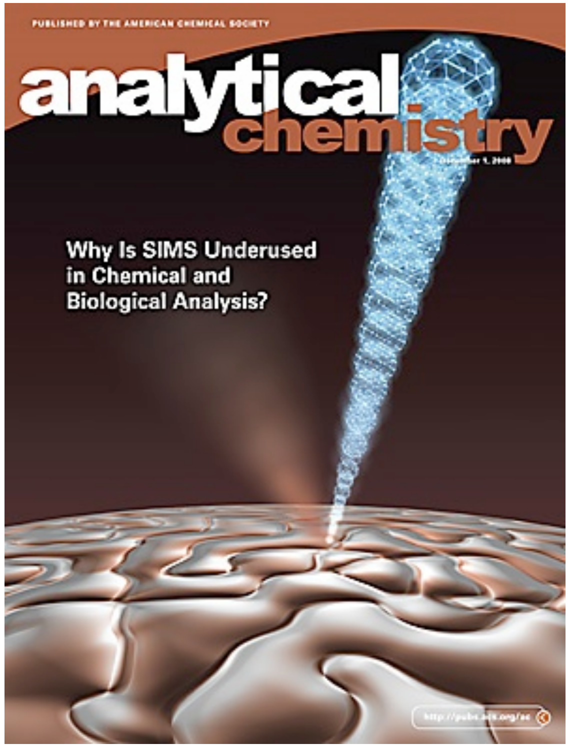
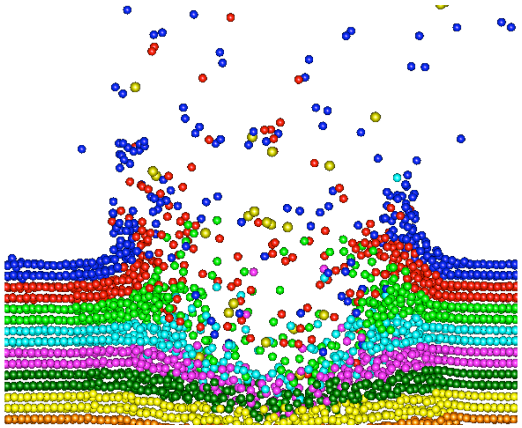
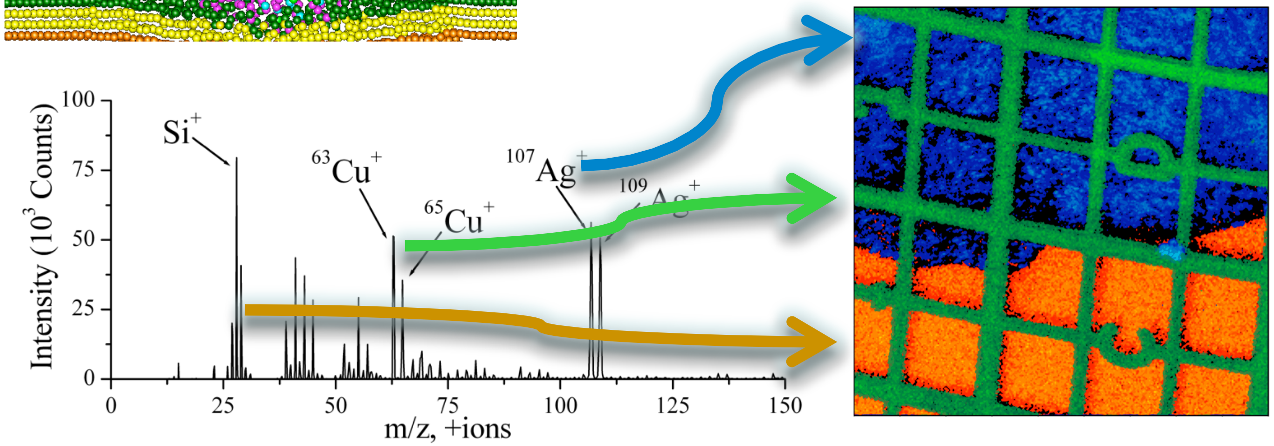
Nanoscale chemical imaging of biomaterials with mass spectrometry: A Tutorial

December 6, 2009





Nicholas Winograd
Department of Chemistry
Penn State University
http://nxw.chem.psu.edu

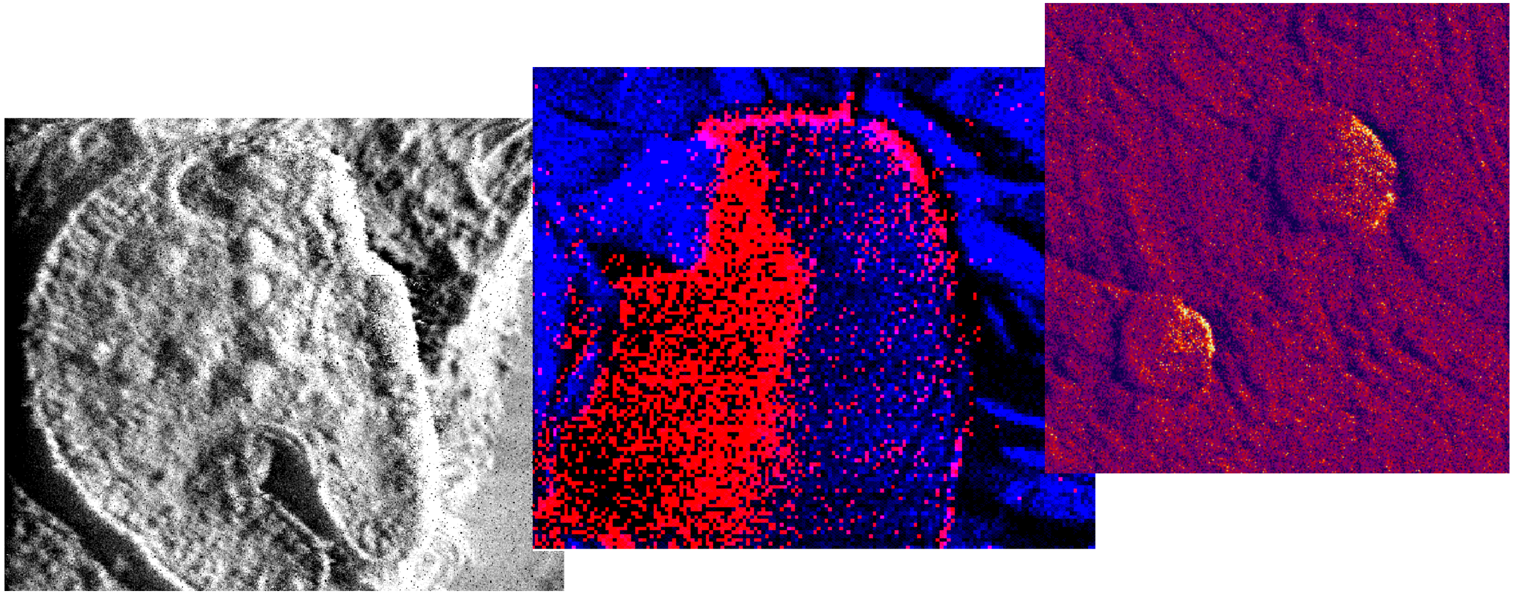


Imaging SIMS – a brief retrospective

- 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
- Availability of imaging cluster sources – SIMS XVIII, Nara 2001.

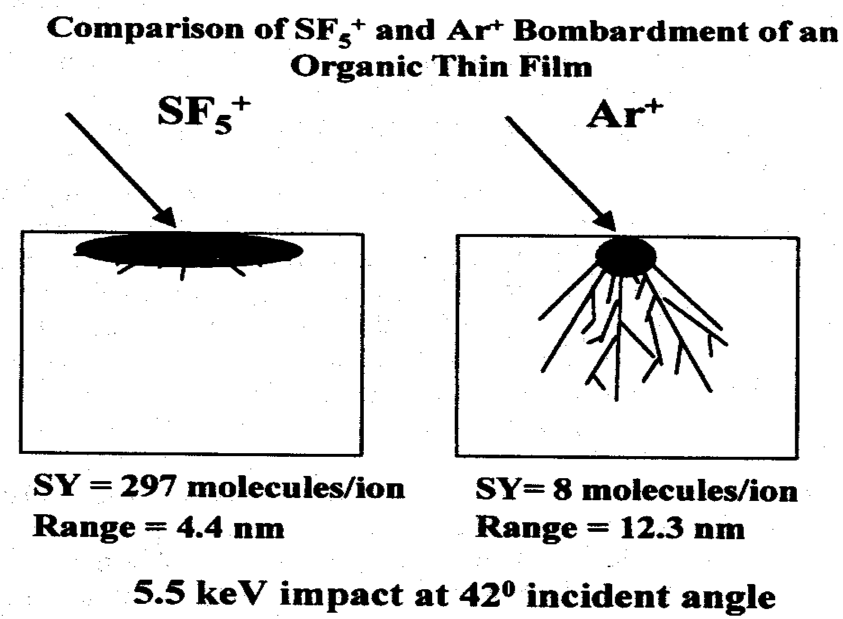
Bioimaging (the killer app?) and the need for cluster sources

- Possible to acquire images at the (sub) cellular level
- Not much stuff in each pixel (10^6 molecules/μm^2)
- 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
- $E_c = E_o(M_c/M_t)$ → energy of atoms< energy polyatomic ion (low penetration depth)
- Dissociation of SF_5^+ → high local E density (sputter yield improved)



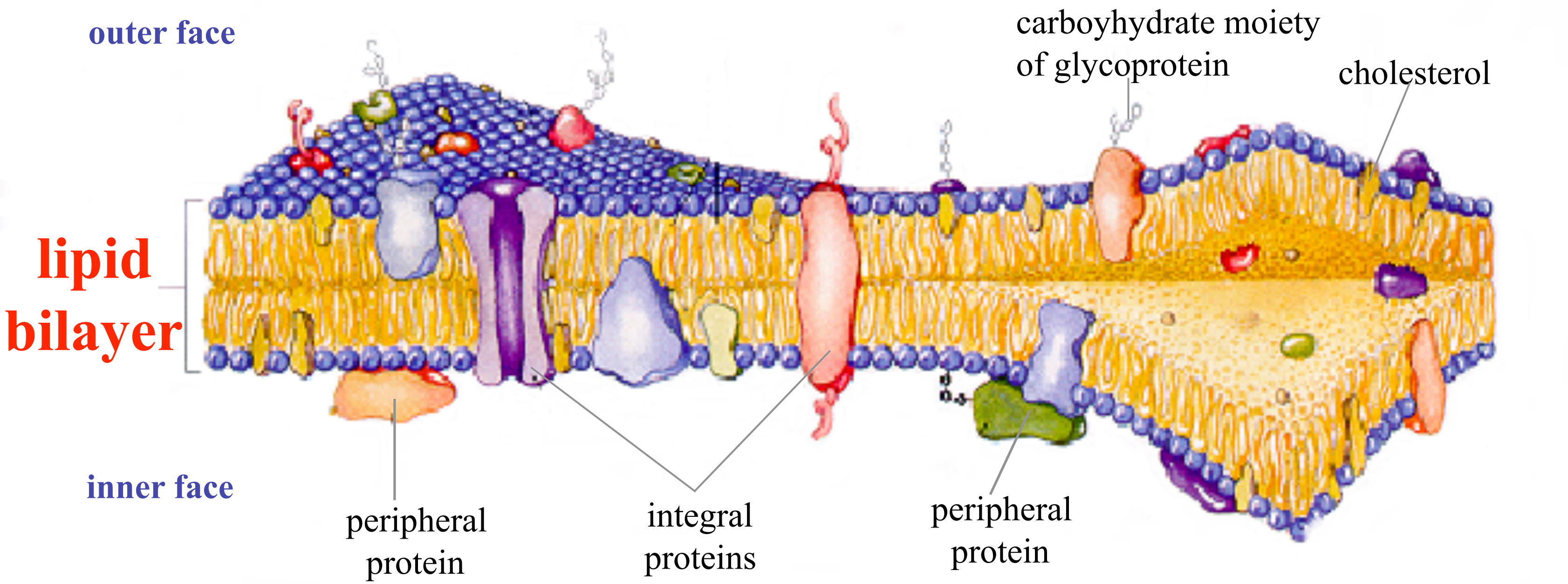
Gillen, G. *Rapid Commun. MS.* **12** (1998) 1303-1312

Cluster projectiles in play

- Au_x^+ ; x=1,3 and sometimes larger numbers
  m/z 197, 591
- Bi_x^{y+} ; x=1,3,5 and y=1,2 ; m/z 209, 627
- Au_{400}^{4+} ; m/z 19,700
- SF_5^+ ; m/z 126
- C_{60}^+,C_{60}^{++},C_{60}^{+++} ; m/z 720
- Argon clusters, where x=500->
- Electrosprayed particles of micron size ; m/z ???

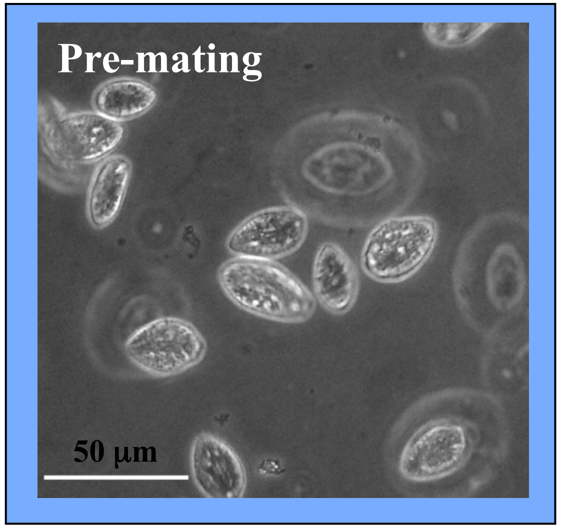
What kind of impact can imaging SIMS make on Biology and the understanding of biological surfaces?

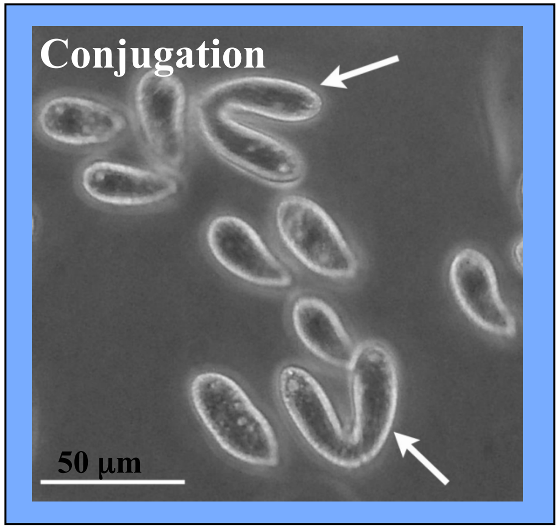
Phospholipids are a good models since they are present at high concentrations in the cell membrane



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.**

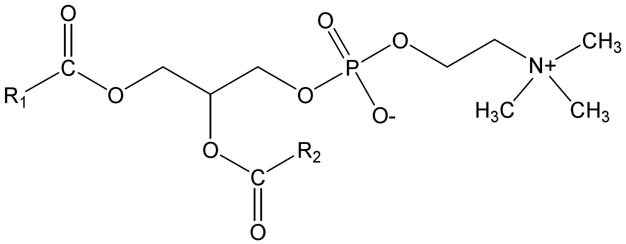


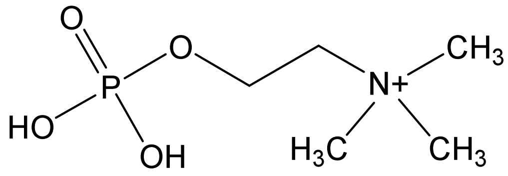


Cells kindly provided by Dr. Craig Van Bell (Edinboro University)

Structures of Lipids and Corresponding Fragment Ions

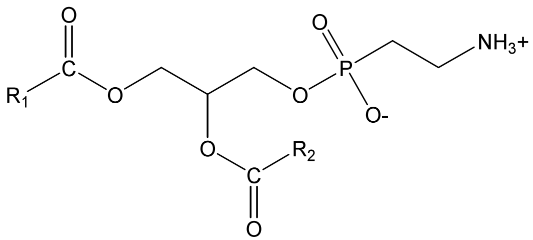
Phosphatidylcholine (PC)

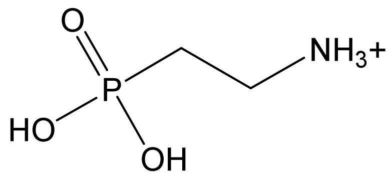




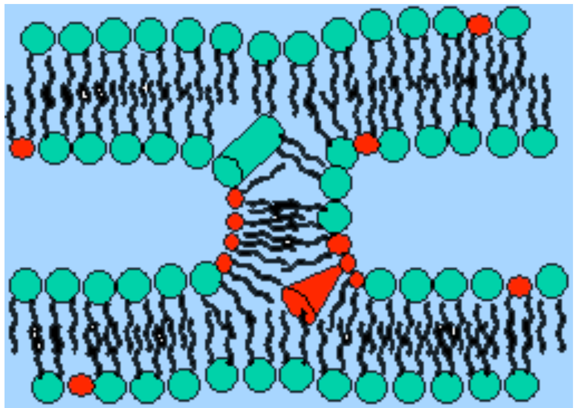
m/z 184

2-aminoethylphosphonolipid (2-AEP)



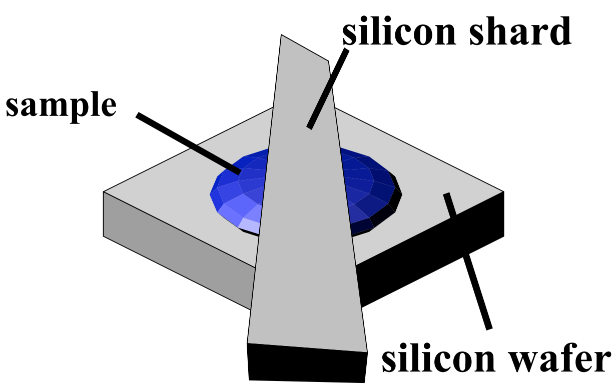


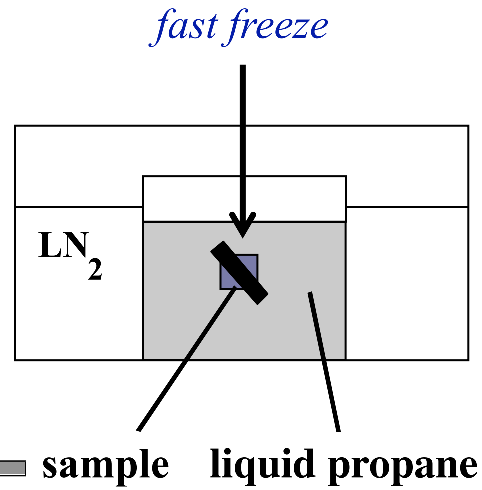
m/z 126

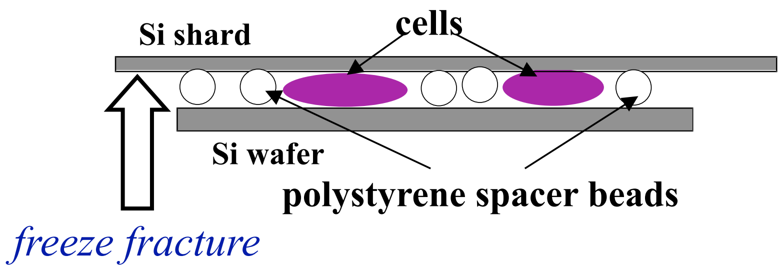


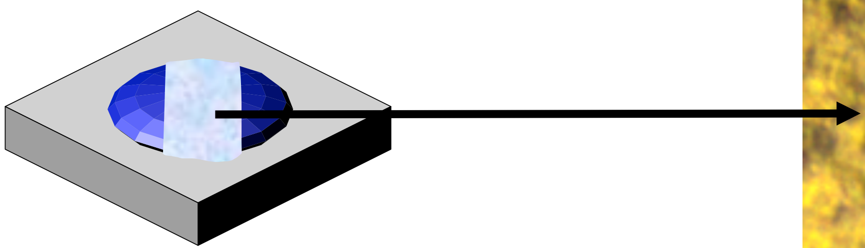
- PC is cylindrical and forms planar surfaces
- AEP is conical and forces curved structures

Sample Preparation for Hydrated Samples

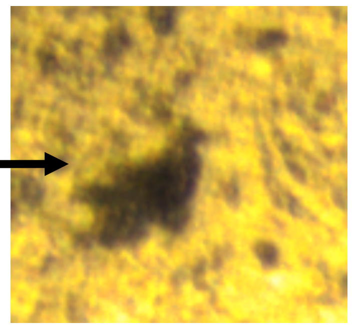






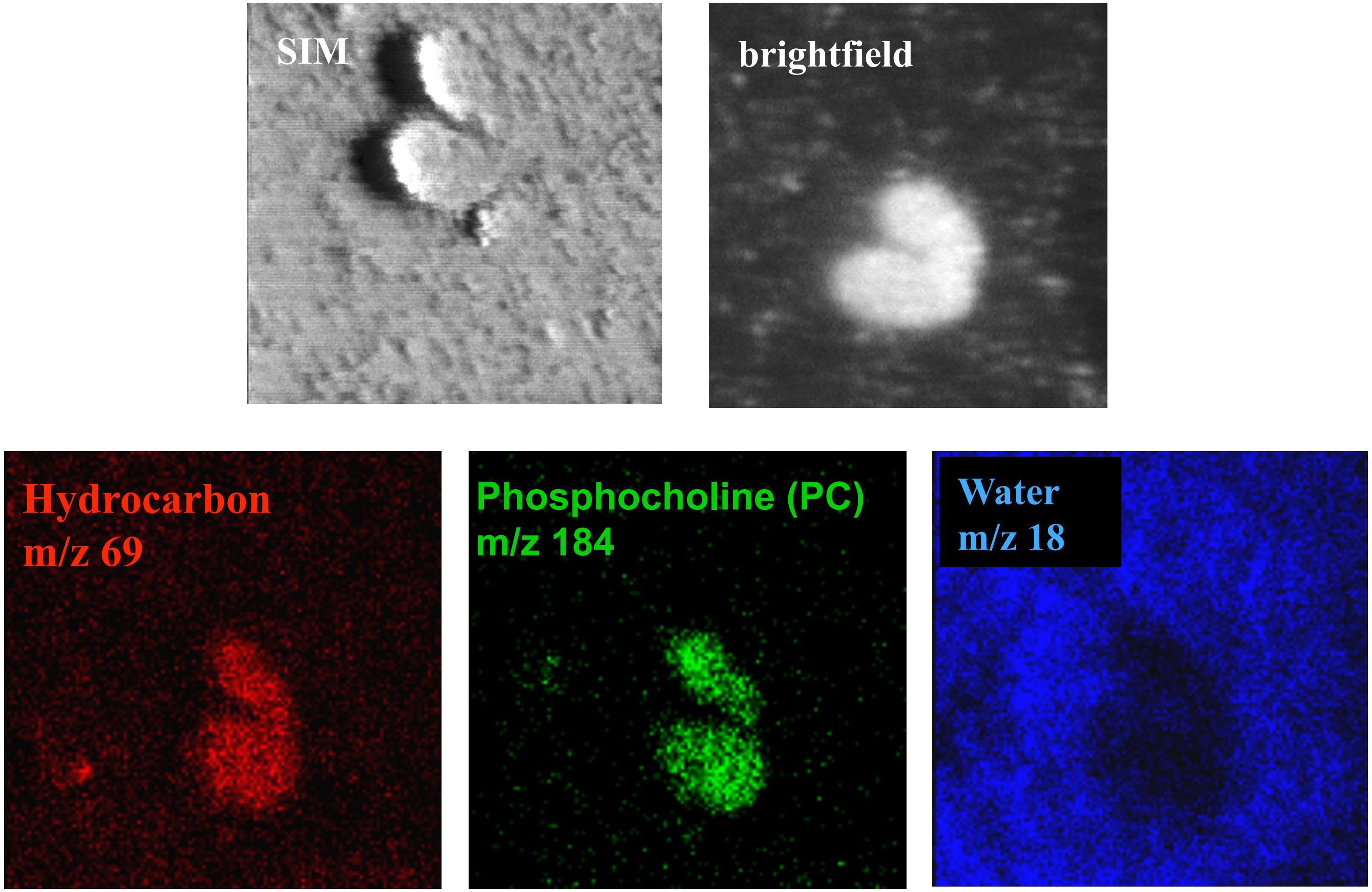


fresh surface for analysis
with TOF-SIMS imaging



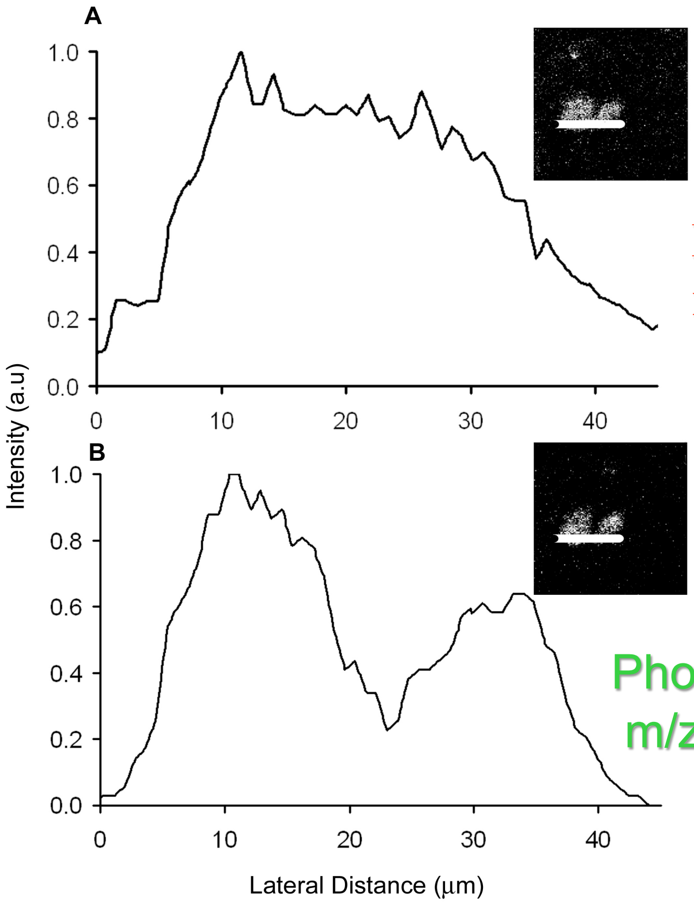
In-situ brightfield image of cells in ice

SIMS Images Demonstrate Lipid Heterogeneity Across Mating Junction (~100 μm field of view)



Ostrowski, Van Bell, Winograd and Ewing, *Science*, **305**, 71 (2004)

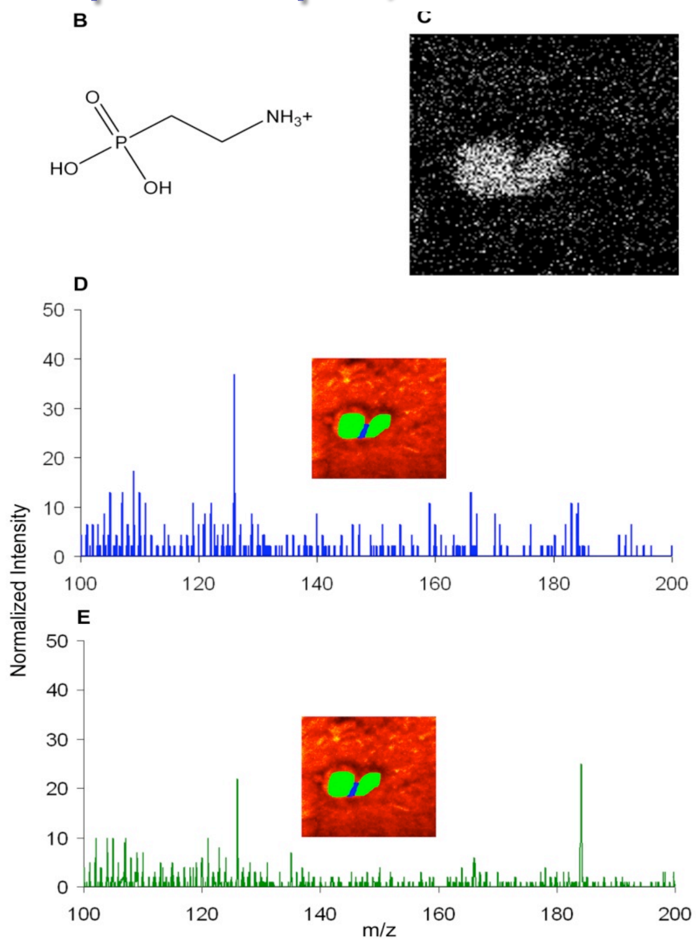
Line Scan Across Junction Demonstrates PC Heterogeneity



Hydrocarbon m/z 69

Phosphocholine m/z 184

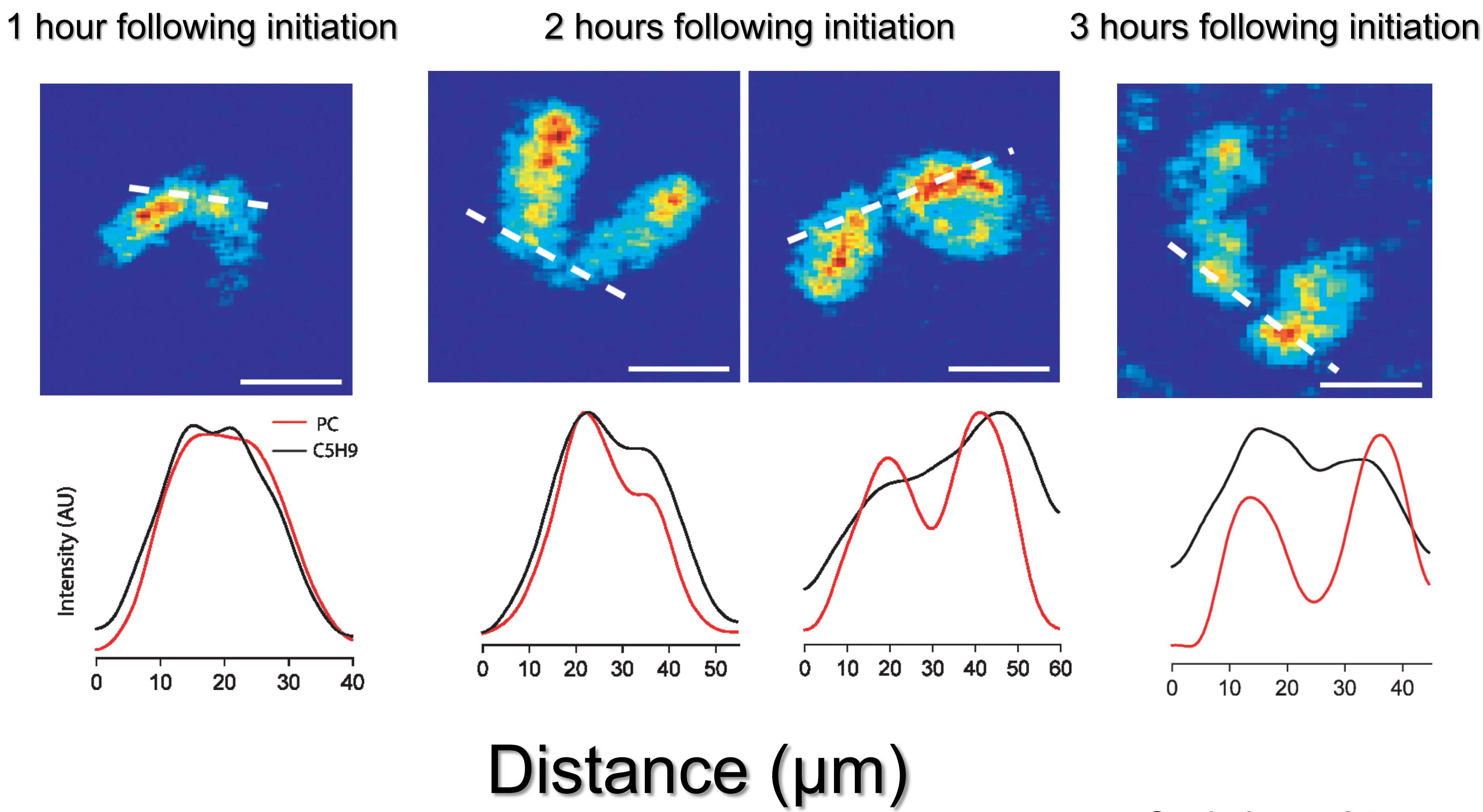
Phosphonolipid, m/z 126



Ostrowski, Van Bell, Winograd and Ewing, *Science*, **305**, 71 (2004)

Does the membrane lipid composition drive its structure or does the structure determine the membrane lipid composition?

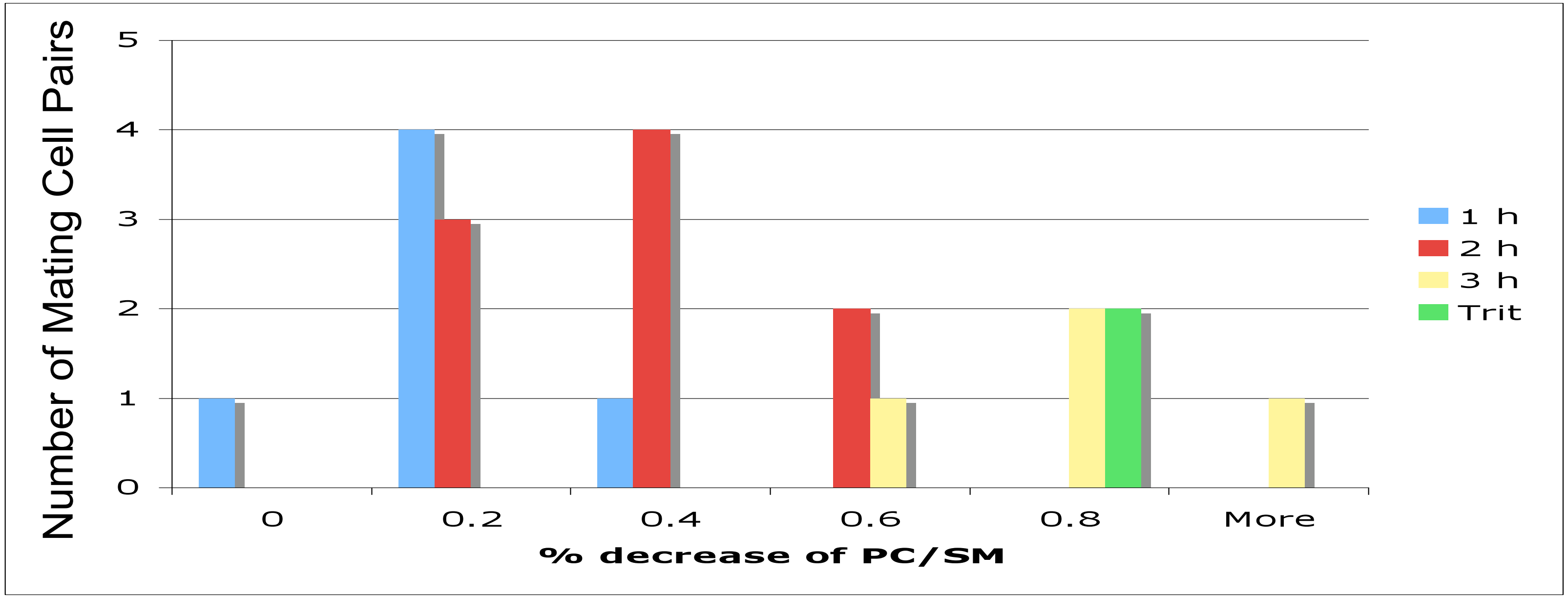
PC depletion is time dependent and not a precondition for fusion



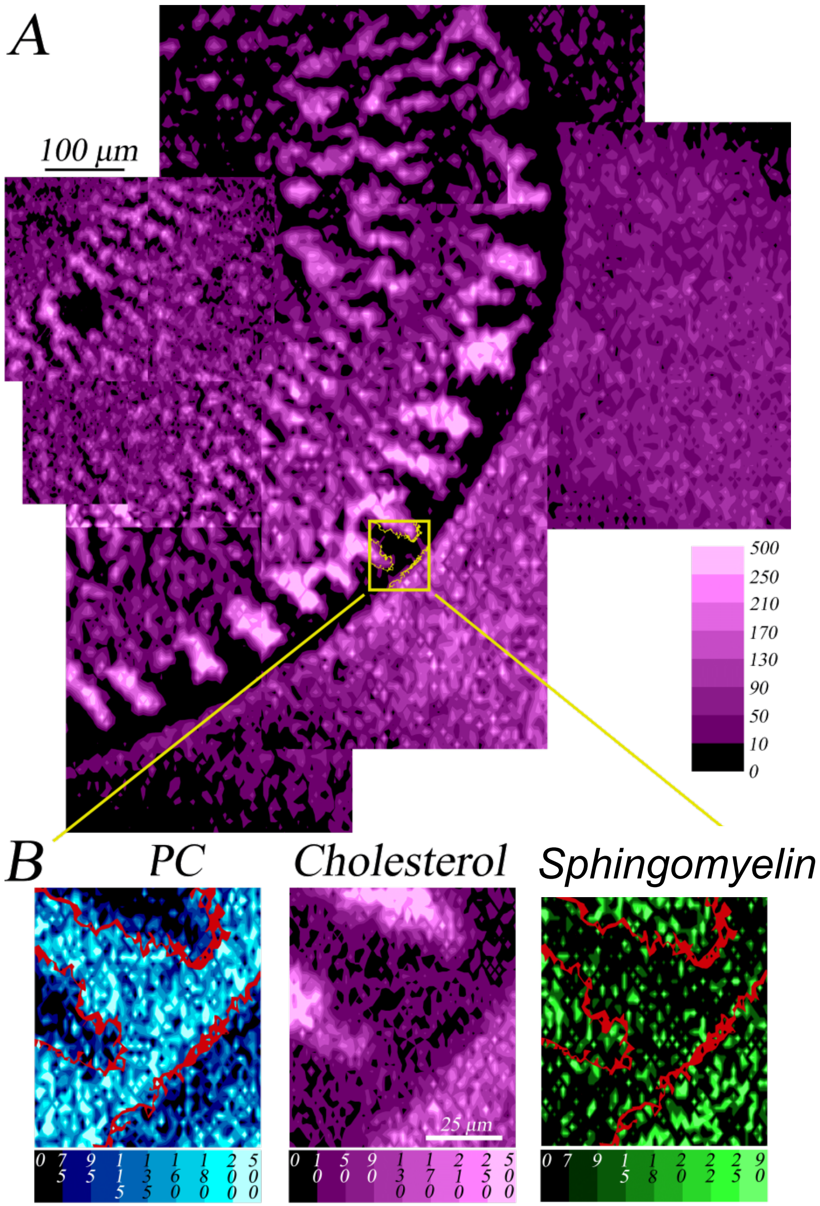
Kurczy, Piehowski and Ewing, submitted

Scale bar = 25 µm

Pore formation in mated *Tetrahymena* drives lipid domain formation

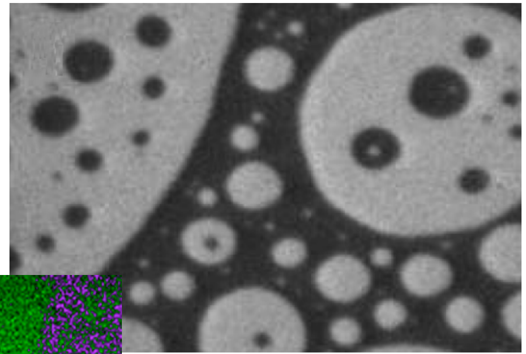


- Cells must be paired before they display domains.
- Domains do not form until the cells have become strongly paired and have begun to form pores.
- PC/SM concentration decreases to make the spontaneous curvature of the contacting layers negative, but this is not a precondition for fusion.

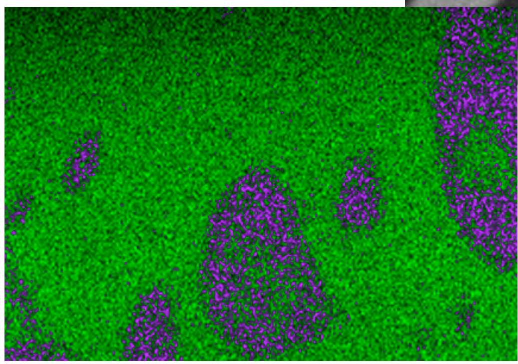


More domains from co-existing liquid lipid phases in Langmuir-Blodgett model systems

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



Stottrup, Stevens, Keller, *Biophys. J.* 88 (2005) 269



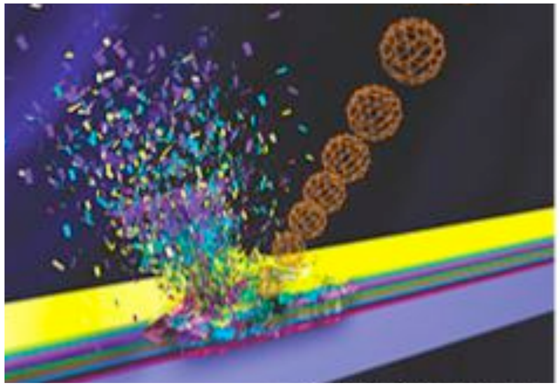
Sostarecz, McQuaw, Zheng, Ewing and Winograd, JACS, 2004, 2007 And Langmuir, 2005.

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

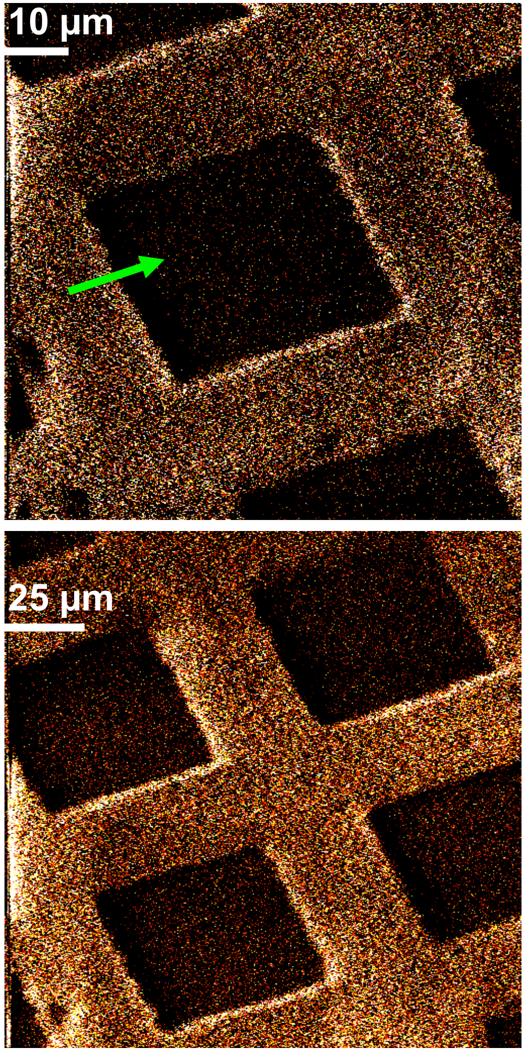


Each Carbon atom carries 1/60th of the total incident kinetic energy

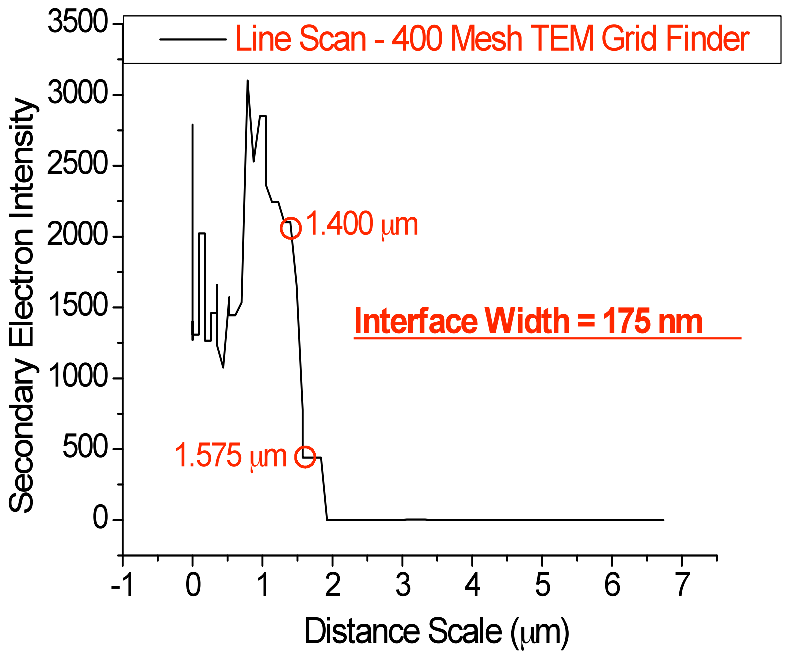
Weibel, Wong, Lockyer, Blenkinsopp, Hill and Vickerman, Anal. Chem., 2005.

Lateral Resolution

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

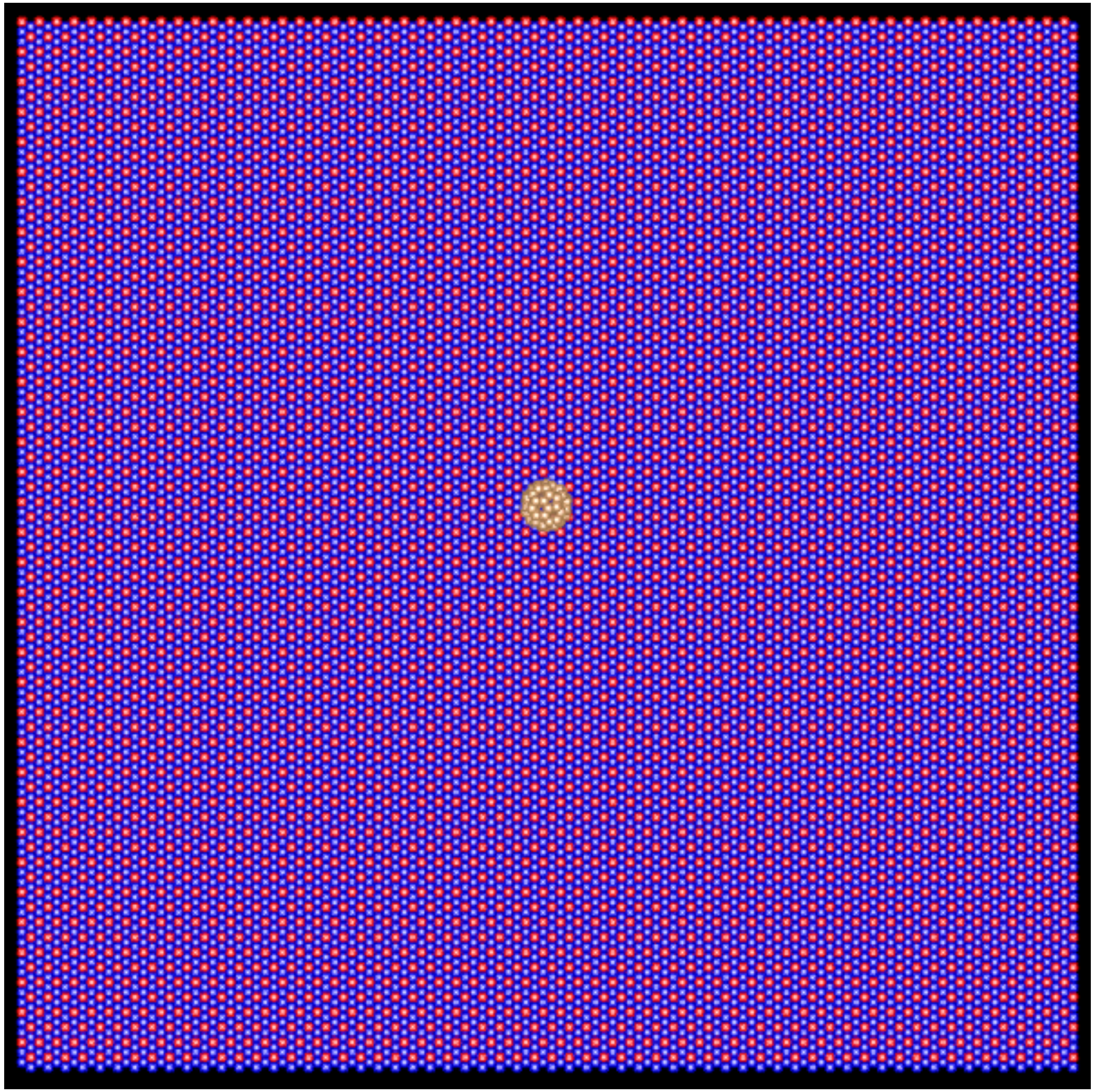


40 keV C_{60}^{+} Lateral resolution – Line Scan Indicated by Green Arrow

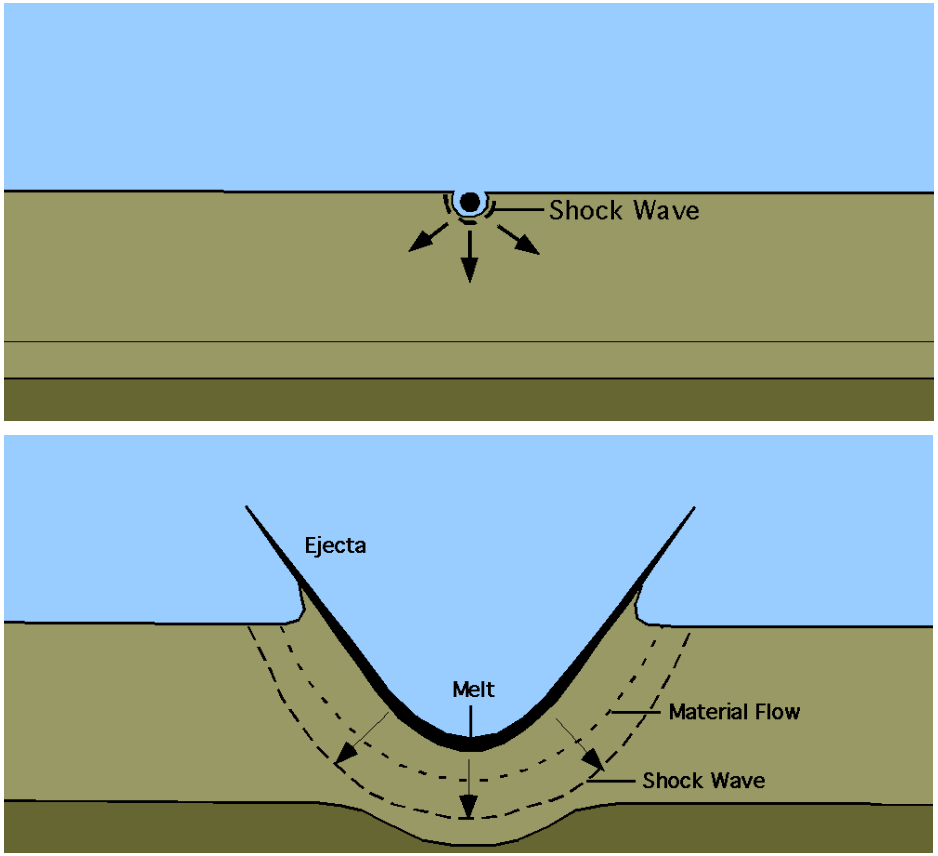


Image, courtesy of Ionoptika

15 keV C_{60} -> Ag(111)



Postawa and Garrison



Meteor Impact might be a close macroscopic analog.

OnTuesday

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

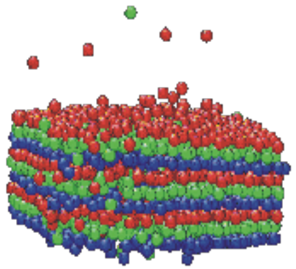
Other key groups:

- Postawa, Krakow
- Urbassek, Kaiserslautern
- Nordlund, Helsinki
- Webb, Surrey
- Matsuo, Yamada and Aoki, Kyoto

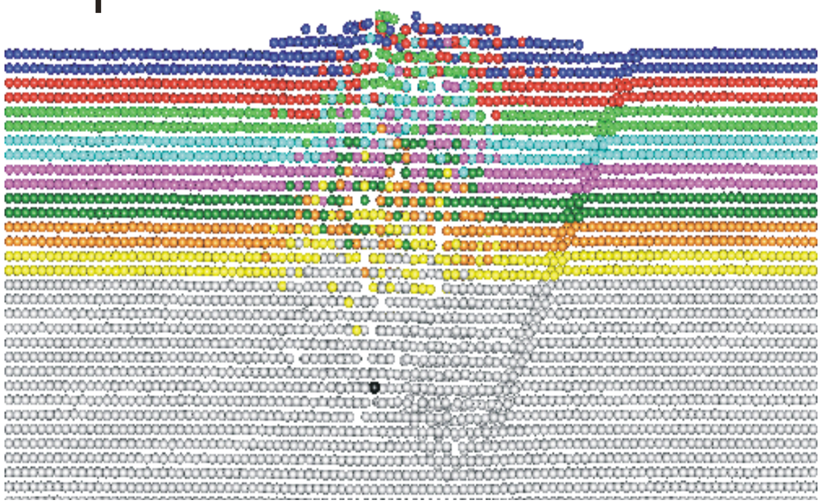
More disruption with Ga – look deep!

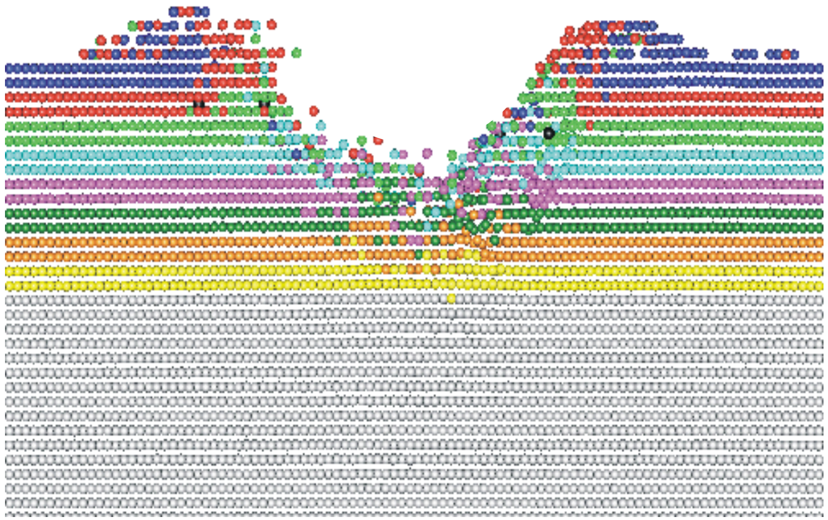
15 keV Ga
Yield 21

15 keV C_{60}
Yield 324



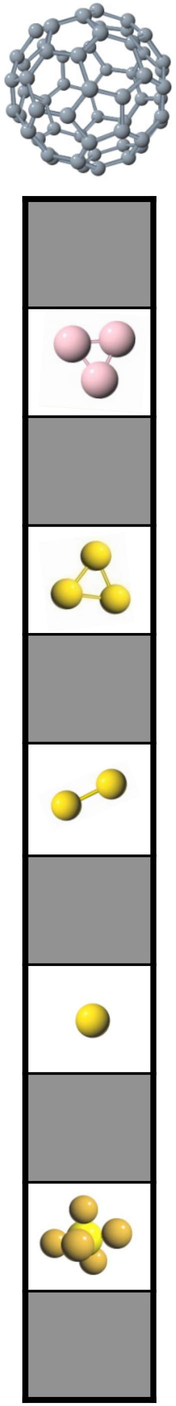
t=29 ps





Larger volume is altered by Ga

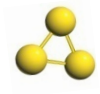
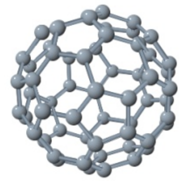
Postawa, Smiley, Winograd, Garrison et al., Anal. Chem., **75** 4402 (2003).



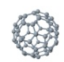
There is new physics associated with this projectile

1. Enormous desorption yields, particularly of soft organic materials, i.e. biomaterials.
2. Molecular depth profiling is feasible by erosion with C_{60} → 3-dimensional imaging.
3. During erosion, topography formation and interface mixing is minimal – think about characterization of complex multilayer structures.

Yield of neutral molecules

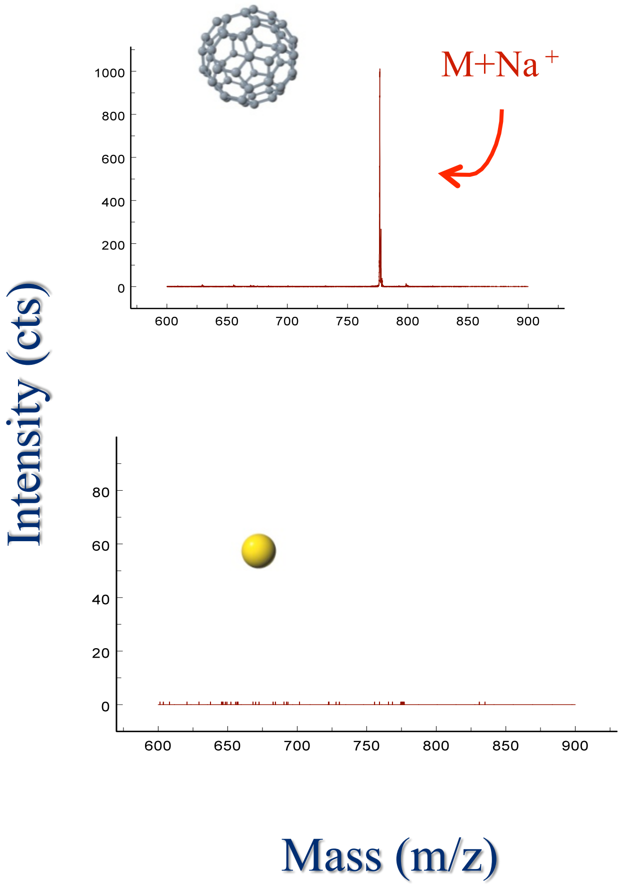
	Au^+ 	Au_2^+ 	Au_3^+ 	C_{60}^+ 
Removed # of H_2O Equivalents	**100**	**575**	**1190**	**2510**

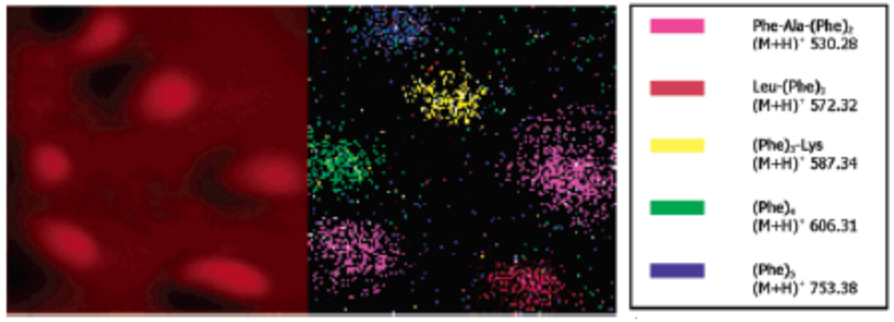
Yields determined by QCM from 500 nm film of amorphous ice deposited onto Silver.

25 keV **gold**, and 20 keV 

Szakal, Kozole, Russo, Garrison and Winograd, Phys. Rev. Lett., 2006.

Yield of ionized molecules

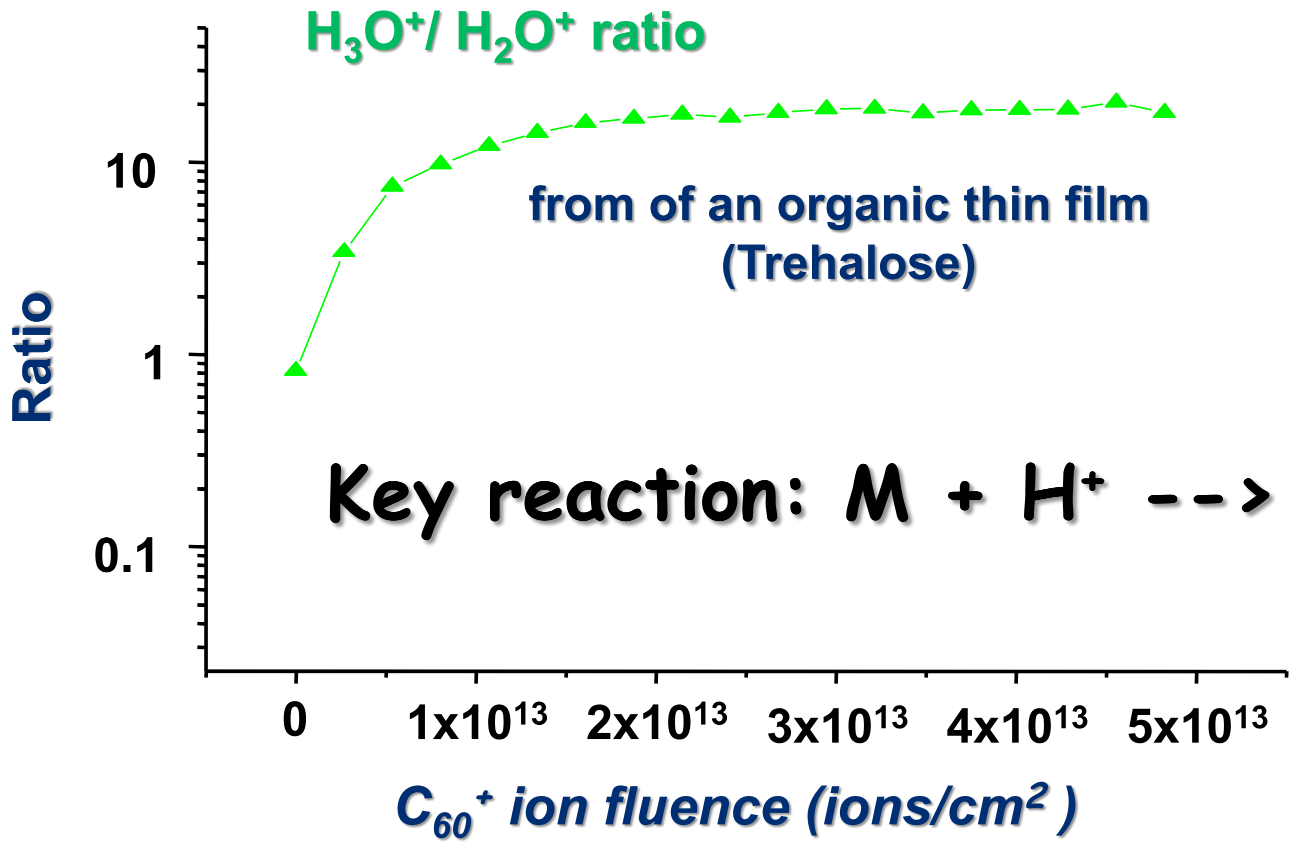


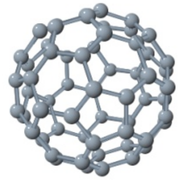


Molecular ion intensity is sufficient for imaging 50 micron resin particles used in solid phase Combinatorial chemistry experiments.

*Xu, Szakal, Martin, Peterson, Wucher and Winograd, JACS, **126**, 3902 (2004).*

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





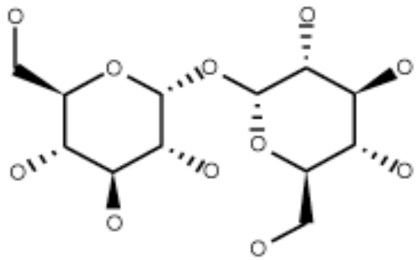
Cheng and Winograd, Anal. Chem., 2005

X. Conlan, N. Lockyer and J. Vickerman, RCMS, 2006

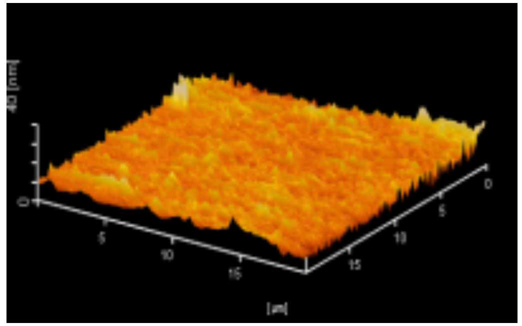
2. Molecular depth profiling feasible in some cases

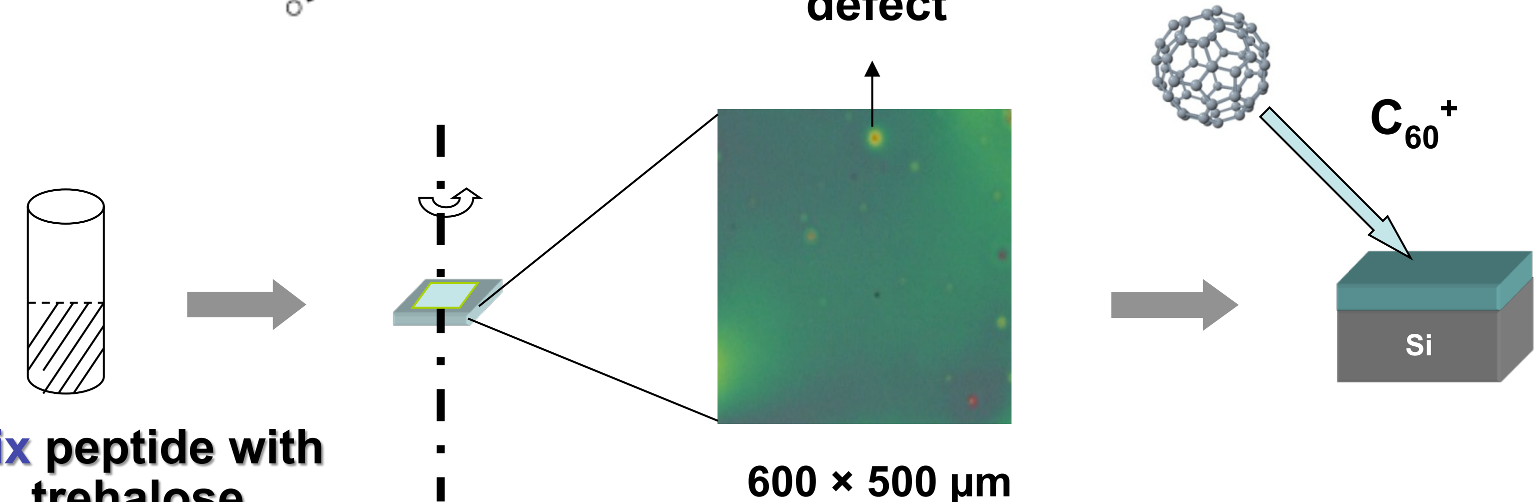
Treholose/Peptide model system

trehalose



2 nm rms surface roughness
as determined by AFM



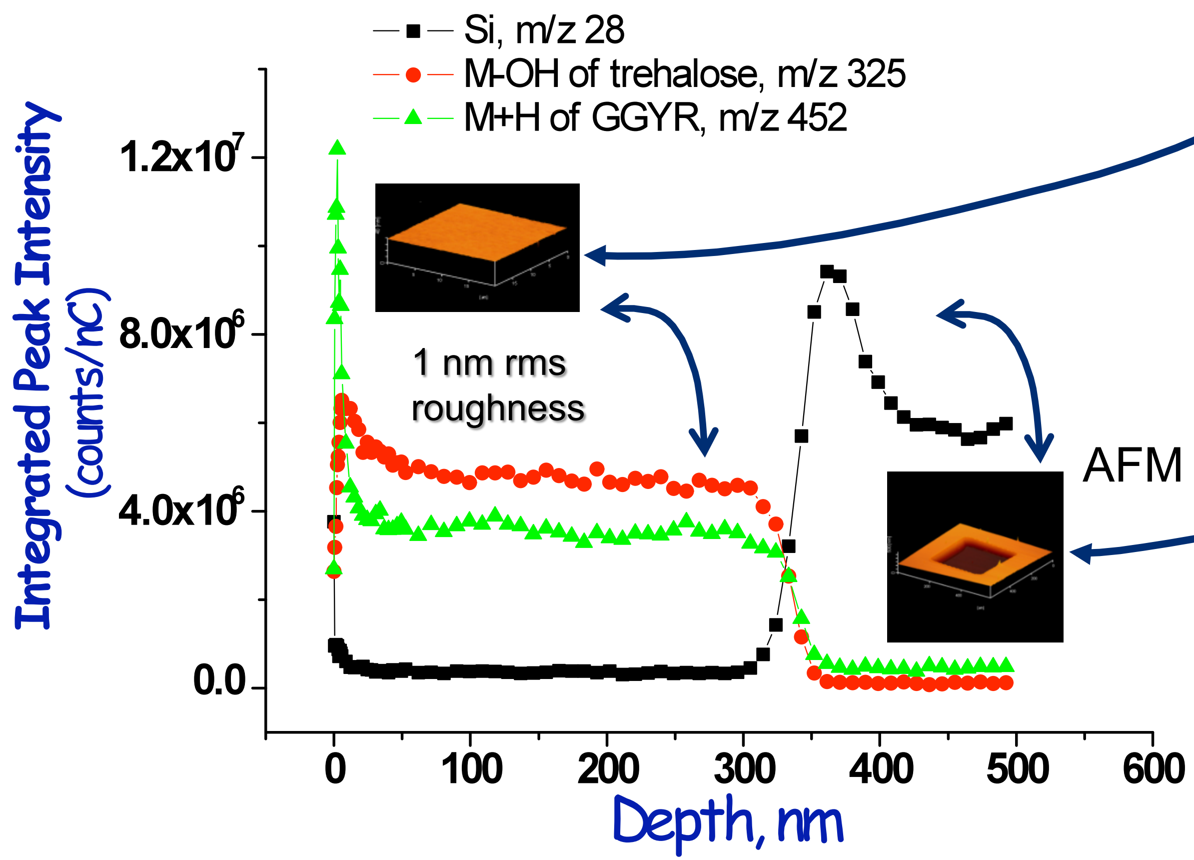


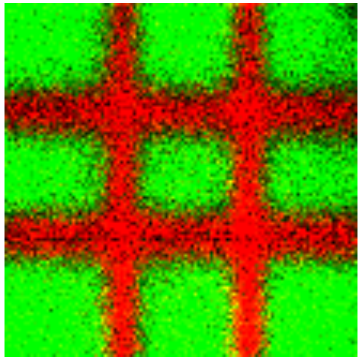
Mix peptide with trehalose

spin coat solution on 5 × 5 mm Si wafer

Cheng and Winograd, Anal. Chem., 2005.

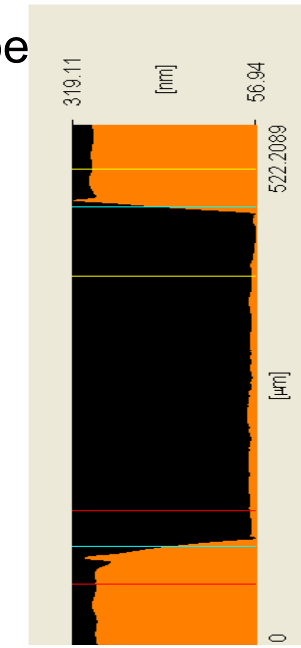
Molecular depth profiling





Peptide array on Si

AFM crater shape

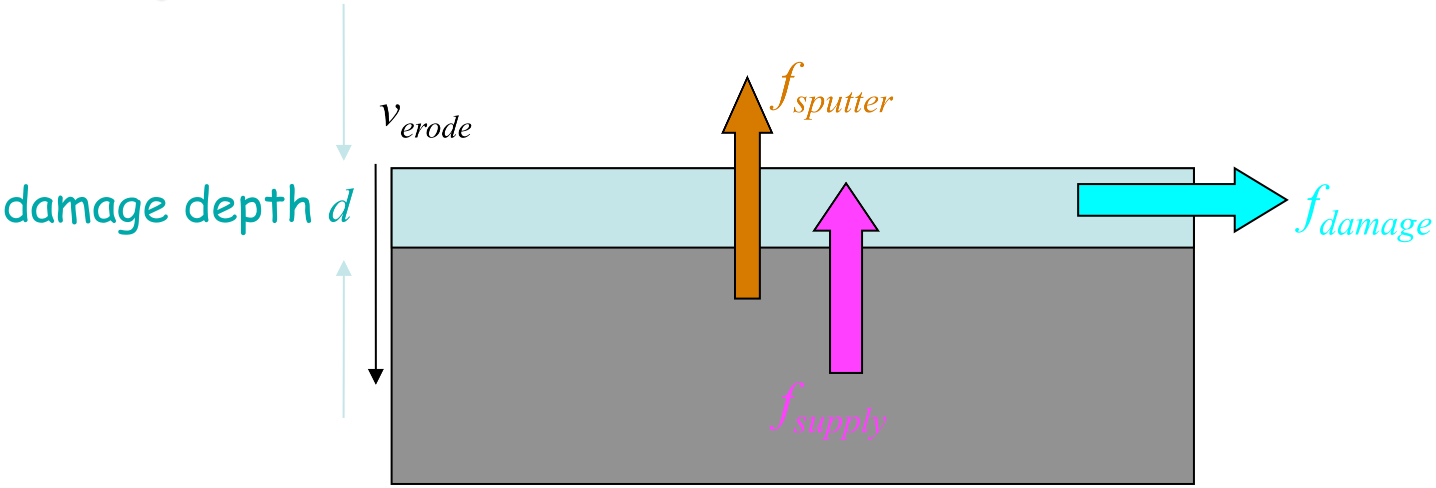


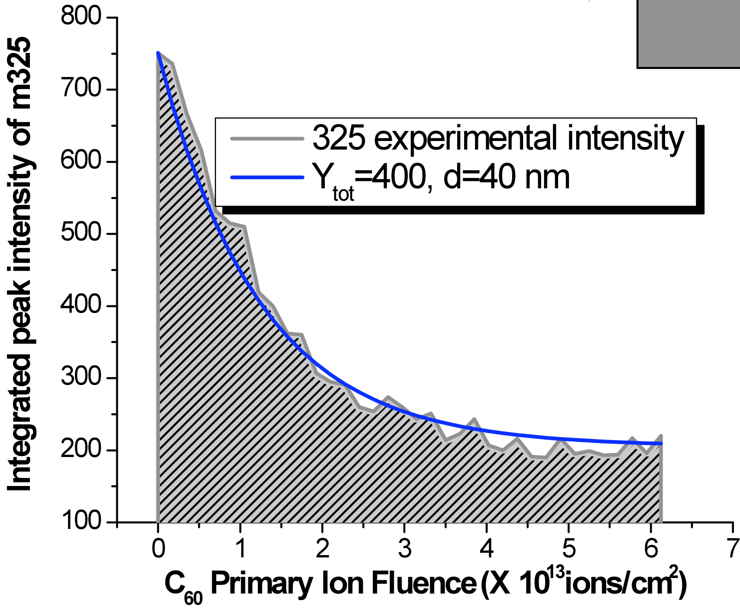
Trehalose + Gly-Gly-Tyr-Arg (100:1) film

Cheng and Winograd, Anal. Chem., 2005.

Erosion Dynamics

$$Y_{tot} >> nd\sigma_D$$





Y_{tot} : total sputter yield

n : number density of molecules

$2.7 \times 10^{21}\, trehalose / cm^3$

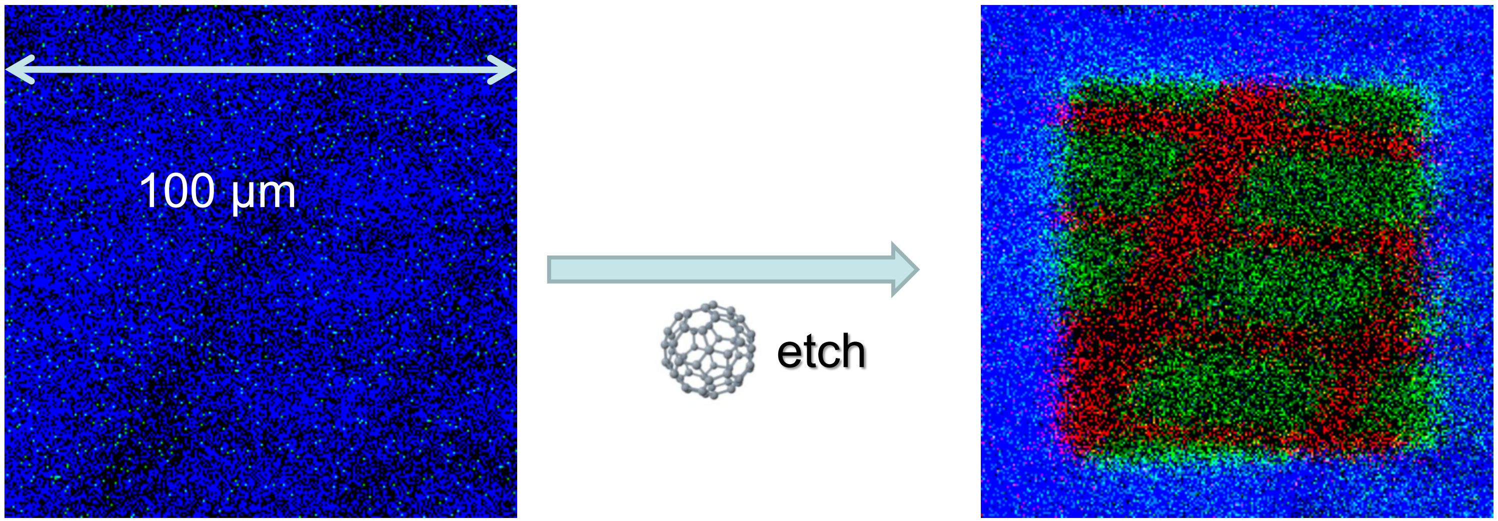
σ_D : damage cross section

$4.4 \times 10^{-14}\, cm^2$

$$S_{steady state} / S_0 = \left(\frac{Y_{tot}}{Y_{tot} + nd\sigma_D} \right)$$

Cheng, Wucher and Winograd, J. Phys, Chem. B., 2006.

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

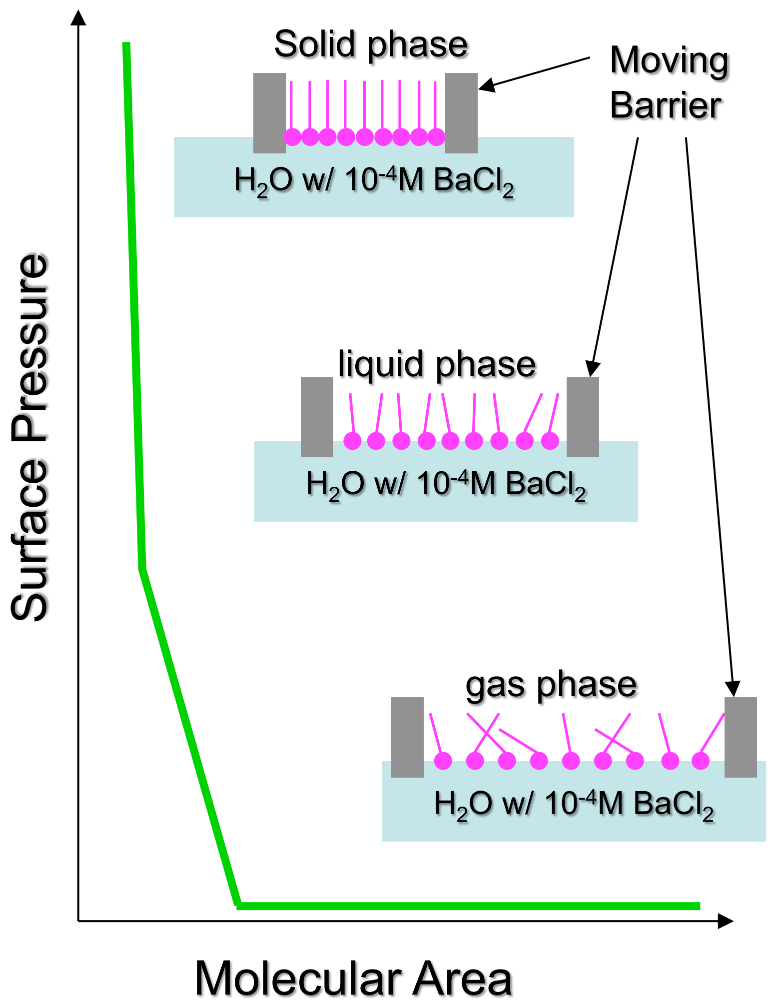


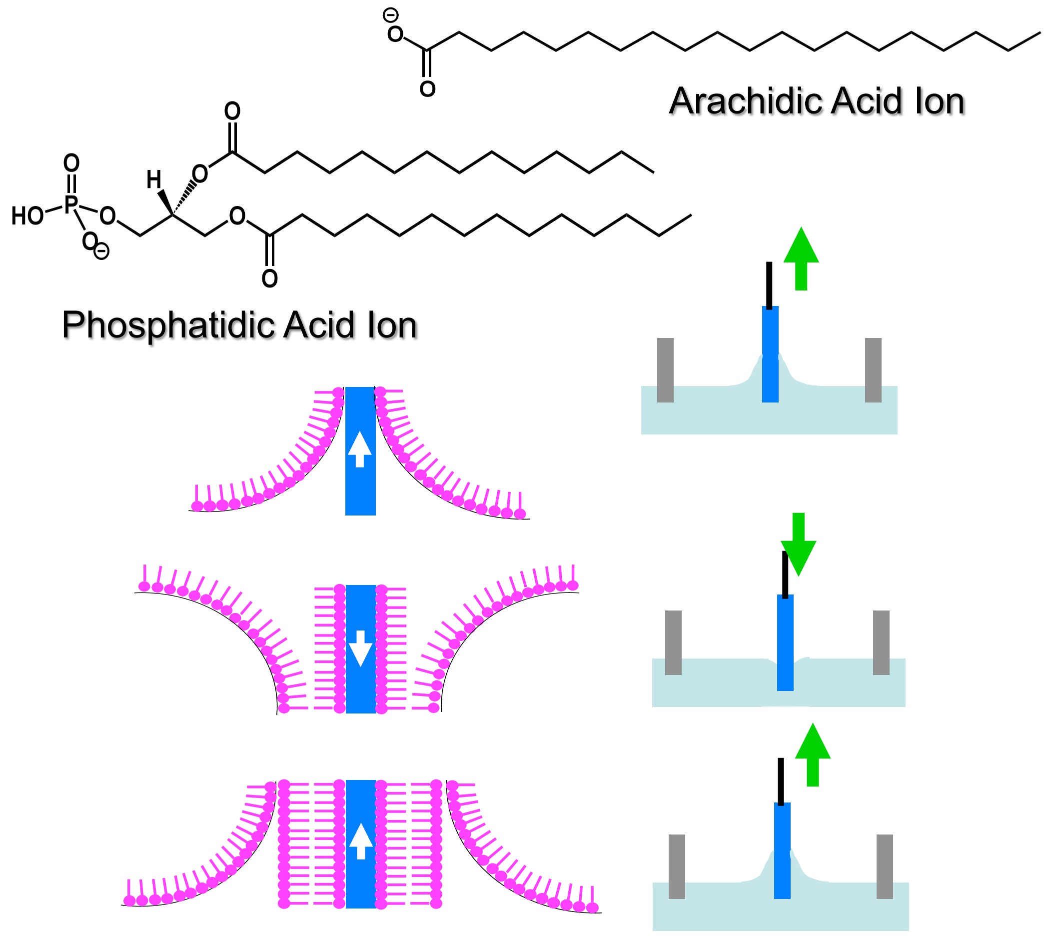
40 keV C_{60}^{+} bombardment of water-ice (m/z 18) covering a patterned film of cholesterol (m/z 369, M-OH^{+}) on silicon (m/z 28).

Piehowski, Ewing and Winograd

3. Depth resolution is a critical issue: Topography and interface mixing

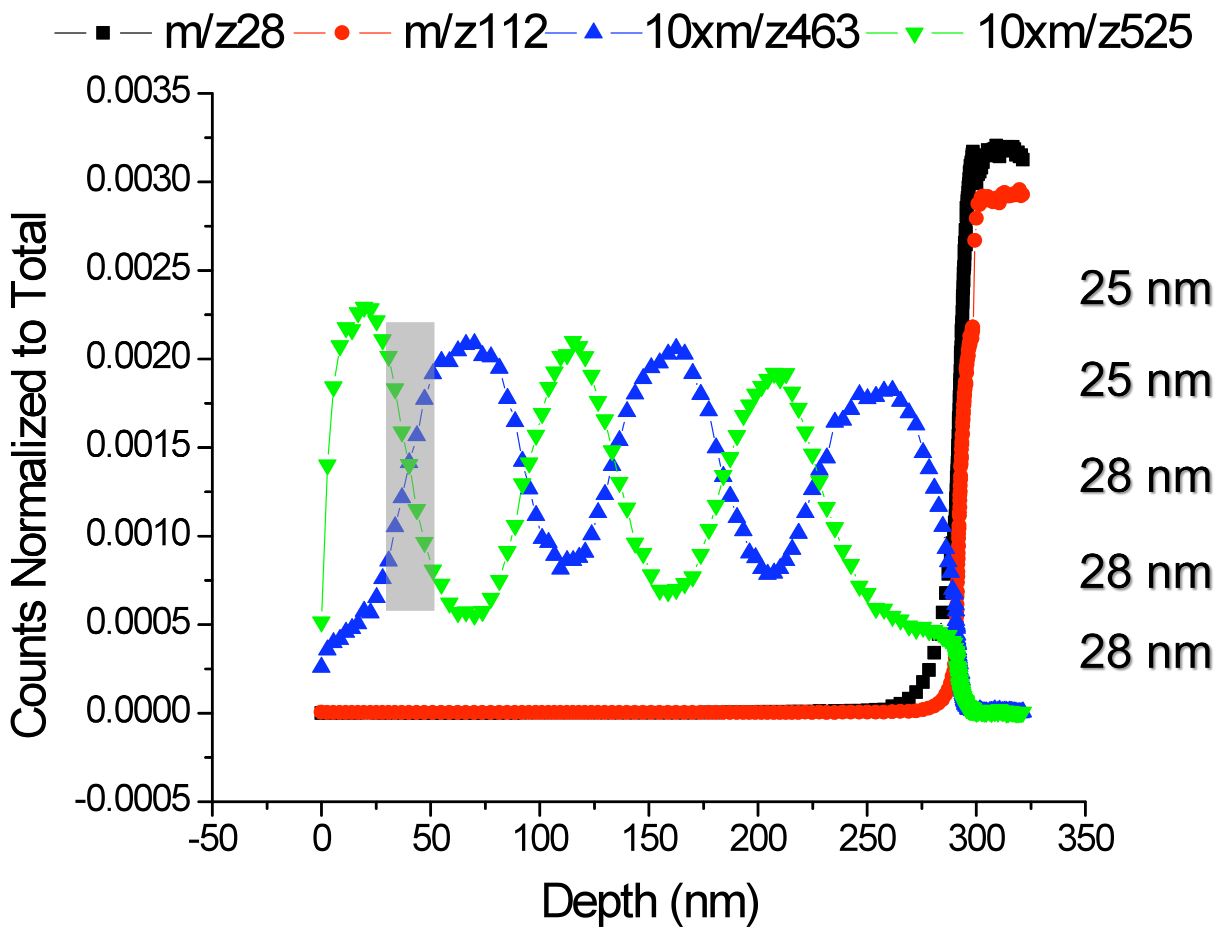
Depth profiling of molecular multilayer structures

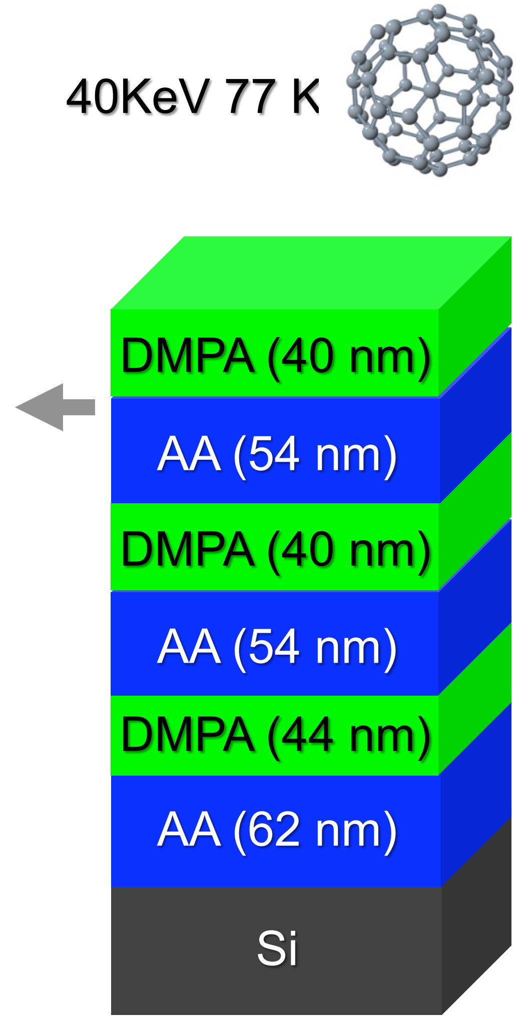




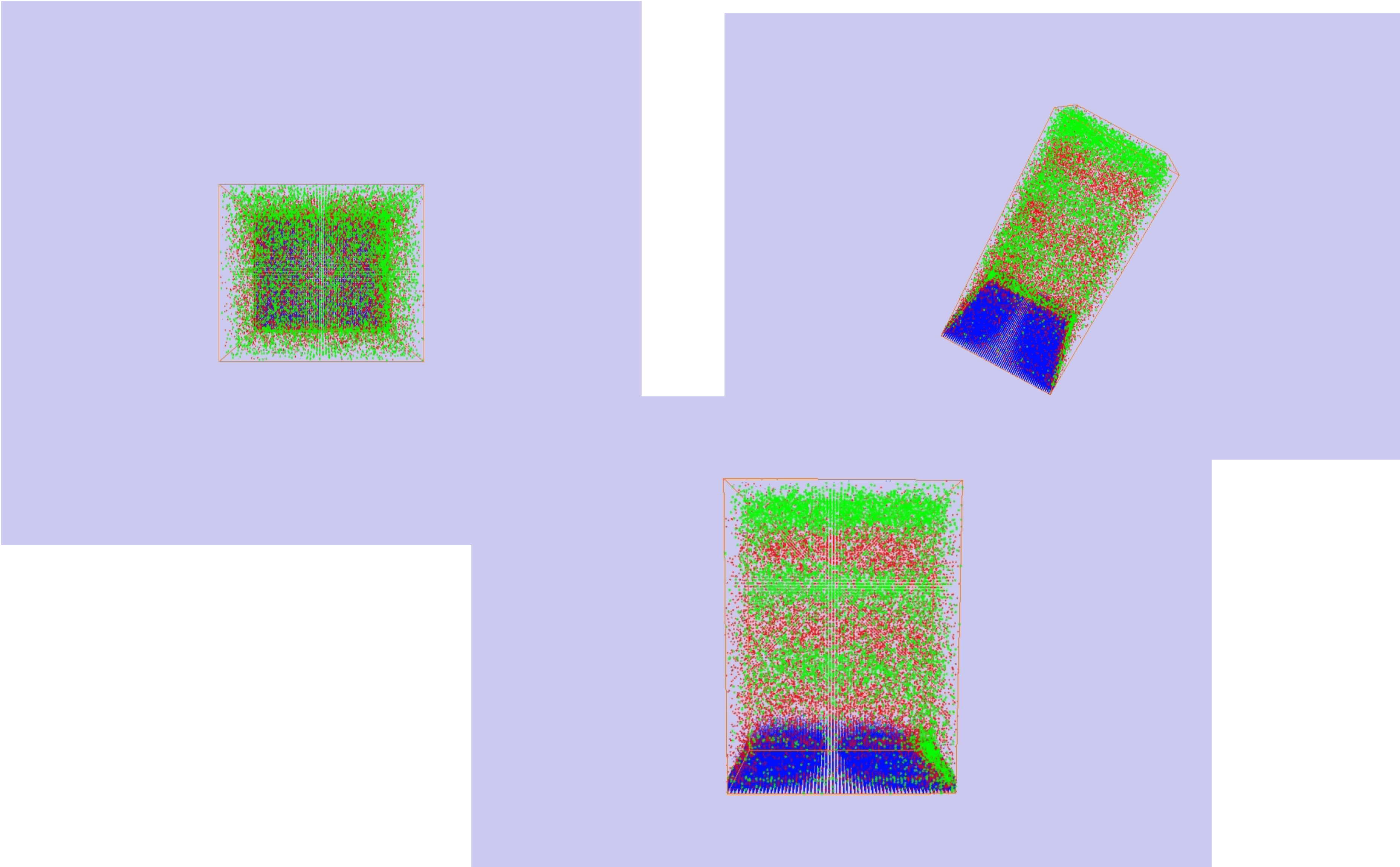
Leiling Zheng, JASMS and Anal Chem, 2008.

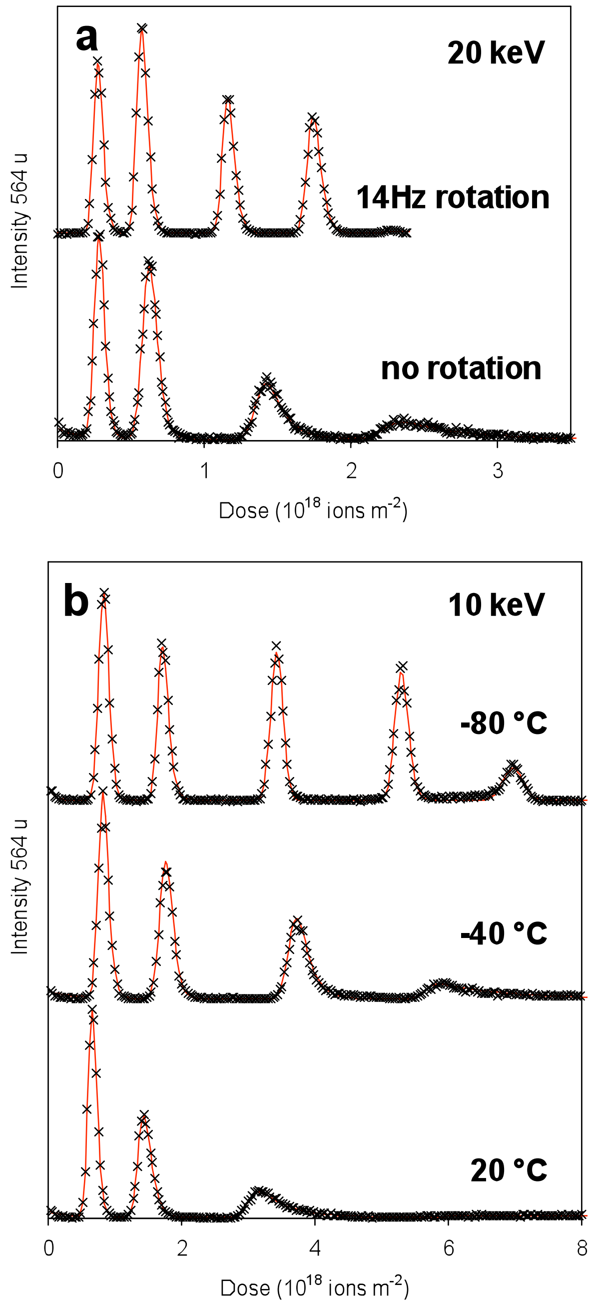
Depth profiling of multilayer structures





In 3-dimensions



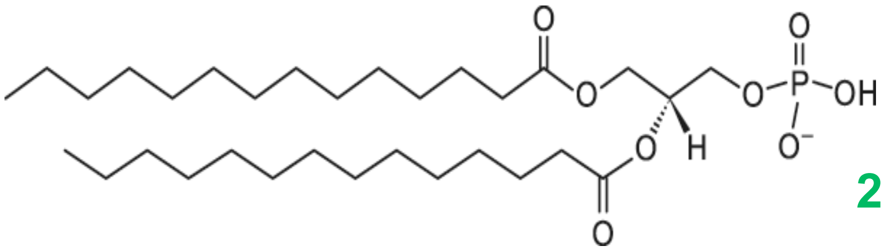


Organic δ-layers serve as a wonderful model system for evaluating the parameters that affect depth resolution

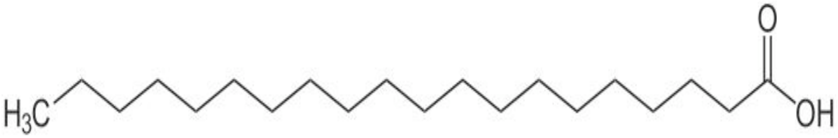
Monolayers of Irgonox 1010 imbedded into Irgonox 3150 at depths of 46, 92 182 and 270 nm. Samples now utilized as a VAMAS standard for interlaboratory comparisons.

Shard, A. G.; Green, F. M.; Brewer, P. J.; Seah, M. P.; Gilmore, I. S. J. Phys. Chem. B 2008, 112, 2596-2605.

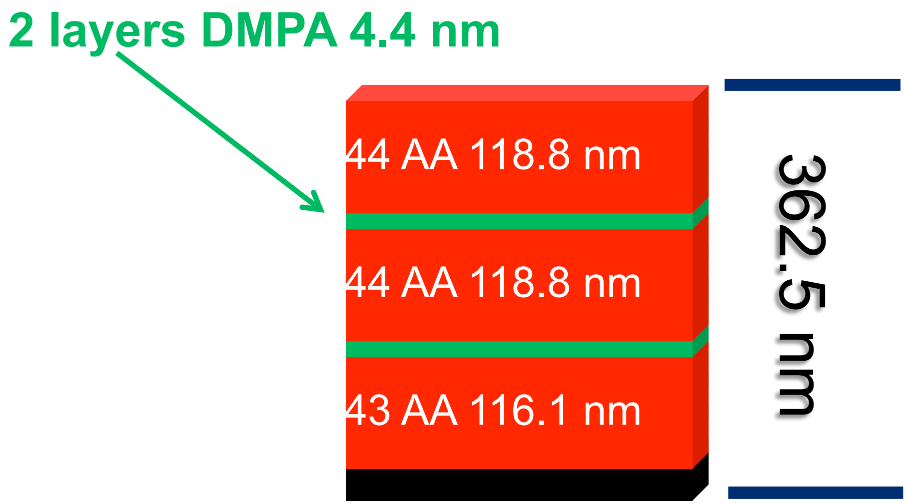
LB δ-layers: membrane bilayer mimics



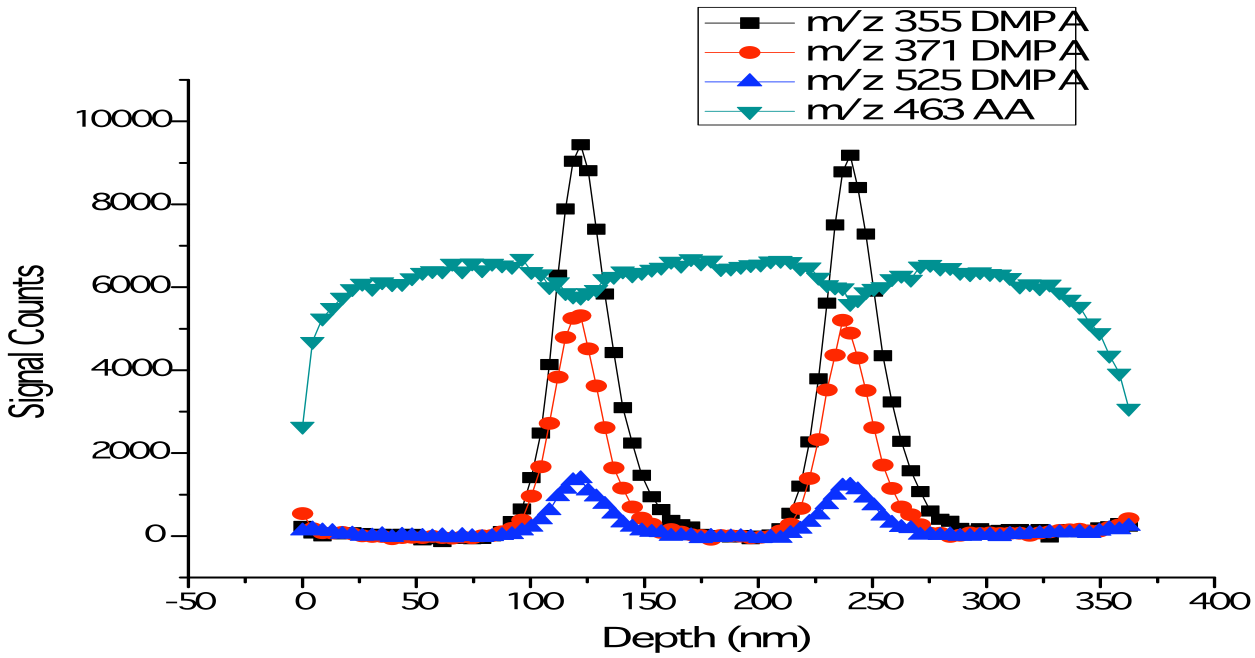
Dimyristoyl Phosphatidate (DMPA)



Arachidic Acid (AA)



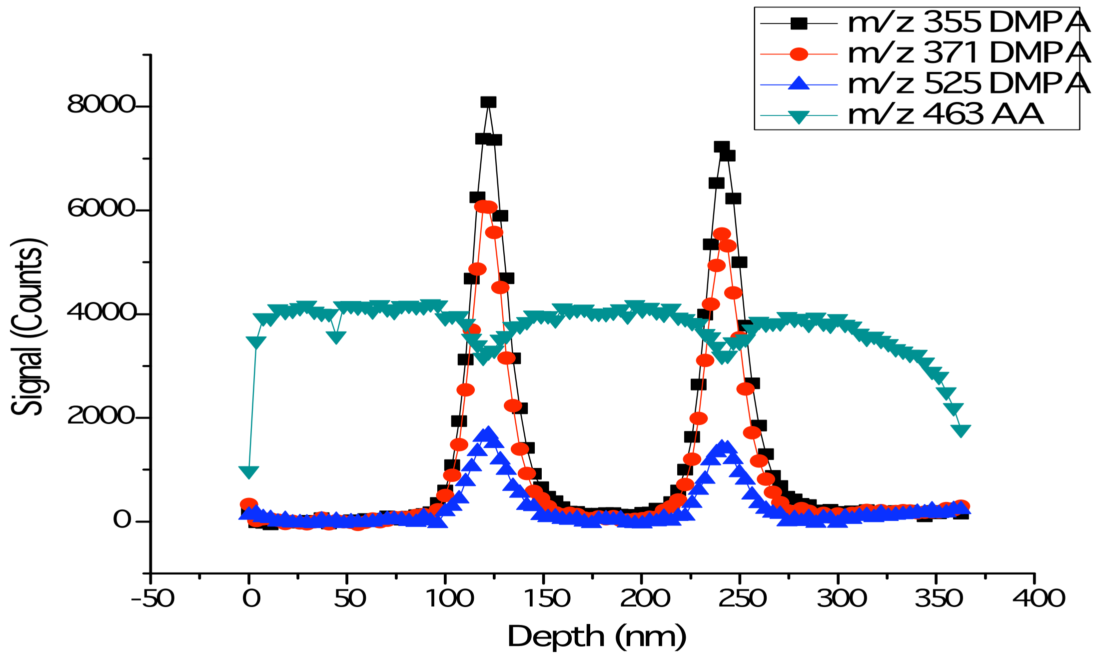
Lipid bilayer at 40° incidence, 298K and 77K



Depth Resolution (FWHM nm)

First Delta Layer (121.0 nm)		Second Delta Layer (244.2 nm)	
RT	LN_2	RT	LN_2
39.3±1.3 nm	25.0±1.1 nm	40.9±1.9 nm	24.8±1.2 nm

Lipid bilayer at 71° incidence, 77K



Depth Resolution (FWHM nm)

First Delta Layer (121.0 nm)		Second Delta Layer (244.2 nm)	
71°	40°	71°	40°
20.5±1.0	25.0±1.1	21.7±1.0	24.8±1.2

Depth Response Function

Dowsett's semi-empirical function

[illegible]

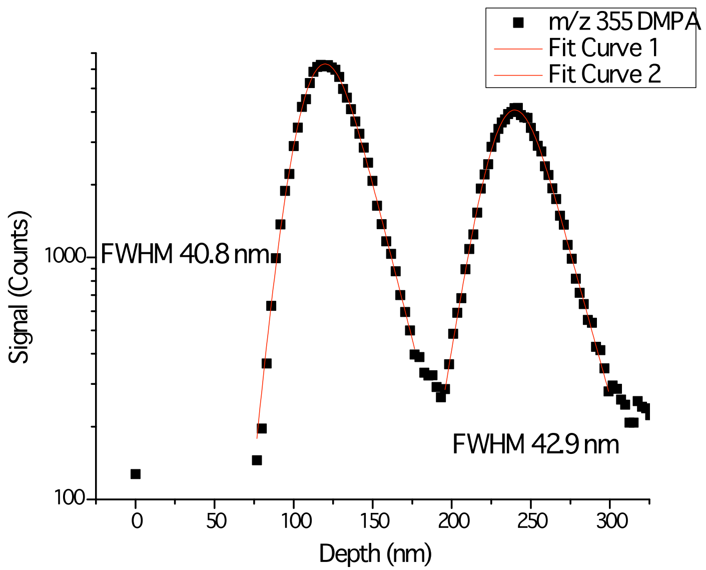
[illegible]

[illegible]

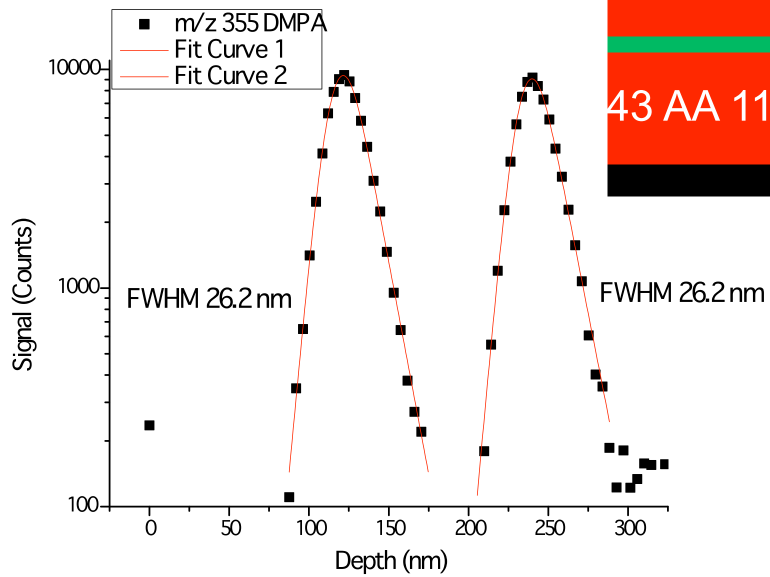
- λ_g **Leading edge growth length – information depth of secondary ions**
- λ_d **Trailing edge decay length – related to ion beam mixing**
- σ **Standard deviation of a central Gaussian connecting the two exponential functions – convolution of all factors effecting depth resolution.**

Dowsett, M. G.; Rowlands, G.; Allen, P. N.; Barlow, R. D. *Surf. Interface Anal.* **1994,** *21* (5), 310-315.

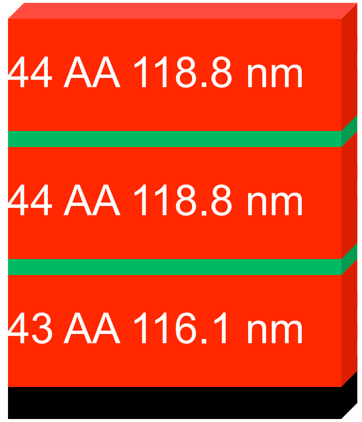
Depth Response Function



Room Temperature

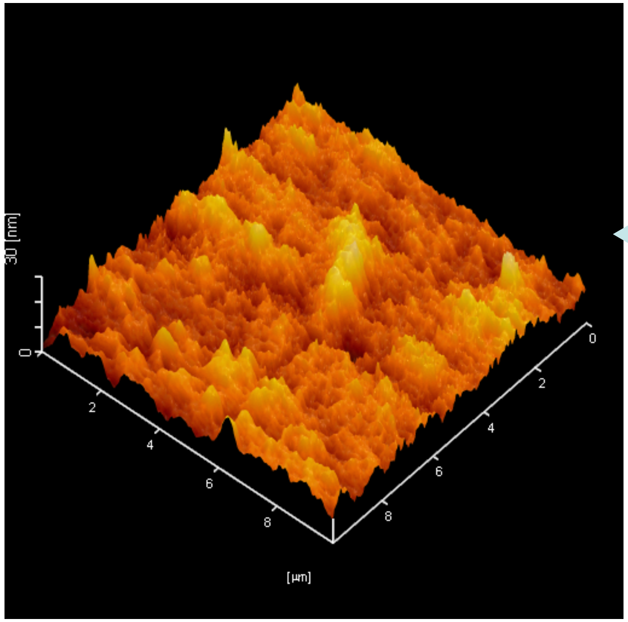


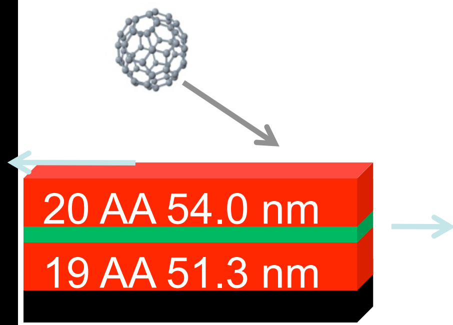
Low Temperature



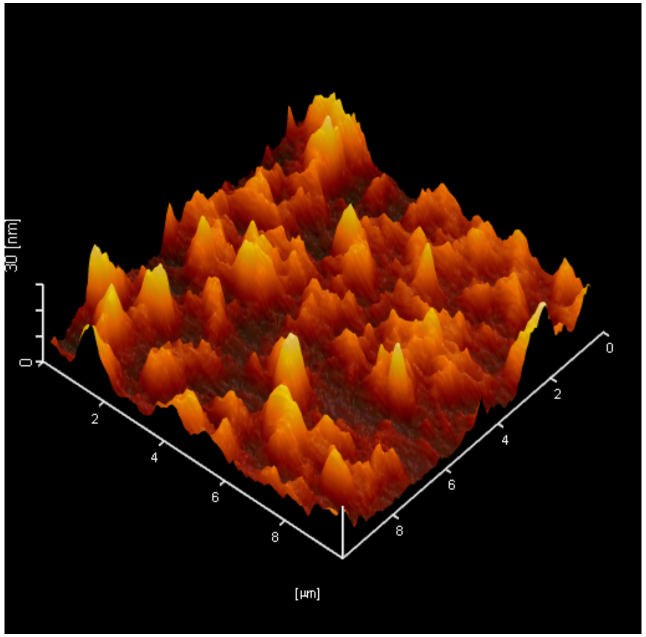
	First Delta Layer (121.0 nm)						Second Delta Layer (244.2 nm)					
	λ_g		λ_d		σ		λ_g		λ_d		σ	
	RT	LN2	RT	LN2	RT	LN2	RT	LN2	RT	LN2	RT	LN2
ave	3.9±2.0	5.7±0.1	14.5±2.1	10.0±1.2	13.0±0.4	7.4±0.2	6.3±4.5	5.6±0.9	15.4±2.5	10.3±1.3	13.5±1.5	7.1±0.4

Surface Roughness





Sample I



RMS 3.5 nm

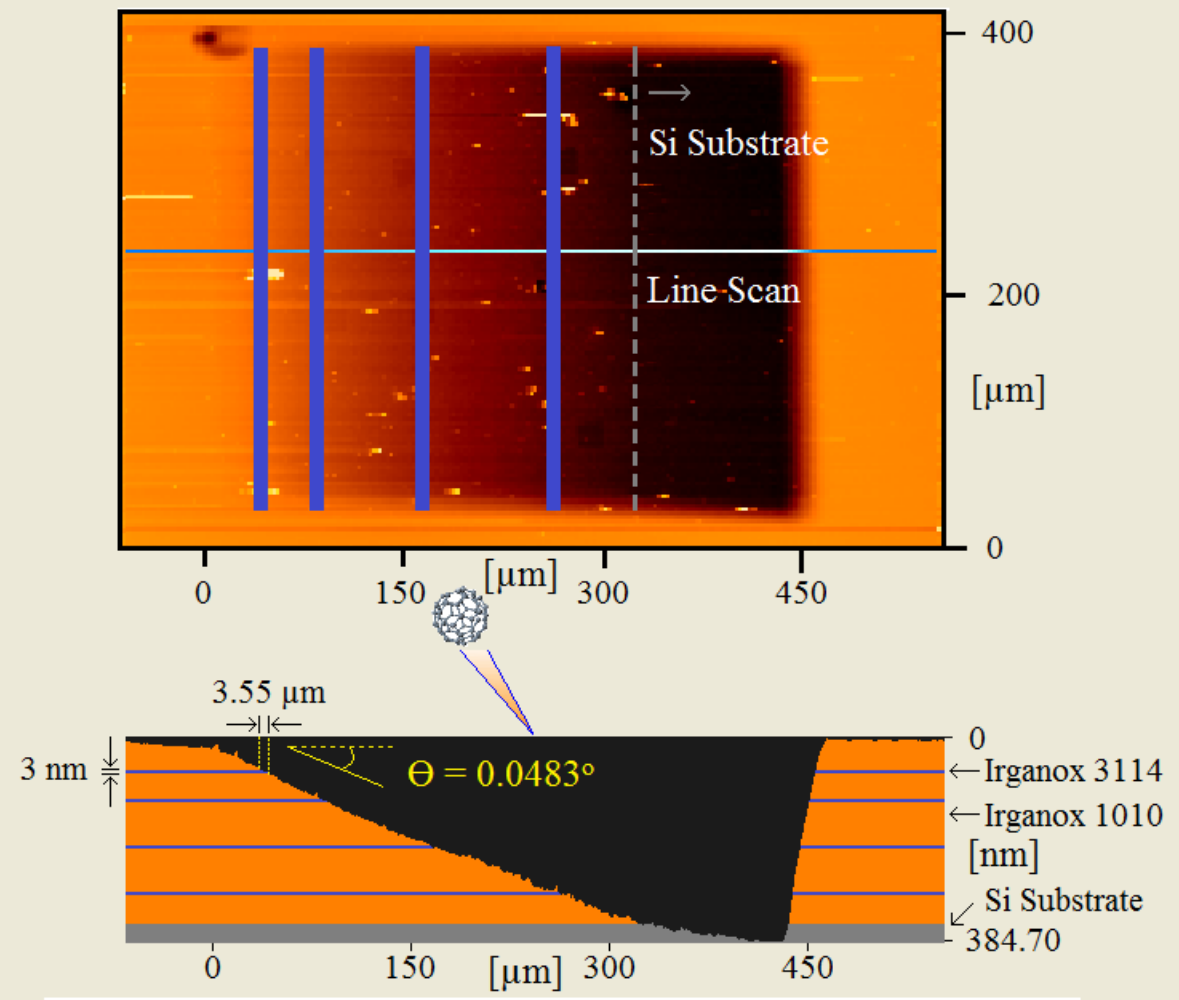
σ=7.7 nm

RMS 4.9 nm

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.
- λ_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

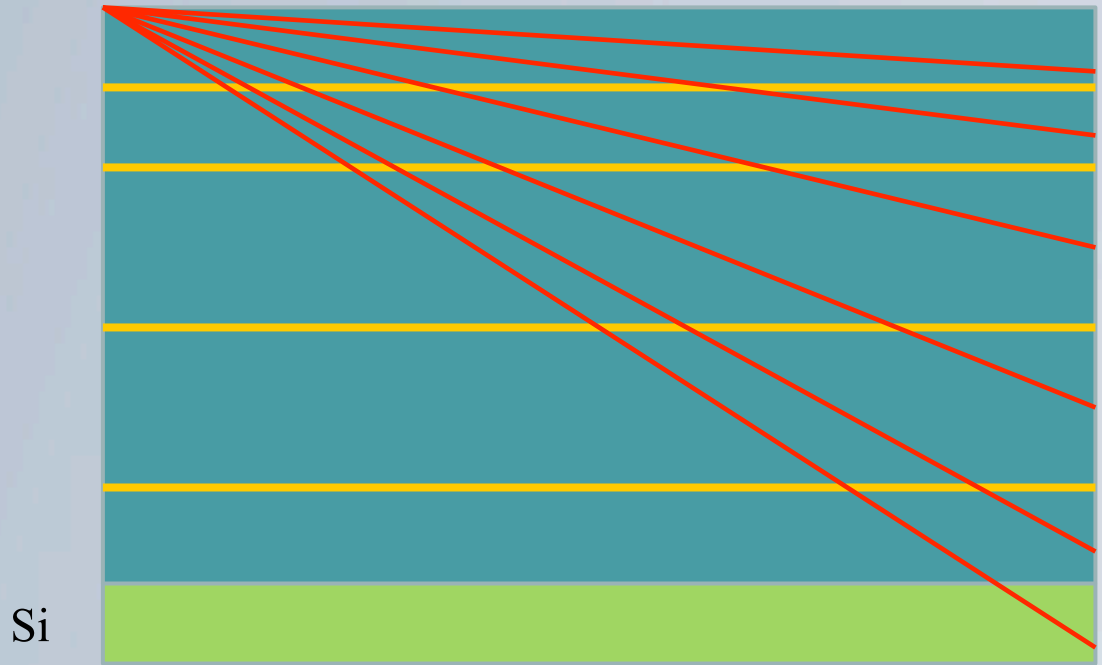
Dan Mao and Andreas Wucher

Depth to SIMS Imaging Transform

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

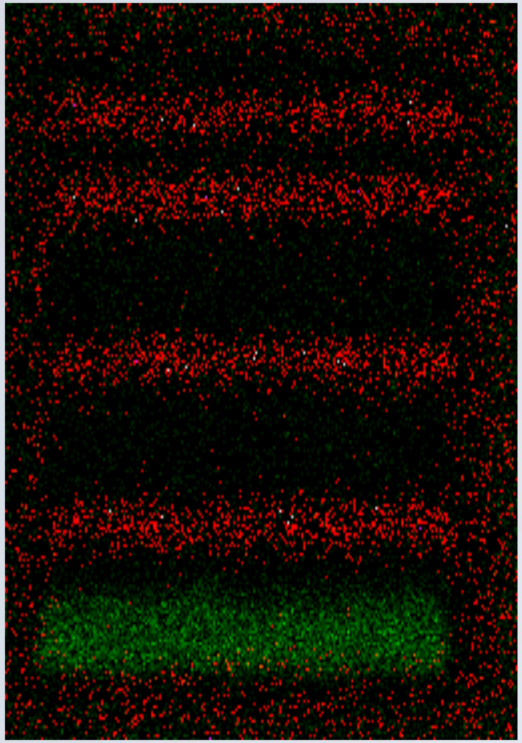
PENNSTATE

SIMS During Wedge



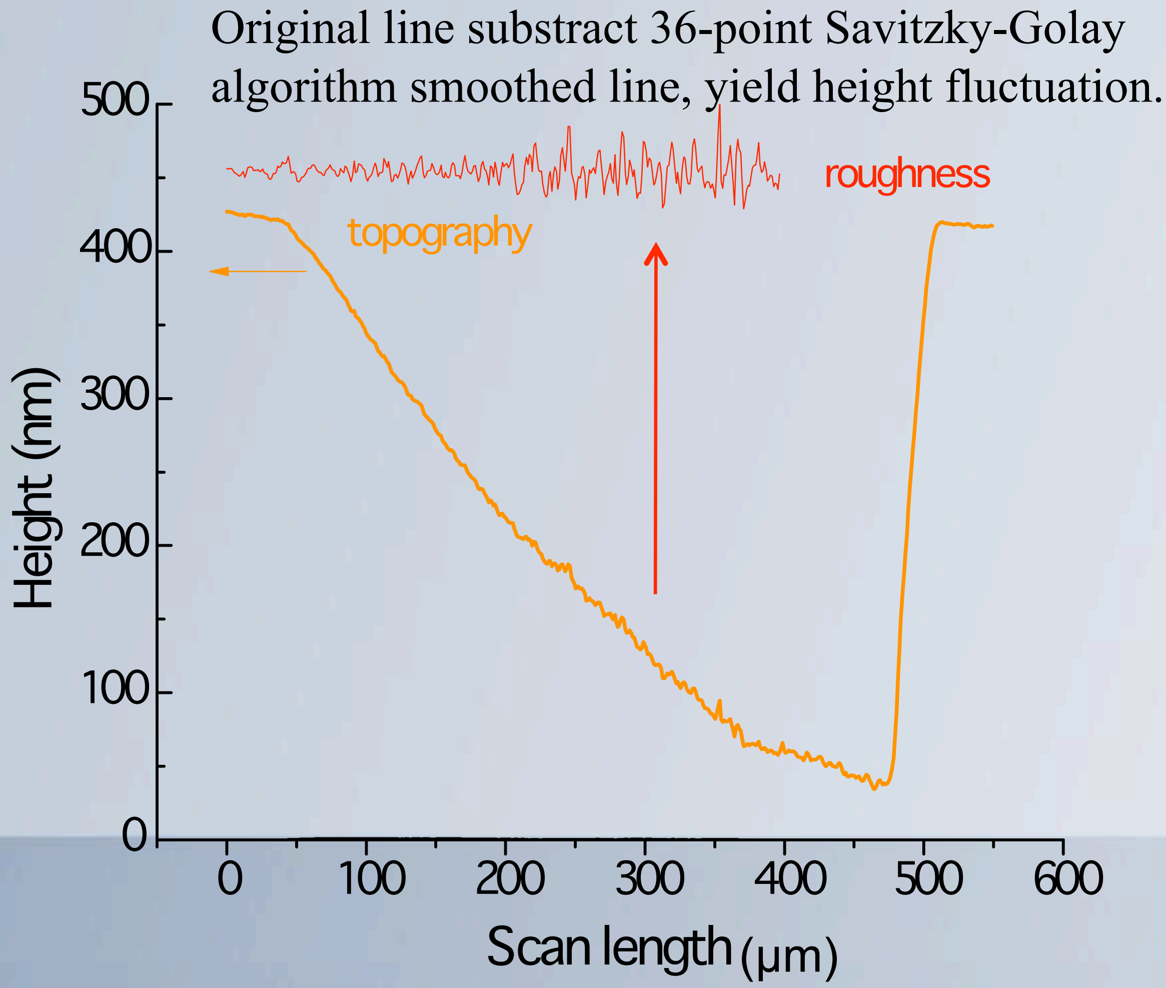
Dark Green: Irganox 1010 / Orange: Irganox 3114
Light Green: Si / Red: Imaging Surface

Red : m/z 42 from Irganox 3114

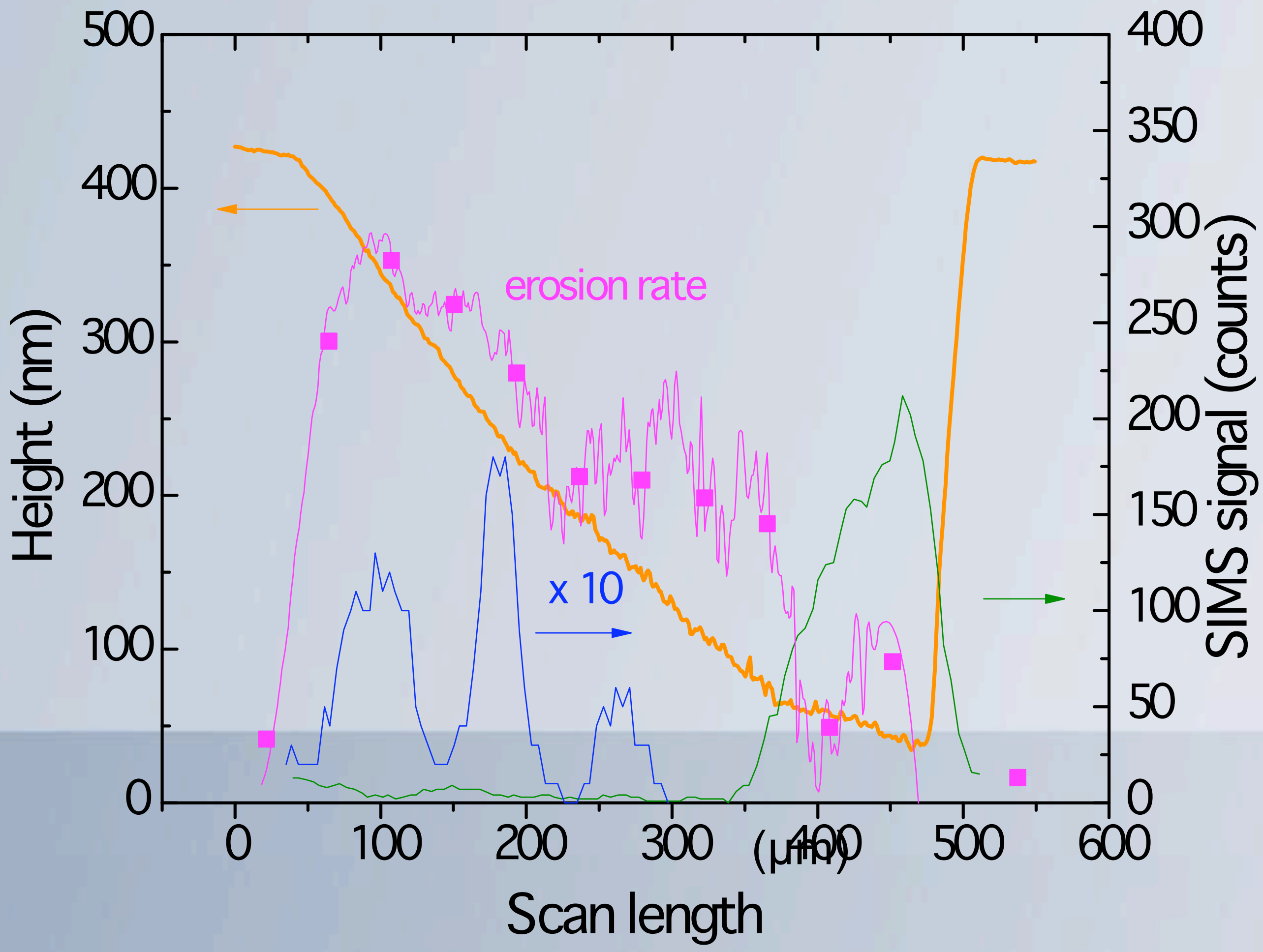


Green : m/z 60 from Si Substrate

AFM Line Scan – Topography evolution



One AFM/SIMS scan provides yield, roughness and erosion rate as a function of depth

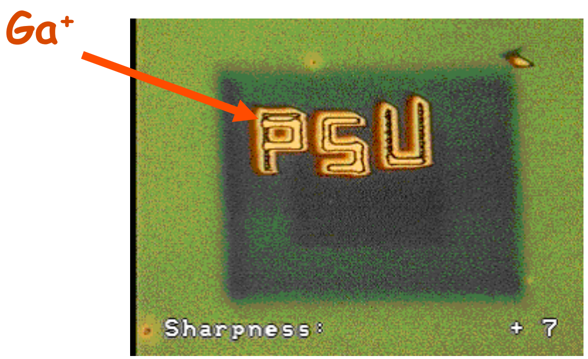


The next critical issues for 3-D imaging

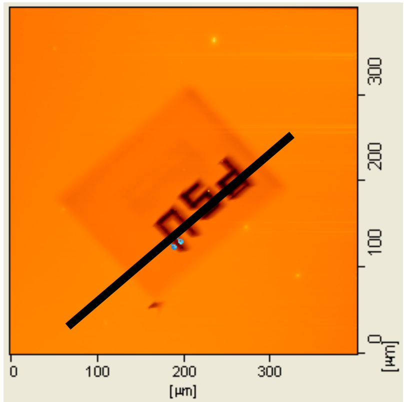
- Erosion rate needs to be known at each fluence. Propose wedges, or possibly *in situ* ellipsometry of some sort.
- For heterogeneous samples, i.e. biological cells, differential erosion rates will complicated the simple notion that images can be stacked.
- Let’s try an example →

Patterned Peptide Film for 3-D Imaging

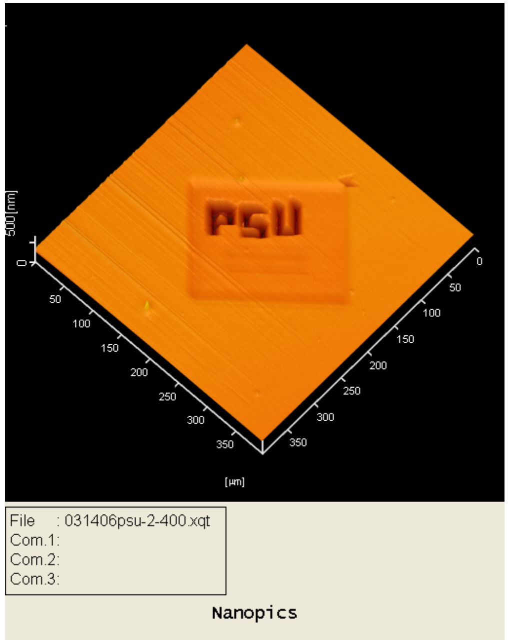
Features written on trehalose (GGYR) thin film with Ga^+ ion bombardment

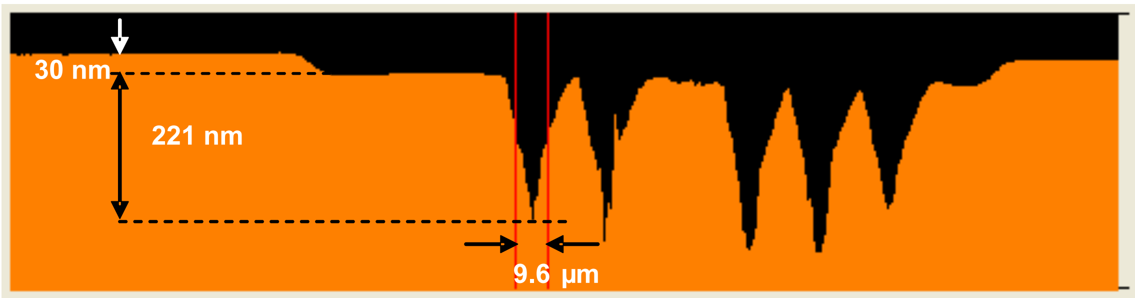


Optical Image



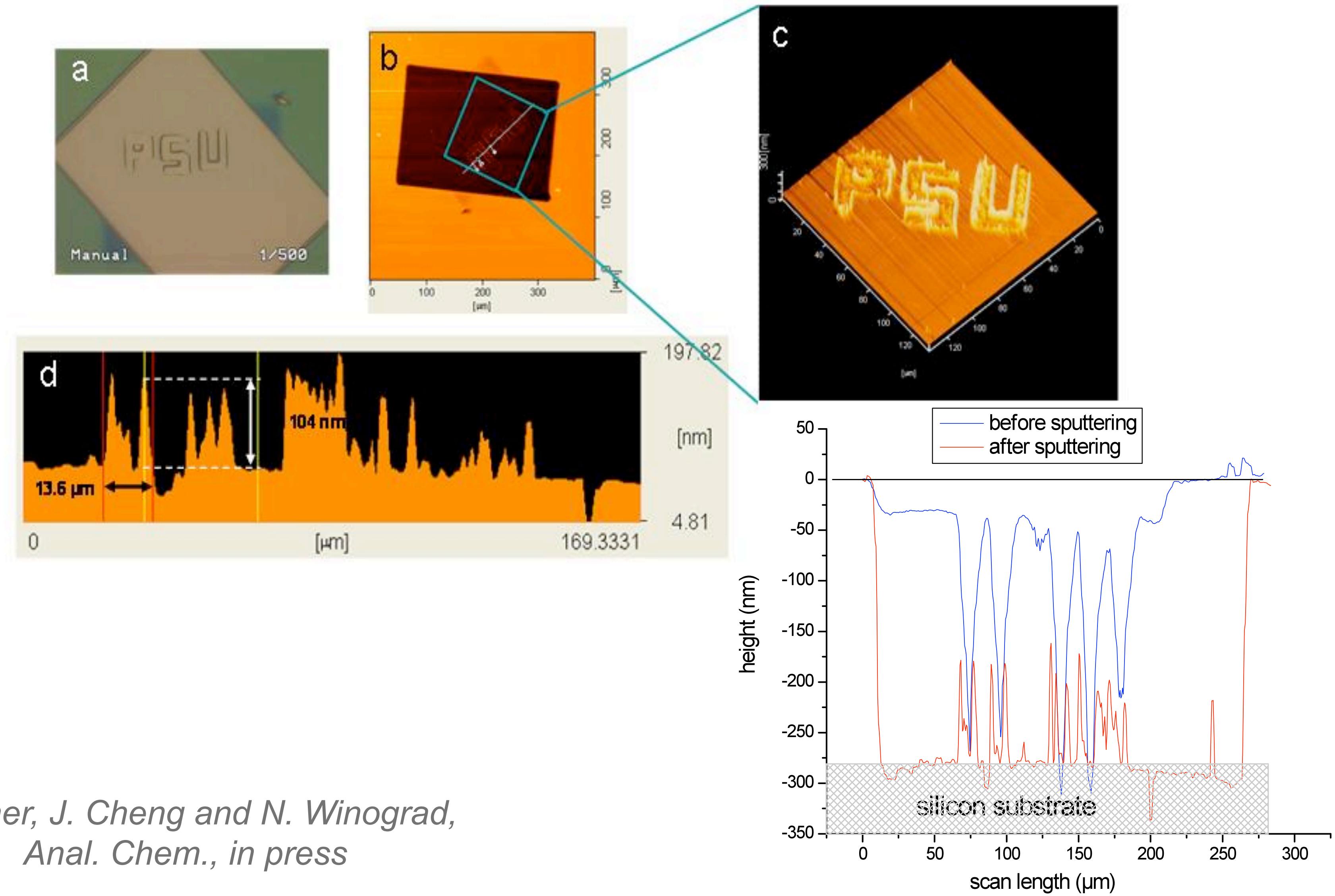
AFM Image





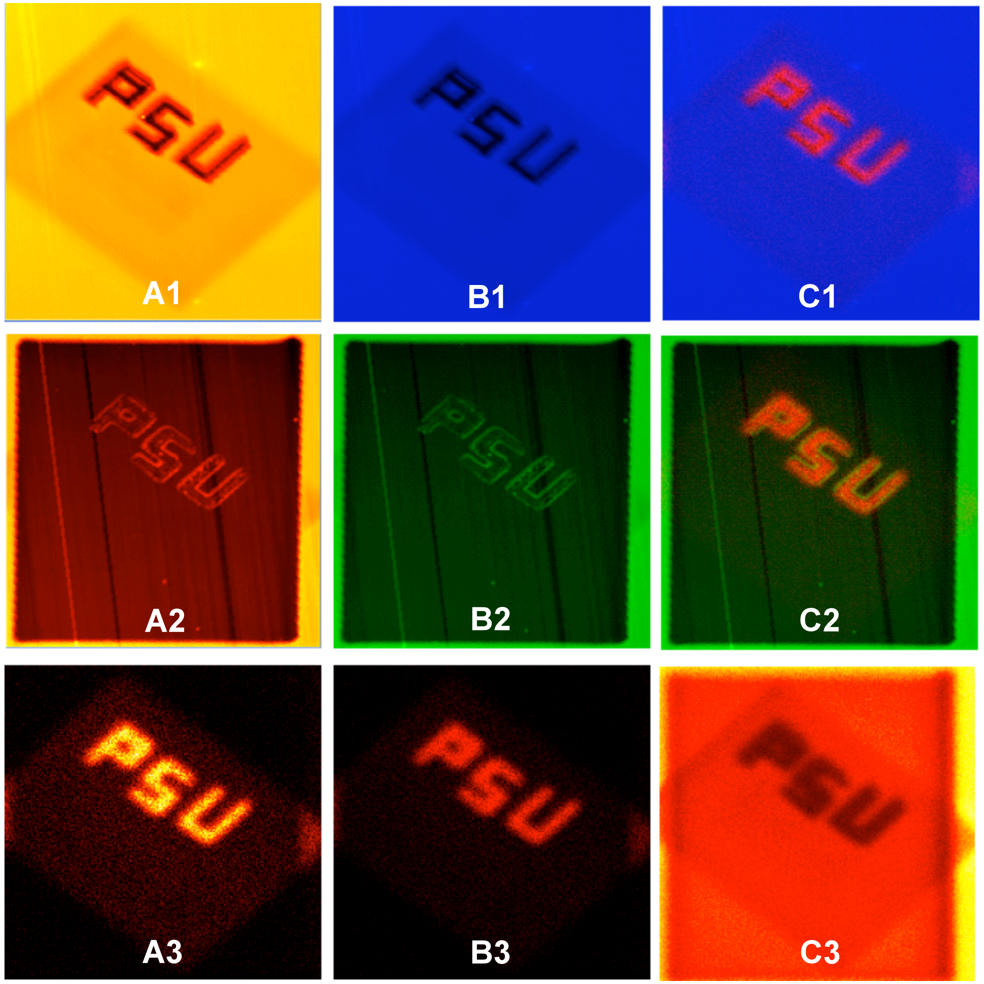
A. Wucher, J. Cheng and N. Winograd, Anal. Chem., 2008

After film erosion to Si substrate



Wucher, J. Cheng and N. Winograd, *Anal. Chem., in press*

Overlay mass spectrometry image with AFM image



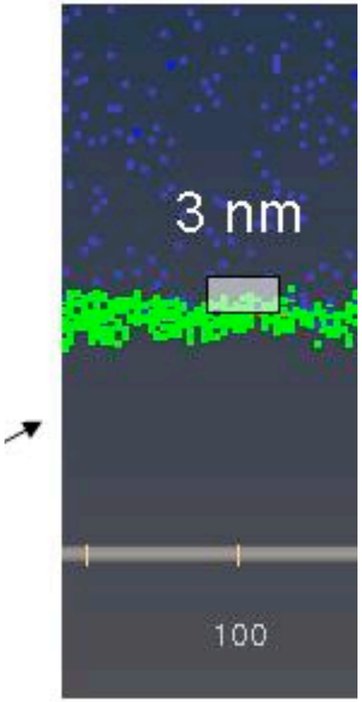
A1,B1: AFM before erosion
A2,B2: AFM after erosion

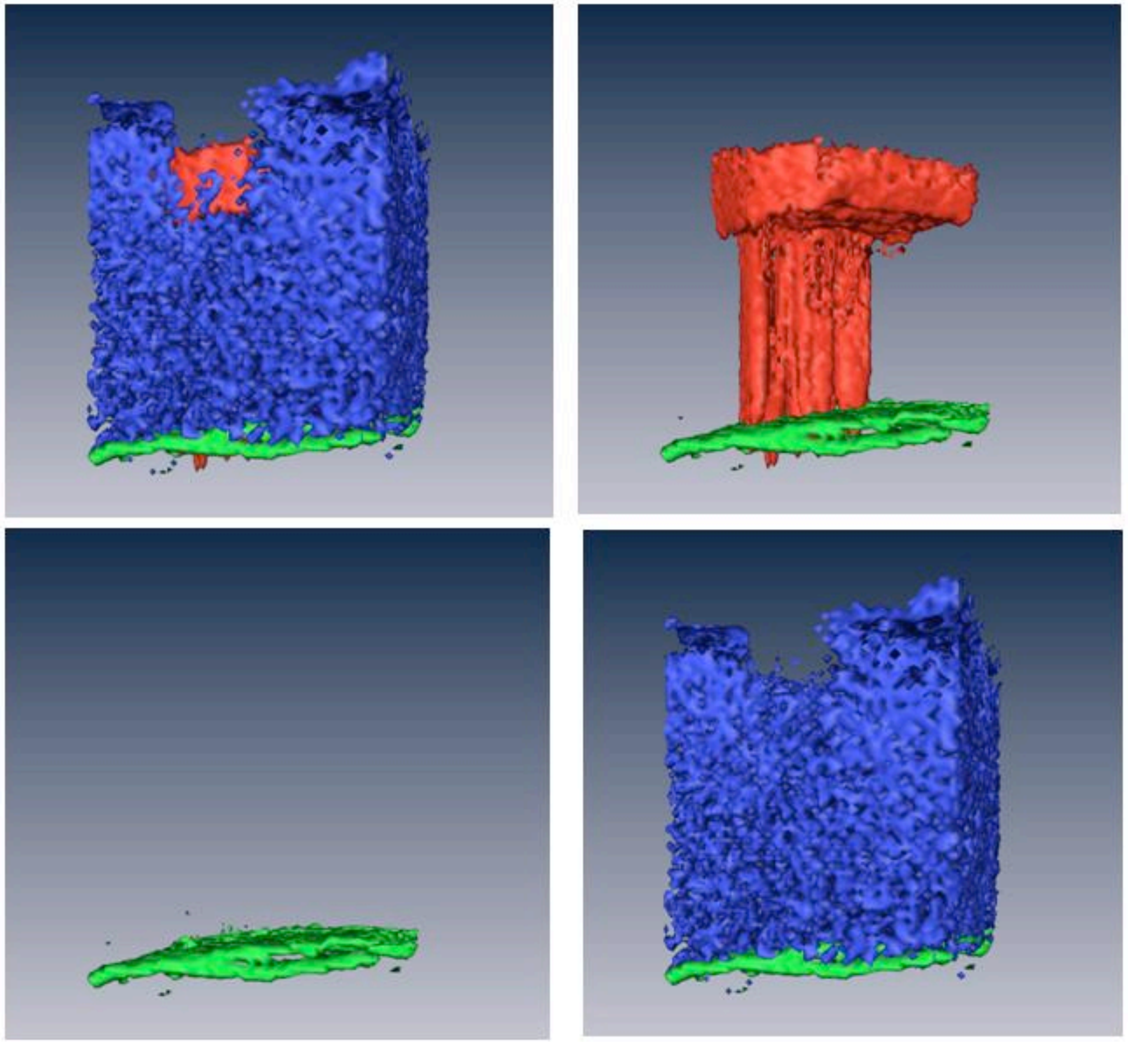
A3,B3: Σ Ga images
C1 = B1+B3
C2 = B2+B3

C3 = Σ total of all ms images

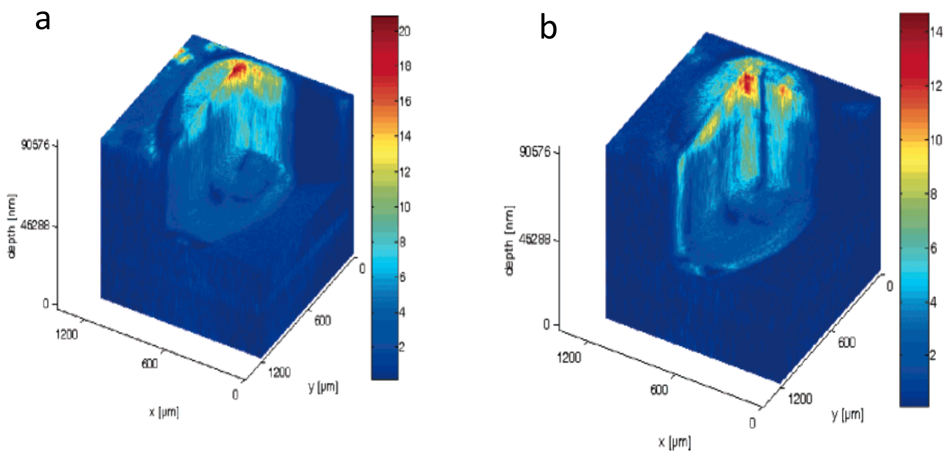
Wucher, J. Cheng and N. Winograd, Anal. Chem., 2008

Depth resolution can approach 3 nm

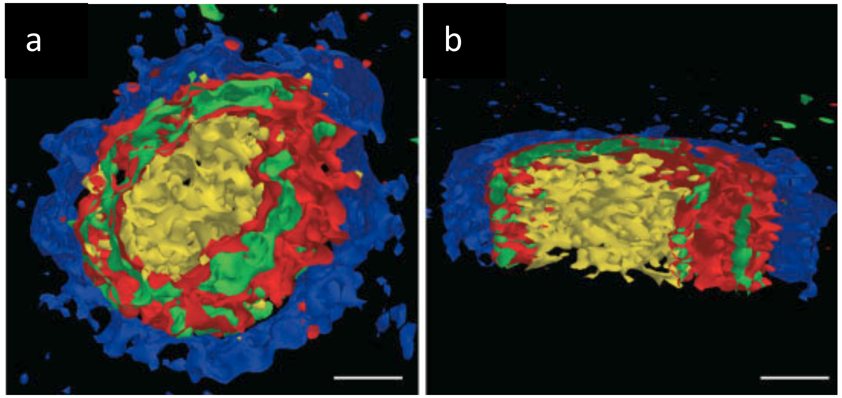




Examples of 3-D imaging are beginning to appear



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



Nygren H, Hagenhoff B, Malmberg P, Nilsson M, Richter K. 2007. Bioimaging TOF-SIMS: High resolution 3D Imaging of single cells. Microscopy Research and Technique 70: 969-74

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

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