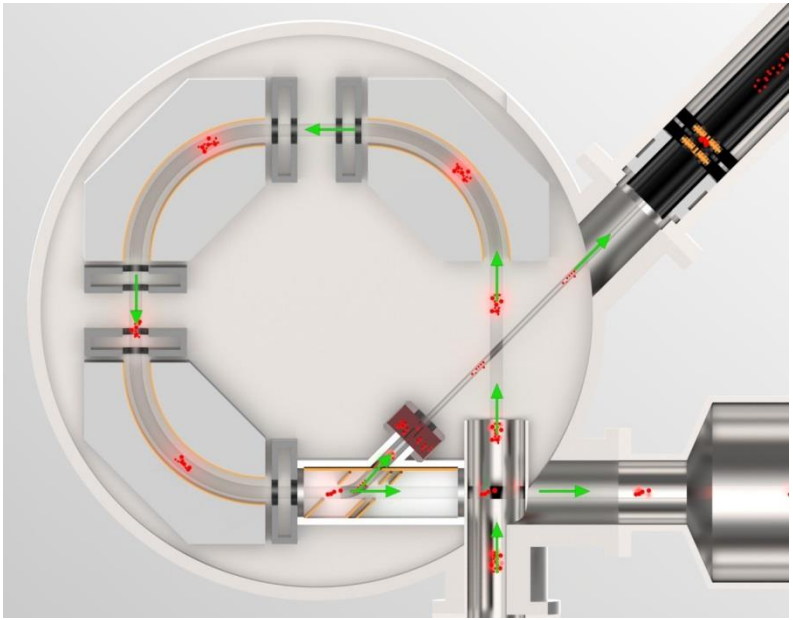
Where do Drug Molecules go Inside of Cells?

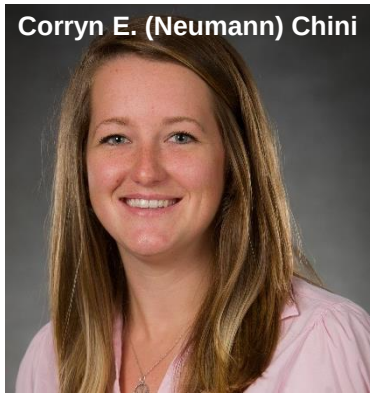
A New Method to Probe the Composition of Cellular Organelles

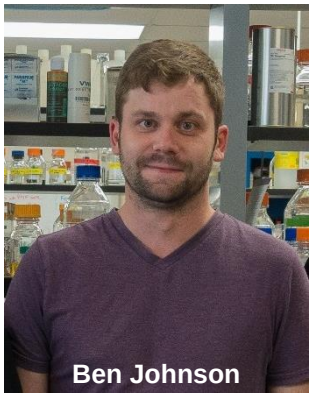


Ashley Ellsworth
Physical Electronics, USA.

Collaborators

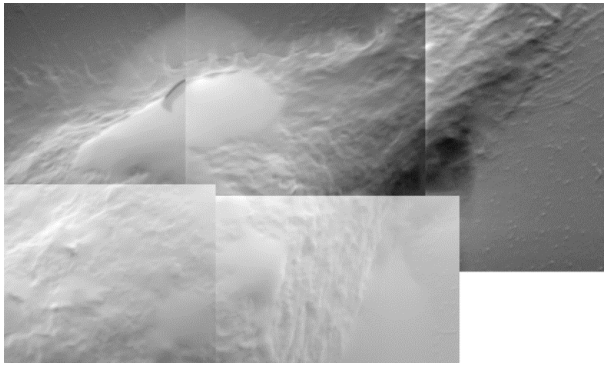
Physical Electronics,
University of Illinois at Urbana-Champaign,
& Colorado State University



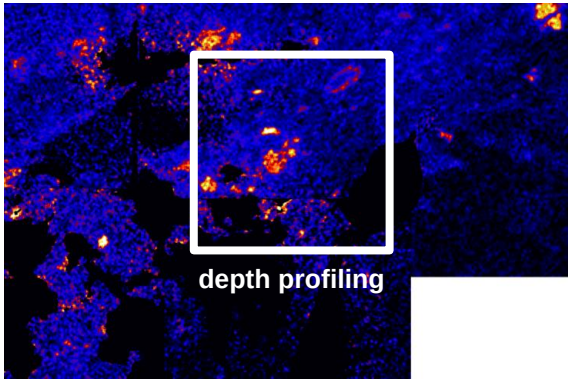


MS 2D Mapping of Lipids & Sterols on MDCK Cell

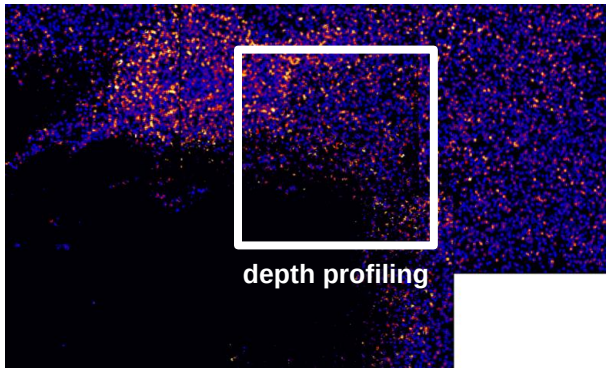
Ion-Induced SE Image of MDCK Cell



^{15}N-sphingolipids

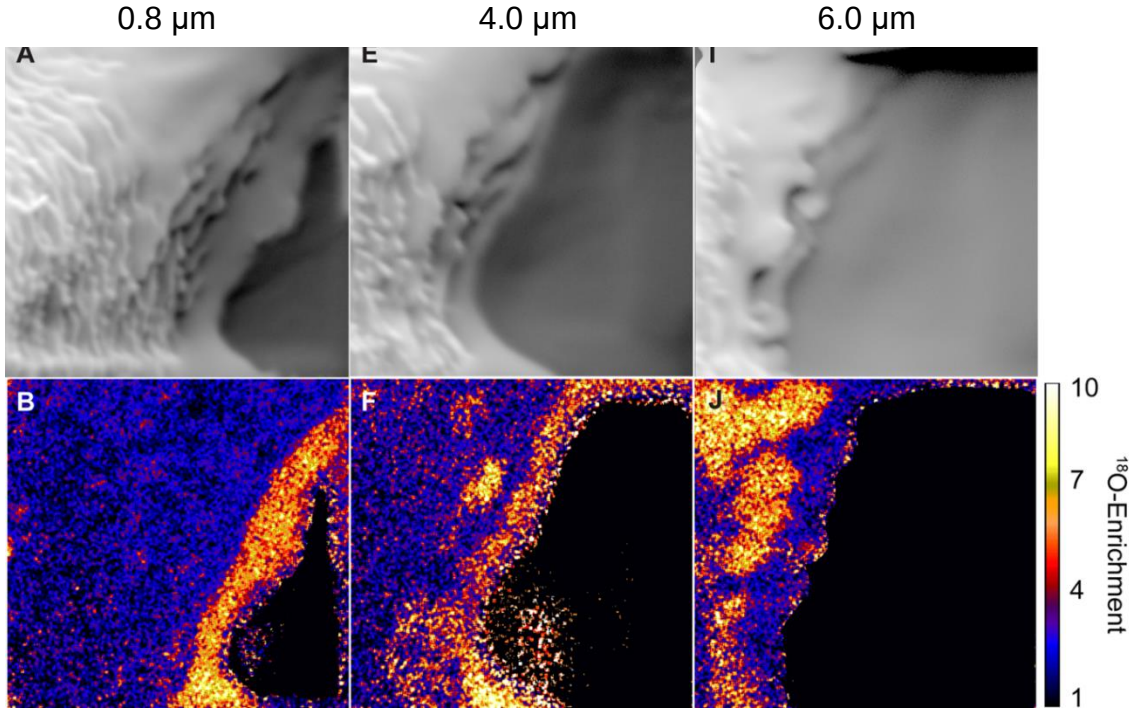


^{18}O-cholesterol



M.L. Kraft & coworkers, *Biointerphases* **11(2)** (2016) 02A309.

MS 3D Mapping of Lipids & Sterols in MDCK Cell

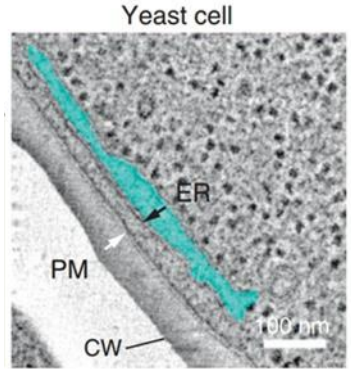


Are the cholesterol-rich regions part of the endoplasmic reticulum, or an organelle involved in cholesterol synthesis and transport??

Identifying specific cellular compartments or structures by MS imaging isn't easy!!

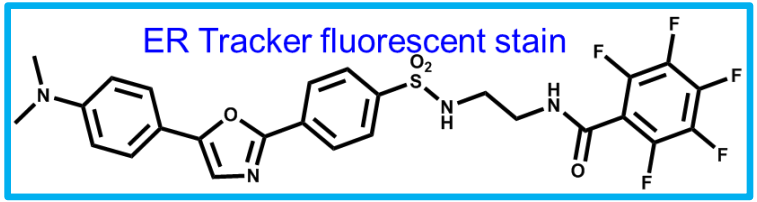
M.L. Kraft & coworkers, *Biointerphases* **11(2)** (2016) 02A309.

Organelle Labeling: Transfected & Stained HEK Cells



yeast cells

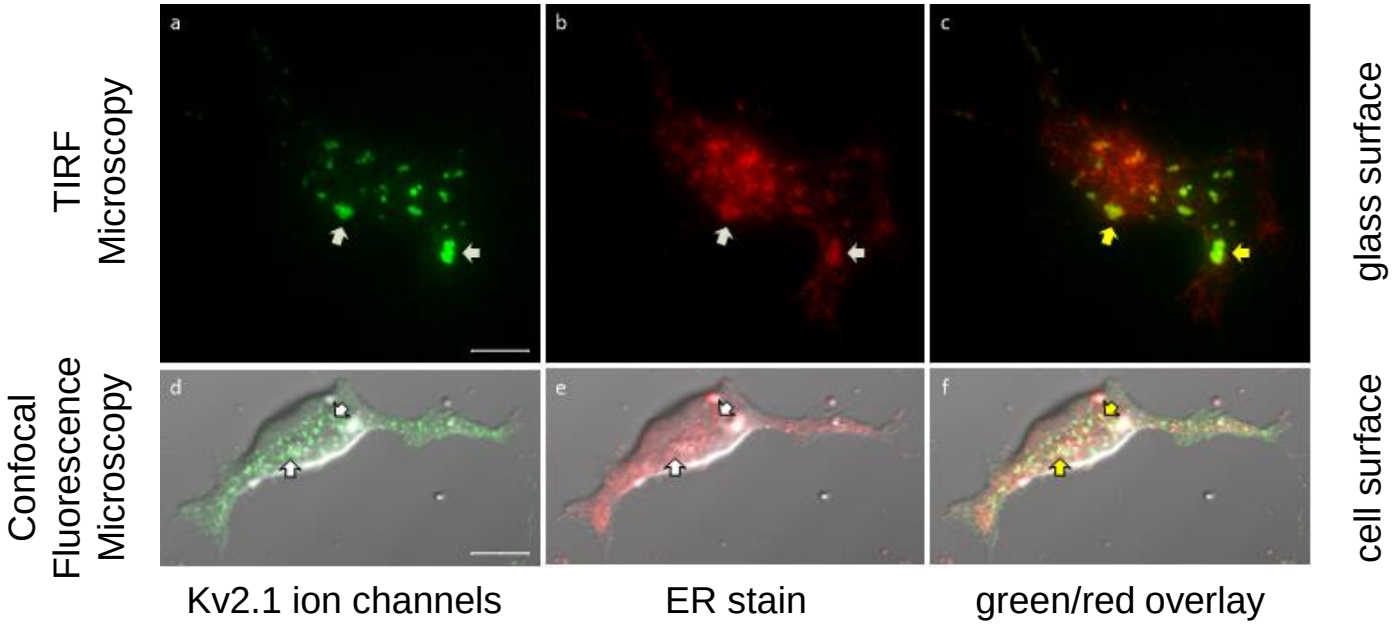
Can we repurpose the ER-Tracker stain for imaging of the endoplasmic reticulum by MSI?



HEK-293 cells were transfected and then stained using the ER-Tracker, and then fixed for analysis.

TIRF and Confocal Fluorescence Microscopy of HEK Cells

The arrows indicate coincidence of the Kv2.1 ion channels and the ER tubules in the stained HEK cells.



The ER tubules measure approximately 0.3 to 2.0 microns in diameter.

MS imaging & tandem MS imaging of HEK cells was performed using a PHI *nanoTOF* II TOF-SIMS Parallel Imaging MS/MS instrument

3D MS^1 / MS^2 Imaging

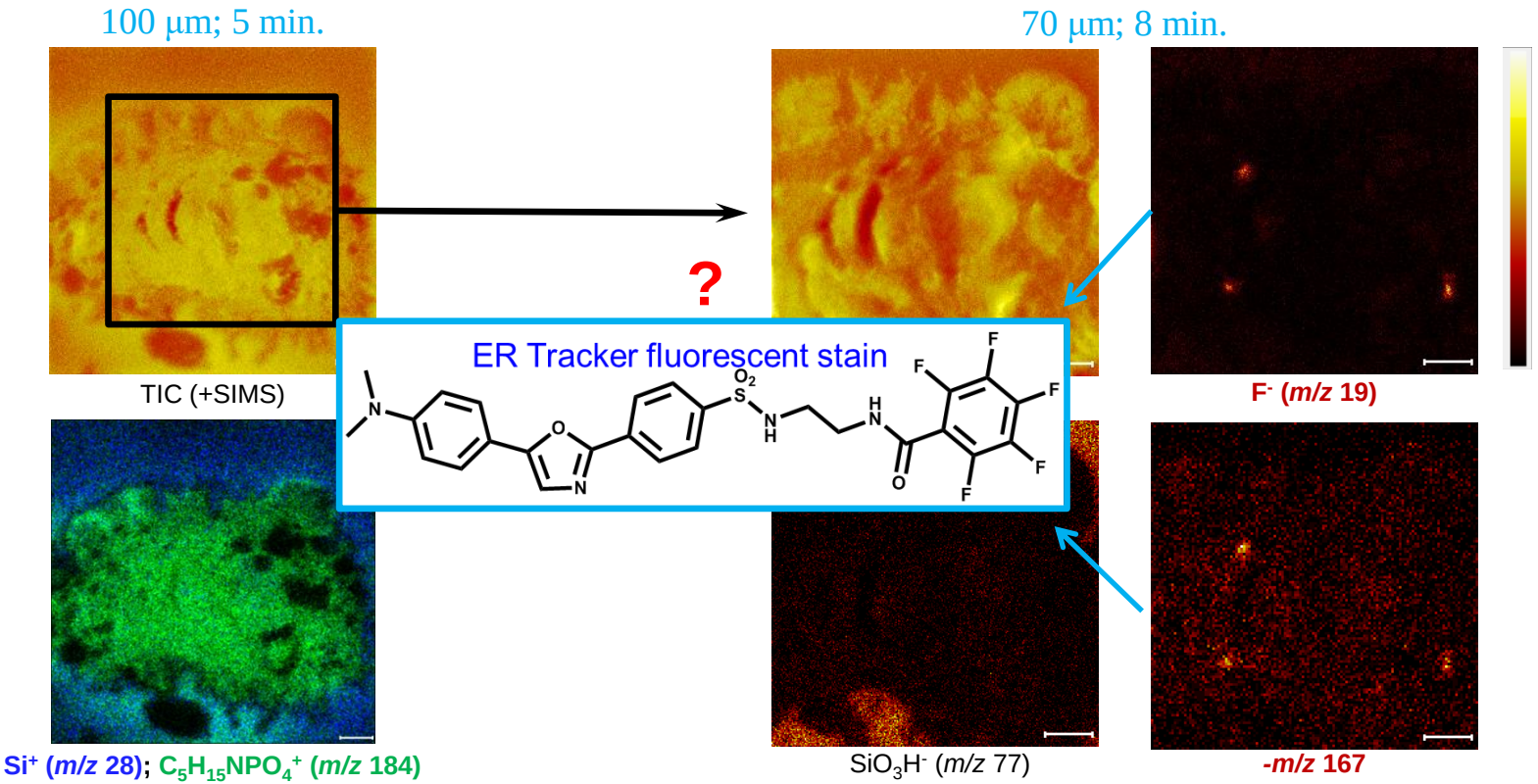
analysis beam
30 keV Bi_3^+, unbunched
3.0 nA, 1.16 x10^{13} ions/cm^2
80 nm beam, 137 nm/pixel

dc sputter beam
5 keV $Ar_{2,500}^+$, 6.0 nA,
8.06 x10^{14} ions/cm^2 (total)

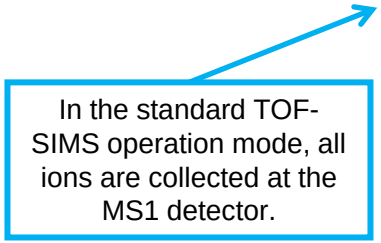
Chini, Fisher, Johnson, Tamkun, Kraft, *Biointerphases* **13** (2018) 03B409.

Potential MS^1 Observation of ER Tubules

No molecular ion signals (i.e. ±*m/z* 580) of the ER-Tracker stain were observed.



PHI *nanoTOF* II Standard TOF-SIMS Mode

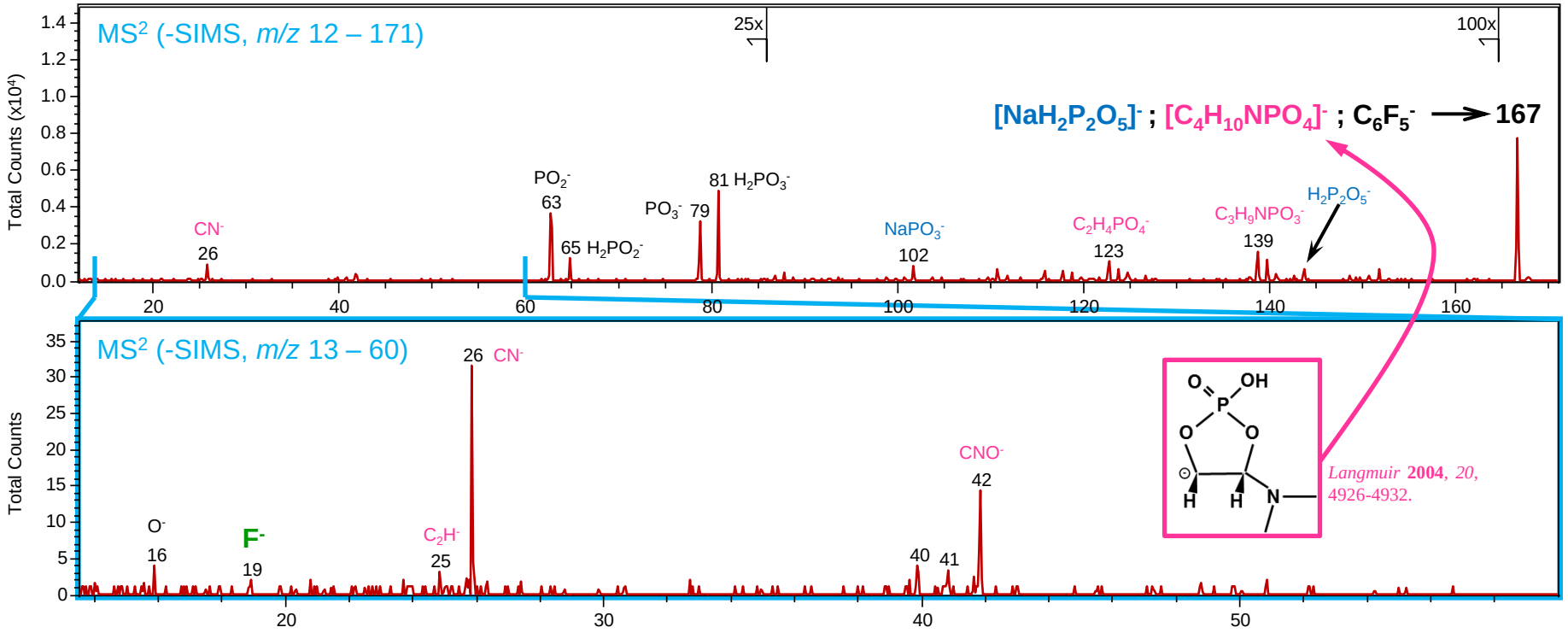


Parallel TOF-SIMS and MS/MS

In MS/MS mode, the precursor ion is deflected into the collision induced dissociation (CID) cell. The resulting fragment ion spectrum is collected with the MS2 detector while the rest of the ions are collected as usual with the MS1 detector.

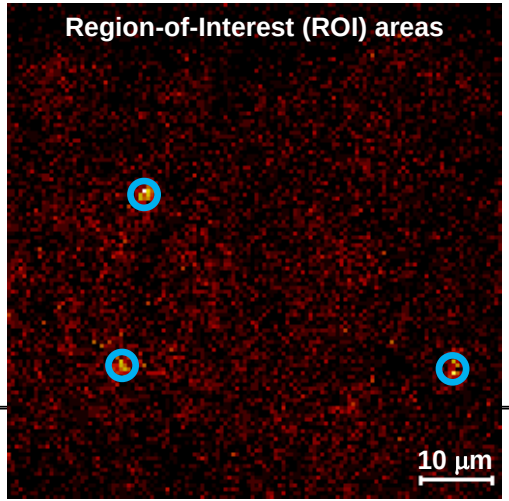
Total Area Tandem MS in Transfected & Stained Cells

MS^2 spectra reveal exogenous F^- in the ER-Tracker-stained cells, potentially from $C_6F_5^-$ ions.

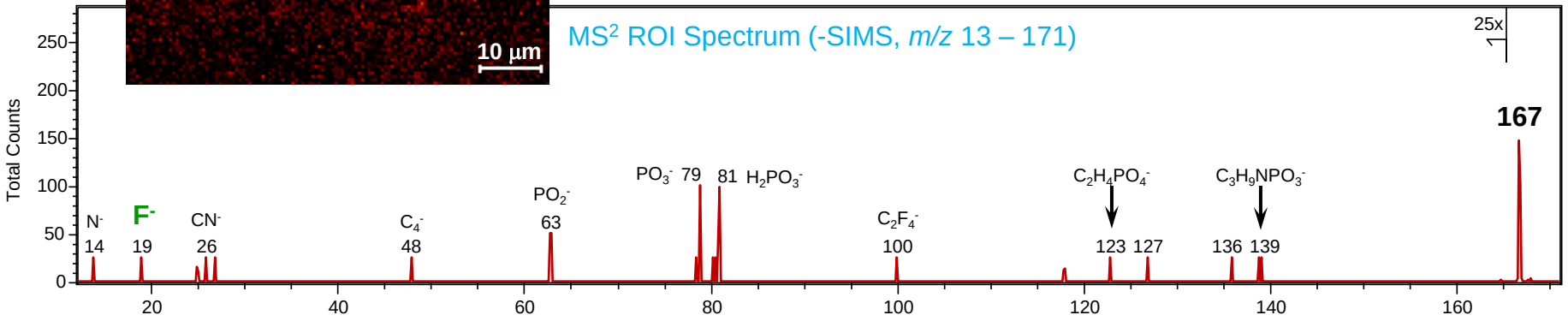


Tandem MS Region-of-Interest (ROI) Spectrum

The F^- in the MS^2 ROI spectrum indicates localization of a fragment of the ER-Tracker.

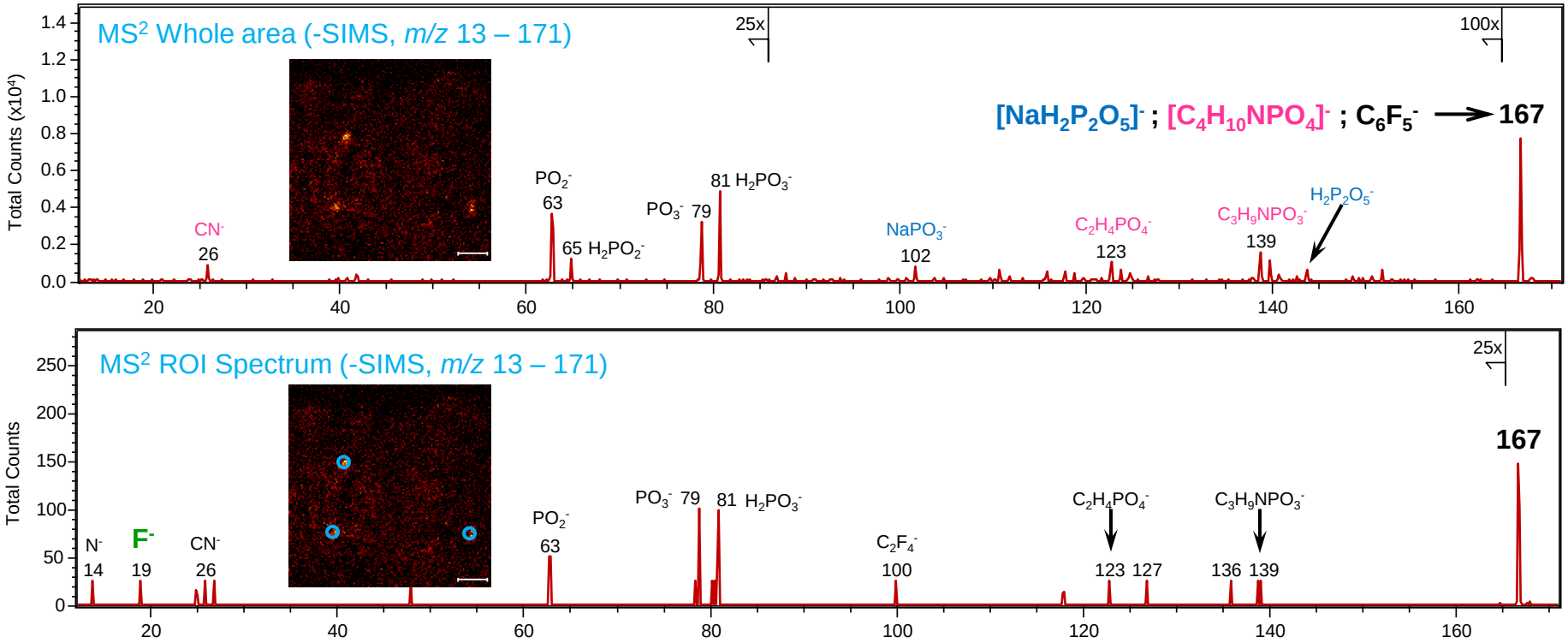


The relative counts of F^- ions are elevated in the ROI product ion (MS^2) spectrum; *potentially, the F^- ions arise from a pentafluorophenyl anion*.



Tandem MS Spectra Comparison

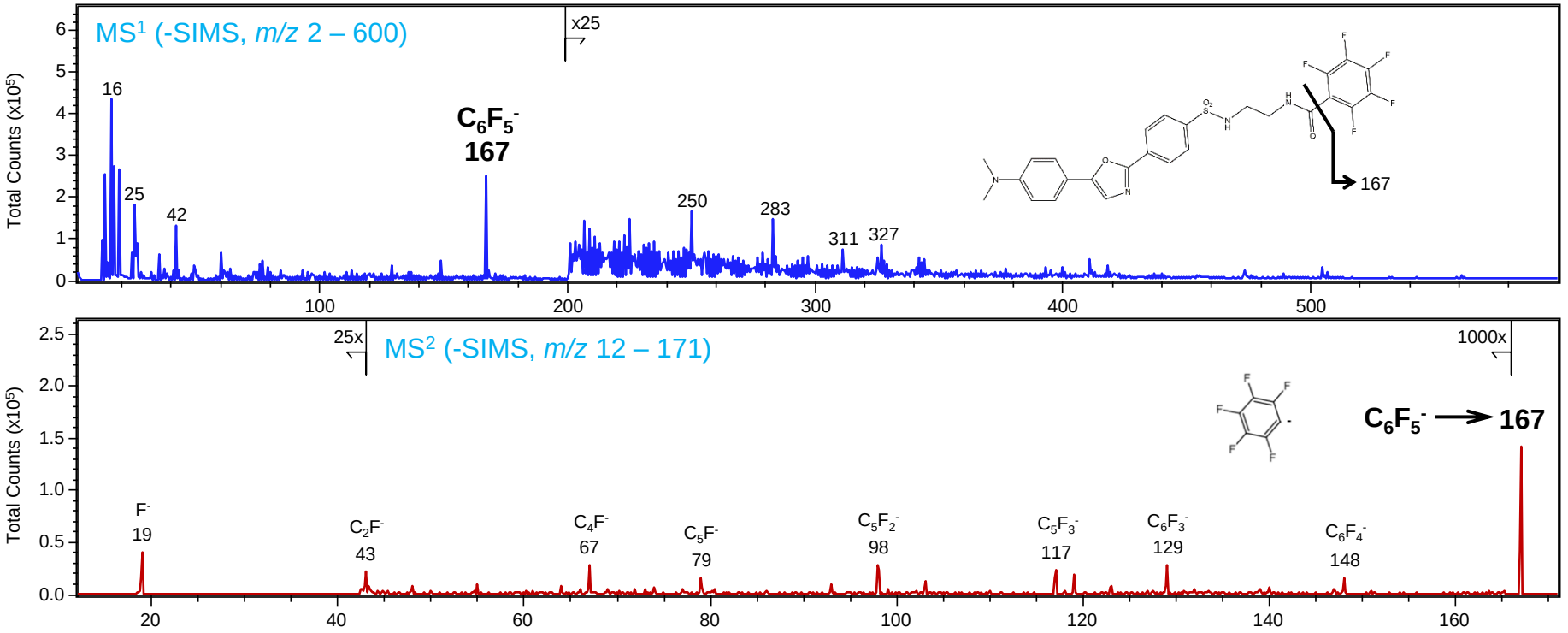
The F^- in the MS^2 ROI spectrum indicates localization of a fragment of the ER-Tracker.



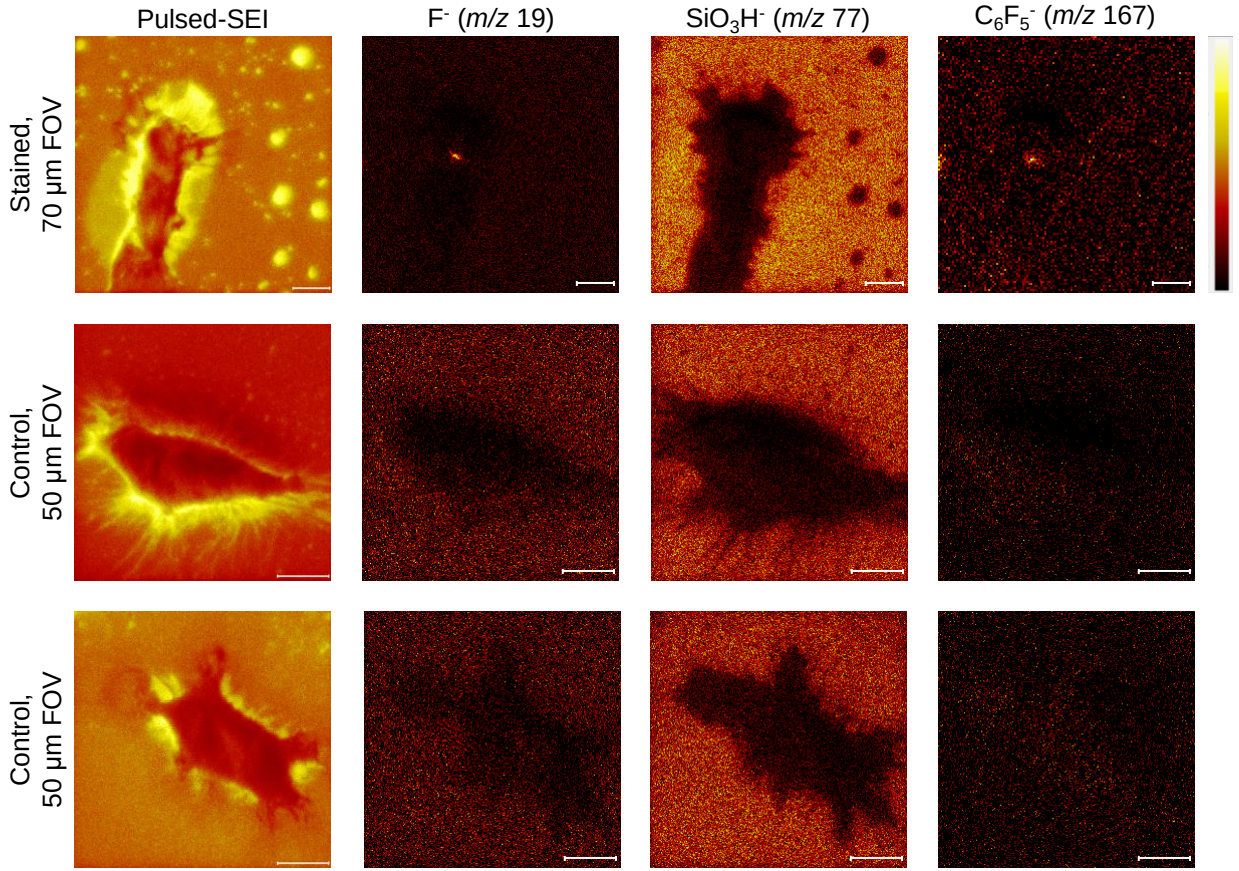
The relative counts of F^- ions are elevated in the ROI product ion (MS^2) spectrum; *potentially, the F^- ions arise from a pentafluorophenyl anion*.

Spectra of the ER-Tracker Reference

No molecular ion observed; MS^2 of -*m/z* 167 confirms a $C_6F_5^-$ composition.



Are ER Features Observed in Unstained Control Cells?

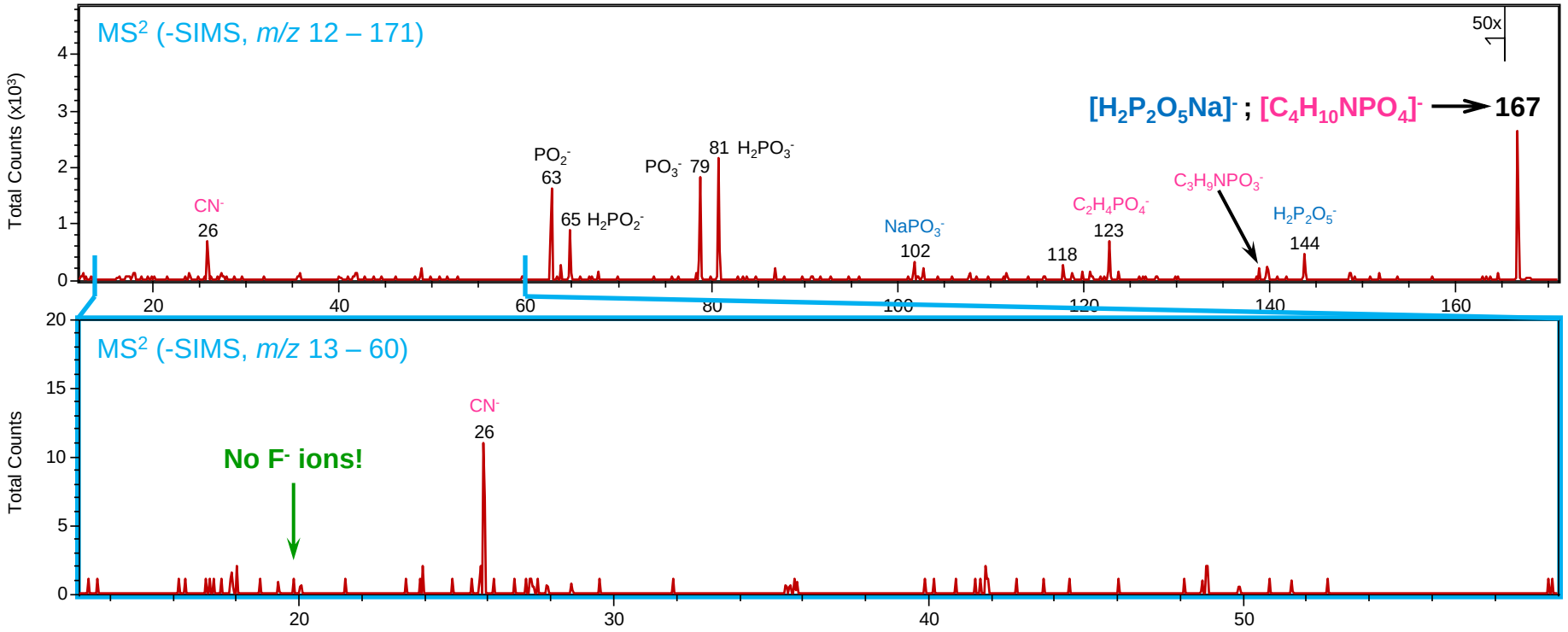


Possible ER tubules and ER-PM junctions are observed in stained cells as noted by localization and correspondence of the F^- and $C_6F_5^-$ ions.

The F^- and $C_6F_5^-$ chemical signatures are not observed in the control cells.

MS^2 of -*m/z* 167 in Control Cell

The product ion spectra show no evidence of the pentafluorophenyl anion.

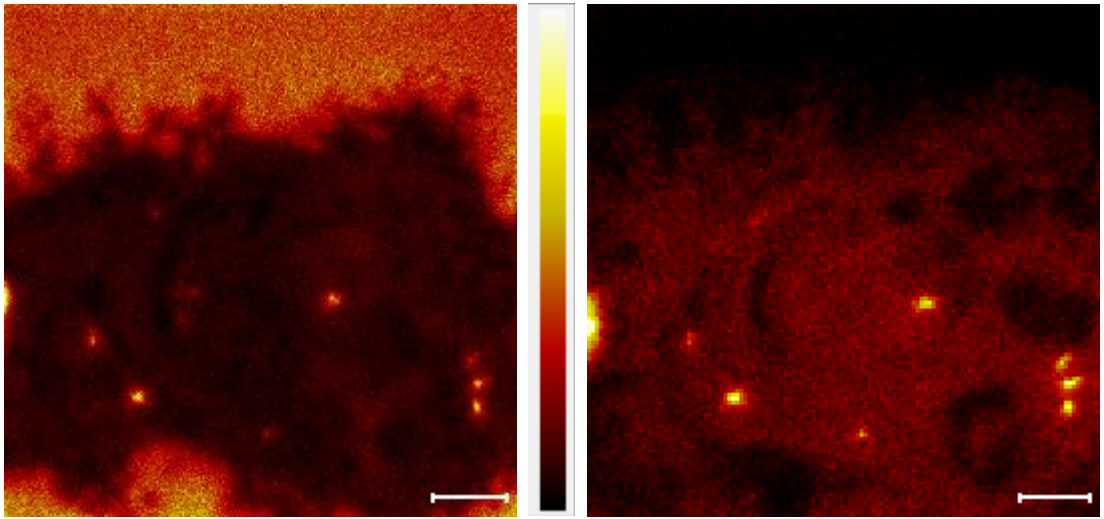


ER Tubules Observed in Both Ion Polarities

What is the source of the $C_5H_5^+$ ions?

-SIMS; 70 μm; 8 min.

+SIMS; 70 μm; 16 min.

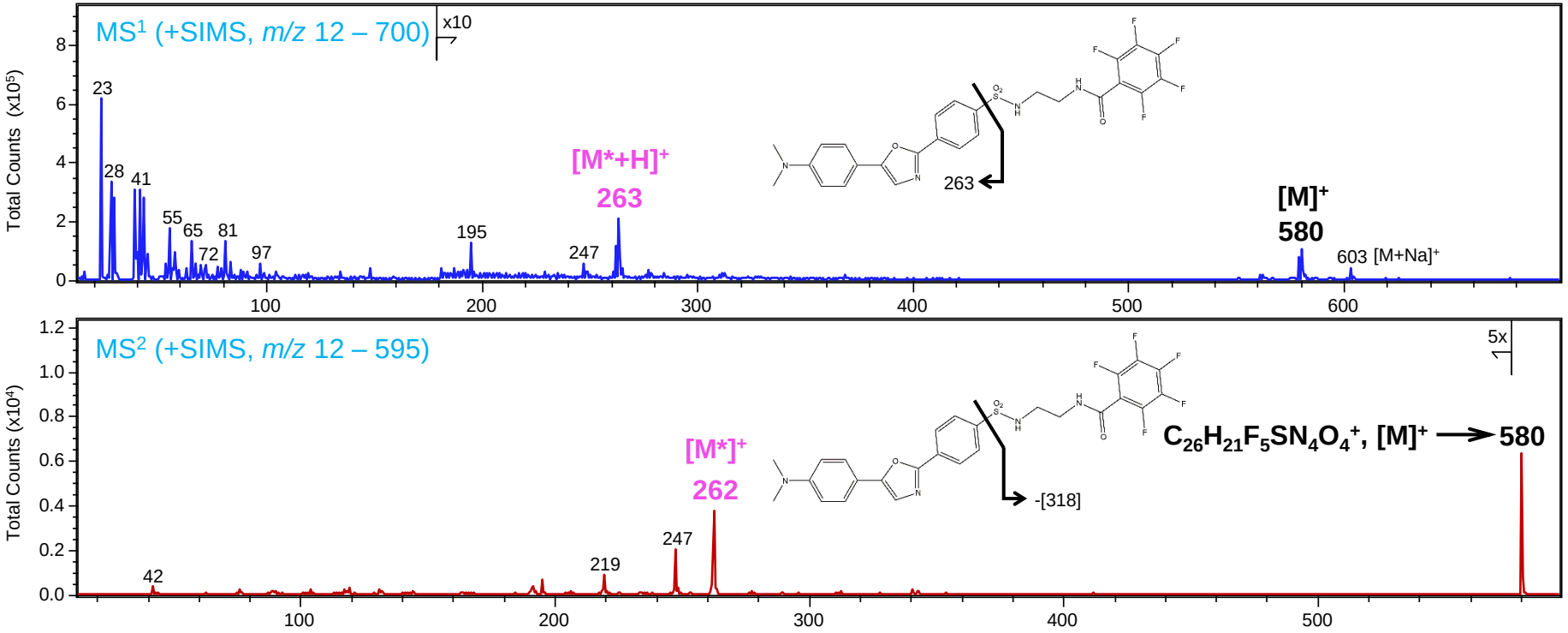


F^- (*m/z* 19)

$C_5H_5^+$ (*m/z* 65) binned

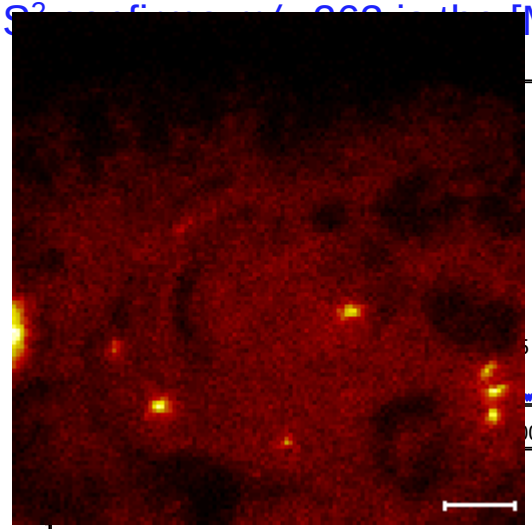
Spectra of the ER-Tracker Reference

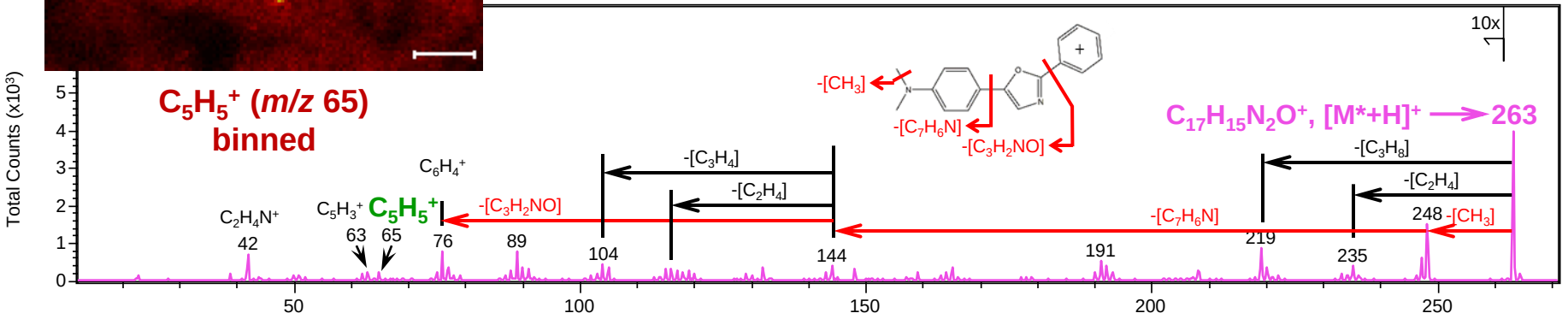
MS^1 reveals the $[M]^+$ and a fragment at *m/z* 263; MS^2 of $[M]^+$ reveals a product ion at *m/z* 262.



Spectra of the ER-Tracker Reference

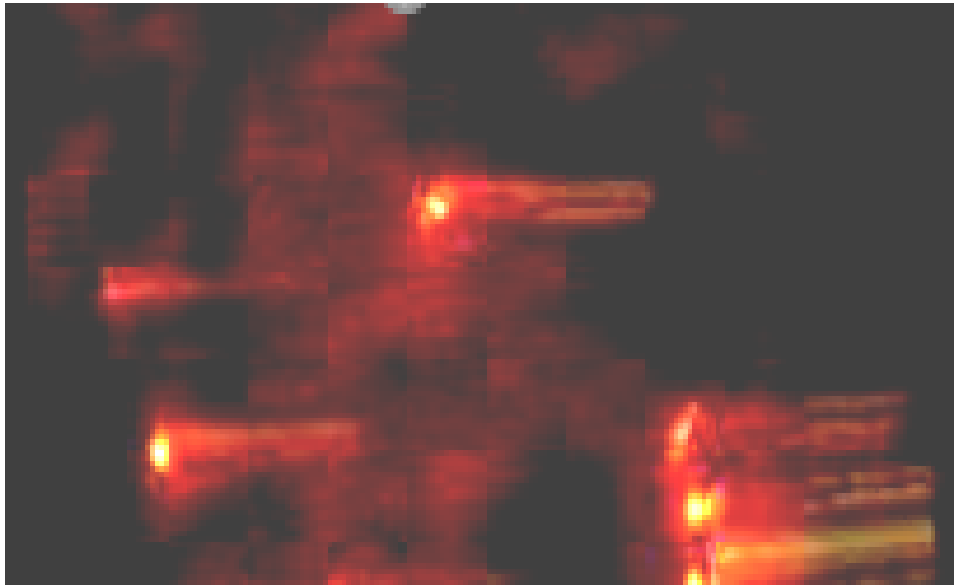
MS² ... [M*+H]⁺ of the dapoxyl dye fragment and gives rise to $C_5H_5^+$ ions.

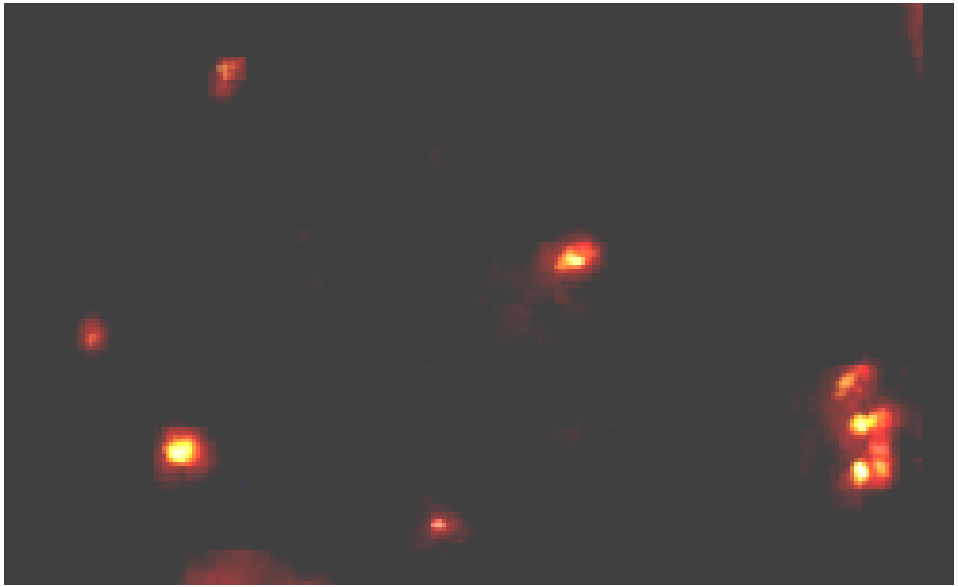




3D Tandem MS Imaging of Transfected/Stained HEK Cell

A 50 µm x 30 µm x 40 nm volume revealing the ER tubules and ER-PM junctions in one cell.

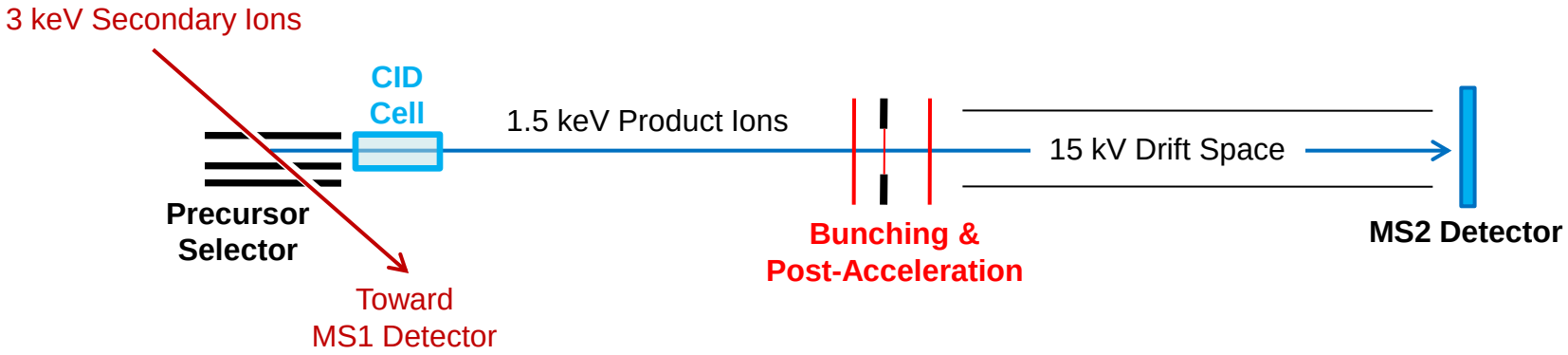




Summary & Outlook

- Organelles must be “labeled” in order to achieve definitive localization of chemistry by MSI
- We employed a combination of staining (ER-Tracker) and tandem MS imaging to directly observe ER tubules and ER-PM junctions in transfected HEK cells
  - high resolving power TOF-SIMS (MS^1) imaging & tandem MS (MS^2) imaging at $\Delta\ell \approx 100$ nm
  - simultaneous collection of MS^1 and MS^2 data from each pixel enhances molecular ID and imaging
  - the same method can be applied to MS imaging of other organelles and cellular structures
- Full 3D MS^1 / MS^2 imaging of the ER in the entire cell volume is underway
- We further aim to identify organelle-specific lipids and differentiate them from e.g. PM lipids
- By extension, it should be possible to identify the location of drugs and metabolites in cells

Tandem MS (MS^2) Spectrometer Schematic

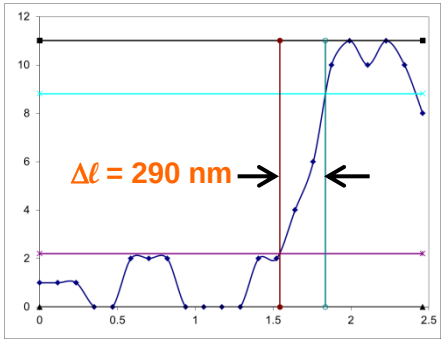


HR² Imaging versus Unbunched Imaging

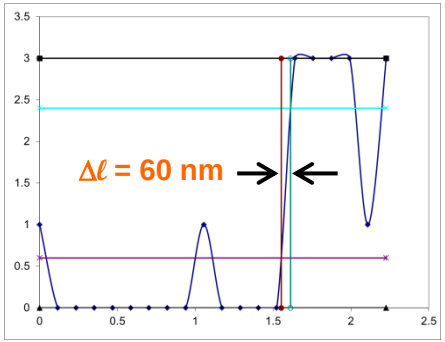
Chemical identification and high lateral resolution together.

Ultimate lateral resolution but with little or no chemical information.

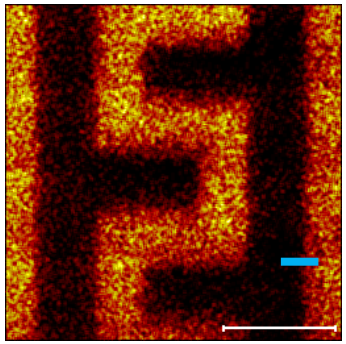
HR²

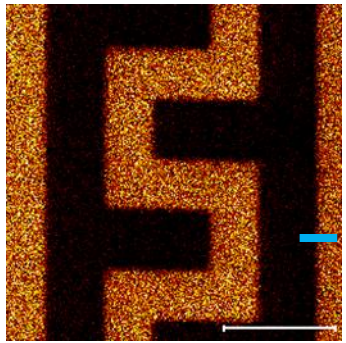


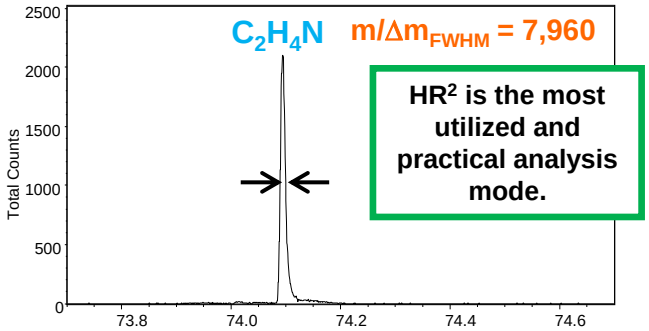
Unbunched



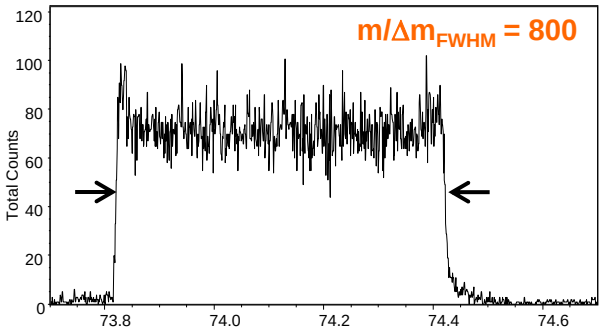
Si⁺ (*m/z* 28); 30 µm FOV





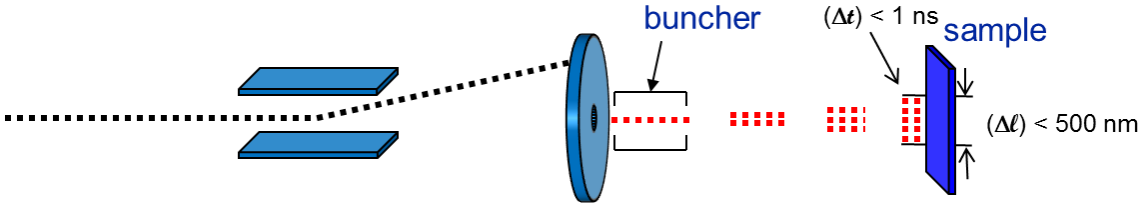


HR² is the most utilized and practical analysis mode.

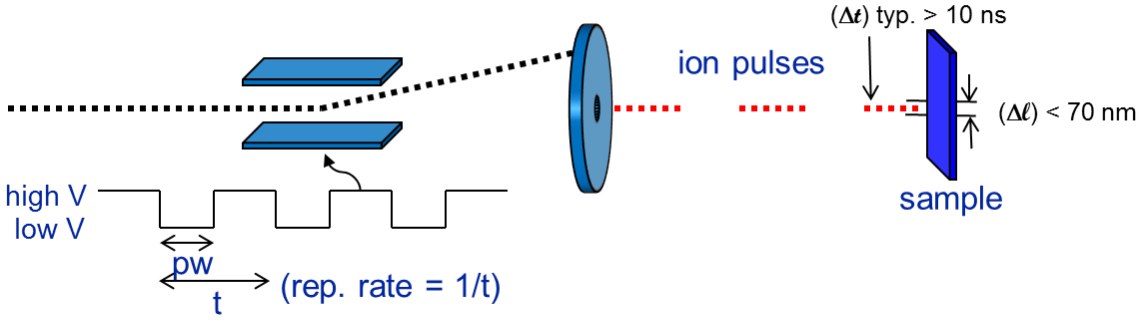


High Resolving Power & Full Tandem MS Resolution

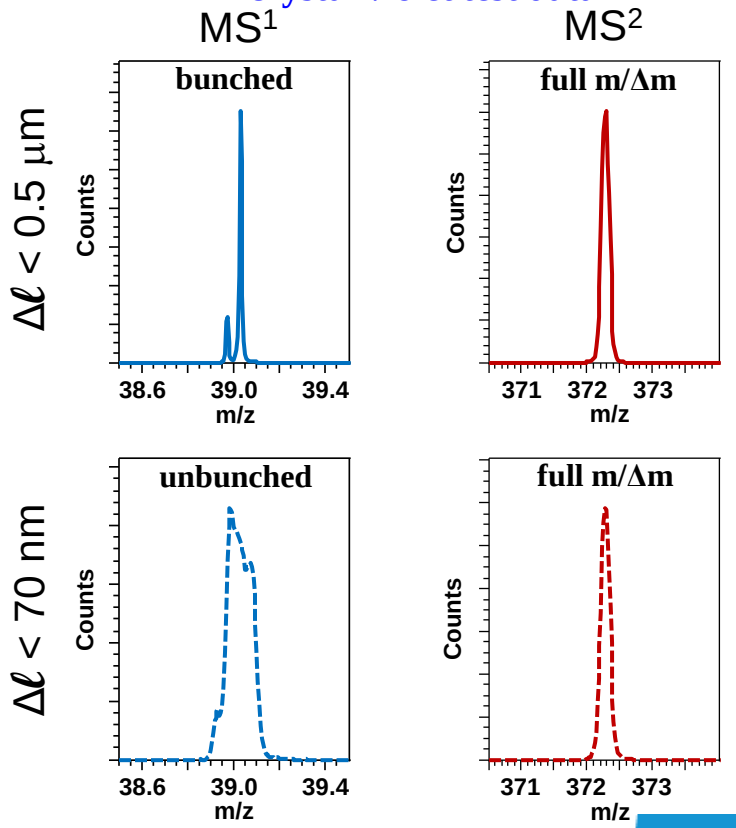
HR² Mode Imaging: (best mass resolution, m/Δm > 10,000)



Unbunched Mode Imaging: (best lateral resolution, Δℓ < 70 nm)



crystal violet test data



What is the Composition at -*m/z* 167 ?

Peak compositions are not confidently identified from the TOF-SIMS (MS^1) data alone.