were obtained by two-photon resonant ionization of benzene. The calculated values were determined using MD with an EAM potential to describe the Ag–Ag interactions, a Brenner potential to describe the hydrocarbon interactions and a Lennard–Jones potential to describe the benzene–silver interactions. Details are provided in [4].

used successfully to establish the mechanism of desorption of bigger molecules from metal surfaces. For example, direct collisions are shown to be responsible for ejection of polystyrene polymer units up to 9000 Da [5]. In general, high action points associated with the bombardment process are found to be responsible for the correlated motion necessary to lift off the molecules without fragmenting them [6]. In most cases, the molecules are found to desorb from within 5–10 nm of the impact point. These observations set some sort of fundamental limit to high lateral resolution imaging experiments.

Although these studies indicate that MD calculations are beginning to provide mechanistic information on more complicated materials, there are still no theoretical models to describe what happens within a bombarded molecular solid. Experiments suggest that the sputtering mechanism may be influenced by reactions within the organic material. In many instances, the kinetic energy distributions exhibit a distribution with stronger thermal character than found for elemental targets [7]. There is promise that modeling can be extended to these more complicated systems in the near future. Preliminary calculations [8] have been completed for three layers of benzene on Ag{111}. A graphical image of one of the trajectories is shown in Fig. 2.

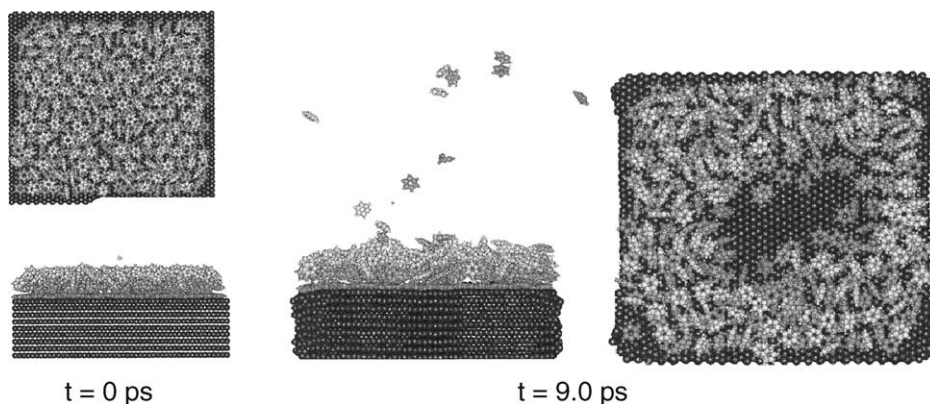


Fig. 2. Computer simulation of the 500 eV Ar bombardment of three layers of benzene on Ag{111}.

For these calculations, it is clear that most of the benzene molecules are desorbed intact leaving a void around the impact site. Moreover, the primary particle exchanges its energy with the film such that there is a lateral spread of the energy. This spread occurs via vibrational and translational energy transfer within the film and desorption is not initiated directly by collisions with substrate metal atoms. These calculations are now possible due to recent improvements in the potential function that include long range van der Waals interactions. With this correction, it also seems possible to begin to model the bombardment of a bulk crystal of benzene. Direct comparison with experiment seems imminent. Thus, even though these studies require a great deal of computer time, the theory is rapidly progressing to the point where more complex solids should be amenable to study.

3. Imaging TOF-SIMS applications

There are of course far too many TOF-SIMS imaging applications to review here. The examples have been chosen from areas where there might be the biggest future impact during the next decade. These include imaging of biological cells, applications in biotechnology and in molecular electronics.

3.1. Biological cells

The lateral resolution and the sensitivity of TOF-SIMS suggest that it is feasible to acquire information

from single biological cells. This goal is particularly important since it is generally recognized that the biochemical properties of individual cells can be quite different than that associated with an ensemble of cells. For example, the neuroscience community is interested in the dynamics of single events during exocytosis, the process by which vesicles of chemical messengers fuse with the cell membrane and release their content into a synapse. Lipid molecules of vesicles and cell membranes have an implicated role in the dynamics of this fusion and release. Traditional biochemistry purification techniques have shown that vesicles of rat adrenal cells contain higher concentrations of phosphatidic acid and lower concentrations of phosphatidyl choline when compared to the cell membrane [9]. Cholesterol has been shown to increase rigidity and thereby reduce the functionality of cells after incubation with amyloid- β protein, the protein that plays a key role in the progression of Alzheimer's disease [10]. Still, domains of native phospholipids have not been observed during dynamic events in cells. Consequently, imaging techniques that resolve subcellular distributions of molecules in cell membranes are required to observe the intricacies of these events.

A very basic model system, then, is to determine the distributions of membrane phospholipids in model systems like liposomes and red blood cells [11]. Since TOF-SIMS is a surface sensitive technique, freeze-fracture methods must be employed to provide a freshly exposed cell membrane surface for analysis. To allow TOF-SIMS imaging of lipids in cultured cell

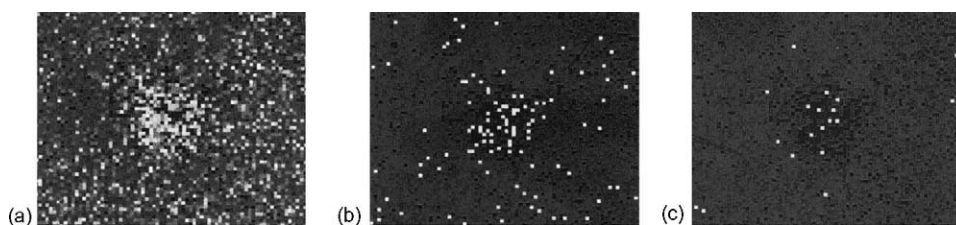


Fig. 3. Positive ion molecule-specific images of freeze-fractured PC12 cells. Ions of interest are plotted as yellow intensity overlaid on water plotted as blue intensity. A 50 μm wide field of view: (a) 69 m/z $[\text{C}_5\text{H}_9]^+$; (b) 184 m/z (phosphocholine headgroup $[\text{C}_5\text{H}_{15}\text{NPO}_4]^+$); (c) 386 m/z (cholesterol molecular ion).

systems, particularly those with neurological function, cell culture techniques have been developed to attach a submonolayer of single cells to a SIMS substrate. In situ fluorescence microscopy has been added to the TOF-SIMS instrument so single cells or organelles can be fluorescently tagged and located in frozen samples prior to TOF-SIMS imaging. Regions of the cells exposed to the surface during freeze fracture exhibit unique chemical signatures in molecule-specific images. For example, the contrast between sodium and potassium ions allows the distinction between intracellular matrix and the extracellular environment. Molecule-specific images of the membrane of a single rat pheochromocytoma (PC12) cell including $[\text{C}_5\text{H}_9]^+$ hydrocarbon fragment, phosphocholine (a fragment of membrane phospholipids), and cholesterol are shown in Fig. 3.

These studies are still at a very early stage. The most difficult part of the work is centered around the sample preparation where it has been found that temperature control during freeze fracture is critical to maintaining the integrity of the spatial distribution of the chemistry. The initial results indicate that imaging is possible, but clearly, more sensitivity is needed to discern the distribution of subcellular features. Some of the possible solutions to these questions are given in Section 4. The importance of determining the location of small molecules in these systems cannot be overstated for elucidating a wide variety of biochemical pathways. If the protocols can be developed a bit further, many biology and pharmaceutical laboratories can be expected to invest in this type of research.

3.2. Biotechnology

Imaging TOF-SIMS experiments, when optimized, have the possibility of completing chemical assays

extremely rapidly. Typically, spectra are acquired at the rate of $10,000 \text{ s}^{-1}$ and if enough information can be acquired from a specific sample in a few cycles, a tremendous throughput can be envisioned. Rapid screening is the underlying issue associated with modern biotechnology research where collections of millions of different chemicals may be under investigation. There are several examples that illustrate the power of TOF-SIMS to contribute to this area in the coming decade. For clinical assays, it is feasible to think about creating biochips which contain many thousands of samples. In Fig. 4, picoliter vials have been etched into a Si wafer at a density of $40,000 \text{ cm}^{-2}$. Using robotics, the vials are filled with 500 fm aliquots of crystal violet molecules and are

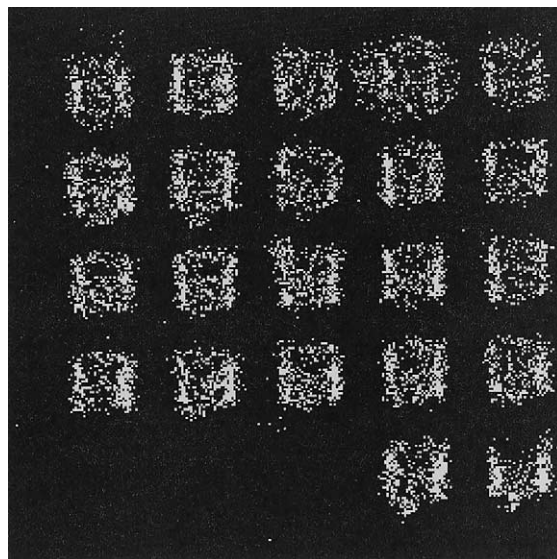


Fig. 4. The TOF-SIMS image of picoliter vials filled with 500 fm of crystal violet over a 100 μm field of view. The $[\text{M} + \text{H}]^+$ ion is monitored.

inserted into the TOF-SIMS machine for imaging [12]. The results suggest that mass spectra may be acquired from each vial in times considerably less than 1 s. One can imagine collecting thousands of clinical samples where similar information is needed from each sample and creating such a surface for rapid screening. Similar ideas may be applied to the area of combinatorial chemistry where diverse chemical libraries of millions of different potential drug molecules are synthesized on 60 μm polymer resin beads. These molecules then need to be identified after screening against appropriate biological receptors. As shown in an accompanying article [13], TOF-SIMS imaging yields readable mass spectra at a rate of better than 400 beads s^{-1} .

Genomics offers perhaps the most exciting challenge for TOF-SIMS imaging. Although the technique is not very good at detecting DNA fragments directly, very clever strategies for detecting DNA binding have been developed. The idea is to bind single-stranded peptide nucleic acid (PNA) of a given nucleic acid sequence to a standard DNA chip [14]. The PNA molecule is identical to DNA except that the phosphate backbone is missing in it. When binding to a complementary strand of DNA occurs, the imaging TOF-SIMS instrument easily detects the phosphate signal.

These types of experiments are still at an early stage, but they clearly show how the combination of imaging and speed makes this technology very exciting in the biotechnology world.

3.3. *Molecular electronics*

Organic molecules have recently been synthesized that change their electrical properties with an applied external electric field. These molecules, known as molecular wires, form the basis for electronic devices consisting of just a few molecules. There is no doubt that TOF-SIMS imaging experiments will play an essential role in characterizing electronic devices constructed from these unique molecules. The initial problem is to attach electrical contacts to spatially confined monolayers such that current can be applied between the contacts. Early experiments have focused on using alkane-thiols on gold substrates as one side of the contact. As it turns out, TOF-SIMS measurements are uniquely suited to characterizing the chemical

reactions that occur when metal atoms react with the organic molecule. For example, when Al atoms are deposited onto a self-assembled monolayer (SAM) of an alkane-thiol, we find that they react either with the surface functional group, with the hydrocarbon backbone, or penetrate the SAM and deposit on the Au substrate. By controlling the nature of the functional group, we have discovered that it is possible to control where the deposited metal ultimately resides [15]. This flexibility allows systematic choice of metals and organic functional groups which optimize the metal–organic contact. An example of this type of chemistry is given in another article in these proceedings [16]. These unique signatures will be invaluable for assessing the chemistry of fabricated devices on the nanometer scale.

4. Prospects for more

The significance of imaging TOF-SIMS experiments to date is certainly tantalizing. To realize the full potential of this methodology, of course, it is clear that advances are needed both with respect to sensitivity and perhaps with respect to finding novel configurations associated with ion/beam surface interactions. There is no shortage of ideas on this front.

4.1. *Cluster ion beams*

It is now well-known that cluster ion sources have the potential to increase ion yields by up to 1000-fold in some cases, and many examples are presented in these proceedings. Although this approach clearly has its advantages for TOF-SIMS measurements, carrying the ideas over the imaging arena is more problematic. Only the liquid metal ion source provides the necessary brightness and probe size necessary for the TOF-SIMS configuration. Yet Ga^+ ions are among the worst projectiles for producing high mass ions. Recently, In^+ liquid metal ion sources have been shown to improve performance by about a factor of four, although these sources still are more finicky to operate. There is promise for Au^+ projectiles, a particularly interesting development since this source is also capable of generating enough current of dimer and trimer ions for imaging purposes [17,18].

Other cluster ion beams such as SF_5^+ ions exhibit enhancement of high mass ions, but so far, sub-micron imaging has not been feasible since the sources require traditional ion optical focusing. Recently, however, a C_{60}^+ ion source has been developed in prototype with a probe size in the $1\ \mu\text{m}$ range [19]. Design improvements may indeed be able to reduce this size further. Developments of this sort will clearly be essential in the coming years to expand imaging options.

There is another strategy that could provide breakthrough capabilities for molecular imaging. In recent work, Gillen and coworkers have shown using a C_8^- ion source that the yield from amino acid thin films is higher than the amount of accumulated ion beam damage. In this situation, molecular information may be acquired in dynamic SIMS mode [20]. So far, they have only used ion sources that can be focused to a few microns, but since such high brightness is not required for their dc ion beam, aperaturing to achieve smaller spot sizes should be feasible. This would open molecular imaging experiments to such instruments as the Cameca NanoSIMS equipment.

4.2. Laser postionization imaging

Ionization probabilities for desorbed molecules are often very small, perhaps less than 10^{-4} . Several groups have attempted to utilize high power lasers to ionize the neutral molecules in the desorption plume, either using a resonant or non-resonant photoionization scheme. Neutral molecules are generally believed to be present in much higher concentration than the secondary ions. Moreover, the influences of matrix ionization effects are often reduced when considering neutral molecule desorption. Although this strategy has not yet found widespread appeal, there are a number of situations where laser postionization experiments yield improved imaging performance. In our laboratory, we utilize a 1 kHz repetition rate ultra-short pulsed laser to non-resonantly photoionize desorbed atoms and molecules using 800 nm radiation. The high repetition rate is extremely important for imaging applications where more than 65,000 mass spectra are required to create the picture. Two examples are shown in Fig. 5. The first case involves the simple situation of a copper grid covering an indium metal substrate.

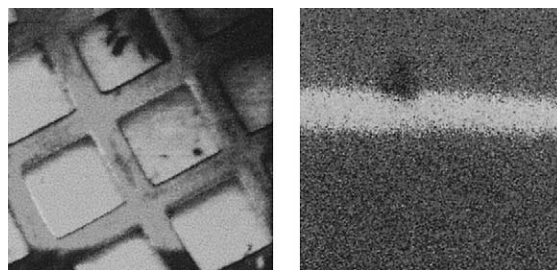


Fig. 5. Laser postionization imaging of atomic species In and Cu (left) and a PEM molecular monolayer (right). See text for details.

Both elements are ionized with the laser with a useful yield of more than 1% so it is feasible to achieve high contrast pictures with several thousand counts in each pixel. The second image is from a SAM of phenethylmercaptan (PEM) adsorbed onto gold. The line is written with indium atoms and the remaining signal consists of characteristic PEM ions. The field of view is $200\ \mu\text{m}$ and the primary 15 keV indium ion dose is 10^{-12} . Aromatic molecules generally yield the most signal, although there have been some very impressive images produced from phospholipid monolayers which have segregated into domains [21]. These experiments are particularly significant since traditional fluorescence labeling alters the behavior of the film formation process. Hence, it is expected that laser-based technology will play an increasing role in imaging, especially as high repetition rate lasers become available at a much lower cost.

4.3. Ion correlation and SIMS

There may be emerging SIMS imaging techniques which provide spatially resolved mass spectra without using focused ion beams at all. In a series of elegant experiments, Schweikert and coworkers [22] have developed ways to determine whether a pair of ions originate from a unique ion impact. When two ions are found to correlate, it is then known that they originated from within several nanometers of each other. Using this scheme, the Schweikert group was able to determine the association of Cs atoms with nanometer-sized Ag particles on a silica substrate—the catalyst used in ethylene oxidation. Other variations on the idea seem possible. For example, there should be a fixed time relation between SIMS ions emitted from the same primary ion, so it ought to be possible to

acquire TOF spectra without using a pulsed beam at all. Finally, there have been preliminary attempts at utilizing the recombination mechanism associated with cluster formation to get an idea of the local chemistry at boundaries between one type of molecule and another. This idea was tested using alkane-thiols on gold surfaces. For this experiment the AuM₂ species were examined using two different thiols, methyl-terminated or hydroxyl-terminated. The mixed dimer, AuM₁M₂, was formed only when the two components were intimately mixed. These types of schemes are appealing since they do not depend upon using a focused beam, and they provide special information in the 1–10 nm domain. Being able to work in this regime is very important for contribution to the emerging field of nanotechnology.

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