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Freeze-Etching and Vapor Matrix Deposition for ToF-SIMS Imaging of Single Cells

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Abstract

Freeze-etching, the practice of removing excess surface water from a sample through sublimation into the vacuum of the analysis environment, has been extensively used in conjunction with electron microscopy. Here, we apply this technique to time-of-flight secondary-ion mass spectrometry (ToF-SIMS) imaging of cryogenically preserved single cells. By removing the excess water which condenses onto the sample *in vacuo*, a uniform surface is produced that is ideal for imaging by static SIMS. We demonstrate that the conditions employed to remove deposited water do not adversely affect cell morphology and do not redistribute molecules in the topmost surface layers. In addition, we found water can be controllably redeposited onto the sample at temperatures below $-100\text{ }^{\circ}\text{C}$ in vacuum. The redeposited water increases the ionization of characteristic fragments of biologically interesting molecules 2-fold without loss of spatial resolution. The utilization of freeze-etch methodology will increase the reliability of cryogenic sample preparations for SIMS analysis by providing greater control of the surface environment. Using these procedures, we have obtained high quality spectra with both atomic bombardment as well as C_{60}^{+} cluster ion bombardment.

Introduction

The role of individual lipid components in membrane processes is a topic of considerable interest in cellular and molecular biology.¹⁻³ A variety of methods including atomic force microscopy (AFM),⁴ NMR,⁵ near-field scanning optical microscopy (NSOM),⁶ electron spin resonance (ESR),⁷ single particle tracking (SPT),⁸ and fluorescence microscopy have been applied to the study of membrane lipid heterogeneity.⁹ Indeed, much of what is known about role of individual lipid components in membrane processes has been obtained with fluorescence techniques. Typically, synthetic fluorescent probes containing fatty acid-like chains are inserted into the membrane. By manipulating the structure of these chains, a preference for various lipid domains is imparted, although the chemical specificity is often restricted. In addition, these bulky fluorophores might perturb the environment and alter intrinsic domain formation events.⁴

Recently, imaging with time-of-flight secondary-ion mass spectrometry (ToF-SIMS) has been shown to be a powerful analytical tool for mapping the distribution of biologically relevant small molecules ($<1000\text{ Da}$) on a surface.¹⁰ This method combines submicrometer

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Supporting Information Available: Images of freeze-etched cells obtained using a C_{60}^{+} primary ion. This material is available free of charge via the Internet at <http://pubs.acs.org>.

spatial resolution, high chemical specificity, and surface sensitivity, making it a promising tool for the study of lipids in cellular membranes. Briefly, a pulsed beam of primary ions is directed at the analysis surface, causing the sputtering of ions and molecules. The ions are extracted into a ToF mass analyzer generating a mass spectrum. To obtain an image, the primary beam is raster-scanned across the surface, recording a mass spectrum for each pixel. Masses of interest are then selected from the spectrum, and their intensities are mapped across the imaging area. When combined with cryogenic sample preparation techniques, ToF-SIMS allows the detailed study of membrane lipids during dynamic processes such as membrane fusion.¹¹⁻¹³

The quality of the information obtained with this technique is dependent upon conservation of the morphological and chemical integrity of samples.¹⁴ However, utilization of ToF-SIMS requires that the biological samples be analyzed in a high vacuum environment. Toward this end, many approaches have been used, including freeze-fracture,¹³ freeze-drying,¹⁵⁻¹⁸ vitrification in trehalose,¹⁹ and imprinting on metal foil.²⁰ We have previously developed a freeze-fracture protocol based on methods used in electron microscopy. In this method, freezing of the sample is performed in liquid ethane to quench the sample, capture dynamic membrane processes, and protect the sample from water crystallization damage. While this technique has the advantage of preserving membranes in their hydrated state, it also has disadvantages. When a fracture is performed, very precise control over the vacuum environment is necessary to maintain a reproducible surface amenable to SIMS analysis. Fracture-induced damage of cells can also occur which may complicate interpretation and degrade the quality of information obtained from SIMS. Lastly, the fracture plane is difficult to reproduce, making many samples unusable as well as making comparison of cells in different populations difficult.

Another challenge facing SIMS imaging is low signal intensity from many biologically interesting molecules. Ionization efficiency measurements of membrane lipids demonstrate that less than 0.01% of molecules desorbed by the primary ion beam impact are ionized.²¹ Improving the ionization efficiency of the target molecules could greatly improve the SIMS signal. Significant increases in sputter yield have been achieved using cluster ion sources for sample bombardment and have attracted the attention of many in the SIMS community.²²⁻²⁴ Many groups have been pursuing ways of improving signals through creative sample preparations, coating samples with thin metal films (metA-SIMS),²⁵ deposition of matrix-assisted laser desorption ionization (MALDI)-type matrices (ME-SIMS),^{15,26-28} and the addition of metal nanoparticles.^{29,30}

In this paper, a sample preparation technique is described utilizing cryogenic sample preparation methods that not only improve the reproducibility of biovacuum interfaces but also increase ionization efficiency. The approach is illustrated using oligodendrocytes and macrophages, and the key attributes of the sample treatment are examined using various lipid model systems. With this method, adventitious sources of surface water-ice, which tend to block the emission of biomolecules, are removed by low-temperature sublimation. Next, a controlled amount of amorphous ice is redeposited back onto the sample surface. This step yields significant signal enhancement when compared to freeze-dried sample preparations and also produces a uniform surface which does not have spatial resolution limited by matrix crystallization.³¹

Experimental Section

Cell Culture

Oligodendrocytes were harvested from 1 day old Sprague–Dawley rat pups and separated from cerebral tissue as previously described.³² The cells were cultured on 5 mm × 5 mm

silicon shards (Ted Pella, Redding, CA) that were coated with 0.2 mg/mL poly(L-lysine) (VWR, West Chester, PA) and 0.1 mg/mL collagen (Sigma-Aldrich, St. Louis, MO). Cells were incubated until maturation was visible under a microscope.

J774 macrophage cells were cultured in sterile, polystyrene cell culture flasks. When confluent, the cells were treated with 25 μ g/mL DiI (Molecular Probes, Eugene OR) in serum-free media (Invitrogen, Carlsbad, CA) for 1 h. Following this treatment, the cells were rinsed with media before being dislodged from the culture dish by gentle tapping. The suspension of cells was added to a new flask containing sterile 5 mm $\times$ 5 mm silicon shards.

Cell Preparation for Imaging

Shards containing maturing oligodendrocytes or macrophages were rinsed for 5 s in 18 M Ω water to remove contamination from excess media. To prevent cell rupture, they were quickly frozen in liquid ethane and stored under liquid nitrogen (LN₂). The samples were introduced to the ultrahigh vacuum (UHV) environment at LN₂ temperatures. Details of this process have been described elsewhere.¹³ Once under vacuum, the sample was warmed 5 $^{\circ}$ C/min to -80° C to remove the water while minimizing crystallization and redistribution of molecules by efflux of water from the surface. This process was monitored using a video camera mounted on a brightfield microscope, to ensure that microscale signs of crystallization did not occur. When the water was removed, the sample was quickly returned to liquid nitrogen temperatures. During the cooling process, some residual water in the vacuum environment was redeposited on the sample, producing a uniform layer of water on top of the cells.

Preparation of Patterned Cholesterol Films

Physical vapor deposition (PVD) films were prepared by subliming cholesterol contained in a crucible by resistive heating of a tungsten filament followed by deposition onto a LN₂-cooled sample stage. The film thickness was monitored using a quartz crystal microbalance (QCM) and subsequently characterized using AFM. AFM images were collected using a Nanopics 2100 instrument (KLA Tencor, San Jose, CA) by scanning a 200 μ m field of view (FOV) in contact mode.

Preparation of Lipid Films

Lipids were dissolved in 9:1 CHCl₃/MeOH solution at concentration of 1 mg/mL. Films were prepared by spin-coating 50 μ L aliquots of lipid solution onto 5 mm² silicon shards at a spin rate of 3000 rpm for 60 s. Dipalmitoylphosphatidylcholine and dilauroylphosphatidylethanolamine were obtained from Avanti Polar Lipids (Alabaster, AL), and cholesterol was obtained from Sigma Aldrich (St. Louis, MO). All lipids were used without further purification. Each lipid spectrum was taken from a sampling area of 132 $\times$ 102 μ m² using 5 $\times$ 10⁵ primary ion pulses. For enhancement measurements, the integrated peak area for the mass of interest was divided by the integrated peak area for the internal standard fragment (m/z 69), yielding a relative intensity.

Mass Spectrometry

Imaging data using In⁺ primary ions were acquired using a Kratos Prism ToF-SIMS spectrometer (Manchester, U.K.) equipped with an In⁺ liquid metal ion source (FEI, Beaverton, OR). The pulsed primary ion source was operated at an anode voltage of 15 kV angled at 45 $^{\circ}$ to the sample. The beam was focused to approximately 200 nm in diameter, and it delivered 1 nA of DC current in 50 ns pulses. The sample was mounted onto a LN₂-cooled analysis stage (Kore Tech. Ltd., Cambridge, U.K.) biased at +2.5 kV, 2.5 in. from a cold trap also cooled with LN₂. An extraction lens, biased at -4.7 kV, collected the

secondary ions which then traveled along a 4.5 m flight path and were detected at a microchannel plate (MCP) detector (Galileo Co., Sturbridge, MA).

Imaging data using C_{60}^+ primary ions were acquired using a BioToF ToF-SIMS spectrometer, described in detail elsewhere.³³ The spectrometer was equipped with a 40 keV C_{60}^+ primary ion source³⁴⁻³⁶ (Ionoptika Ltd., Southampton, U.K.). The source was operated with a 300 μm diameter beam defining aperture, yielding approximately 20 pA of DC current.

Mass spectrometry images were acquired by raster-scanning the primary ion beam across the sample region and collecting a mass spectrum for each pixel. Using in-house imaging software, Visual C++, molecule-specific images were created by selecting a mass peak of interest from the total ion mass spectrum and plotting the intensity of this mass at each pixel in the image. The intensity of each peak could then be displayed in false color, and multiple images were overlaid. Signal intensities can be relatively compared in mass specific images using the line scan function built into the software. A line scan is a plot of the summed pixel intensities for a given chemical map as a function of the lateral distance of the line.

The instrument was also equipped with a channeltron detector (Burle, Lancaster, PA) positioned approximately 0.5 cm from the sample which was used to generate scanning ion micrographs (SIM). Scanning ion images were obtained by using the channeltron to measure the current of charged species and electrons emitted from the sample during direct current ion bombardment.

Results and Discussion

Single Cell Imaging

Analyzing the native morphology and molecular distributions is crucial to determining the biological role of individual molecules. Several cryogenic methods have been developed to achieve this difficult end.^{14,37} In the method presented here, the samples are plunge-frozen in ethane to eliminate sample damage due to water crystallization and then stored under LN_2 until analysis. A sample preparation and transfer chamber designed in-house¹³ is utilized to fracture the sample sandwich and move the sample to the analysis stage without significant warming. With careful control of the temperature and pressure during this process, a clean fracture surface can be generated. The limitations of this approach are that control over the conditions can be difficult to maintain through the transfer process and that the plane of fracture is unpredictable. This can result in fractures through the center of a cell or through pure ice, leaving the cell damaged or completely buried.

Previous attempts in our laboratory to remove excess surface water through sublimation have been unsuccessful. Warming of freeze-fractured liposomes results in images with lipid spread homogeneously across the surface.¹³ It appears that large water sublimation fluxes can redistribute molecules across surfaces. We have found here that significant amounts of water can be removed from the surface without the redistribution of lipids, by ensuring that the sample temperature does not exceed $-80^\circ C$ before being returned to LN_2 temperature. Images of cells that have been prepared in this fashion are shown in Figure 1.

A mature oligodendrocyte in culture, exhibiting the characteristic, delicate cellular processes, is shown in Figure 1A. A brightfield image of a similar oligodendrocyte in vacuum after undergoing the freeze-etching process is shown in Figure 1B. The SIM image shown in Figure 1C of the same cell shown in part B demonstrates that the cell maintains its morphology. A SIMS image overlay, where the green pixels represent m/z 184 phosphatidylcholine (PC) and blue represents m/z 28 from the Si substrate, is shown in

Figure 1D. From this image, we can see that there is not a measurable redistribution of lipid molecules from the cell to the adjacent surface, as previously seen when warming samples. 13,38

To demonstrate the damage that occurs when warming is not controlled, Figure 1E and F show a cell that has been damaged by the warming process. The brightfield image in part E shows that the delicate processes of the cell have been destroyed. In addition, the SIMS overlay shown in part F demonstrates that there is significant smearing of the PC signal that occurs. In fact, the resulting image in these cases rarely resembles what is observed in the microscope.

Deposition of Water

To test the effect of water redeposition on the sample, a simple model system consisting of a patterned thin film of cholesterol was used, and this was characterized by SIMS and AFM. The film was created using physical vapor deposition of cholesterol onto a Si substrate with a finder grid in the center. After the deposition, the finder grid was removed, leaving a relief pattern on the Si. SIMS images of the resulting film, before (A and B) and after (C and D) the deposition process, are shown in Figure 2.

Images A and C in Figure 2 show all of the ions collected from the surface on the same thermal intensity scale. Deposition of water was confirmed by monitoring the increase of m/z 18, representing $[\text{H}_2\text{O}]^+$, using SIMS (data not shown). When qualitatively comparing images A and C, it is evident that more ions are collected from the cholesterol islands after deposition. The inverse is true for the signal from the Si substrate. Figure 2B and D shows SIMS overlay images that represent cholesterol (green) and Si (blue). The ionization effect on cholesterol is demonstrated through the use of a line scan shown in Figure 2E. A more thorough investigation of these ionization effects can be found later. The data in image D shows that there is no measurable change in the distribution of cholesterol as a result of water deposition. Due to the limitations of spot size of the In^+ source, measurements of lateral resolution from this analysis have been limited to approximately $1\text{ }\mu\text{m}$, and no change in resolution was measured after deposition. To more fully demonstrate the preservation of film features, the samples were also analyzed using AFM.

Representative AFM images are shown in Figure 3. Images A and B show the same feature on the film before and after being placed in vacuum and having water deposited and then removed, respectively. Image B shows that there are no changes resulting from the deposition and removal process. The representative line scans shown correspond to the same feature on the film, and measurements of feature size confirm that there is no change.

There is no evidence of damage from deposition or removal, which implies that a matrix can be vapor-deposited without imposing a limit on spatial resolution. This result has important implications for imaging of single cells with SIMS, as it increases the signal without compromising sample preservation. Various compounds, other than water, might be deposited in vacuum to yield greater ionization increases.

Signal Enhancement

Water sublimed from the sample is not completely removed from the vacuum environment. Thus, the subsequent return of the sample to liquid nitrogen temperature results in the deposition of some water on the sample. Redeposition can be monitored from the intensity of the mass peak at m/z 18, in the mass spectrum of freeze-etched samples. Of great potential interest, water deposition was found to enhance the signal from several lipid molecules.

To quantify the enhancement observed, films of the lipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), and cholesterol (CH) were analyzed with SIMS using an In^+ primary ion beam (Table 1). The intensity of characteristic fragments^{39,40} was measured by integrating the peak areas and normalizing them to the intensity of m/z 69 (C_5H_9^+). The (C_5H_9^+) fragment has been used as an internal standard to account for small variations in secondary-ion signals that can occur during SIMS analysis as a result of primary beam current fluctuation, sample topography, and secondary-ion collection efficiency.^{16,41} With this analysis, variability of less than 10% is achieved using many different samples.

Signal enhancement is observed for all characteristic fragments measured in the analysis; however, as is seen in previous studies,^{25,30,31,42} the amount of enhancement varied for each fragment analyzed. Some signals, such as m/z 184, resulting from the PC headgroup, have already been extensively mapped in previous SIMS imaging experiments.^{15,16,20,37} Other ions have not produced sufficient signal intensity for the production of detailed images. Of particular interest to lipid mapping experiments are the signal enhancements observed for characteristic lipid tail fragments, lipid molecular ions, and the m/z 224 fragment (PC headgroup plus glycerol). Significant increases in the tail group signal make it possible to image unique distributions of acyl chains in lipid models such as liposomes or supported bilayers. Also significant is the appearance of the molecular ion peak for dipalmitoylphosphatidylcholine (DPPC). Ion bombardment using In^+ is known to induce a large amount of fragmentation upon impact, and the DPPC molecular ion is not observed in dry lipid preparations. The cold samples of DPPC exhibit a considerable signal corresponding to $[\text{M} + \text{H}]^+$. Enhancement of the 224 fragment is also of significance to biological imaging experiments because reference spectra⁴³ demonstrate that this fragment can be used to differentiate between PC derived from phospholipid species and PC derived from sphingomyelin, which has not been possible in previous imaging studies.

Role of Water Deposition in Signal Enhancement

It has been previously established that PC ionization can proceed by intermolecular proton abstraction and that the presence of water in frozen hydrated samples increases ionization efficiency.⁴⁴ In an attempt to differentiate the effect of the presence of water from the effect of cooling the sample, a cold trap was placed in the analysis chamber to remove water from the chamber before the film was cooled on the stage. When the trap is employed, the signal enhancement observed from cooling is significantly decreased by $20 \pm 8\%$, and the change in ionization efficiency was not significant (Student's t -test, $p = 0.145$). When the trap is not used, the enhancement observed is significant ($51 \pm 6\%$, Student's t -test, $p = 0.002$). This is strong evidence that small amounts of water deposition cause the increase in ionization efficiency. Results are summarized in Figure 4.

Surface Stability during Analysis

To demonstrate the stability of the surface that results from the low-temperature deposition of a water matrix, multiple regions were analyzed across the surface of a DPPC film over the course of 3 h. The intensity of m/z 18, standardized to total secondary-ion intensity, was used to monitor the deposition of water (data not shown). After an initial 20 min of cooling, the enhancement effect is saturated. Assuming a sticking coefficient of unity, 20 min corresponds to 1.6×10^{13} water molecules/ cm^2 . Following the initial increase, the signal intensity of characteristic DPPC fragments varied by no more than 10%.

Conclusion

A novel alternative approach is presented to prepare reproducible biovacuum interfaces amenable to ToF-SIMS imaging analysis. This process exposes cells with similar surface environments, making cell-to-cell comparisons feasible. During the preparation process, the cell remains frozen, thus preserving the native state of the membrane as well as the spatial distribution of the lipid molecules. Matrix deposition occurs at low temperatures, resulting in a matrix which does not impose a limit on lateral resolution.

Deposition of water from the vacuum environment serves to enhance the ionization efficiency of characteristic lipid fragments as well as high mass ions. Current evidence suggests that water interacting directly with the biological surface may donate protons, resulting in the observed enhancement effect. If so, it should be feasible to deposit other compounds that may be better proton donors to further increase the enhancement observed with water-ice. The deposition of water also serves to enhance the ionization of cholesterol, though the role of water in the mechanism is currently not known.

The enhancement effect is stable over a period of hours, resulting in an “analysis window” in which the results of different images can be directly compared. The ability to produce a consistent and uniform surface environment will greatly improve the reproducibility of SIMS imaging, and it is essential to obtaining quantitative information from SIMS analyses.

These procedures yield high quality spectra both for atomic bombardment in the low dose mode and with cluster bombardment. We believe this strategy adds to the options available for producing cryogenically preserved single cells with high spatial integrity for SIMS analysis.

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References

1. Simons K, Ikonen E. *Nature*. 1997; 387:569–572. [PubMed: 9177342]
2. Simons K, Toomre D. *Nat Rev*. 2000; 1:31–41.
3. Huttner WB, Zimmerberg J. *Curr Opin Cell Biol*. 2001; 13:478–484. [PubMed: 11454455]
4. Burns AR. *Langmuir*. 2003; 19(20):8358–8363.
5. Veatch SL, Polozov IV, Gawrisch K, Keller SL. *Biophys J*. 2004; 86(5):2910–2922. [PubMed: 15111407]
6. Burgos P, Yuan CB, Viriot ML, Johnston LJ. *Langmuir*. 2003; 19(19):8002–8009.
7. Ge MT, Gidwani A, Brown HA, Holowka D, Baird B, Freed JH. *Biophys J*. 2003; 85(2):1278–1288. [PubMed: 12885671]
8. Ritchie K, Iino R, Fujiwara T, Murase K, Kusumi A. *Mol Membr Biol*. 2003; 20:13–18. [PubMed: 12745919]
9. Mukherjee S, Maxfield FR. *Annu Rev Cell Dev Biol*. 2004; 20:839–866. [PubMed: 15473862]
10. Johansson B. *Surf Interface Anal*. 2006; 38(11):1401–1412.
11. Roddy TP, Donald M, Cannon J, Meserole CA, Winograd N, Ewing AG. *Anal Chem*. 2002; 74:4011–4019. [PubMed: 12199568]
12. Roddy TP, Donald M, Cannon J, Ostrowski SG, Winograd N, Ewing AG. *Anal Chem*. 2002; 74:4020–4026. [PubMed: 12199569]
13. Cannon DMJ, Pacholski ML, Winograd N, Ewing AG. *J Am Chem Soc*. 2000; 122:603–610.

14. Chandra S, Bernius MT, Morrison GH. *Anal Chem.* 1986; 58:493–496. [PubMed: 3963401]
15. McDonnell LA, Piersma SR, Altelaar AFM, Mize TH, Luxembourg SL, Verhaert PDEM, Minnen Jv, Heeren RMA. *J Mass Spectrom.* 2005; 40:160–168. [PubMed: 15706616]
16. Monroe EB, Jurchen JC, Lee J, Rubahkin SS, Sweedler JV. *J Am Chem Soc.* 2005
17. Peteranderl R, Lechene C. *J Am Soc Mass Spectrom.* 2004; 15:478–485. [PubMed: 15047053]
18. Marxer CG, Kraft ML, Weber PK, Hutcheon ID, Boxer SG. *Biophys J.* 2005; 88(4):2965–2975. [PubMed: 15695628]
19. Parry S, Winograd N. *Anal Chem.* 2005; 77:7950–7957. [PubMed: 16351142]
20. Nygren H, Eriksson C, Malmberg P, Sahlin H, Carlsson L, Lausmaa J, Sjoval P. *Colloids Surf, B.* 2003; 30:87–92.
21. Ostrowski SG, Szakal C, Kozole J, Roddy TP, Xu JY, Ewing AG, Winograd N. *Anal Chem.* 2005; 77(19):6190–6196. [PubMed: 16194078]
22. Winograd N. *Anal Chem.* 2005; 77:143A–149A.
23. Xu J, Ostrowski S, Szakal C, Ewing AG, Winograd N. *Appl Surf Sci.* 2004; 231(2):159–163.
24. Toubol D, Kollmer F, Niehus E, Brunelle A, Laprevote O. *J Am Soc Mass Spectrom.* 2005; 16:1608–1618. [PubMed: 16112869]
25. Altelaar AFM, Klinkert I, Jalink K, deLange RPJ, Adan RAH, Heeren RMA, Piersma SR. *Anal Chem.* 2006; 78:734–742. [PubMed: 16448046]
26. Wu KJ, Odom RW. *Anal Chem.* 1996; 68:873–882. [PubMed: 21619183]
27. Adriaensen L, Vangaever F, Lenaerts J, Gijbels R. *Rapid Commun Mass Spectrom.* 2005; 19(8): 1017–1024.
28. Altelaar AFM, van Minnen J, Jimenez CR, Heeren RMA, Piersma SR. *Anal Chem.* 2005; 77(3): 735–741. [PubMed: 15679338]
29. Marcus A, Winograd N. *Anal Chem.* 2006; 78(1):141–148. [PubMed: 16383321]
30. Kim Y-P, Oh E, Hong M-Y, Lee D, Han M-k, Shon HK, Moon DW, Kim H-S, Lee TG. *Anal Chem.* 2006; 78:1913–1920. [PubMed: 16536428]
31. Luxembourg SL, McDonnell LA, Duursma MC, Guo XH, Heeren RMA. *Anal Chem.* 2003; 75(10):2333–2341. [PubMed: 12918974]
32. McCarthy KD, DE Vellis J. *J Cycl Nucleotide Res.* 1978; 4:15–26.
33. Braun RM, Blenkinsopp P, Mullock SJ, Corlett C, Willey KF, Vickerman JC, Winograd N. *Rapid Commun Mass Spectrom.* 1998; 12(18):1246. [PubMed: 9772767]
34. Wong SCC, Hill R, Blenkinsopp P, Lockyer NP, Weibel DE, Vickerman JC. *Appl Surf Sci.* 2003; 203:219–222.
35. Weibel D, Wong S, Lockyer N, Blenkinsopp P, Hill R, Vickerman JC. *Anal Chem.* 2003; 75(7): 1754–1764. [PubMed: 12705613]
36. Fletcher JS, Conlan XA, Jones EA, Biddulph G, Lockyer NP, Vickerman JC. *Anal Chem.* 2006; 78(6):1827–1831. [PubMed: 16536417]
37. Colliver TL, Brummel CL, Pacholski ML, Swanek FD, Ewing AG, Winograd N. *Anal Chem.* 1997; 69:2225–2231. [PubMed: 9212701]
38. Severs, NJ.; Shotton, DM., editors. *Rapid Freezing, Freeze-Fracture, and Deep Etching.* Wiley-Liss; New York: 1995.
39. Murphy, RC. *Mass Spectrometry of Lipids.* Vol. 7. Plenum Press; New York: 1993.
40. Murphy, RC. *Mass Spectrometry of Phospholipids: Tables of Molecular and Product Ions.* Illuminati Press; Denver: 2002.
41. Ostrowski SG, Bell CTV, Winograd N, Ewing AG. *Science.* 2004; 305:71–73. [PubMed: 15232100]
42. Delcorte A. *Appl Surf Sci.* 2006; 252(19):6582–6587.
43. McQuaw CM, Zheng LL, Ewing AG, Winograd N. *Langmuir.* 2007; 23(10):5645–5650. [PubMed: 17417886]
44. Roddy TP Jr, Ostrowski SG, Ewing AG, Winograd N. *Anal Chem.* 2003; 75:4087–4094. [PubMed: 14632121]

Figure 1.

(A) Brightfield image of rat oligodendrocyte in culture. (B) Brightfield image of an oligodendrocyte cultured on silicon and cryogenically preserved using the described sample preparation method. (C) SIM image of rat oligodendrocyte. (D) SIMS image of a preserved oligodendrocyte, m/z 184 (PC) green, m/z 28 (Si) blue, intensity scale 0–4. (E) Brightfield image of oligodendrocyte that has been warmed to allow crystallization. (F) SIMS image of a corresponding area shown in (E), m/z 184 (PC) green, m/z 28 (Si) blue, intensity scale 0–2. Images were obtained using an In^+ primary ion beam; all scale bars are 20 μm .

Figure 2.

(A) Total ion image of a patterned cholesterol film prior to deposition. (B) SIMS image overlay from (A) m/z 369 (Cholesterol) green, m/z 28 (Si). (C) Total ion image of patterned cholesterol film after water deposition. (D) SIMS image overlay from (C) m/z 369 (Cholesterol) green, m/z 28 (Si). Thermal intensity scale from black to white, 0–20 counts. Scale bars represent 25 μm . (E) Line scans displaying the intensity of the cholesterol ion as a function of distance across the image “before” and “after” water deposition as indicated by the lines in part B and D.

Figure 3.

(A) 3D-AFM image of patterned cholesterol film before water deposition, FOV $100\ \mu\text{m}^2$. To the right is an average cross section of the film. (B) 3D-AFM image of patterned cholesterol film after water deposition and removal, FOV $100\ \mu\text{m}^2$. To the right is an average cross section of the same region as above.

Figure 4.

Histogram summarizing results of deposition experiments utilizing a coldfinger to control the deposition of water. Control intensity represents the signal intensity of m/z 184 (PC headgroup) on a dry lipid film. “Cooled with Trap” intensity represents signal intensity of m/z 184 when a coldfinger was cooled in the sample chamber prior to sample cooling, thus limiting the deposition of water. “Cooled No Trap” intensity represents the signal intensity of m/z 184 (PC headgroup) after cooling the stage without employment of a coldfinger.

Normalized Intensity of Characteristic Lipid Fragments before (Control) and after Water Matrix Deposition (Cold Film)^a

Table 1

fragment (<i>m/z</i>)	PC fragments				CH fragments			PE fragments			tail groups	[M + H] ⁺
	184	86	224	166	369	385	124	142	575	735		
control intensity	0.67	0.99	0.047	0.13	0.18	0.11	0.072	0.087	0.044	0		
control standard deviation	0.045	0.039	0.0043	0.007	0.043	0.012	0.0033	0.0041	0.0031	0		
cold film intensity	1.0	1.9	0.11	0.24	0.28	0.21	0.114	0.121	0.08	0.00179		
cold film standard deviation	0.11	0.039	0.021	0.034	0.031	0.025	0.0057	0.0085	0.0054	0.00077		
% enhancement	51	94	141	84	56	95	59	40	82			

^aData were obtained using In⁺ as the primary ion with all intensities normalized to *m/z* 69. Cold films were exposed to approximately 0.1 L (1 Langmuir) = 10⁻⁶ Torr s).