Nanoscale chemical imaging of biomaterials with mass spectrometry: A Tutorial

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Imaging SIMS - a brief retrospective

- Molecular desorption, static SIMS and quadrupole mass analyzers – Benninghoven 1968-1982
- Fast atom bombardment – Barber 1976
- TOF-SIMS – Standing and Benninghoven 1981
- Liquid metal ion source for imaging – Briggs 1988
- Cluster ion sources – Appelhans, Delmore, Schweikert 1989
Bioimaging (the killer app?) and the need for cluster sources

- Possible to acquire images at the (sub) cellular level
- Not much stuff in each pixel (10^6 molecules/μm²)
- Restricted mass range with SIMS often limits assay to fragment ions
Polyatomic Ion Sources have transformed SIMS in less than 6 years

- Low penetration depths and high sputter yields result in less accumulated beam damage
- $E_c = E_o(M_c/M_i)$ → energy of atoms $<\text{ energy polyatomic ion (low penetration depth) }$
- Dissociation of $\text{SF}_5^+$ → high local E density (sputter yield improved)

Comparison of $\text{SF}_5^+$ and $\text{Ar}^+$ Bombardment of an Organic Thin Film

$\text{SF}_5^+$

SY = 297 molecules/ion
Range = 4.4 nm

$\text{Ar}^+$

SY = 8 molecules/ion
Range = 12.3 nm

5.5 keV impact at 42° incident angle

Cluster projectiles in play

- $\text{Au}_x^+; x=1, 3$ and sometimes larger numbers $\text{m/z} 197, 591$
- $\text{Bi}_x^+; x=1, 3, 5$ and $y=1, 2$; $\text{m/z} 209, 627$
- $\text{Au}_{400}^{4+}; \text{m/z} 19, 700$
- $\text{SF}_5^+; \text{m/z} 126$
- $\text{C}_{60}^+, \text{C}_{60}^{++}, \text{C}_{60}^{+++}; \text{m/z} 720$
- Argon clusters, where $x=500->$
- Electrosprayed particles of micron size; $\text{m/z} ???$
What kind of impact can imaging SIMS make on Biology and the understanding of biological surfaces?
Phospholipids are a good models since they are present at high concentrations in the cell membrane.
Examining Lipid Heterogeneity Using *Tetrahymena*

- Mating involves formation of hundreds of fusion pores in a ~8 µm membrane junction region.
- Entire junction region may have a different lipid composition from the cell body.

Cells kindly provided by Dr. Craig Van Bell (Edinboro University)
Structures of Lipids and Corresponding Fragment Ions

**Phosphatidylcholine (PC)**

**2-aminoethylphosphonolipid (2-AEP)**

- PC is cylindrical and forms planar surfaces
- AEP is conical and forces curved structures
Sample Preparation for Hydrated Samples

- Sample
- Silicon shard
- Silicon wafer

Polystyrene spacer beads

Cells

Freeze fracture

Fast freeze

LN$_2$

Sample

Liquid propane

Fresh surface for analysis with TOF-SIMS imaging

In-situ brightfield image of cells in ice
SIMS Images Demonstrate Lipid Heterogeneity Across Mating Junction (~100 μm field of view)

Line Scan Across Junction Demonstrates PC Heterogeneity

Phosphonolipid, m/z 126

Hydrocarbon, m/z 69

Phosphocholine, m/z 184

Does the membrane lipid composition drive its structure or does the structure determine the membrane lipid composition?
PC depletion is time dependent and not a precondition for fusion

1 hour following initiation

2 hours following initiation

3 hours following initiation

Distance (μm)

Kurczy, Piehowski and Ewing, submitted

Scale bar = 25 μm
Pore formation in mated *Tetrahymena* drives lipid domain formation

- Cells must be paired before they display domains.
- Domains do not form until the cells have become strongly paired and have begun to form pores.
- PC/SM concentration decreases to make the spontaneous curvature of the contacting layers negative, but this is not a precondition for fusion.
Investigating lipid interactions
Identifying contents of liquid phases
Understanding lipid “raft” formation

More domains from co-existing liquid lipid phases in Langmuir-Blodgett model systems

- Investigating lipid interactions
- Identifying contents of liquid phases
- Understanding lipid “raft” formation

Need more counts!!!!
The higher yields, reduced
damage accumulation and
submicron imaging capabilities
associated with cluster projectiles
promise to greatly expand the
mass range and applicability of
these type of studies.
Buckyballs ($C_{60}$) have been just the ticket to allow molecule-specific imaging in the 600-1000 m/z range for lipid profiling.

The primary ion is focused to a submicron spot to define the x,y coordinate of the impact point.

Each Carbon atom carries 1/60th of the total incident kinetic energy.

Lateral Resolution

40 keV $C_{60}^+$ Secondary Electron Images from a TEM Grid Finder

40 keV $C_{60}^+$ Lateral resolution – Line Scan Indicated by Green Arrow

Interface Width = 175 nm

Image, courtesy of Ionoptika
15 keV $C_{60} \rightarrow$ Ag(111)
Meteor Impact might be a close macroscopic analog.

Interstate 40 at exit 233
35 miles east of Flagstaff,
20 miles west of Winslow,
in Arizona, USA.
On Tuesday

- BJG - MD simulation theory and examples
- Arnaud Delcorte – Optimal cluster size

Other key groups:
- Postawa, Krakow
- Urbassek, Kaiserslautern
- Nordlund, Helsinki
- Webb, Surrey
- Matsuo, Yamada and Aoki, Kyoto
More disruption with Ga – look deep!

15 keV Ga
Yield 21

15 keV C_{60}
Yield 324

$t=29$ ps

Larger volume is altered by Ga

There is new physics associated with this projectile

1. Enormous desorption yields, particularly of soft organic materials, i.e. biomaterials.

2. Molecular depth profiling is feasible by erosion with $C_{60} \rightarrow 3$-dimensional imaging.

3. During erosion, topography formation and interface mixing is minimal - think about characterization of complex multilayer structures.
## Yield of neutral molecules

<table>
<thead>
<tr>
<th>Removed # of H₂O Equivalents</th>
<th>Au⁺</th>
<th>Au₂⁺</th>
<th>Au₃⁺</th>
<th>C₆₀⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>575</td>
<td>1190</td>
<td>2510</td>
</tr>
</tbody>
</table>

Yields determined by QCM from 500 nm film of amorphous ice deposited onto Silver.

25 keV gold, and 20 keV ☻

Yield of ionized molecules

Molecular ion intensity is sufficient for imaging 50 micron resin particles used in solid phase Combinatorial chemistry experiments.

Dynamically created pre-formed ions (DCPI): Proton buildup from previous hits.

**Key reaction:** $M + H^+ \rightarrow MH^+$

**H$_3$O$^+$ / H$_2$O$^+$ ratio**

- From an organic thin film (Trehalose)

**Graph:**
- **X-axis:** $C_{60}^+$ ion fluence (ions/cm$^2$)
- **Y-axis:** Ratio

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X. Conlan, N. Lockyer and J. Vickerman, RCMS, 2006
2. Molecular depth profiling feasible in some cases
Treholose/Peptide model system

- Trehalose
- 2 nm rms surface roughness as determined by AFM

Mix peptide with trehalose

Spin coat solution on 5 × 5 mm Si wafer

Molecular depth profiling

Erosion Dynamics

\[ Y_{tot} \gg n d \sigma_D \]

damage depth \( d \)

\( v_{erode} \)

\( f_{sputter} \)

\( f_{supply} \)

\( f_{damage} \)

**Erosion Dynamics**

\[ Y_{tot} : \text{total sputter yield} \]
\[ n : \text{number density of molecules} \]
\[ \sigma_D : \text{damage cross section} \]

\[ S_{steady\ state} / S_0 = \left( \frac{Y_{tot}}{Y_{tot} + n d \sigma_D} \right) \]

This protocol opens new possible sample preparation techniques since ice overlayers can be removed by ion beam etching.

40 keV C$_{60}^+$ bombardment of water-ice (m/z 18) covering a patterned film of cholesterol (m/z 369, M-OH$^+$) on silicon (m/z 28).

Piehowski, Ewing and Winograd
3. Depth resolution is a critical issue: Topography and interface mixing
Depth profiling of molecular multilayer structures

Depth profiling of multilayer structures

Counts Normalized to Total

Depth (nm)

- m/z28
- m/z112
- 10x m/z463
- 10x m/z525

DMPA (40 nm)
AA (54 nm)
DMPA (44 nm)
AA (62 nm)
Si

40 KeV 77 K
In 3-dimensions
Organic δ-layers serve as a wonderful model system for evaluating the parameters that affect depth resolution.

Monolayers of Irgonox 1010 imbedded into Irgonox 3150 at depths of 46, 92, 182 and 270 nm. Samples now utilized as a VAMAS standard for interlaboratory comparisons.

LB \( \delta \)-layers: membrane bilayer mimics

Dimyristoyl Phosphatidate (DMPA)

Arachidic Acid (AA)

2 layers DMPA 4.4 nm

44 AA 118.8 nm

44 AA 118.8 nm

43 AA 116.1 nm

362.5 nm
Lipid bilayer at 40° incidence, 298K and 77K

Depth Resolution (FWHM nm )

<table>
<thead>
<tr>
<th></th>
<th>First Delta Layer (121.0 nm)</th>
<th>Second Delta Layer (244.2 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>LN₂</td>
</tr>
<tr>
<td></td>
<td>39.3±1.3 nm</td>
<td>25.0±1.1 nm</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>LN₂</td>
</tr>
<tr>
<td></td>
<td>40.9±1.9 nm</td>
<td>24.8±1.2 nm</td>
</tr>
</tbody>
</table>

44 Layers AA 118.8 nm
43 layers AA 116.1 nm
Lipid bilayer at 71° incidence, 77K

Depth Resolution (FWHM nm)

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<th></th>
<th>First Delta Layer (121.0 nm)</th>
<th>Second Delta Layer (244.2 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angle (°)</td>
<td>71°</td>
<td>40°</td>
</tr>
<tr>
<td>Depth Resolution (nm)</td>
<td>20.5±1.0</td>
<td>25.0±1.1</td>
</tr>
<tr>
<td></td>
<td>21.7±1.0</td>
<td>24.8±1.2</td>
</tr>
</tbody>
</table>

44 Layers AA 118.8 nm
44 Layers AA 118.8 nm
43 Layers AA 116.1 nm
Depth Response Function

Dowsett’s semi-empirical function

\[ \frac{1}{G} = \frac{1}{g} - \frac{1}{d} + \frac{1}{\sigma} + \frac{1}{\lambda_g} \]

\[ \frac{1}{G} = \frac{1}{g} + \frac{1}{d} \]

\[ \frac{1}{G} = \frac{1}{\sigma} - \frac{1}{\lambda_d} \]

- \( \lambda_g \) Leading edge growth length – information depth of secondary ions
- \( \lambda_d \) Trailing edge decay length – related to ion beam mixing
- \( \sigma \) Standard deviation of a central Gaussian connecting the two exponential functions – convolution of all factors effecting depth resolution.

Depth Response Function

First Delta Layer (121.0 nm)  Second Delta Layer (244.2 nm)

<table>
<thead>
<tr>
<th></th>
<th>λ_g</th>
<th>λ_d</th>
<th>σ</th>
<th></th>
<th>λ_g</th>
<th>λ_d</th>
<th>σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>3.9±2.0</td>
<td>5.7±0.1</td>
<td>14.5±2.1</td>
<td>RT</td>
<td>10.0±1.2</td>
<td>6.3±4.5</td>
<td>15.4±2.5</td>
</tr>
<tr>
<td>LN2</td>
<td>13.0±0.4</td>
<td>7.4±0.2</td>
<td>10.0±1.2</td>
<td>LN2</td>
<td>13.5±1.5</td>
<td>10.3±1.3</td>
<td>7.1±0.4</td>
</tr>
</tbody>
</table>

**Room Temperature**

**Low Temperature**

<table>
<thead>
<tr>
<th>m/z 355 DMPA</th>
<th>Fit Curve 1</th>
<th>Fit Curve 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>44 AA 118.8 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43 AA 116.1 nm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FWHM 40.8 nm

FWHM 42.9 nm

FWHM 26.2 nm

FWHM 26.2 nm

 ave
Surface Roughness

RMS 3.5 nm
σ=7.7 nm
RMS 4.9 nm

Sample I

By Nanopics 2100 scanning area 10 x 10 µm
For L-B δ-layer systems

- Low temperature and glancing angles improves the depth resolution.
- AFM measurements and the asymmetric shape of response signal indicate mixing is the main factor determining the depth resolution.
- $\lambda_g$ is temperature independent.
- Mechanism behind the temperature effect and topography formation needs to be understood in detail. WEDGES!
Wedge sculpting with $C_{60}$ allows yield and topography vs fluence to be determined at each point.

A wedge angle of 0.05 allows enormous lateral magnification.

Dan Mao and Andreas Wucher
Depth to SIMS Imaging Transform

Simple trigonometry transforms a 3 nm delta layer into a 9 μm stripe in the xy plane.
SIMS During Wedge

Red: m/z 42 from Irganox 3114

Green: m/z 60 from Si Substrate

Dark Green: Irganox 1010 / Orange: Irganox 3114
Light Green: Si / Red: Imaging Surface
AFM Line Scan – Topography evolution

Original line substract 36-point Savitzky-Golay algorithm smoothed line, yield height fluctuation.
One AFM/SIMS scan provides yield, roughness and erosion rate as a function of depth.
The next critical issues for 3-D imaging

• Erosion rate needs to be known at each fluence. Propose wedges, or possibly *in situ* ellipsometry of some sort.

• For heterogeneous samples, i.e. biological cells, differential erosion rates will complicated the simple notion that images can be stacked.

• Let’s try an example →
Patterned Peptide Film for 3-D Imaging

Features written on trehalose (GGYR) thin film with Ga⁺ ion bombardment

Overlay mass spectrometry image with AFM image

A1, B1: AFM before erosion
A2, B2: AFM after erosion
A3, B3: ∑ Ga images
C1 = B1+B3
C2 = B2+B3
C3 = ∑ total of all ms images

Depth resolution can approach 3 nm
Examples of 3-D imaging are beginning to appear

Fletcher JS, Lockyer NP, Vaidyanathan S, Vickerman JC. 2007. TOF-SIMS 3D biomolecular imaging of Xenopus laevis oocytes using buckminsterfullerene (C_{60}) primary ions. Analytical Chemistry 79: 2199-206

And so...

- Phenomena associated with cluster mass spectrometry are changing the name of the game, both with respect to instrumentation and applications.
- 3-D imaging is the next big thing...
- Best conditions for good molecular depth profile, and depth resolution are being elucidated.
- Fundamentals of temperature dependence and topography formation still a mystery.
- Instrumentation poised for a change.