Published in final edited form as:

Appl Surf Sci. 2008 December 15; 255(4): 1298–1304. doi:10.1016/j.apsusc.2008.05.139.

Which is more important in bioimaging SIMS experiments—The sample preparation or the nature of the projectile?

M.E. Kurczyk^a, P.D. Piehowski^a, S.A. Parry^a, M. Jiang^b, G. Chen^b, A.G. Ewing^{a,c}, and Nicholas Winograd^{a,*}

^a Department of Chemistry, Penn State University, 104 Chemistry Building, University Park, PA 16802, United States

^b Department of Biology, Penn State University, 224 Life Sciences Building, University Park, PA 16802, United States

^c Department of Chemistry, Gothenburg University, Kemivägen 4, SE-41296 Gothenburg, Sweden

Abstract

Sample preparation is central to acquiring meaningful molecule-specific images with SIMS, especially when submicron lateral resolution is involved. The issue is to maintain the distribution of target molecules while attempting to introduce biological cells or tissue into the high vacuum environment of the mass spectrometer. Here we compare freeze-drying, freeze-etching, freeze-fracture and trehalose vitrification as possible strategies for these experiments. The results show that the prospects for successful imaging experiments are greatly improved with all of these methods when using cluster ion bombardment, particularly C_{60}^+ ions, not only due to increased sensitivity of this projectiles, but also since it removes contamination overlayers without insult to the underlying chemistry. The emergence of 3-dimensional imaging capabilities also suggests that sample preparation should not perturb the 3-dimensional morphology of the cell, a situation not generally possible during freeze-drying. Hence, sample preparation and projectile type are strongly coupled parameters for bioimaging with mass spectrometry.

Keywords

Bioimaging; Cluster SIMS; Freeze-fracture; Freeze-etching; C_{60} ; Vitrification

1. Introduction

There is a fundamental problem with employing mass spectrometry for bioimaging experiments, and that is, the desorbed molecules – be they derived from laser ablation or ion beam sputtering – originate from the near surface region of the sample and must be detected in a vacuum environment. Since live cells or tissue do not appreciate a vacuum very much, a great deal of effort has been devoted to sample preparation so that representative chemical information can be obtained within the confines of the mass spectrometer [1–6]. The emergence of cluster projectiles with SIMS has implications for bioimaging since bombardment often leaves relatively little chemical damage on molecular surfaces, even after rather high fluences. In some cases, molecular depth profiling and 3-dimensional imaging are feasible with submicron resolution [7,8]. These properties open new avenues for sample preparation since the surface of the sample need not necessarily be presented to the ion beam in pristine condition.

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

Our laboratory has been exploring many different ways of dealing with cells and tissue for imaging mass spectrometry. The “gold standard” in our view is freeze-fracture of frozen hydrated cells. With this approach, a thin aqueous layer containing a small number of cells is quenched in liquid ethane to form an amorphous ice structure at temperatures below 150 K [9]. Rapid cooling is essential to avoid crystallization, which can vault molecules many microns away from their original positions. This method is effective, but very tedious. We have made many comparisons to freeze-drying techniques, where water is removed from the material slowly by evacuation at room temperature or at reduced temperature. This approach almost always leads to artifacts, although they can be minimized through careful control of temperature and pressure. Other methods are currently under development, including one that utilizes trehalose as a matrix to stabilize peptides, lipids and whole cells [10] and one that involves freeze-etching frozen hydrated cells in the mass spectrometer at 150 K. A major point that has increased the flexibility of sample preparation techniques is the fact that contamination overlayers – be they ice-like or biological junk – may be removed by cluster bombardment in the mass spectrometer without insult to the arrangement of underlying molecules. In this paper, these methods will be compared using model systems, and several different cultures including tetrahymena, glial, and macrophage cell lines. The bottom line, with respect to the title of this presentation, is that the sample preparation and the nature of the projectile are intimately entangled, and new opportunities are emerging.

2. Experimental methods

Tetrahymena thermophila of complementary mating types B2086 (II) and Cu428.1 (VII) were grown overnight in PPYGF medium (2% proteose peptone, 0.1% yeast extract, 0.2% glucose, and 0.003% ferric EDTA) at 300 K with shaking. The cells were washed twice by centrifugation (400 g × 5 min), resuspended, and shaken overnight in 10 mM Tris-HCl, pH 7.4 at 300 K. To initiate conjugation, the starved, complementary strains were mixed and incubated at 300 K. After allowing the cells to mix for several hours, small aliquots (5 µL) of the cell suspension were placed on 5 mm × 5 mm silicon wafers and either fast frozen or freeze-dried.

Glial cells were harvested from 1-day-old Sprague-Dawley rat pups and separated from cerebral tissue as previously described [11]. The cells were cultured on 5 mm × 5 mm Si shards (Ted Pella) that were coated with 0.2 mg/mL poly(L-lysine) (VWR) and 0.1 mg/mL collagen (Sigma-Aldrich). Cells were incubated until maturation was visible under a microscope.

Macrophages were cultured on sterile polystyrene cell culture flasks and incubated at 37 °C and 5% CO₂. Two flasks containing cells were treated with 25 µg of Dye/mL of serum-free RPMI media (Invitrogen, Carlsbad, CA) for 1 h, one flask was treated with 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine (DiI) and the other was treated with 1,1'-dioctadecyl-3,3',3'-tetramethylindodicarbocyanine (DiD). The cells were then rinsed 5 times in the serum-free media, dislodged from their respective flasks, and placed in a Petri dish containing 5 mm × 5 mm silicon wafers (Ted Pella, Redding, CA). The cells were allowed to settle onto the Si wafers for all sample preparation methods.

Mouse embryos were dissected according to appropriate available protocols [12]. Transverse slices were performed in PBS buffer under a dissection microscope using a scalpel. The tissue was rinsed in distilled water and frozen with liquid N₂.

In this paper, we focus only on a few characteristic ions that indicate that the cells have not been destroyed. These include the phosphocholine headgroup fragment, C₅H₁₅NPO₄⁺, at *m/z* 184.07, a common SIMS signal from most cells, due to the natural abundance of phosphatidylcholine and sphingomyelin in cell membranes. In addition, the positive ion signal at *m/z* 69.07 signal, from C₅H₉⁺, is a fragment from the lipid tail groups, and is ubiquitous for

most sample preparations. The positive ion signals m/z 369.4 and 385.4, $M+H-H_2O$ and $M-H$, respectively, identify cholesterol in SIMS. The m/z 28 is used to locate signal from the Si substrates commonly used in the cell experiments.

Imaging data using In^+ bombardment was 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 keV angled at 45° to the sample. The beam was focused to approximately 200 nm in diameter, and delivered 1 nA of current in 50 ns pulses. The sample was mounted onto a liquid N_2 cooled analysis stage (Kore Tech. Ltd., Cambridge, U.K.) biased at +2.5 keV, 2.5 in. from a cold trap also cooled with liquid N_2 . 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 with C_{60}^+ , Au^+ , Au_3^+ bombardment was acquired using a homebuilt ToF-SIMS spectrometer, described in detail elsewhere [13]. The spectrometer was equipped with a 40 keV C_{60}^+ and a 25 keV Au^+ primary ion sources (Ionoptika Ltd., South-ampton, U.K.) [14]. The C_{60}^+ source was operated with a 300 μm diameter beam defining aperture, yielding approximately 20 pA current. The Au^+ probe was focused to about 250 nm and delivered from 100 to 300 pA of current. Au_3^+ delivered 10–25 pA of current and was focused to 1 μm .

3. Results and discussion

3.1. Freeze-drying

The simplest strategy for preserving cells for analysis in vacuum is freeze-drying of cells cultured on a flat substrate such as a Si substrate. This procedure involves removing the substrate from the culture media, with no rinsing, followed by plunging the chip directly into liquid N_2 coolant. Once frozen, the substrate is transferred to a vacuum chamber for several hours at a modest pressure of 10^{-6} torr where the water is removed by sublimation. The chamber remains at room temperature during this process, but the water sublimates before any melting occurs.

An example, using the tetrahymena cell line, is illustrated in Fig. 1. In this situation, the freeze-dried cells generally retain their two dimensional morphology, as seen in the brightfield optical image and the SEM images shown in Fig. 1(a and b), respectively. However, the total ion SIMS image, and the mass selected images at m/z 184 and m/z 28 are extremely weak in the location of the cells as seen in Fig. 1(c and d). It is clear from the optical and SEM image that residual material from the culture medium has deposited on and around the cells. Acquiring a mass spectrum from the pixels in the cell region gives a spectrum that is dominated by Na and PDMS. This material is blocking the emission of the key secondary ions, especially m/z 184, which should be of high intensity in the region of the cell.

It is certainly possible to quickly water rinse the cells to rid the freeze-dried product of residual culture material. There is significant danger of altering the morphology of the cells, due to swelling induced by osmotic pressure, as illustrated in the example presented in Fig. 2. In this instance, the optical brightfield image of the rinsed tetrahymena cells shown in Fig. 2(a) suggest that they have lysed open. The absence of residual material, however, allows membrane-specific ions such as m/z 184 to be present in much higher intensities as seen in Fig. 2(b). The m/z 184 signal also strongly localizes to the cell. Since the cells have experienced a great deal of trauma during the rinsing process, detailed biological studies would be quite suspect.

Even when the procedure is highly optimized by quickly plunge-freezing the rinsed tetrahymena cells into liquid C_2H_6 , and slowly freeze-drying to minimize water vapor wind

phenomenon, there are still often experimental artifacts that complicate any interpretation. An example is shown in Fig. 3, where the SEM image of these types of cells suggests that they are well-preserved. The SIMS images, however, clearly show that even though the m/z 184 diagnostic signal is nicely localized, the presence of m/z 69, an ubiquitous hydrocarbon ion which identifies hydrocarbon material that most likely originated in the cell, suggests leakage from cell into the surrounding areas.

Finally, it is interesting to examine the response of freeze-dried cells to cluster bombardment, even though the possibility of cell damage must still be kept in mind. A comparison of the results of total ion imaging with several different projectiles is shown in Fig. 4. Note that the amount of signal arising from the cell itself goes from nearly zero using the In^+ or Au^+ projectile, to values larger than seen from the Si substrate for Au_3^+ and C_{60}^+ bombardment. This type of effect has been reported previously for polymer beads on Si substrates [15]. The effect arises due to the much higher relative sputtering yield enhancement observed for soft materials versus harder materials such as Si.

In summary, freeze-drying in its various forms often yields cells that appear to be well-preserved, but many artifacts both obvious and subtle can occur during the process. The emergence of cluster bombardment has opened many new possibilities since greatly improved signal intensities are usually observed from the cell location. It is also interesting to note that many of these artifacts are not observed until the lateral resolution is reduced to below several microns.

3.2. Trehalose vitrification

To minimize cell collapse and cell damage, we have sought to find a stabilizing matrix that helps to keep things in their original location. The disaccharide trehalose is known to preserve dehydrated biological tissue and cells [10]. Even entire trehalose producing organisms have been found to survive natural desiccation for years [16]. Hence, this material is an excellent candidate for use such as a stabilizing matrix, and we have worked to add this step to the freeze-drying process. The main advantage is that the 3-dimensional integrity of the cell membrane is preserved while maintaining the possibility of achieving submicron lateral resolution.

An example of glial cells prepared for SIMS imaging via trehalose vitrification is shown in Fig. 5. The procedure is quite straightforward. Trehalose is added directly to the culture media. After several hours incubation, the culture media is rinsed away with a PBS buffer solution with 50–100 mM trehalose. Then there is a final rinse in a 50–100 mM trehalose, 0.3% glycerol, water solution. Since disaccharides are natural cryoprotectants, fast-freezing is not necessary. The cells are then frozen in liquid nitrogen, and dried in vacuum at 10^{-6} torr. In Fig. 5(a), the total ion SIMS image from 20 keV Au^+ ion bombardment shows detailed morphological structure, with the cell nucleus bulge clearly visible. In Fig. 5(c), the m/z 184 ion is shown to be localized with the cell, cell processes and nuclear bulge in the membrane without any apparent artifacts. The preparation is also amenable to C_{60}^+ ion bombardment. As shown in Fig. 5(b) the total ion image exhibits much less contrast than Au^+ bombardment since the yields are approximately the same from the cell and the substrate, but the chemical image shown in Fig. 5(d) exhibits larger counts of m/z 184, albeit with somewhat poorer lateral resolution.

Practically then, trehalose offers a platform where cells can be analyzed at room temperature in their native morphology. The disaccharide matrix offers to minimize the financial and time investments incident to analysis of frozen samples. While amorphous ice provides the most native cellular preservation, frozen samples are particularly susceptible to condensation *in vacuo*, which can prevent resolving and profiling fine cellular domains, such as those found in the cellular membrane. A matrix which facilitates long term storage and analysis and 3-

dimensional preservation, may be the model platform for time intensive-studies, such as depth profiling of biological cells.

3.3. Freeze-etching

Another strategy for maintaining the 3-dimensional morphology of cells is to prepare them in a frozen-hydrated state. We have explored several approaches to this challenging sample preparation method. The simplest procedure is to culture the cells directly on a Si substrate, wash the cell surface quickly, within a few seconds, with distilled water to remove the culture medium, and then plunge freeze the entire construct into liquid C_2H_6 . The plunge freezing minimizes ice crystallization which can vault molecules many microns away from their original location. This sample may then be stored under liquid nitrogen indefinitely. For SIMS imaging, the cold sample is removed from the liquid nitrogen and inserted directly into the cooled sample stage of the mass spectrometer. During this transfer, ice crystals inevitably deposit on the cell surface, preventing a meaningful analysis. However, in vacuum, we have found it is possible to slowly warm the sample at 5 K/min to a final temperature of 190 K. At this point, the water on top of the cell is slowly subliming without allowing crystallization. After about 1 h, a time determined by monitoring the process with a video camera, the sample is recooled to less than 100 K for imaging. Although a small amount of ice condenses on the sample surface during cooling, positive SIMS ion yields are found to be enhanced by about a factor of 2 due to the presence of this layer [17].

An example of images produced with this procedure is shown in Fig. 6 using rat oligodendrocytes as a model. Note that the optical image in Fig. 6(a) shows well-defined morphology with processes of $\sim 1 \mu m$ in diameter clearly seen extending from the cell body. These features are retained for the total ion image, and the molecule-specific image shown in Fig. 6(c and d), respectively. Overall, this approach is slightly less complicated than *in situ* freeze-fracture, and provides results that are generally as reliable.

3.4. C_{60} -etching

The observation that C_{60} bombardment allows molecular depth profiling in many cases suggests that the preparation techniques discussed above may be modified and improved. The basic idea is to culture cells in the laboratory environment and transfer them directly to the mass spectrometer at cryogenic temperatures. The C_{60} etching is then utilized to remove any residual contamination overlayer without insult to the underlying material.

An example of the application of this idea is shown in Fig. 7. For the top two images, Fig. 7(a and b), a 300 nm film of cholesterol was deposited onto a Si substrate covered with a Cu grid by physical vapor deposition. After deposition, the grid was removed to produce the resulting patterned film with an interface width between the Si and cholesterol of about $2 \mu m$. The film was then inserted into liquid N_2 , and transferred in air to the mass spectrometer. The initial image shows a rather uniform coverage of m/z 18, with the underlying pattern barely visible. After etching with a dose of $10^{13} C_{60}^+$ ions/cm², the water overlayer is completely removed, exposing the patterned cholesterol film underneath.

For the two images on the right hand side, Figs. 7(c and d), respectively, a macrophage cell is cultured directly onto a Si wafer and flash-frozen as described above. After transfer to the mass spectrometer, the image is largely covered with water-ice as indicated by the blue color at m/z 18. After etching with a dose of $10^{13} C_{60}^+$ ions/cm², the m/z 184 ion is clearly revealed and is well-localized to the macrophage. These results certainly suggest that it is no longer necessary to worry as much about contamination overlayers during the sample preparation process.

The C₆₀-etching method may also be useful for a number of freeze-dried samples. In preliminary experiments with a freeze-dried mouse embryo, we find that the drying process results in the smearing of the m/z 184 signal and the m/z 369 signal as shown in Fig. 8(a–c). Using the hypothesis that the redistribution chemistry may be less violent within the cell, we utilized C₆₀ etching to remove the redistributed layer to uncover a less disturbed portion of the sample. To estimate the sputter depth, values for sputter yield and molecular density from previous experiments involving trehalose, were utilized. Therefore, the fluence corresponds to the removal of roughly 30–40 nm of material. As a result of sputtering, a decrease in m/z 184 was observed as expected, while the m/z 369 signal increased from nonexistent. At this point, as is clear from Fig. 7(d–f) that both the m/z 184 signal and the m/z 369 signal are well localized to the cell. This preliminary result suggests that the redistribution of cholesterol is largely a surface phenomenon, and may remain undisturbed in the bulk of the tissue. These observations will obviously need to be tested more rigorously, but they suggest that the use of cluster bombardment may open new ways to preserve chemical localization.

3.5. Freeze-fracture of frozen-hydrated cells

We believe that the safest way to preserve the 3-dimensional cell morphology and chemical arrangement to submicron precision is by freeze-fracture of frozen-hydrated cells directly within the confines of the mass spectrometer. There is considerable evidence that the presence of water as well as preserving the native bilayer structure, provides an optimal environment for ionization [18–20]. This strategy has already led to a number of successful applications including the quantitative assay of cholesterol in macrophage cells [21] and the observation of lipid rearrangement in the highly curved membranes of mating tetrahymena cells [22]. However, there are still artifacts with this technique that need to be considered. An example is shown in Fig. 9 where different freeze-fracture situations result either in undesirable topography or an unacceptable levels of sample charging. Three situations are compared using the m/z 69 species to represent ubiquitous hydrocarbon, and the m/z 184 peak to indicate membrane composition. If the ice layer is too thick, as seen in Fig. 9(a and b), the samples charge very badly under atomic ion bombardment as indicated by very weak signals. Current efforts to neutralize this sample charging, using an electron flood gun, have been unsuccessful. If the ice layer is too thin, as seen in Fig. 9(c and d), the morphology of the cell creates shadowing effects which make quantitative interpretation very difficult. Moreover, these samples are subject to rapid warming during transfer which often is accompanied by chemical smearing as noted in Fig. 9(c). Only when the ice thickness is on the order of the same thickness of the cell, in this case about 10 μm , is a uniform distribution of m/z 69 observed across the cell, indicating the non-uniform distribution of m/z 184 has biological significance. This last situation provides the cleanest representation of the chemistry of cells that we have seen using any of the other sample preparation protocols.

Until now, this cell line has only been examined with 15 keV In⁺ ion bombardment, due to instrumental constraints in our laboratory. We are anxious to utilize C₆₀⁺ ion bombardment to image these cells for comparison purposes. If what we have learned from the earlier discussions holds true for the freeze-fracture methodology, the use of C₆₀ should greatly reduce the charging problems associated with thicker ice films and should obviate the need for very thin ice layers.

4. Conclusions

Here we have described a number of preparation techniques under development in our laboratory applicable to SIMS imaging experiments on single biological cells. It is essential to appreciate that the strategy for these types of systems is very different than for the characterization of tissue slices where the image field of view and lateral resolution

requirements may differ by orders of magnitude. The strategies described here are by no means the only possible approaches. Other workers have proposed rinsing with ammonium acetate [23] to remove remnants of the culture medium, and others have used fixative agents [24,25] and imprints of cells in metal foil [26] to maintain chemical localization. All of these methods deserve further research to broaden the tools available for SIMS users.

There are a few issues which are coming to the forefront of our thinking. First, it is clear that 3-dimensional imaging has enormous potential, given the properties of cluster bombardment. For 3-dimensional imaging to work properly, the 3-dimensional structure of the cell must be preserved to a very high level of precision, given that the depth resolution of SIMS in this modality is on the order of 20 nm or less. Hence, we are skeptical that simple freeze-drying methods will be good enough to allow reliable data to be acquired. Secondly, the advent of cluster bombardment opens new possibilities for sample preparation. The removal of contamination overlayers without insulting the underlying cell is certainly an important characteristic when dealing with cryogenic samples, or when using sugar to prevent cell rupture. In summary, then, successful SIMS imaging at the single cell level is critically dependent upon the choice of sample preparation protocols. There are many options available, but the emergence of cluster ion beams adds new dimensions to these options. Hence, both sample preparation and the nature of the projectile are equally important to acquiring successful information.

Acknowledgments

The authors acknowledge the National Institute of Health under grant # EB002016-13 for financial support of this research.

References

1. Chandra S, Morrison GH. *Biology of the Cell* 1992;74:31–42. [PubMed: 1511245]
2. Colliver TL, Brummel CL, Pacholski ML, Swanek FD, Ewing AG, Winograd N. *Analytical Chemistry* 1997;69:2225–2231. [PubMed: 9212701]
3. Cannon DM, Pacholski ML, Winograd N, Ewing AG. *Journal of the American Chemical Society* 2000;122:603–610.
4. Marcus A, Winograd N. *Analytical Chemistry* 2006;78:141–148. [PubMed: 16383321]
5. Nygren H, Eriksson C, Malmberg P, Sahlin H, Carlsson L, Lausmaa J, Sjoval P. *Colloids and Surfaces B: Biointerfaces* 2003;30:87–92.
6. Altelaar AFM, Klinkert I, Jalink K, Lange RPJ, Adan RAH, Heeren RMA, Piersma SR. *Analytical Chemistry A*. 2005
7. Wucher A, Cheng J, Winograd N. *Analytical Chemistry* 2007;79:5529–5539. [PubMed: 17583913]
8. Fletcher JS, Lockyer NP, Vaidyanathan S, Vickerman JC. *Analytical Chemistry* 2007;79:2199–2206. [PubMed: 17302385]
9. Severs, Nicholas JJ.; Shotton, David M. *Rapid Freezing, Freeze Fracture and Deep Etching*. Wiley, John & Sons; 1995.
10. Parry S, Winograd N. *Analytical Chemistry* 2005;77:7950–7957. [PubMed: 16351142]
11. McCarthy KD, Vellis JDE. *Journal of Cyclic Nucleotide Research* 1978;4:15–26. [PubMed: 25289]
12. Kathleen, Molyneaux. Tissue was prepared under the appropriate protocols. Case Western Reserve University;
13. Braun RM, Blenkinsopp P, Mullock SJ, Corlett C, Willey KF, Vickerman JC, Winograd N. *Rapid Communications in Mass Spectrometry* 1998;12:1246–1252. [PubMed: 9772767]
14. Fletcher JS, Conlan XA, Jones EA, Biddulph G, Lockyer NP, Vickerman JC. *Analytical Chemistry* 2006;78:1827–1831. [PubMed: 16536417]
15. Xu J, Ostrowski S, Szakal C, Ewing AG, Winograd N. *Applied Surface Science* 2004;231–2:159–163.

16. Crowe JH, Crowe LM. *Nature Biotechnology* 2000;18:145–146.
17. Piehowski PD, Kurczy ME, Willingham D, Parry SA, Heien ML, Winograd N, Ewing AG. *Langmuir*. in press.
18. Roddy TP, Cannon DM Jr, Ostrowski SG, Ewing AG, Winograd N. *Analytical Chemistry* 2003;75:4087–4094. [PubMed: 14632121]
19. Prinz C, Malm J, Hook F, Sjoval P. *Langmuir* 2007;23:8035–8041. [PubMed: 17569548]
20. Conlan XA, Lockyer NP, Vickerman JC. *Rapid Communications in Mass Spectrometry* 2006;20:1327–1334. [PubMed: 16555365]
21. Ostrowski SG, Kurczy ME, Roddy TP, Winograd N, Ewing AG. *Analytical Chemistry* 2007;79:3554–3560. [PubMed: 17428032]
22. Ostrowski SG, Bell CTV, Winograd N, Ewing AG. *Science* 2004;305:71–73. [PubMed: 15232100]
23. Wu KJ. *SIMS Workshop* 2007;20
24. Levi-Setti R, Gavrilov KL, Neilly ME, Strick R, Strissel PL. *Applied Surface Science* 2006;252:6907.
25. Liu Q, Guo Z, He L. *Analytical Chemistry* 2007;79:3535–3541. [PubMed: 17428031]
26. Sjoval P, Lausmaa J, Nygren H, Carlsson L, Malmberg P. *Analytical Chemistry* 2003;75:3429–3434. [PubMed: 14570193]

Fig. 1.

Freeze-dried tetrahymena cells directly from culture medium. The images shown are (a) optical brightfield, (b) SEM, (c) total positive ions and (d) m/z 28 (blue) and m/z 184 (green-barely visible). The field of view is 100 μm . All SIMS images were recorded using 15 keV In^+ ion bombardment (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 2. Tetrahymena cells, rinsed in 18 Mohm water, and then freeze-dried. (a) Optical brightfield. (b) m/z 28 (blue) and m/z 184 (green). The field of view is 100 μm . The SIMS image was recorded using 15 keV In^+ ion bombardment (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 3. Freeze-dried tetrahymena cells revealing hidden damage (a) SEM. (b) m/z 28 (blue), m/z 184 (green), and m/z 69 (red). Field of view is 100 μm . The SIMS image was recorded using 15 keV In^+ ion bombardment (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 4.

Total ion SIMS images of rinsed and freeze-dried tetrahymena cells. The primary ion is (a) 15 keV In^+ , (b) 20 keV Au^+ , (c) 20 keV Au_3^+ and (d) 20 keV C_{60}^+ . The field of view is 100 μm in (a and b), and 250 μm in (c and d) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 5.

Mixed Glia–Oligodendrocytes and Astrocytes lyophilized in trehalose. From the total ion SIMS image acquired using Au⁺ bombardment in (a), the morphology of the cell is seen to be preserved. The total ion SIMS image in (b) was acquired using C₆₀ bombardment. The molecule-specific images from Au⁺ and C₆₀⁺ bombardment are shown in (c and d), respectively. The field of view is 390 μm (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 6.

(a) Brightfield image of rat oligodendrocyte cultured on Si substrate and cryogenically preserved in a vacuum environment. (b) SEM image of the same cell showing good morphological preservation. (c) Total ion image of cell shown in (b). (d) SIMS image of rat oligodendrocyte, m/z 184 (green), m/z 28 (blue). Field of view is 75 μm in all cases. SIMS and SEM Images were obtained using In^+ primary ion (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 7.

Application of C_{60}^+ bombardment to remove water layer. (a) Patterned cholesterol with water overlayer, m/z 18 (blue). (b) Smaller crater etched away with C_{60}^+ revealing cholesterol pattern using a dose of 10^{13} ions/cm², m/z 28 (red), m/z 369 (green). (c) Macrophage on Si with water overlayer. (d) Smaller crater etched away showing cholesterol localization using a dose of 10^{13} ions/cm² (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Fig. 8. SIMS images of a transverse slice from a mouse embryo using 40 keV C_{60}^+ projectiles. (a and d) total ion image, (b and e) m/z 184, and (c and f) m/z 369. The upper three images were taken directly on the slice. The lower three images were recorded after sputtering with a dose of $10^{13} C_{60}^+$ ions/cm².

Fig. 9. Chemical images of mating tetrahymena cells taken with differing ice thicknesses. (a, c and d) m/z 69, and (b, d and f) m/z 184. The sample (a and b) was fractured from a sandwich structure that produced a layer of ice thick enough to exhibit charging. The sample (c and d) contained a very thin ice layer that induced topographical artifacts. The sample in (e and f) has an ice thickness that produces results free of artifacts.