



**VU BIOMOLECULAR MULTIMODAL IMAGING CENTER**  
*HuBMAP Tissue Mapping Center*

PIs: Richard M. Caprioli, Ph.D. & Jeffrey M Spraggins, Ph.D.  
Mass Spectrometry Research Center  
Vanderbilt University

*2019 HuBMAP Annual Meeting*



VANDERBILT  
UNIVERSITY

VANDERBILT  UNIVERSITY  
MEDICAL CENTER

  
TU Delft

**COORDINATION CORE**



Richard Caprioli<sup>1</sup>  
MPI / Director



Jeffrey Spraggins<sup>1\*</sup>  
MPI / Imaging MS



Danielle Gutierrez<sup>1\*</sup>  
Project Leader / Omics



Maureen Casey<sup>1</sup>  
Center Admin.

**KIDNEY - ORGAN SPECIFIC PROJECT**



Mark deCaestecker<sup>2</sup>  
OSP Team Leader / MxIF



Raymond Harris<sup>2</sup>  
OSP Team Leader



Agnes Fogo<sup>2</sup>  
Pathology Assessment



Elizabeth Neumann<sup>1\*</sup>  
IMS/Microscopy



Maya Brewer<sup>2</sup>  
Tissue Processing



David Anderson<sup>1</sup>  
Sample Preparation



Jamie Allen<sup>1</sup>  
Preanalytical Analysis



Jennifer Harvey<sup>1</sup>  
Histology

**DATA ANALYSIS CORE**



Raf Van de Plas<sup>3</sup>  
DAC Team Leader / Fusion



Ken Lau<sup>4</sup>  
Cell Segmentation



N. Heath Patterson<sup>1</sup>  
Image Registration



Lukasz Migas<sup>3</sup>  
Signal Processing



Kavya Sharman<sup>1\*</sup>  
Segmentation/Omics



Tina Tsui<sup>1</sup>  
Tools/Automation

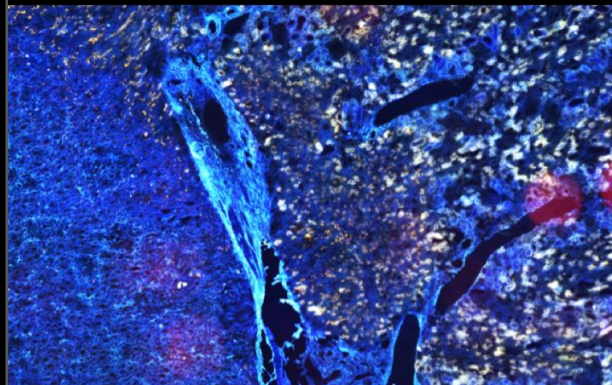
\*HuBMAP Annual Meeting Attendee

# VU BIOMIC / Project Overview

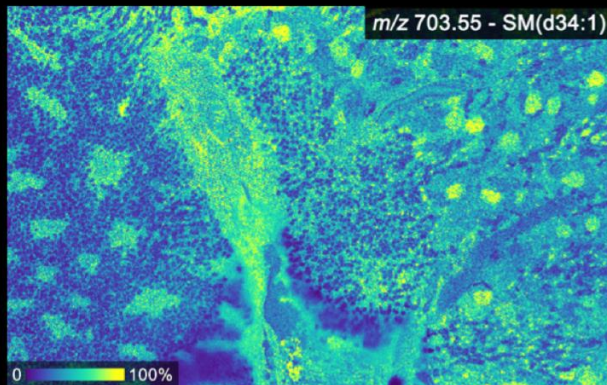
*Mission: to build a platform of integrated technologies for imaging and molecular analysis that enables the construction of comprehensive 3-D molecular atlases of human tissues.*

## MULTIMODAL MOLECULAR IMAGING – Human Kidney

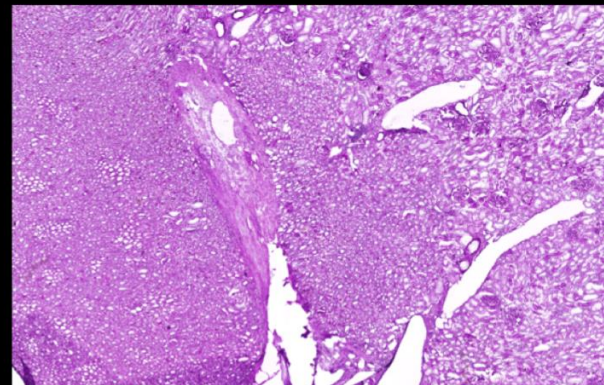
Autofluorescence Microscopy  
Image Registration



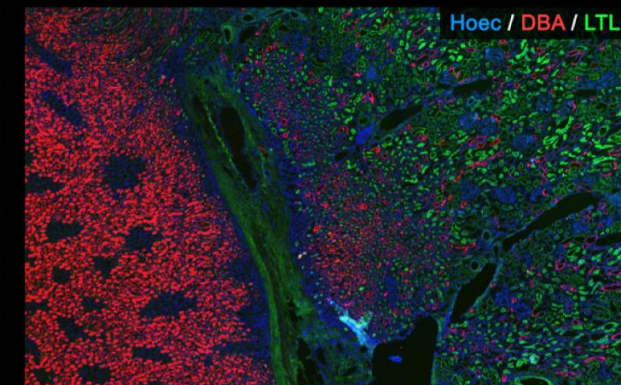
IMAGING MASS SPECTROMETRY  
Highly Multiplexed / Unlabeled



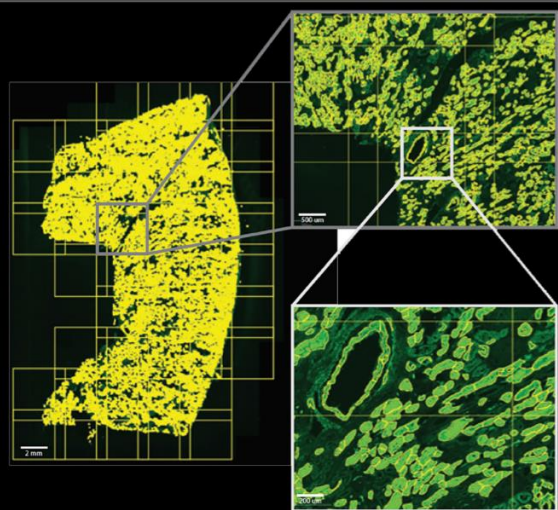
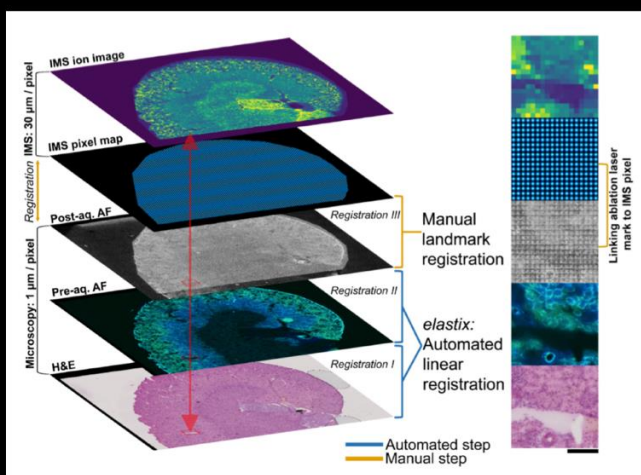
Stained Microscopy  
Tissue Morphology



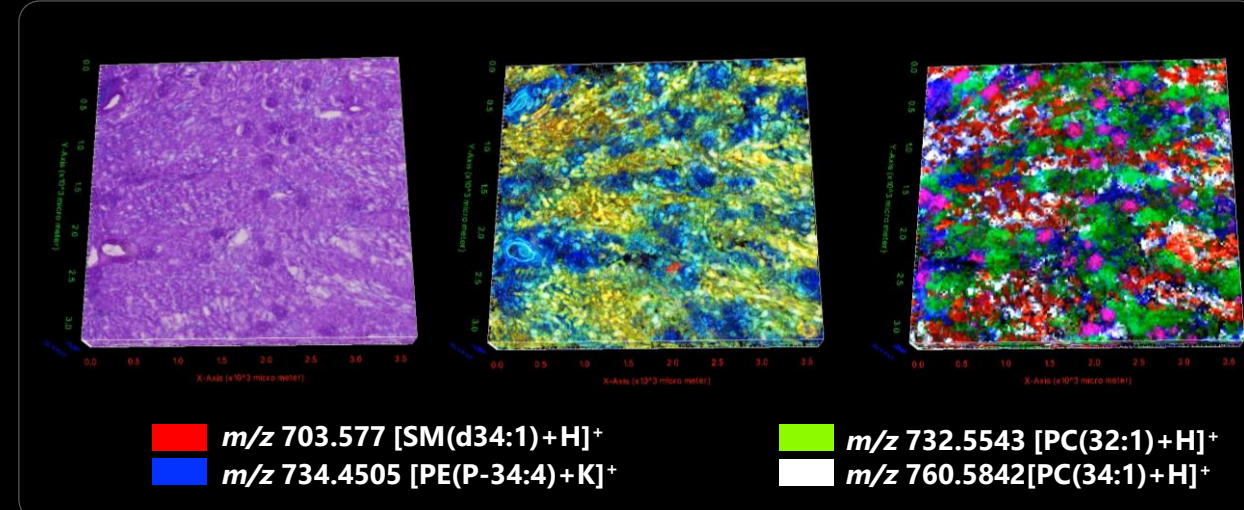
Multiplexed IF Microscopy  
Cell-Type Differentiation



## COMPUTATIONAL TOOLS – Data Integration



## 3-D MOLECULAR IMAGING – Human Kidney



# VU BIOMIC / Year 2 Deliverables

*Mission: to build a platform of integrated technologies for imaging and molecular analysis that enables the construction of comprehensive 3-D molecular atlases of human tissues.*

## 2-D Molecular Imaging Data

- Data Types
  - 15 Autofluorescence Microscopy Images
    - Location: 10 Cortex / 5 Medulla
    - Spatial Resolution: 1  $\mu\text{m}$
  - 15 Multiplexed Immunofluorescence Microscopy Images
    - Location: 10 Cortex / 5 Medulla
    - Spatial Resolution: 1  $\mu\text{m}$
  - 16 Imaging Mass Spectrometry datasets
    - 8 Positive Ion Mode / 8 Negative Ion Mode
    - Spatial Resolution: 10  $\mu\text{m}$
    - >125 Lipid/Metabolite Identifications

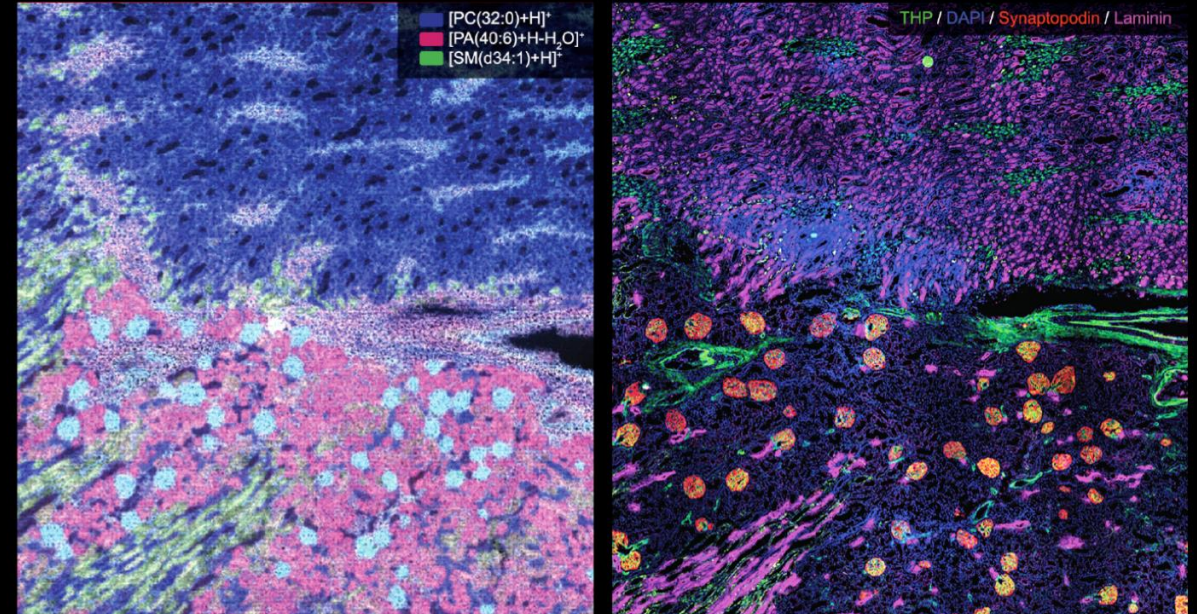
## 3-D Molecular Imaging Data

- 1 3-D Multimodal Molecular Imaging Dataset
  - Location: Cortex
    - 50 serial tissue sections
  - 50 Autofluorescence Microscopy Images
    - Spatial Resolution: 1  $\mu\text{m}$
  - 25 Imaging Mass Spectrometry Datasets
    - Spatial Resolution: 10  $\mu\text{m}$
    - >125 Lipid / Metabolite Identifications
  - 25 Multiplexed Immunofluorescence Microscopy Images
    - Spatial Resolution: 1  $\mu\text{m}$
    - 4 Markers

## Sharable Tools

- Multimodal Image Registration
- Whole Slide Image Segmentation

## OFFICIAL DATA UPLOAD - VAN0001-RK-1-21\_24



## Multimodal Imaging Data

- IMS: Positive Ion Mode / 10  $\mu\text{m}$  Spatial resolution / OME-TIFF, imZML
- AF Microscopy: 1  $\mu\text{m}$  Spatial resolution / OME-TIFF
- PAS Stained Microscopy: 1  $\mu\text{m}$  Spatial resolution / OME-TIFF
- Multiplexed IF Microscopy: 4 Channel / 1  $\mu\text{m}$  Spatial resolution / OME-TIFF

## Metadata

- Clinical / Pathology / Spatial / Instrumental

## Protocols

- 11 protocols all linked using protocols.io

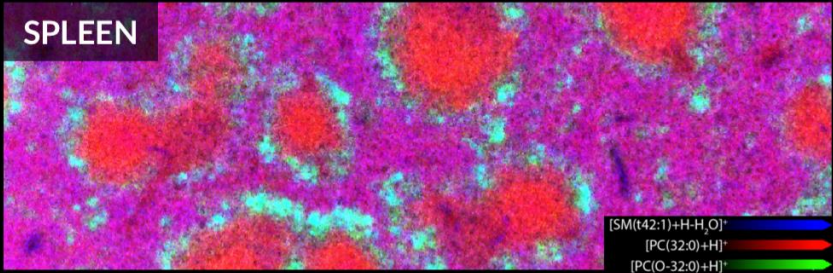
# VU BIOMIC / Collaborative Projects

## Common Tissue Project

- Apply BIOMIC pipeline to a common tissue for comparison across the HuBMAP consortium.

## VU-UF Collaboration

- Collect high content molecular and multimodal imaging data from spleen tissue.
- Apply IMS to other tissues within the HuBMAP Consortium.



## VU-UCSD Collaboration

- Multi-site analysis of human kidney tissue
- Register and integrate lipid/protein imaging with in situ RNA-based DART-FISH.

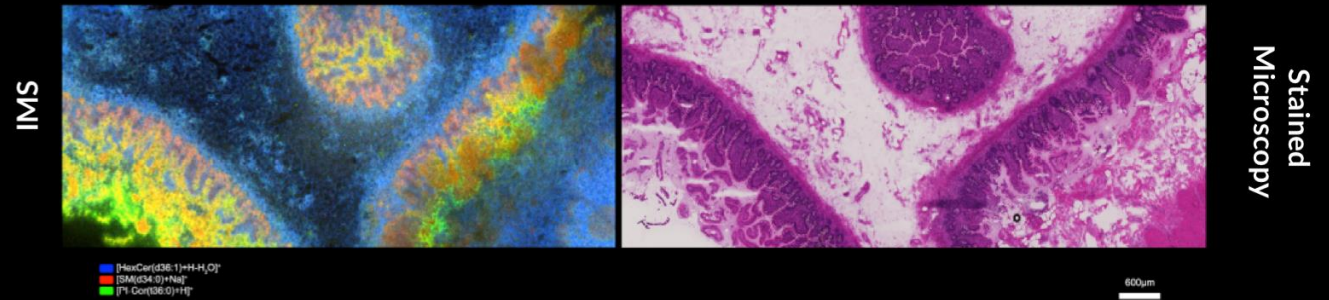
## VU-Purdue Collaboration

- Compare MS-based analyses of kidney tissue
- Register and integrate MALDI lipid imaging with nano-DESI metabolite imaging to provide greater molecular coverage.

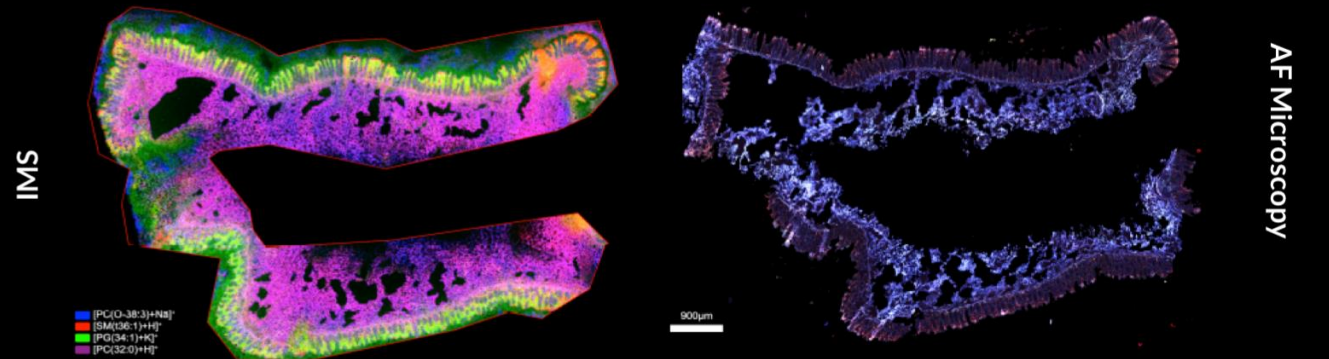
## VU-IU Collaboration

- Develop a multi-subject average and variability atlas of kidney anatomy for CCF.
- CT and MR images from ImageVU.
- Constructed from a cohort of ~500 individuals

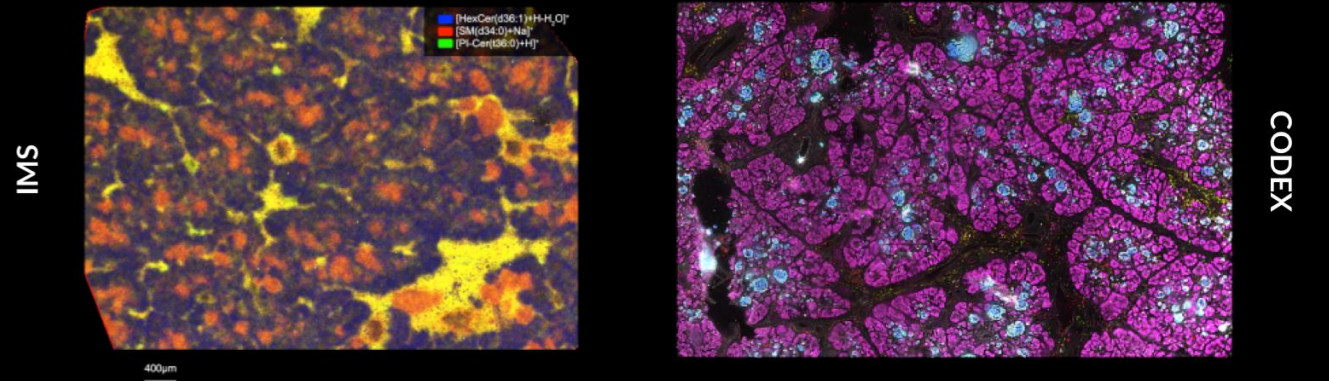
## SMALL INTESTINE



## COLON



## PANCREAS



## What should the priorities for the Consortium in Year 2?

- Data Collection and Sharing – In short-term we should focus on getting 2-D analysis right before attempting 3-D.
- Streamline data ingest including linking data with protocols, metadata, and QC outputs.
- Multimodal data visualization –Providing a means for users to interact with and query data will be critical for ensuring HuBMAP data are impactful.
- Improving diversity of samples (e.g. age / sex / race).
- Get input from physicians/surgeons/clinicians, etc. to see how HuBMAP data and technologies could be adapted to be useful to them.

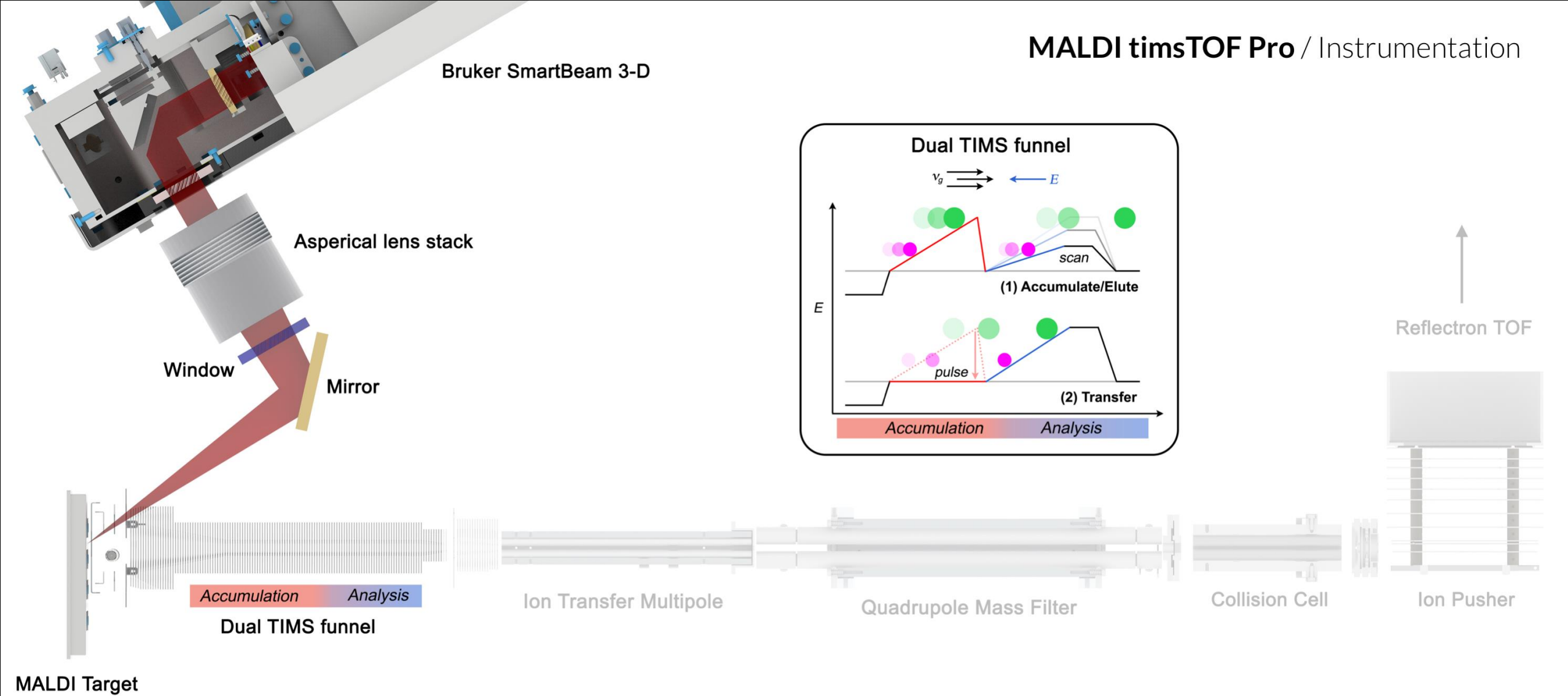
**Data Generation > Data Ingest > Data Retrieval > Visualization > Analysis**



# **HIGH PERFORMANCE MOLECULAR IMAGING PLATFORM**

PROTOTYPE MALDI timsTOF Pro

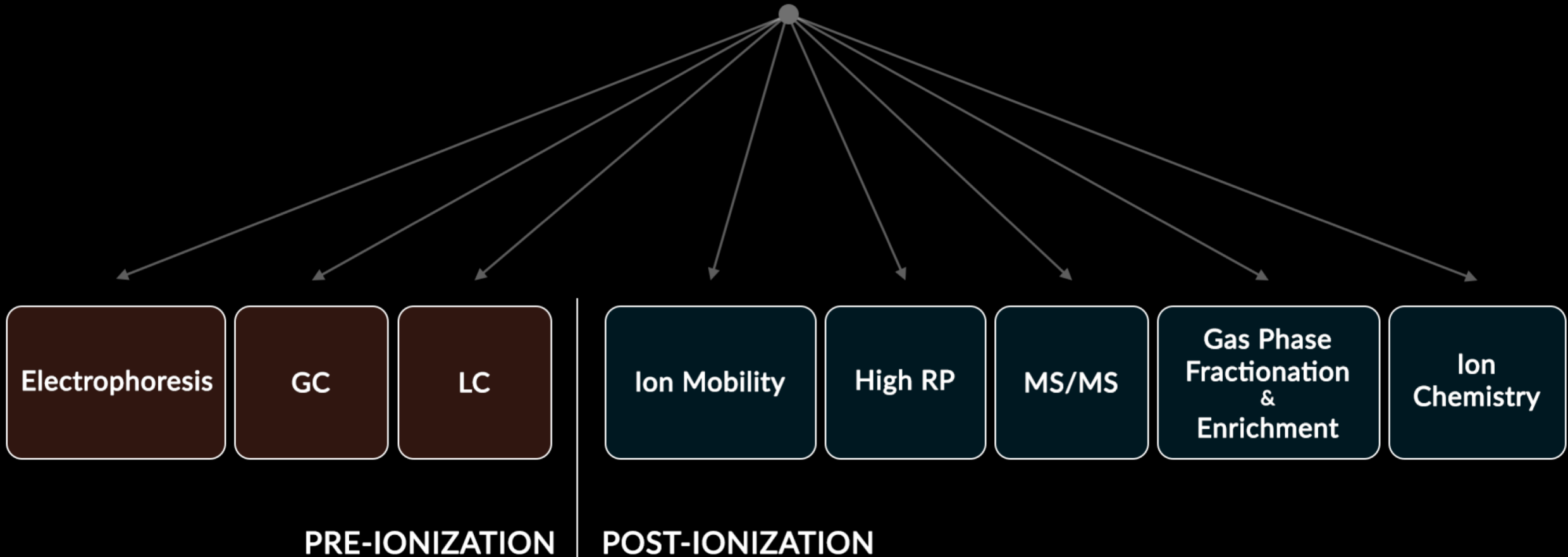
# MALDI timsTOF Pro / Instrumentation



Tunable IM resolving power and throughput | ~50 – 200 RP mobility resolution | 20 – 2 px/s acquisition speed



## COMPLEX BIOLOGICAL MIXTURES

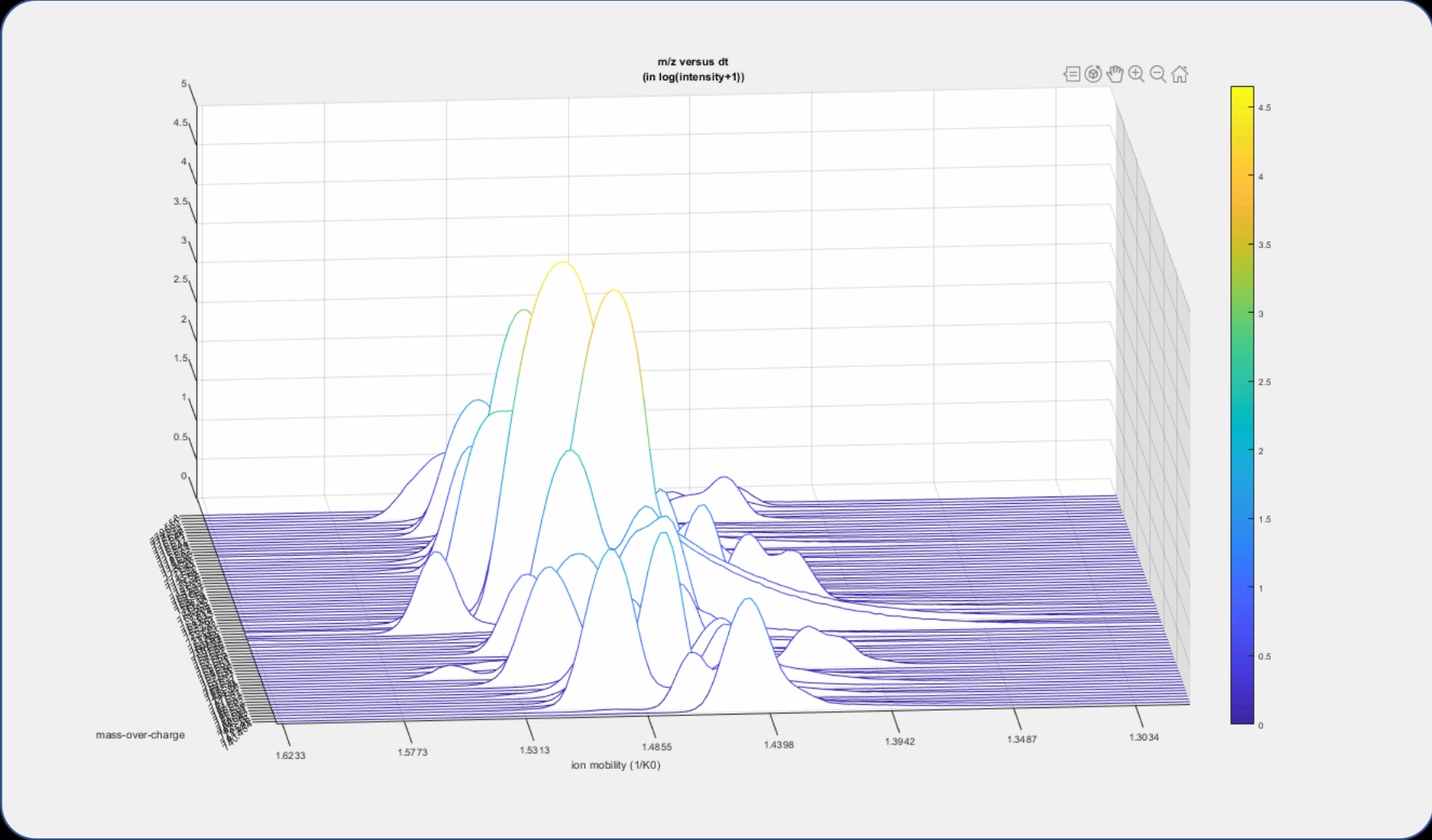


-Throughput and spatial fidelity requirements make pre-ionization approaches impractical for most imaging MS applications.

-Post-ionization strategies are completely decoupled from surface sampling and are performed on time scales compatible with IMS.

# MALDI timsTOF Pro / TIMS Imaging MS

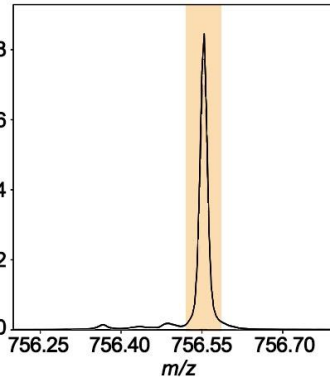
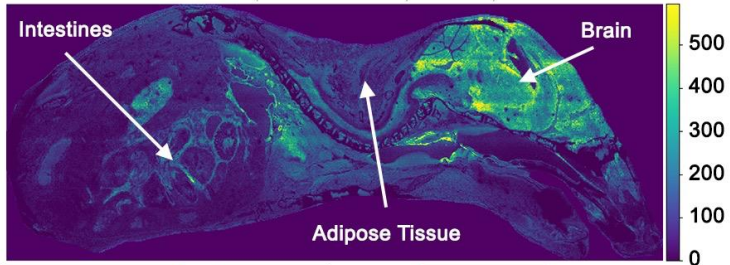
High Specificity Imaging



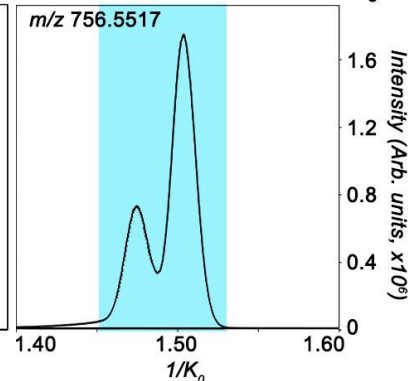
# MALDI timsTOF Pro / TIMS Imaging MS

High Specificity Imaging

$m/z$  756.5517 |  $1/K_0$  1.45 - 1.53

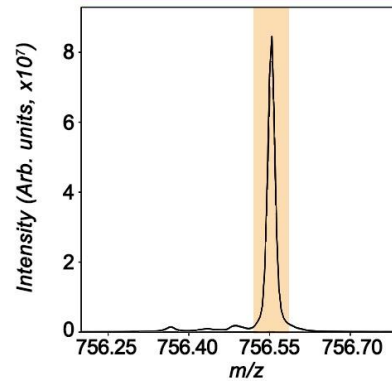
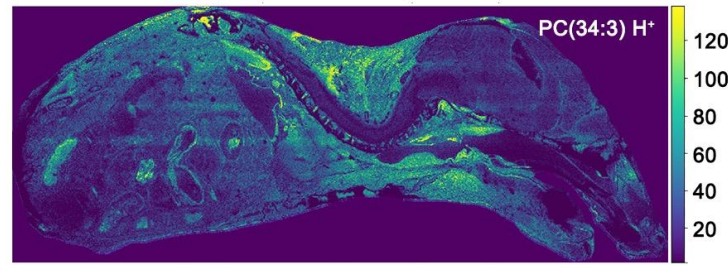


MASS SPECTRUM

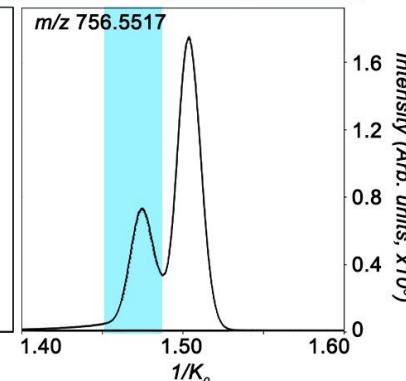


EXTRACTED ION MOBILOGRAM

$m/z$  756.5517 |  $1/K_0$  1.45 - 1.48

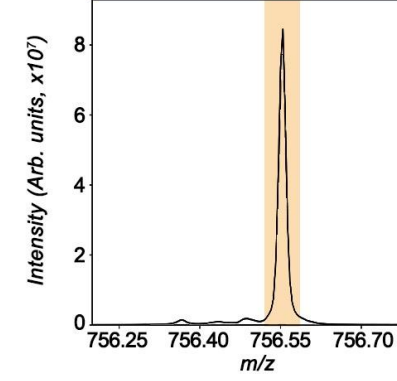
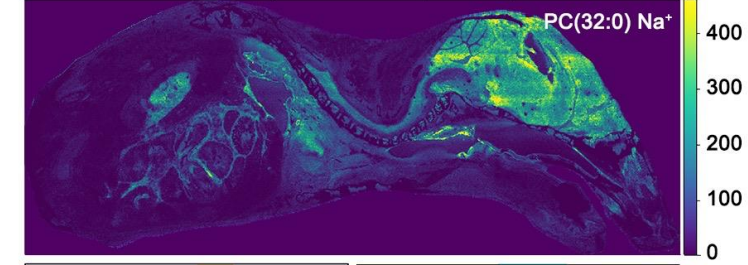


MASS SPECTRUM

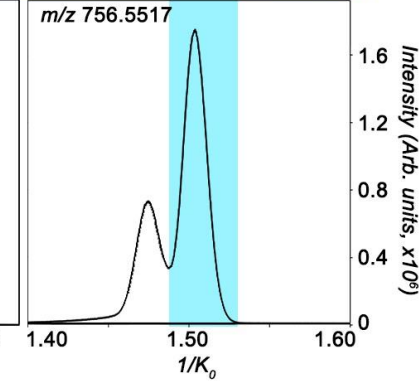


EXTRACTED ION MOBILOGRAM

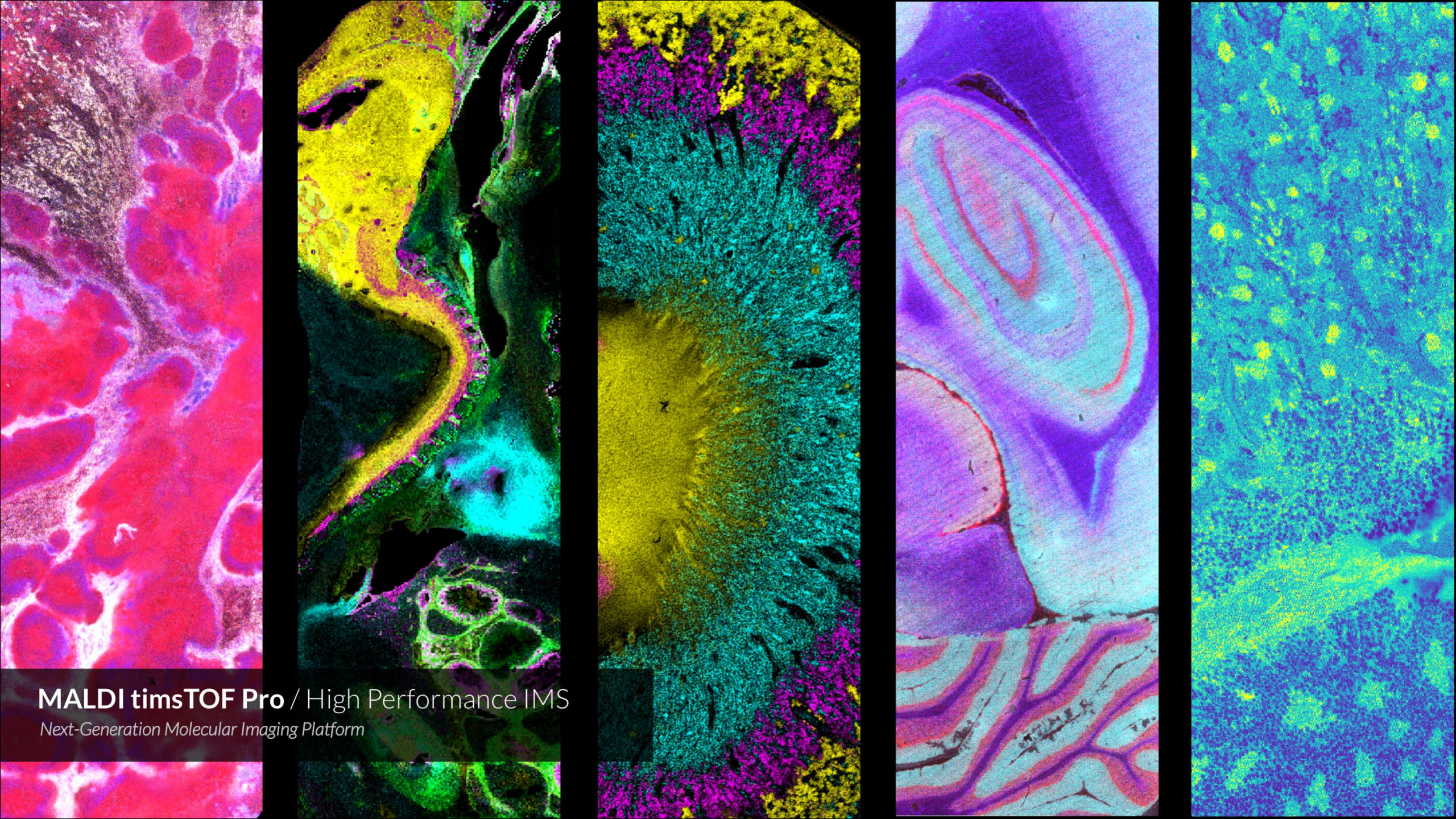
$m/z$  756.5517 |  $1/K_0$  1.48 - 1.53



MASS SPECTRUM



EXTRACTED ION MOBILOGRAM



**MALDI timsTOF Pro / High Performance IMS**

*Next-Generation Molecular Imaging Platform*