Supplemental Information

Three Dimensional Secondary Ion Mass Spectrometry Imaging (3D-SIMS) of *Aedes aegypti* ovarian follicles

Anthony Castellanos[1], Cesar E. Ramirez[1], Veronika Michalkova[2], Marcela Nouzova[2,3], Fernando G. Noriega[2,4] and Francisco Fernández-Lima[1,4]*

[1]Department of Chemistry and Biochemistry, Florida International University, Miami, Florida, 33199, United States; [2]Department of Biological Sciences, Florida International University, Miami, Florida, 33199, United States; [3] Institute of Parasitology, Biology Centre CAS, Ceske, Budejovice, Czech Republic; and [4]Biomolecular Sciences Institute, Florida International University, Miami, Florida, 33199, United States.

Corresponding author:* Francisco A. Fernández-Lima, Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8th St AHC4-233, Miami, FL 33199, USA; *e-mail:* fernandf@fiu.edu

Keywords: SIMS, lipids, mass spectrometry, mosquitoes, ovary, imaging

Table of contents:

With an aerial view of the follicles in Figure 3, nurse cell-like features are more easily observed as in the case of the sucrose-fed female follicle (Figure S1). Several advantages of 3D-MSI are exemplified here. Heterogeneously distributed organelles, that would otherwise be missed by analyzing a single 2D slice, can be observed using appropriate sputter parameters. In contrast to histological staining, any number of molecular species can be simultaneously probed.When accurate sample height is crucial for the understanding of the sample material, techniques such as atomic force microscopy can be used to calibrate the z-axis. Because such a requirement was not necessary in this study, the z-axis here should be considered to be arbitrary units. For a more comprehensive explanation, readers are referred to the work of Robinson et. al.[1]

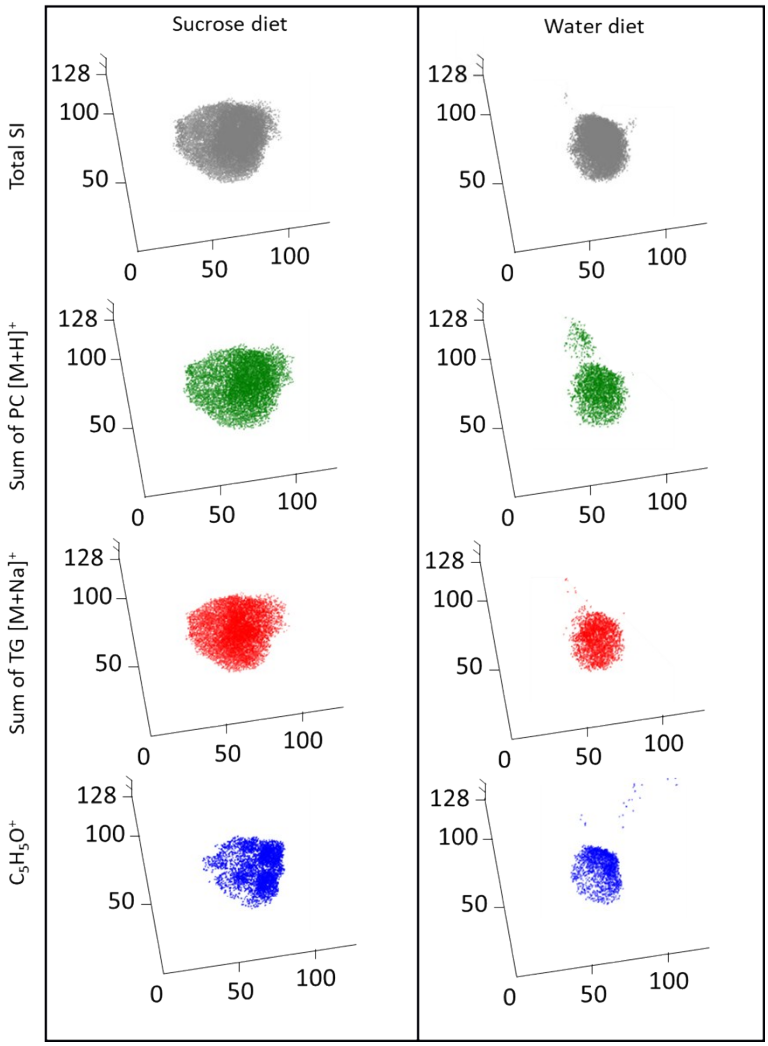

Figure S1. Software reconstructed 3D images for non-flat surfaces of water- and sucrose-diet ovarian follicles. The X and Y axis are shown in pixels (1pixel = 1.56 µm).

In the case of phosphatidylcholine analyzed by 3D-TOF-SIMS, significant differences in relative abundance were not seen. This observation is somewhat counterintuitive considering the relative sizes of water- and sucrose-diet follicles (see Figure 4). It is hypothesized here that PCs remain in relatively similar amounts for our experimental parameters, and that the follicular epithelium expands and contracts to accommodate triacylglyceride reserves.

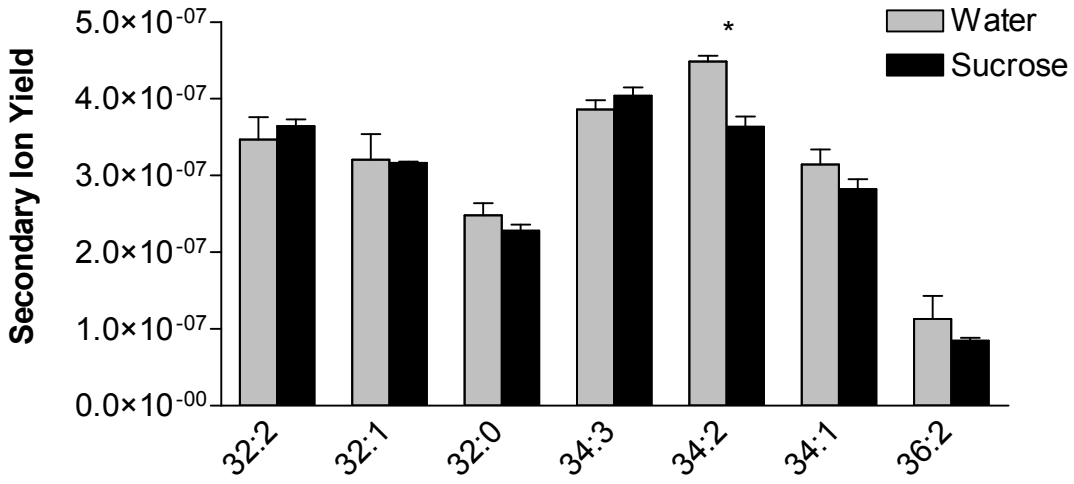

Figure S2. Comparison of sum PC signals in the 3D-MSI. Analysis is comprised of two individual follicles each feeding conditions (i.e., water- and sucrose-fed females). Bars represent the mean and the standard error of the mean (SEM) of duplicate measurements. Asterisks denote significant difference (unpaired t-test: * $p<=0.05$).

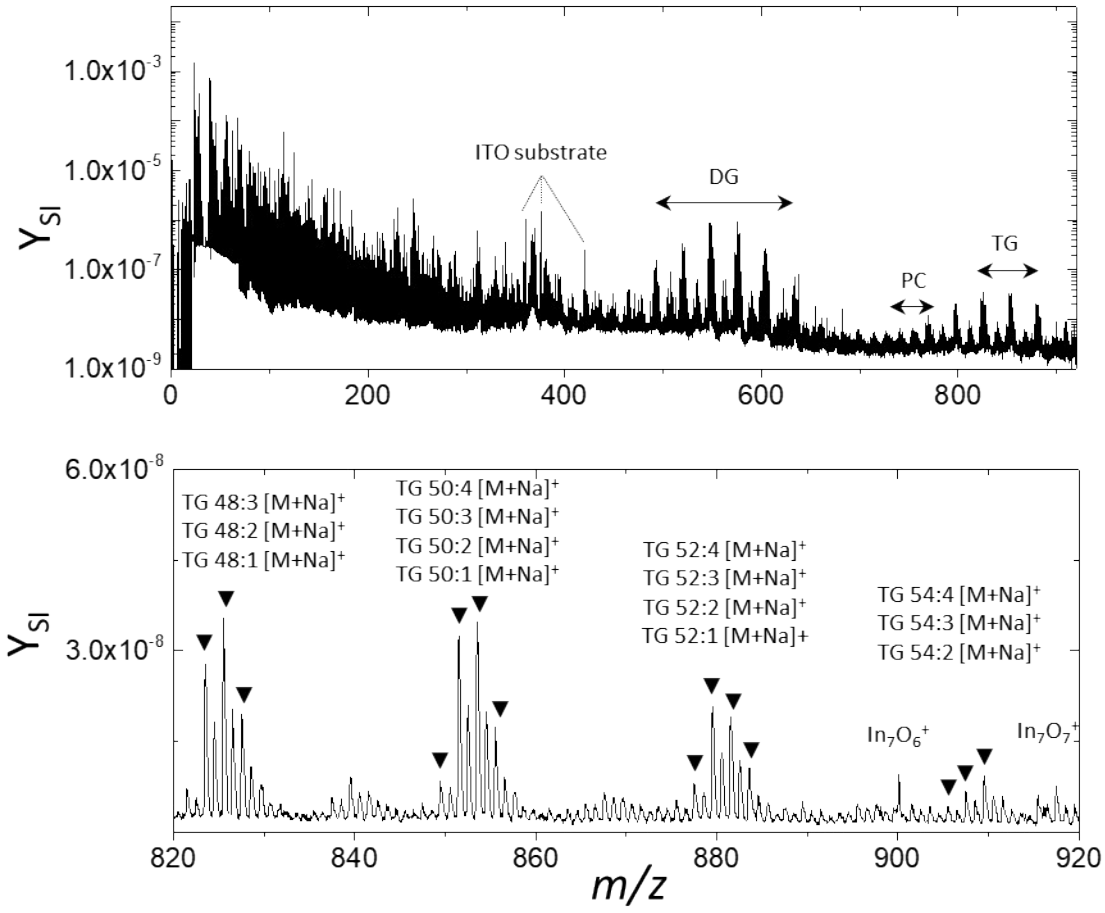

Figure S3. Top) Typical sum spectrum of a 3D-MSI analysis of a single ovarian follicle. The spectrum shown was acquired in positive polarity from a sucrose-fed adult female insect. Bottom) Signals corresponding to diacyglycerides (DG), triacyglycerides (TG) and phosphatidylcholine are labeled. TG are denoted with triangles. Indium tin-oxide signals corresponds to the glass substrate.

References:

1. M. A. Robinson, D. J. Graham and D. G. Castner, *Analytical Chemistry*, 2012, **84**, 4880-4885.