# Mass Spectrometry Imaging of Membrane Lipids in Cryogenically Preserved Single Cells

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## Introduction

Many techniques have been developed with low detection limits to allow the investigation of chemical species on a single cell level. For example, electrochemical techniques have been used to study single release events during exocytosis, in which neurotransmitter-containing vesicles fuse with the cell membrane and release their contents for intercellular communication. The lipids in vesicles and cell membranes are believed to be a driving force in vesicle fusion and release. Still, domains of native phospholipids have not been observed during exocytosis in cells. Consequently, imaging techniques that resolve subcellular distributions of cell membrane molecules in their natural hydrated environment are required to observe the intricacies of these events. Static time of flight secondary ion mass spectrometry (ToF-SIMS) has been used to image distributions of relatively large molecules across sample surfaces including domains of lipids in Langmuir-Blodgett films.[1] To image intact cell membranes in the ultrahigh vacuum (UHV) environment of the ToF-SIMS instrument, it is necessary to freeze the cells in their hydrated state and maintain their frozen state throughout analysis. Since ToF-SIMS is a surface sensitive technique, freeze-fracture methods are used to provide a freshly exposed cell membrane surface for imaging. Initial experiments using imaging static ToF-SIMS have measured distributions of small molecules (like cocaine and dimethylsulfoxide) in frozen-hydrated single paramecium multimicronucleatum cells.[2] More recently, unique phospholipids have been imaged in fusing multilamellar liposomes (>50μm).[3] Cell preparation techniques have been improved upon to expose membranes of cells to the surface during freeze-fracture, without condensed water or residual matrix contamination of the cellular surfaces. In this paper, we present SIMS images of single paramecium and rat pheochromocytoma (PC12) cells (approx. 10 μm) to demonstrate the capability of static ToF-SIMS to image cell membrane phospholipids.

## Experimental

PC12 cells (ATCC, Manassas, VA), were cultured to confluence in collagen-coated flasks. Cells were removed from the flask, centrifuged to a pellet, and reconstituted in medium to concentrate the cells. 100 μL of a suspension of these cells was placed onto a silicon wafer (Ted Pella, Redding, CA). Another piece of silicon was used to cover the sample, and the entire assembly was frozen in liquid ethane. Paramecia multimicronucleatum (Figure 1) were obtained from Carolina Supply Company (Burlington, NC). A droplet of paramecia in spring water was placed onto a copper substrate where 25 μm polystyrene beads were placed to prevent crushing of the cells. Another copper piece was placed over the sample, and the entire assembly was frozen in liquid nitrogen. The freeze-fracture methods employed after the sample was introduced to the UHV were described previously in detail.[3] The freeze-fractured frozen-hydrated samples were then moved to a liquid nitrogen cooled ToF-SIMS analysis stage. ToF-SIMS analyses were performed in a Kratos (Manchester, U.K.) Prism ToF-SIMS spectrometer with a FEI (Beaverton, OR) indium liquid metal ion gun (LMIG). The

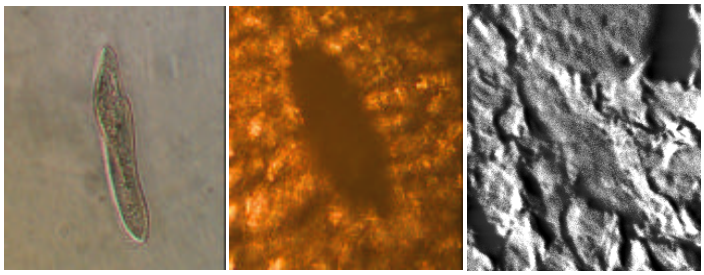


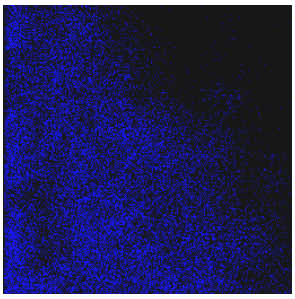
*Figure 1. Phase contrast micrograph of a paramecium in water on a glass coverslip (left). Reflected brightfield micrograph of a freeze-fractured paramecium in ice on the ToF-SIMS analysis stage (center). Scanning ion image of the same freeze-fractured paramecium. ~150 mm f.o.v. (right)*

instrument also used a channeltron detector (Burle, Lancaster, PA) positioned approximately 0.5 cm from the sample to record scanning ion micrographs (SIM), similar to scanning electron micrographs.

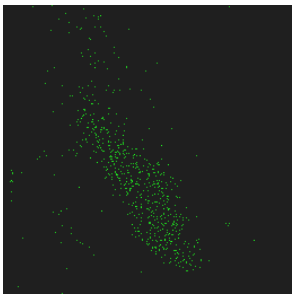
**Results and Discussion**

Images of paramecia have been collected using the latest freeze-fracture techniques and retrospective ToF-SIMS imaging to demonstrate the ability of the techniques to image phospholipids in single cells. Figure 2 contains positive SIMS images of the single freeze-fractured paramecium shown in the brightfield and scanning ion images in Figure 1. As expected, water is not localized to the paramecium. The upper right corner of the sample is shadowed, due to a significant change in sample morphology as seen in the SIM in Figure 1. Fragments of common membrane lipids, including phosphatidylcholine at 184 m/z and phosphatidylethanolamine at 142 m/z, are localized to the cell.

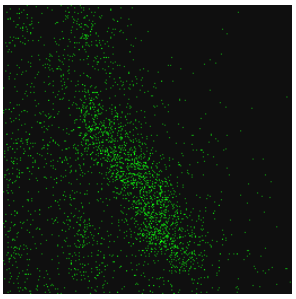
*Figure 2. ToF-SIMS images of water and phospholipids of a single freeze-fractured paramecium (~150 ㎜ f.o.v.).*



+18,19 m/z water (0-3 count scale)



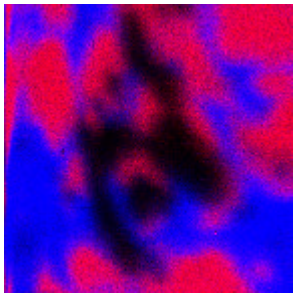
+184 m/z phosphatidylcholine fragment (0-2 count scale)



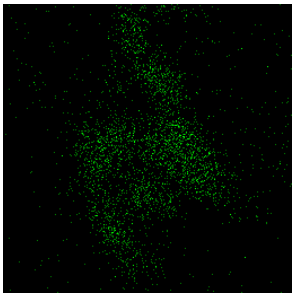
+124 m/z phosphatidylethanolamine fragment (0-2 count scale)

In addition to paramecia, PC12 cells, frozen in cell culture medium and freeze-fractured, have been imaged on a silicon substrate. As shown in Figure 3, the high sodium content (blue) of the cell culture medium is in contrast to the underlying silicon signal (red). In the depression of sodium and silicon signals, PC12 cells exhibit similar phospholipid signals (green) to the paramecium. These images demonstrate the ability of ToF-SIMS imaging to analyze phospholipids in single cell membranes. In the future, these techniques will be used to study subcellular distributions of lipids in membranes to correlate molecular composition with biological function.

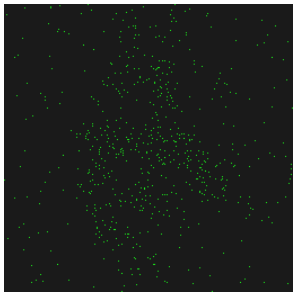
*Figure 3. ToF-SIMS images of water and phospholipids of freeze-fractured PC12 cells (~70 ㎜ f.o.v.).*



+23 m/z sodium (blue, 0-50 count scale)
+28 m/z silicon (red, 0-33 count scale)



+184 m/z phosphatidylcholine fragment (0-2 count scale)



+124 m/z phosphatidylethanolamine fragment (0-2 count scale)


1. Leufgen, K. M.; Rulle, H.; Benninghoven, A.; Sieber, M. *Langmuir* **1996**, 12, 1708.
2. Colliver, T. L.; Brummel, C. L.; Pacholski, M. L.; Swanek, F. D.; Ewing, A. G.; Winograd, N. *Anal. Chem.* **1997**, 69, 2225.
3. Cannon, D. M.; Pacholski, M. L.; Winograd, N.; Ewing, A. G. *J. Am. Chem. Soc.* **2000**, *122*, 603-610.