Abstract # (to be added by the organizers)

High-Resolution Secondary Ion Mass Spectrometry (SIMS) Imaging of Intracellular Lipid Distributions

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Lipid metabolism and intracellular transport regulate the relative abundances of lipids and cholesterol within organelles. Thus, disturbances in lipid metabolism and transport can result in abnormal lipid distributions within cells and variations in organelle size and composition. These changes and are often a hallmark of lipid-mediated health defects. Thus, assessing intracellular lipid distribution may provide insight into lipid-mediated health defects. Imaging with a Cameca NanoSIMS in depth profiling mode has enabled high-resolution visualization of the three-dimensional distributions of isotope-labeled sphingolipids and cholesterol metabolically incorporated into cells. However, identifying organelles can be challenging because SIMS depth profiling distorts their shapes in the z-direction, and organelles must contain distinct stable isotopes or nonnative elements to enable identification with a NanoSIMS. Therefore, we have developed strategies that facilitate improved visualization of organelles. First, we used the secondary electron images acquired during secondary ion mass spectrometry (SIMS) depth profiling to predict the cell morphology and reshape the 3D NanoSIMS depth profiling images. This revealed distinct arrangements of the lipids and permits identifying the shapes and sizes of intracellular compartments. Second, we created a cell line that expresses organelle-specific proteins fused to small enzymes that covalently attach to small-molecule ligands. Functionalizing these ligands with fluorophores and distinctive elements or isotopes enables detection of organelles with SIMS and fluorescence microscopy. 3D NanoSIMS depth profiling images of cells expressing the organelle-specific protein construct, labeled with our custom ligands, and reshaped using the novel depth correction strategy will allow assessment of the distributions of lipids in organelles.