

# Advanced Multimodal Spatially-Resolved Mass Spectrometry Methods for Untargeted Spatial Metabolomics and Untargeted Spatial Proteomics on the Same Tissue Section

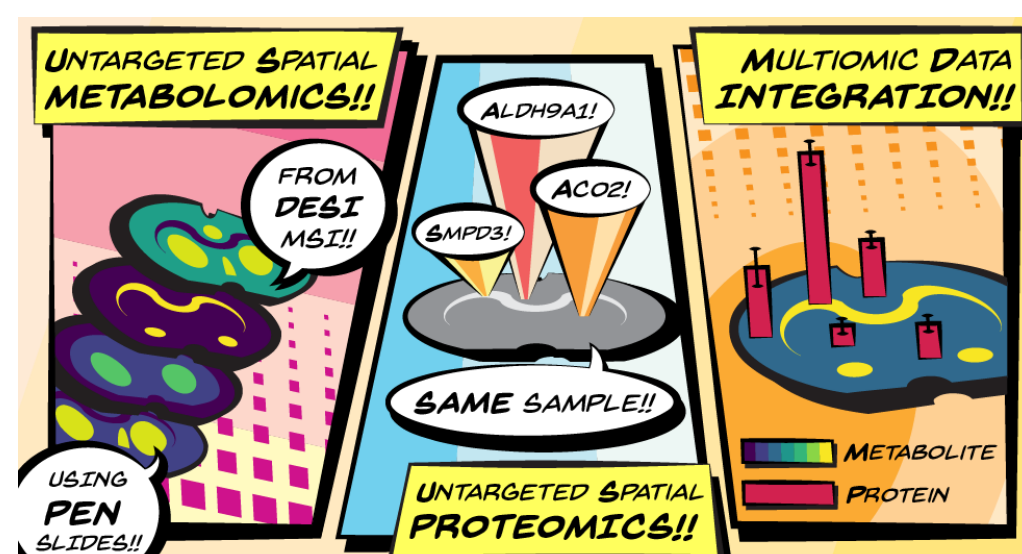
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**PNNL@ASMS**  
 BALTIMORE  
 SHORT COURSES MAY 31 & JUNE 1

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## Overview

- Spatially-resolved omics approaches that can measure biomolecules within tissue anatomical regions are powerful new tools for correlation of protein and metabolite abundances
- Our team has been making advancements in combining mass spectrometry imaging (MSI)-based spatial metabolomics with informed microscale proteomics interrogation for deep profiling of specific cell populations and tissue substructures

## Previously on...

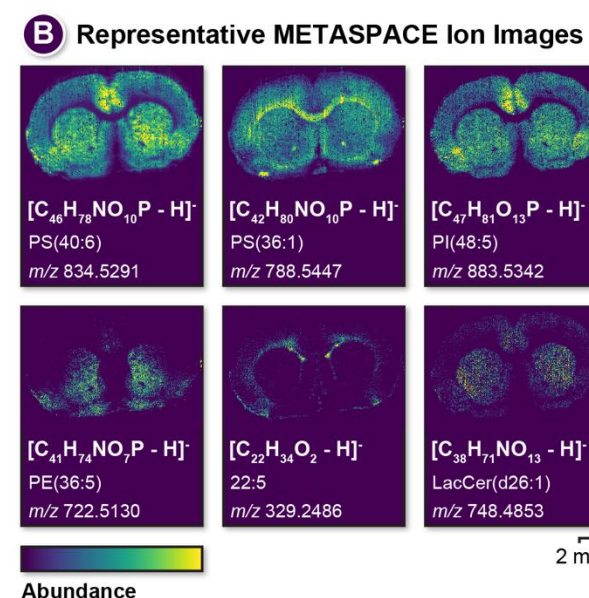
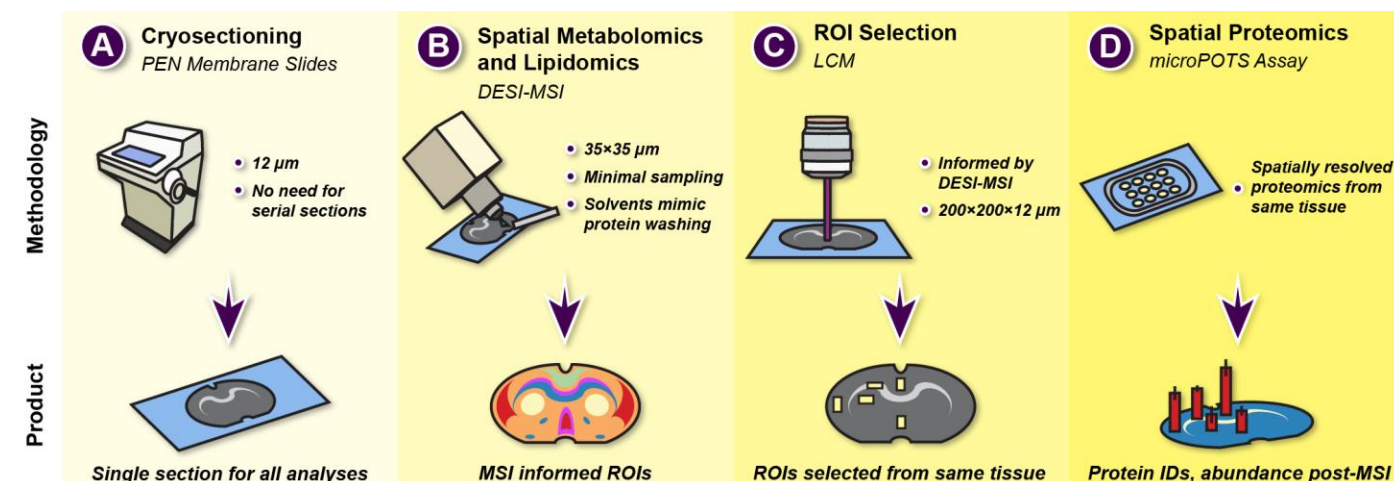


We combined desorption electrospray ionization (DESI)-MSI, for spatial metabolomics, with laser capture microdissection (LCM) our microscale Processing in One pot for Trace Samples (microPOTS) approach, for spatial proteomics. This enabled untargeted spatial multiomics from the same tissue section.

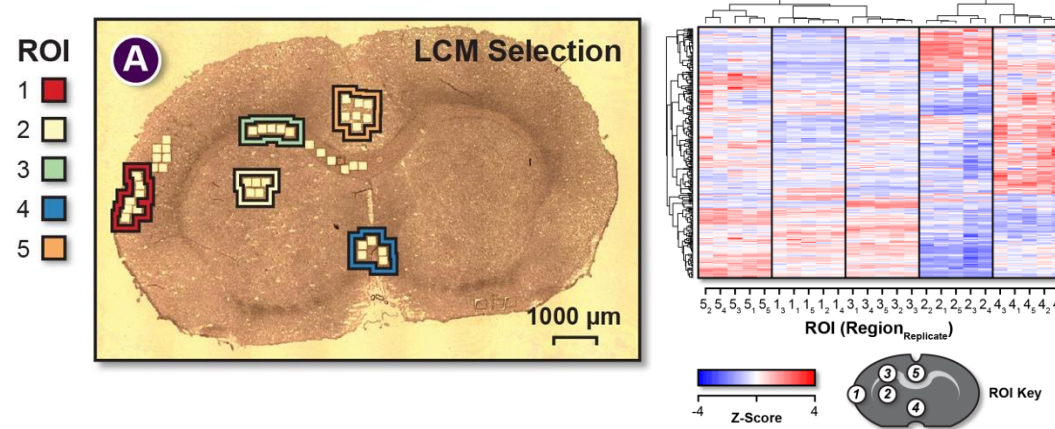


Vandergrift et al., *Anal Chem* 2025, 97, 392

- Compatibility between polyethylene naphthalate (PEN) membrane slides and DESI-MSI analyses
- Non-destructive sampling by DESI-MSI in advance of spatial proteomics



Waters SELECT SERIES™ MRT DESI XS  
 Thank you to Waters and Bindesh Shrestha!



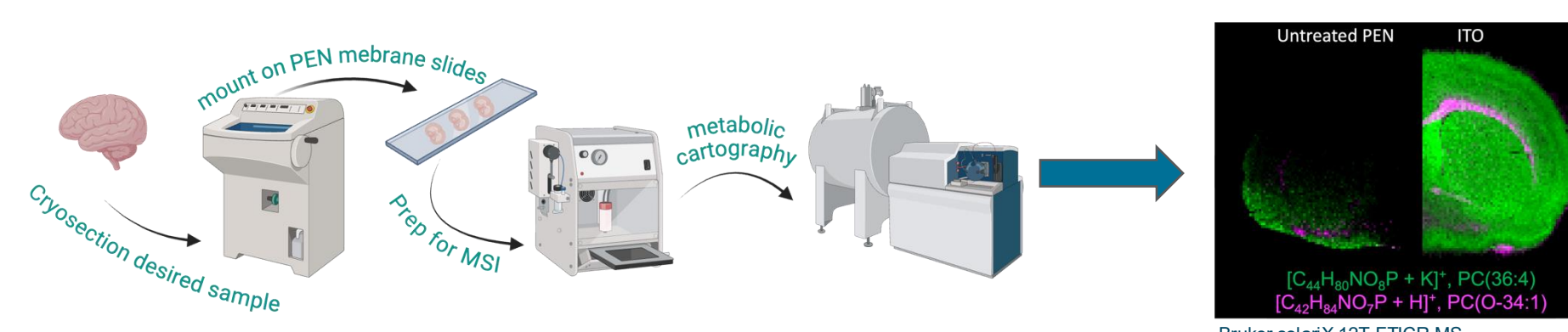
Spatial metabolomics detected ~180 metabolites and lipids (as annotated against HMDB in METASPACE, 20% FDR).

Spatial proteomics detected ~4000 proteins per voxel after DESI-MSI on the same tissue section. Each region of interest (ROI) had a distinct protein composition, and our LCM-microPOTS approach demonstrated consistent intra-ROI protein expression.

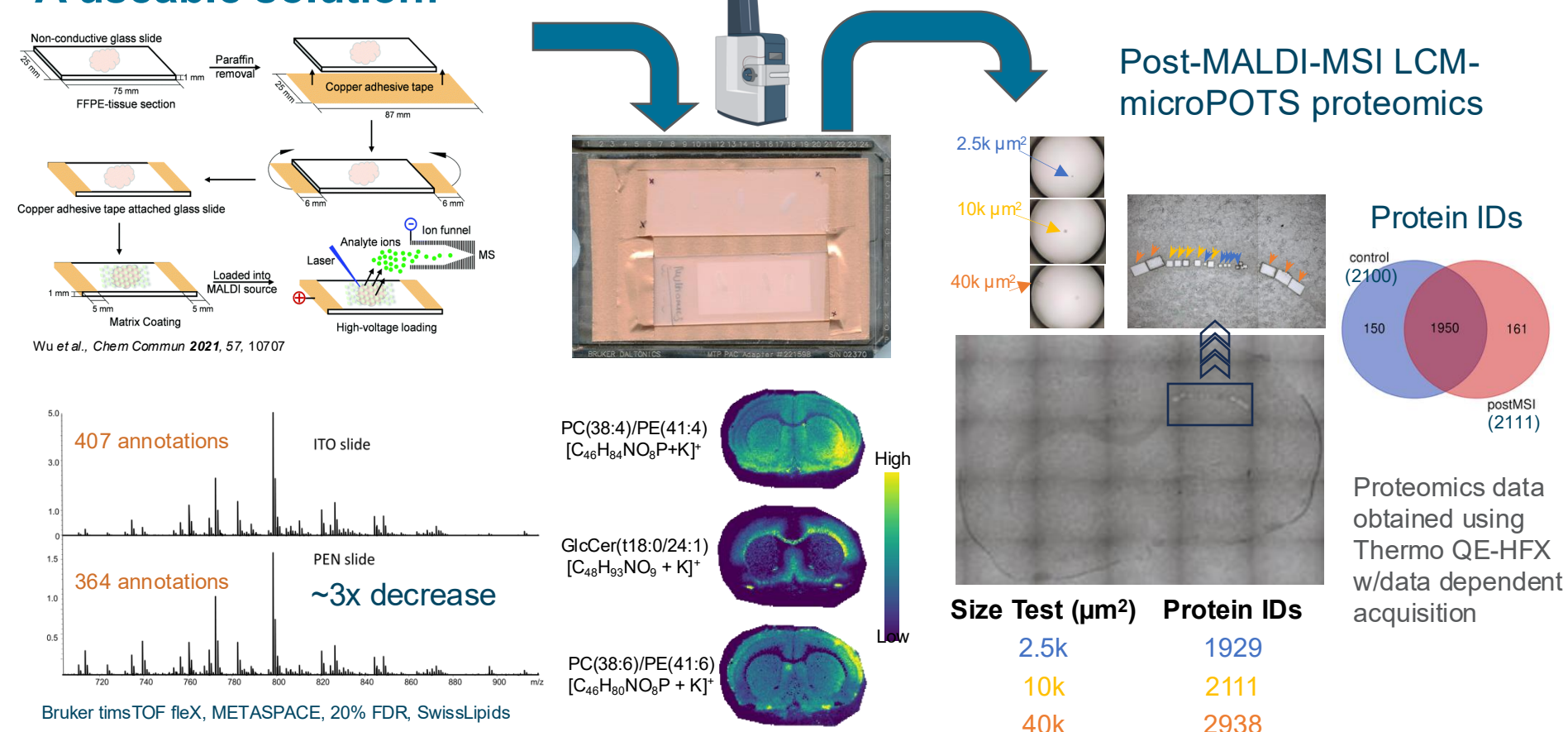
## On our current episode...

### The Challenge:

Conductive substrates (e.g., indium tin oxide [ITO]-coated glass slides) improve performance MALDI-MSI performance by dissipating surface charge during desorption/ionization, whereas LCM performance is optimal with thermoplastic membranes (e.g., PEN) that enable facile release of dissected tissue from glass slides.

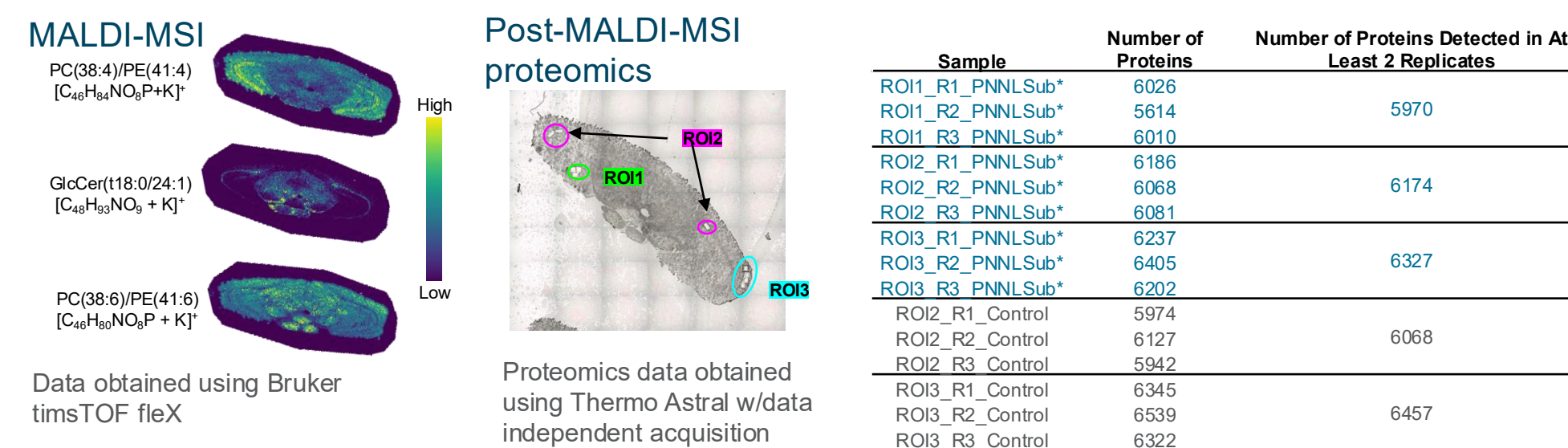


### A useable solution:



We used this approach on plant tissue, too!  
**MOB am 09:30**, Marija Velickovic Optimization of spatial metabolomics and proteomics analyses from a single section of poplar root tissue

### A new solution: Proprietary PNNL substrate\*



## Summary

- We developed approaches that enable sensitive untargeted spatial proteomics informed by upstream non-destructive MSI imaging-based spatial metabolomics on the same sample.
  - DESI-MSI can utilize PEN membrane slides as is.
  - Modifications to mounting PEN membrane slides is required for MALDI-MSI.

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