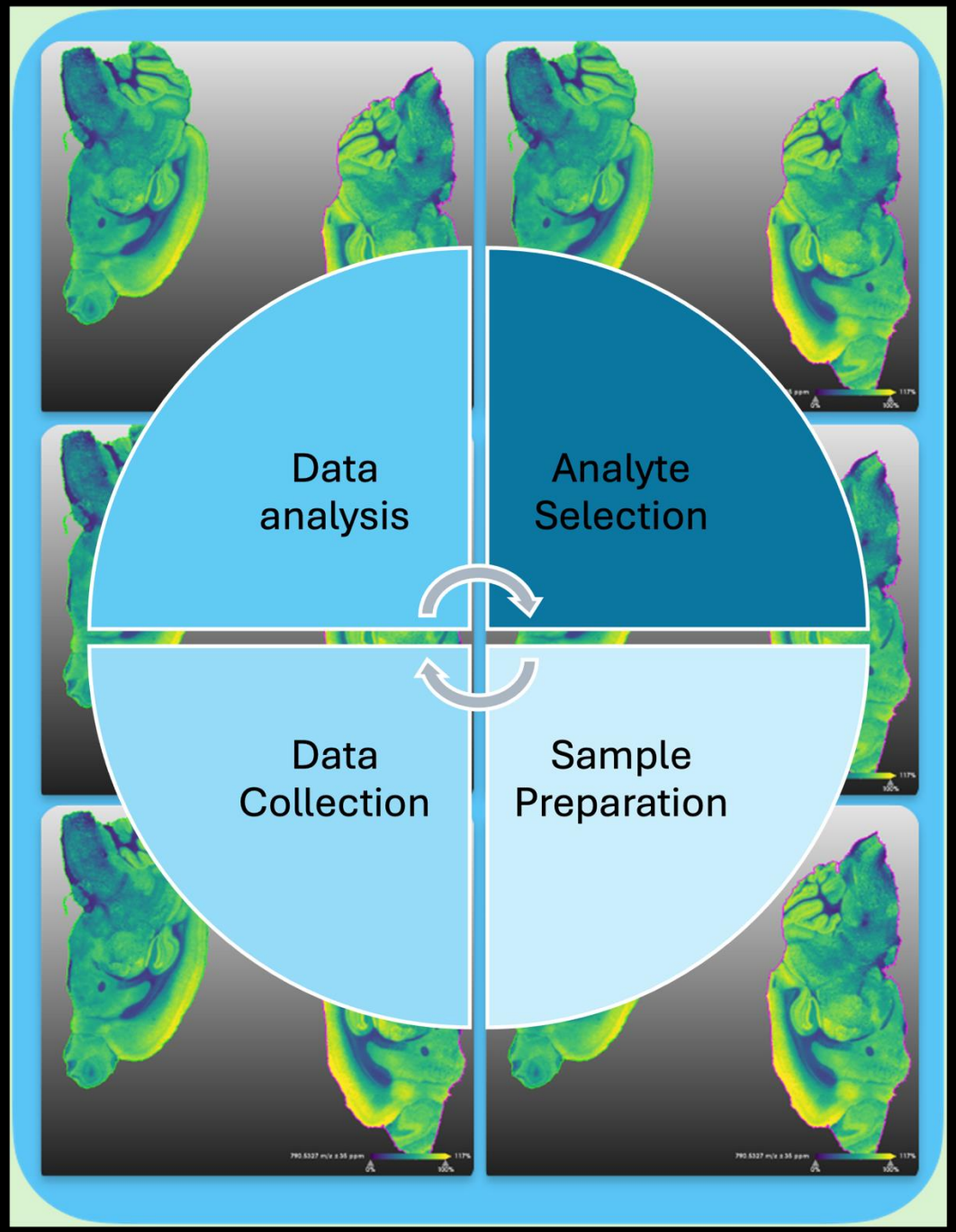
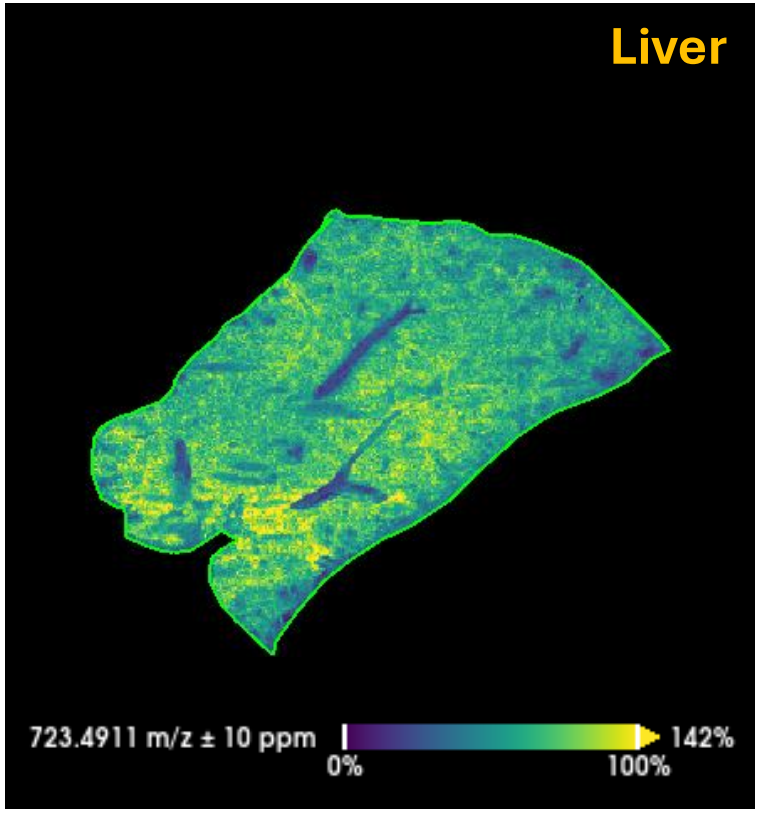


Introduction to MALDI and DESI Imaging

Georgia Charkoftaki (Yale University)
Peggi Angel (MUSC)
Madeline Colley (Vanderbilt)
Christina Ferreira (Purdue University)
David Muddiman (NC State University)

Mass Spectrometry Imaging (MSI) : A novel technology for spatial omics



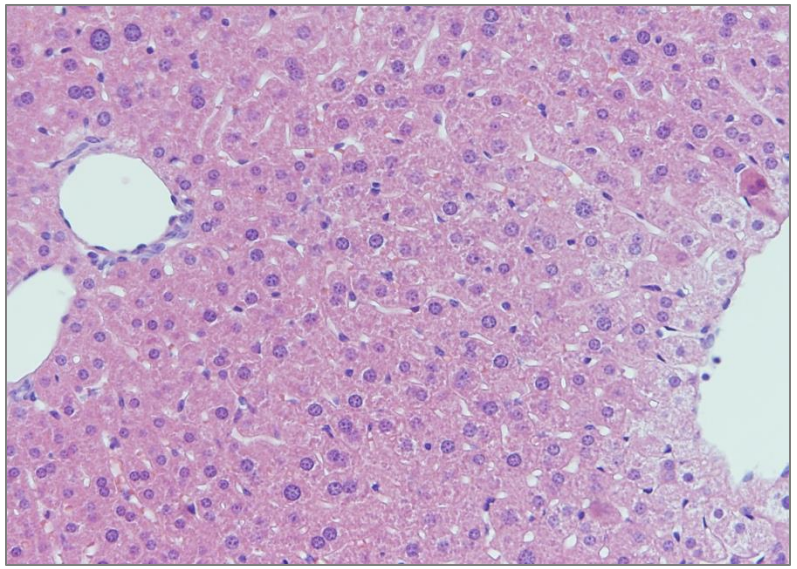
- Mass spectrometry imaging (MSI) allows **visualization of biomolecule distributions within tissues**

- **MALDI MSI** involves the local extraction, desorption, and ionization of the biomolecules in a spatially correlated manner, using **the matrix-assisted laser desorption/ionization (MALDI) technique**

- MALDI MSI can be employed to **image a broad variety of biomolecules ranging from low-molecular-weight metabolites (~100-1000 Da), lipids (~300-1500 Da) and proteins (<70 kDa)**

MALDI MSI: Beyond Classic “Read-Outs”

Classic staining techniques

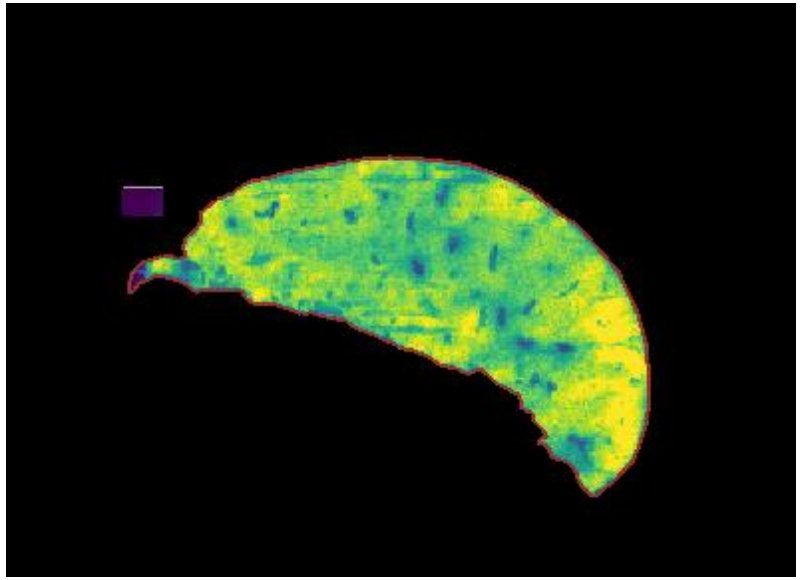


- Antibody dependent:

(i) functionality, (ii) specificity and (iii) validation

- Performance of antibodies is application-dependent

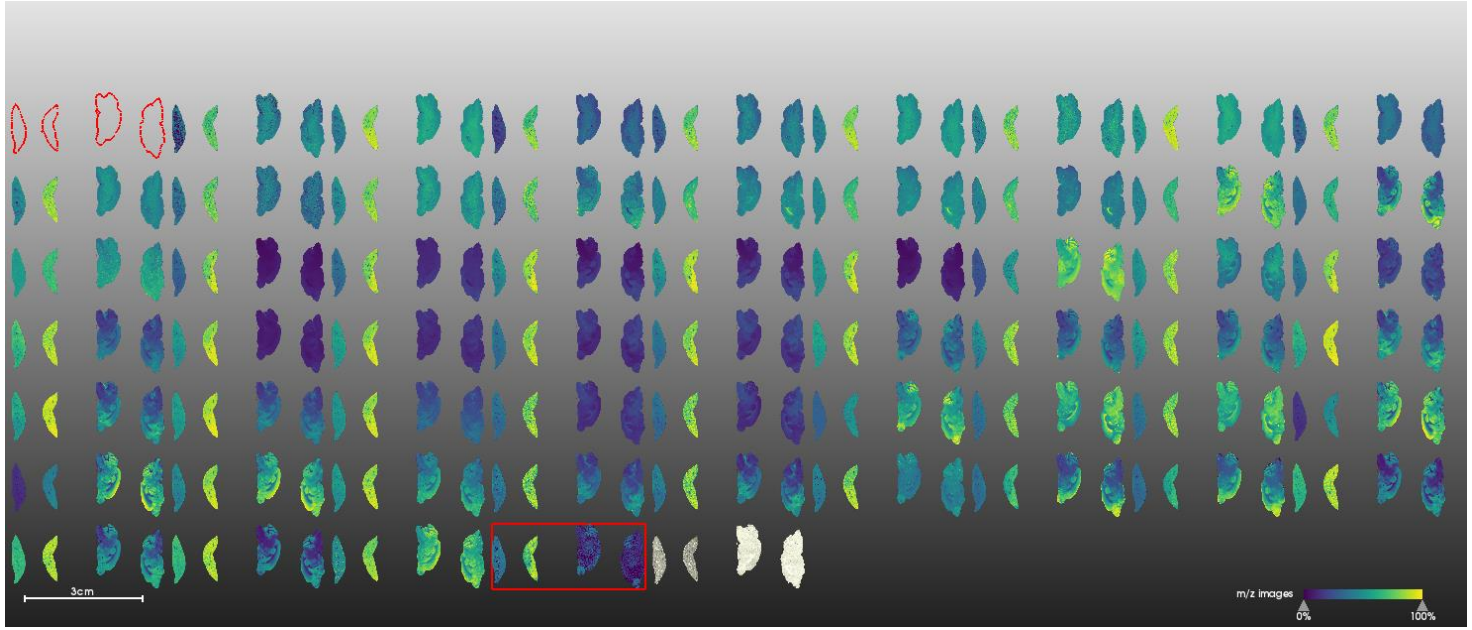
MALDI MSI



- Broad variety of molecule classes, e.g., lipids, proteins and low molecular weight metabolites, can be mapped
- No labeling agents
- Spatial distribution

MALDI MSI Workflow

(fresh frozen tissues)



Tissue sectioning: Cryostat
Tissue thickness
(10-15 µm)

Matrix application

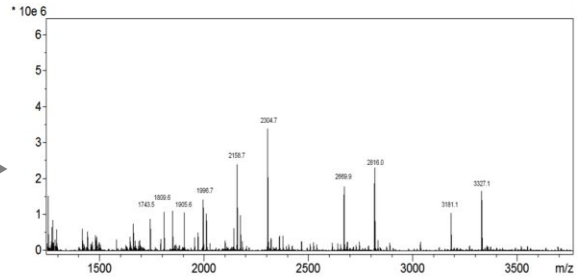
Laser beam

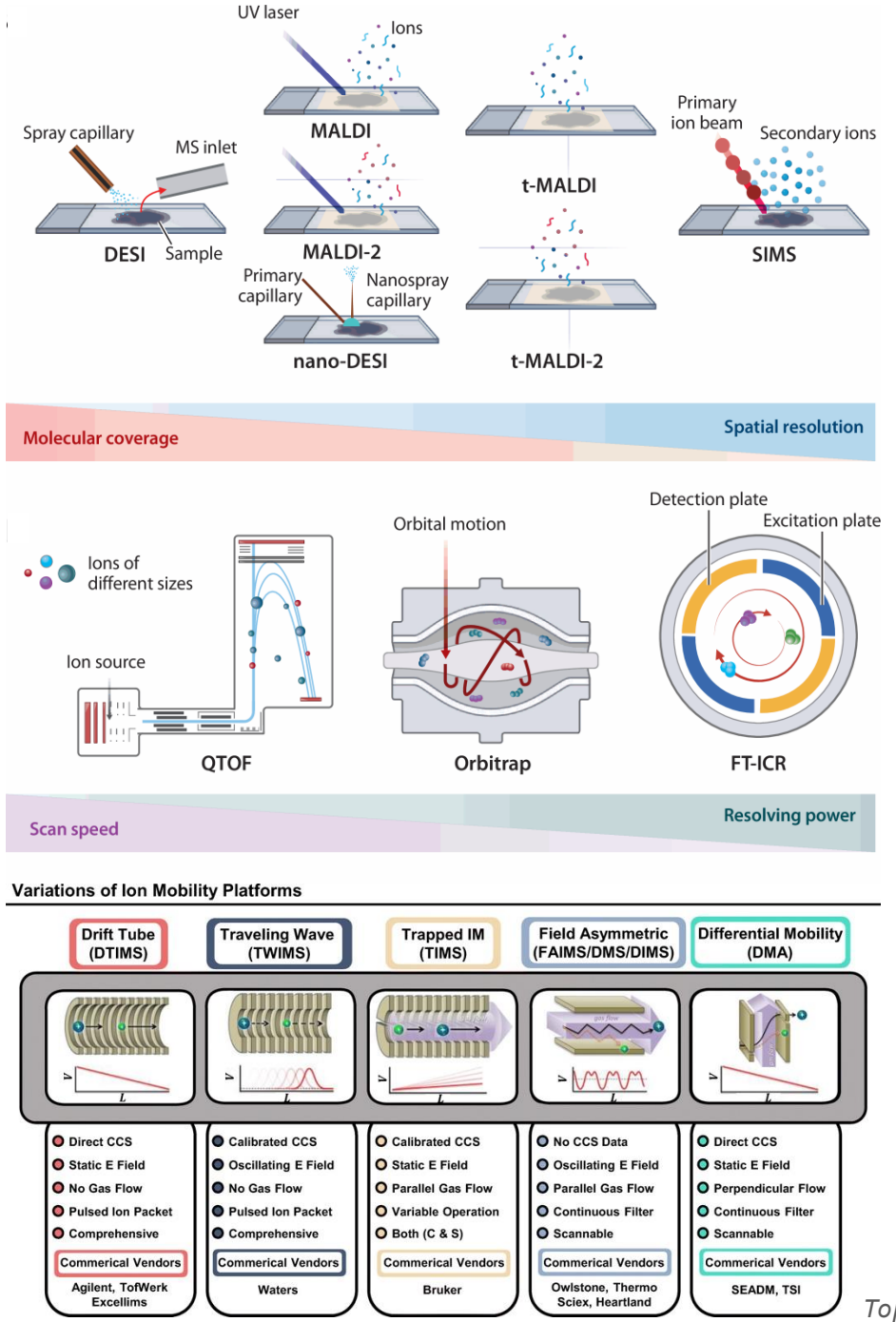
Ions

Mass Spectrometer

Detector

Data analysis

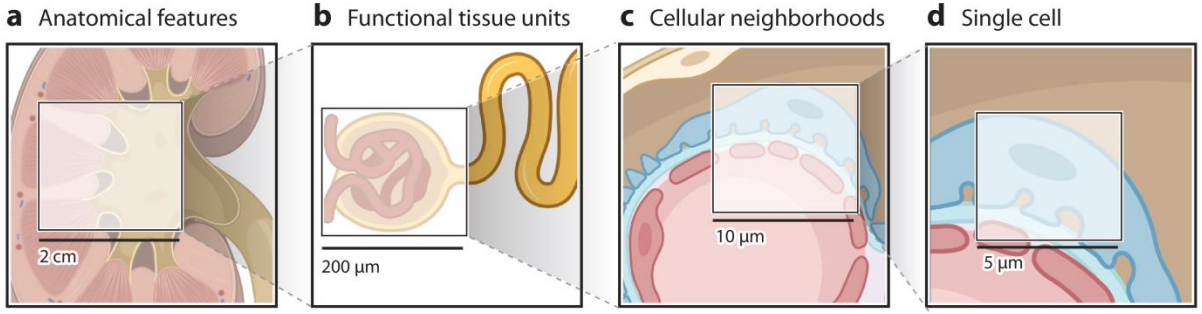




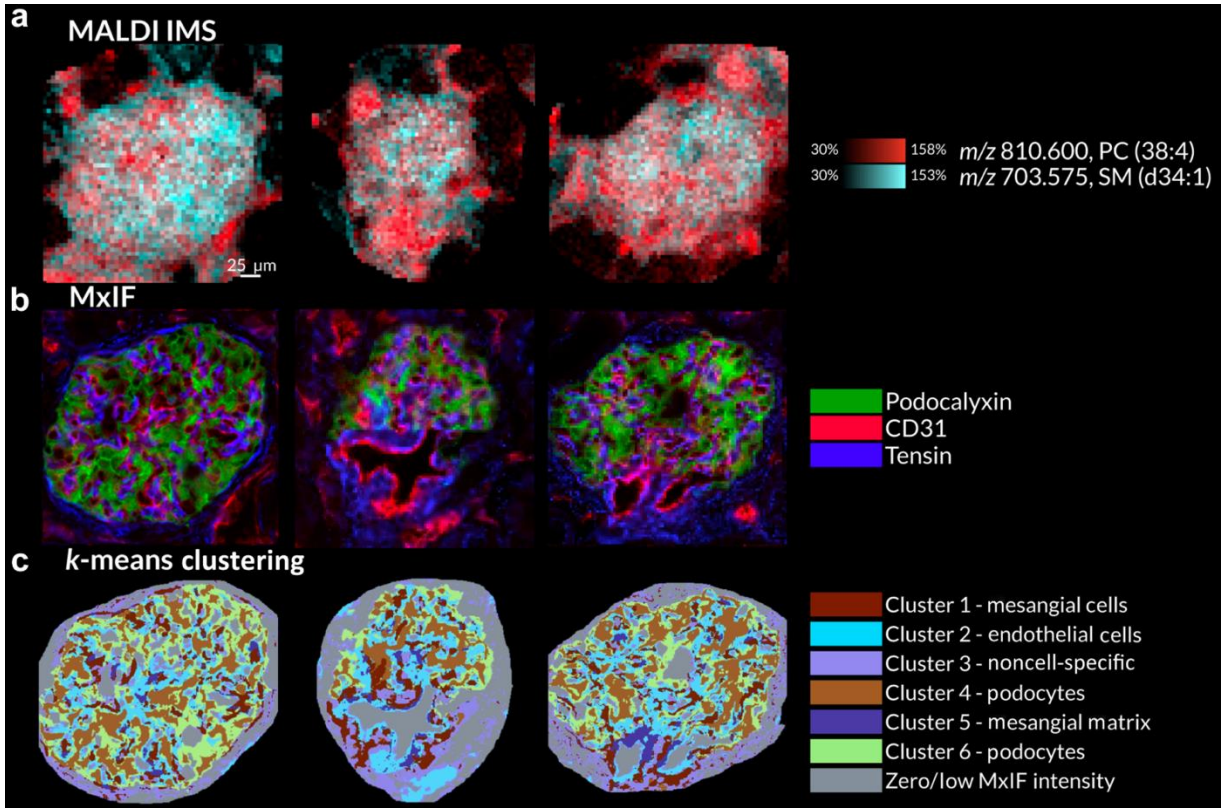
Instrumentation is Finding the Correct Balance

- There is always a question to be asked with any available instrument!
- Finding the right instrument depends on your goals and questions; no instrument is one-size-fits-all
  - Analyte type
  - Analyte validation
  - Spatial resolution
  - Ionization pathway
  - Acquisition time
  - Sample prep compatibility
  - Cost $$$$

Top: Colley, ME, et al. 2024. Annu. Rev. Anal. Chem. 17:1-24
Bottom: Dodds, JN, at al. 2019. JASMS. 30(11).



Colley ME, et al. 2024
Annu. Rev. Anal. Chem. 17:1–24



Top: Colley, ME, et al. 2024. Annu. Rev. Anal. Chem. 17:1-24
Bottom: Esselman AB, et al. 2024. Kidney International. 107(2):332-337

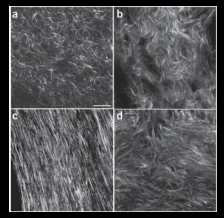
Multimodal Imaging Complements MSI

- Microscopy measurements off biological ground truth (Pathology interpretation, protein biomarkers, RNA biomarkers, etc)
- Microscopy measurements > MSI, can offer understanding of cell boundaries, cell neighborhoods and disease state
- Microscopy can let you know the limitations of your MSI spatial resolution (how many microscopy pixels are there per MSI? How many cells exist under that one pixel?)

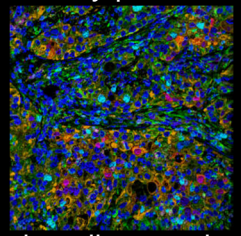
- Multimodal imaging and external validation strengthens biological interpretations!

Targeted Spatial Biology using Multimodal Cyclical Enzyme Imaging

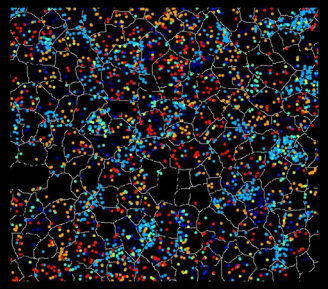
Second Harmonic Generation Microscopy

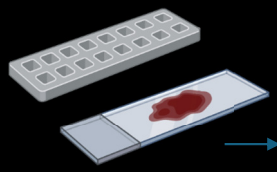


Single or multiplexed antibody platforms

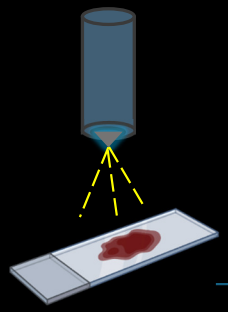


Single cell transcriptomics



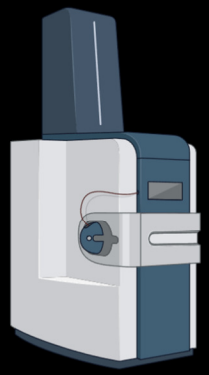


e.g., digital record, remove coverslip



Enzyme spraying

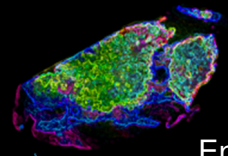
Change the enzyme, change the Omics



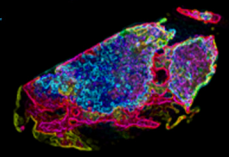
Imaging MS
Glycomics
Matrisomics

Enzyme 1: Target N-glycans PNGase F Powers…Drake, 2013

Enzyme 2: Target Extracellular Microenvironment Collagenase Angel, 2018



Enzyme 3: Target General Proteome Trypsin Reid…Caprioli 2008



Remove products & repeat with a different enzyme

…Enzyme n

- Cyclical enzyme imaging

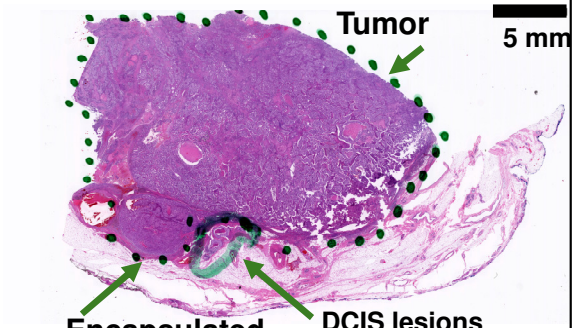
Images from
Chen et al, Nature Protocols, 2012 **7**:654
Ionpath.com; https://www.genengnews.com/topics/omics/spatial-transcriptomics-puts-more-biology-on-the-map/

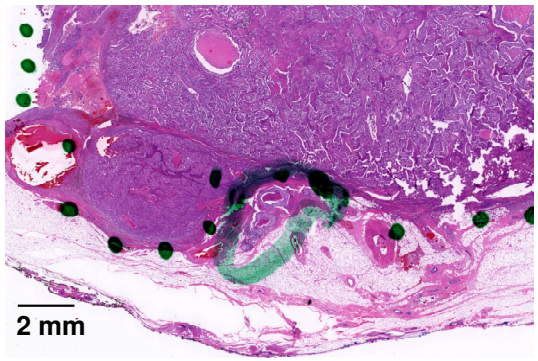
Multiplexed Glycomic-Cell Marker for the Triple Negative Breast Cancer Microenvironment

- Cellular- ~core fucosylation
- Stroma- ~outer arm + branched

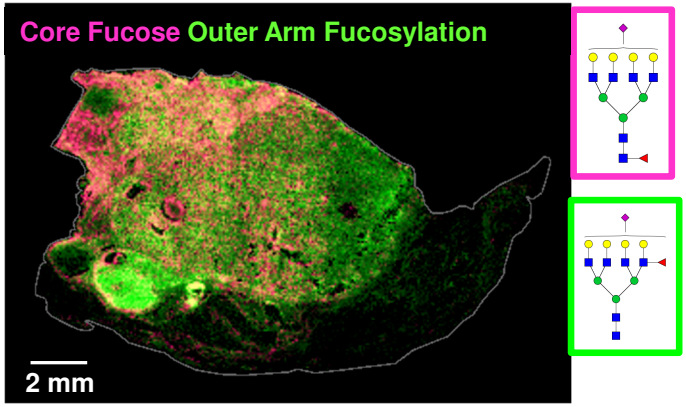
Scott et al, Angel, 2025 Mol Canc Res

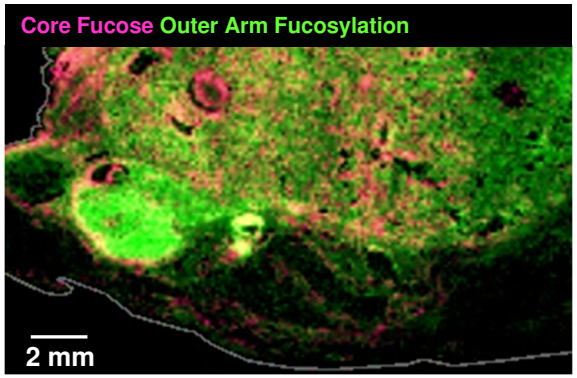
A) Hematoxylin & Eosin with pathologist annotation



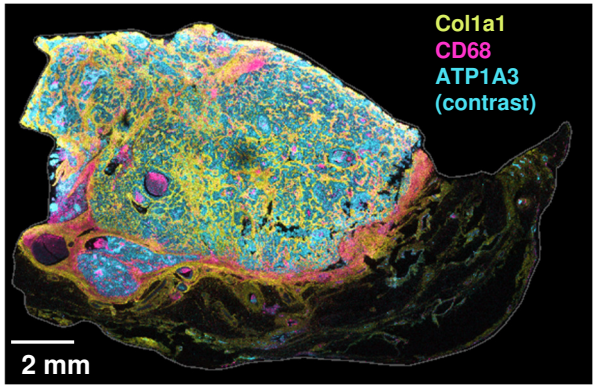


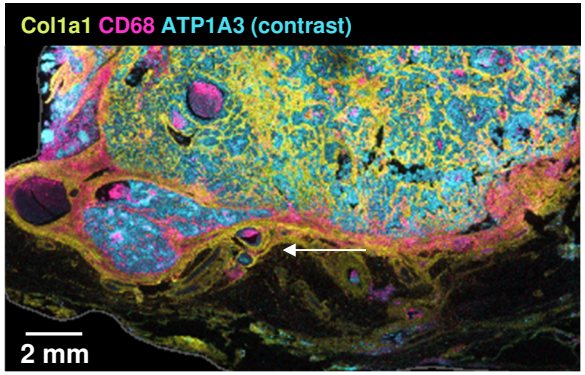
B) Co-registered multiglycomic imaging





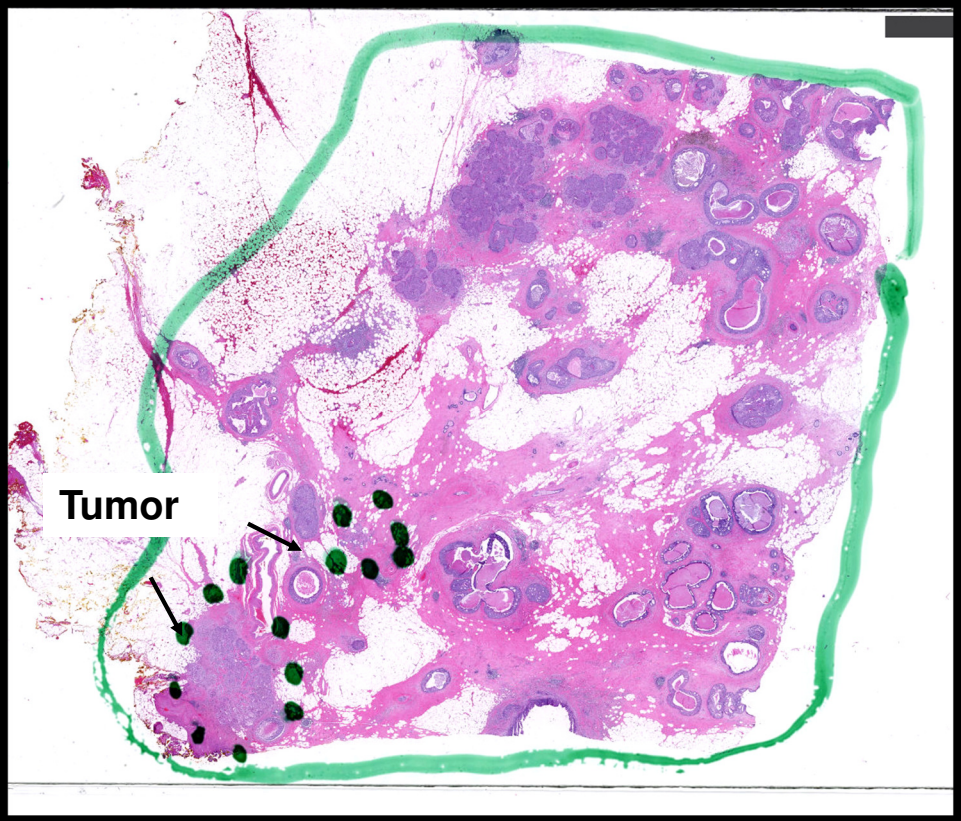
C) Mass Spectrometry labile tagging



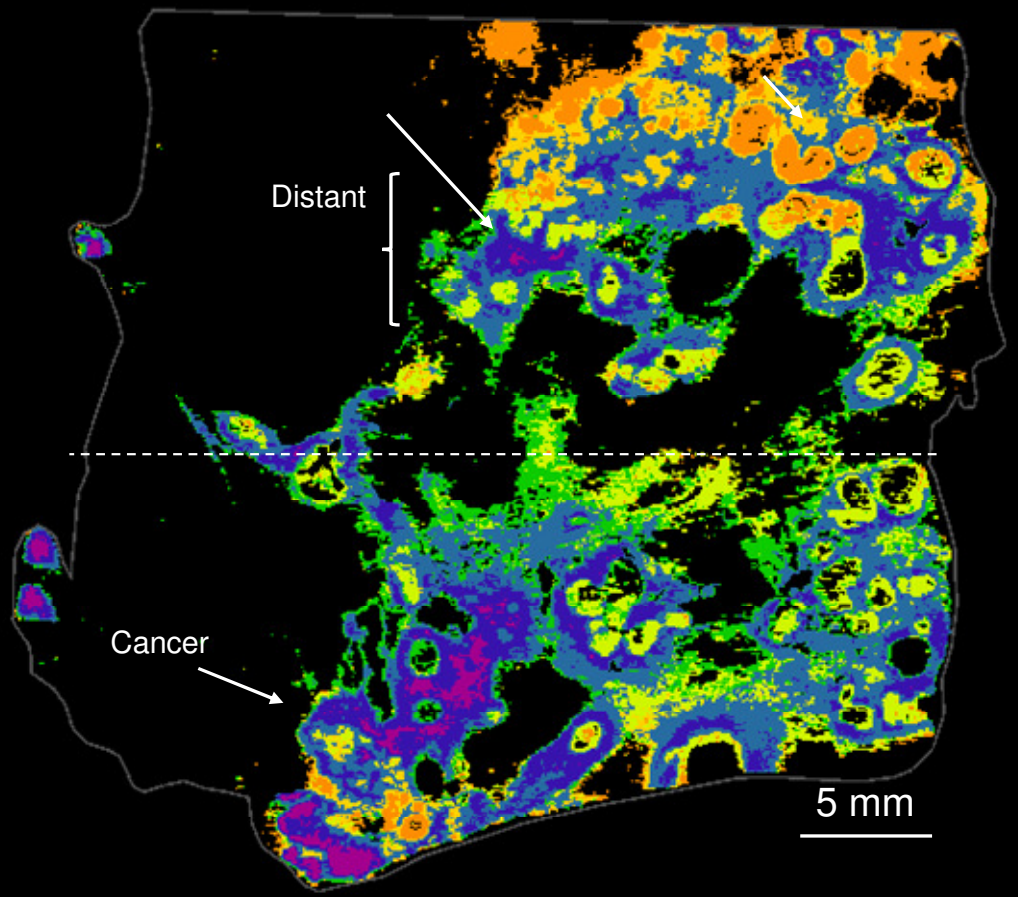


Legend, sugar composition Fucose; N-acetylglucosamine (GlcNAc); Sialic acid; Mannose; Galactose

Pathology of Ductal Carcinoma In Situ (DCIS): Extracellular Microenvironment



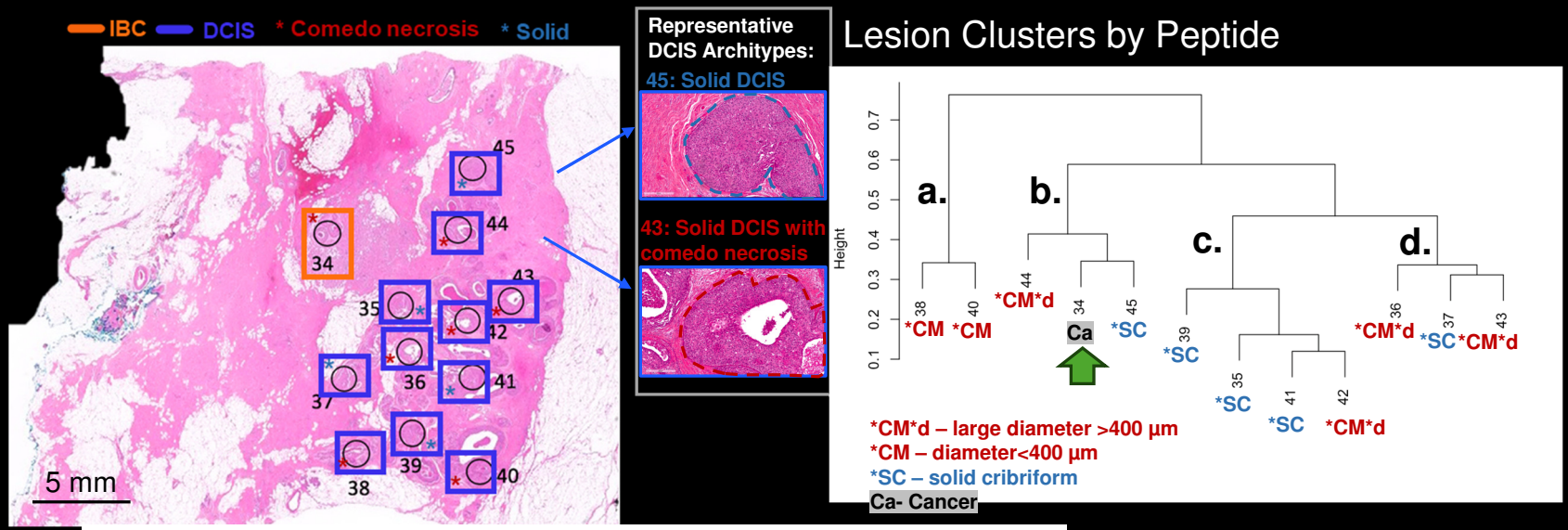
DCIS/IBC Glycoroteomic Image Segmentation



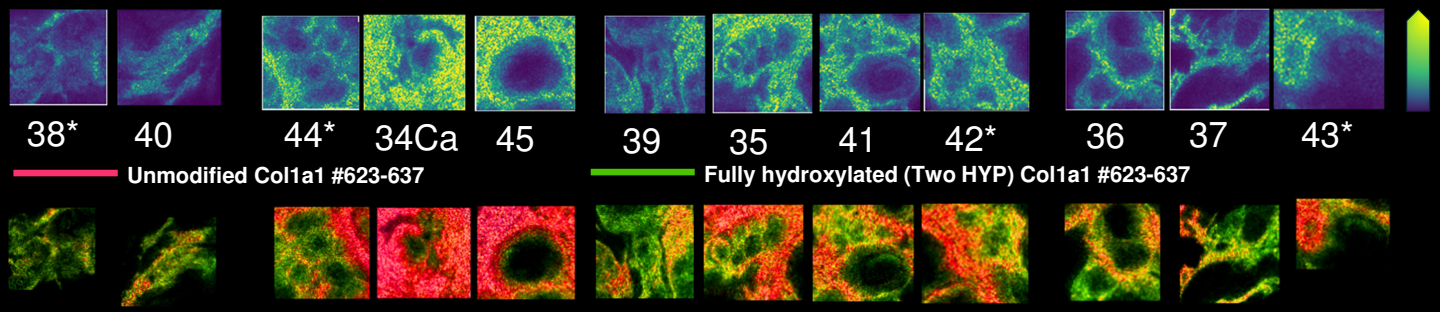
Age Dx: 71
Sx: Partial mastectomy with ax. node dissection
Path: TNBC stage III , poorly differentiated, nuclear grade 3

unpublished

Defining Ductal Carcinoma in situ Lesion Proteomic Pathology Within Same Patient Sections



Col1a1 Amino acid #623-637 GPAGERGEQGPA 1125.528 m/z Δ3.6 ppm

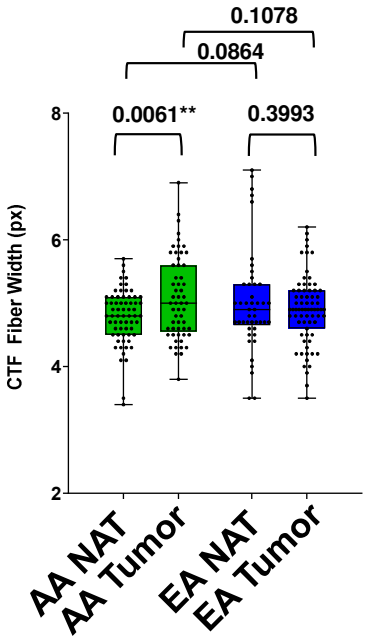


unpublished

Multimodal, Multiplexing Fiber Width and Peptide Correlation in Breast Cancer

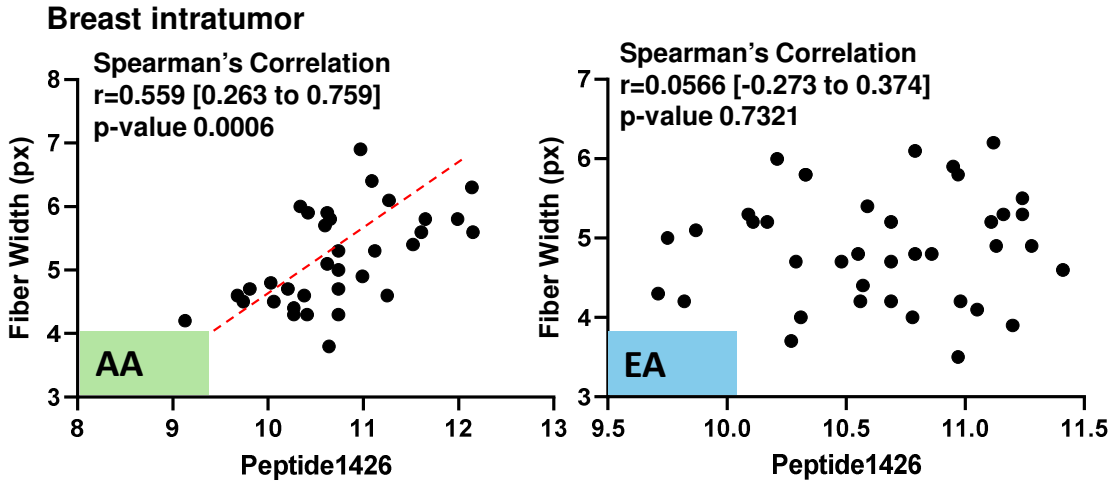
Same slide:

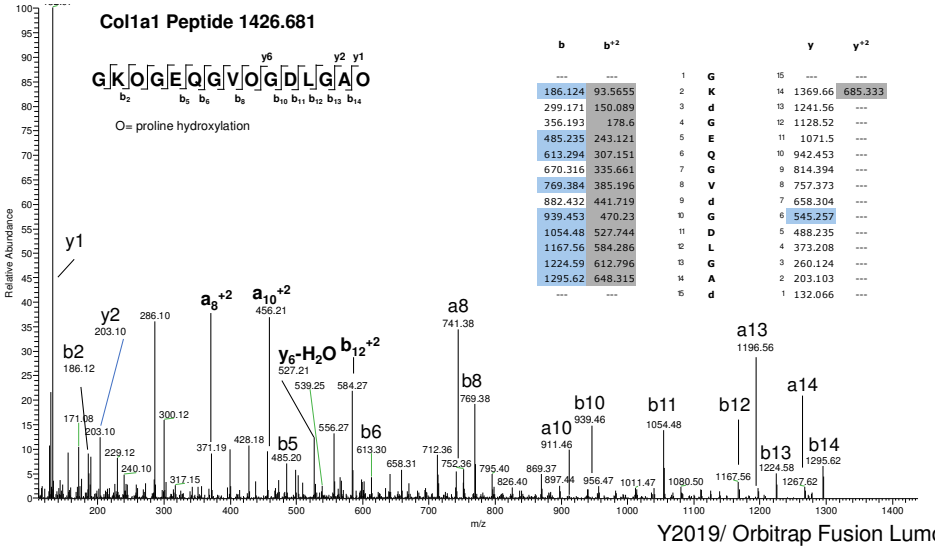
H&E
↓
Second Harmonic Generation Microscopy
↓
Remove coverslip
↓
(N-glycan imaging)
↓
Targeted ECM Imaging
↓
Matching tissues for sequencing



	AA NAT	AA Tumor	EA NAT	EA Tumor
n	57	65	71	45

Welch's t-test; Grubbs outlier test, α=0.05; Graphpad v10.0.1





Taylor, HB et al, Angel, Matrix Biology, 2025

Y2019/ Orbitrap Fusion Lumos

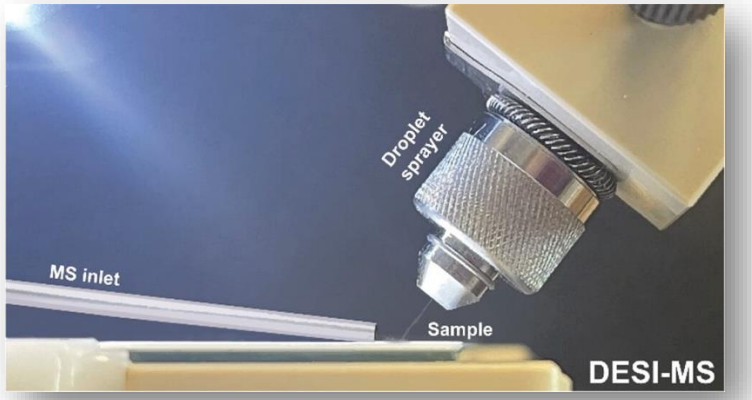
DESORPTION ELECTROSPRAY IONIZATION (DESI) MSI

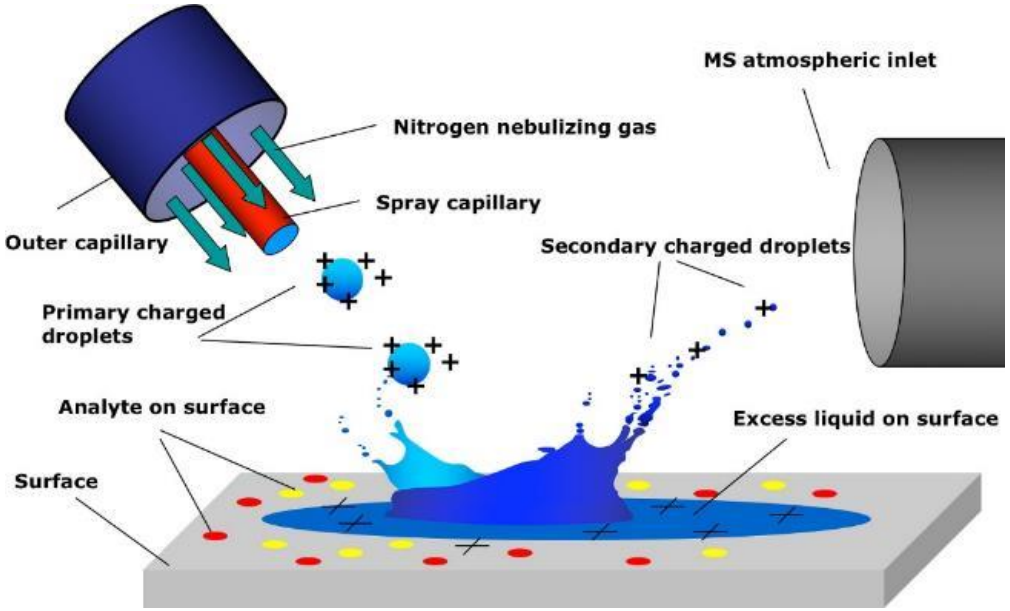
Can be performed in samples of any thickness as far as they fit under the ion source

No need of matrix deposition to assist ionization

Reagents spiked in the sprayer solvent or onto the sample surface can be used for online derivatization (reactive DESI)

DESI-MSI can be coupled to a triple quadrupole mass spectrometer for targeted and sensitive analysis



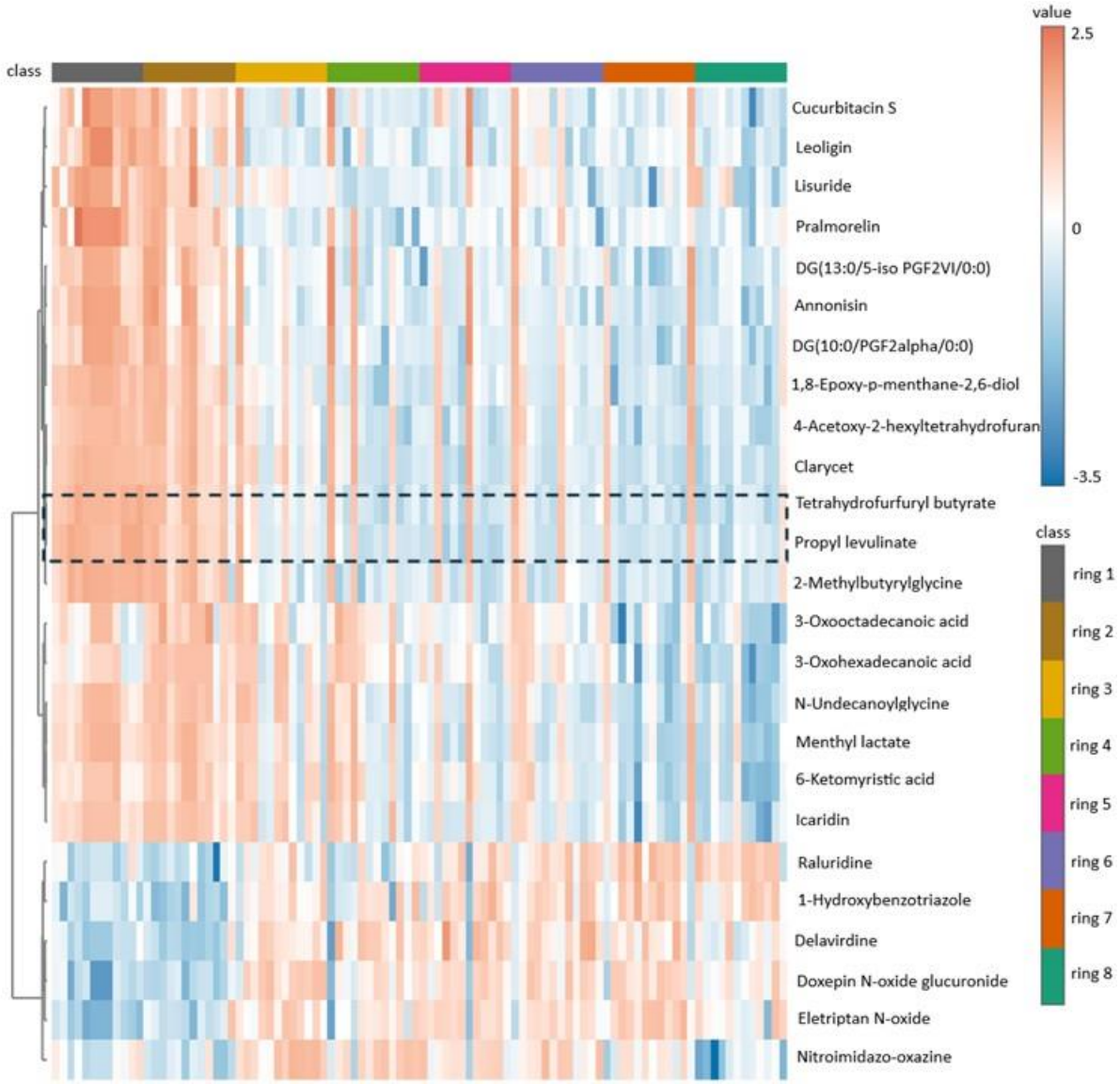


Detection Technologies. Ambient mass spectrometry. Cooks RG, Ouyang Z, Takats Z, Wiseman JM. ***Science***. 2006 Mar 17;311(5767):1566-70.

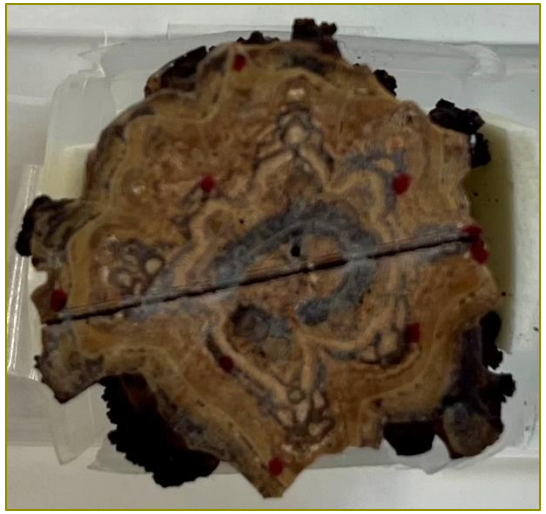
PURDUE UNIVERSITY

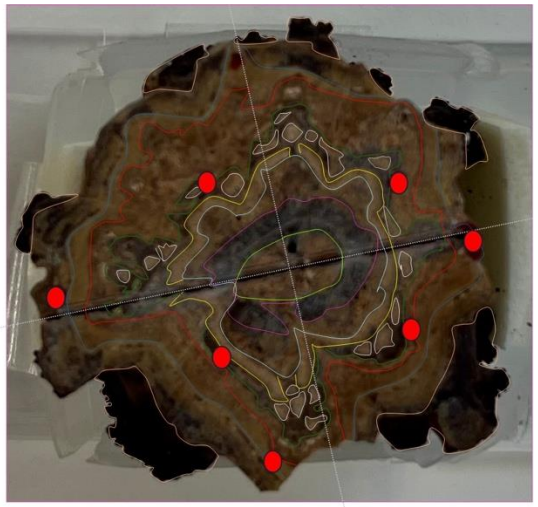
Sample Processing Takes Place During The Analysis

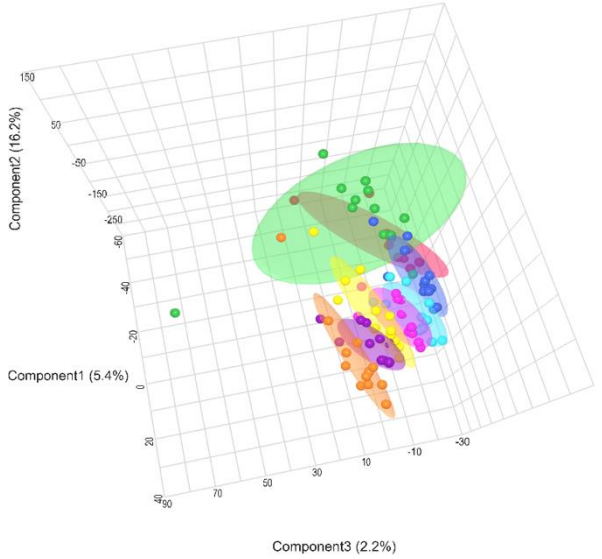
DESI-MSI of a thick sample surface (kidney stone)



Chemical composition of kidney stone rings mapped by DESI-MSI







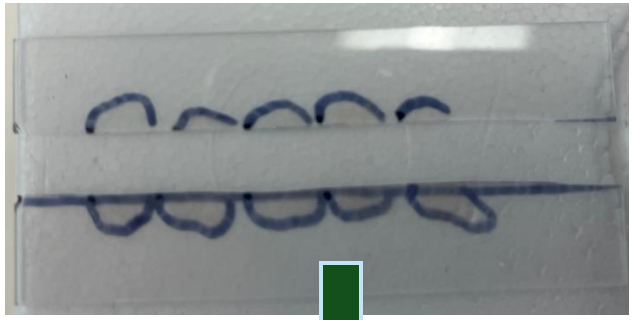
- Data analysis was performed by selecting 3 regions of interest (ROI) for each of the 4 quadrants for the sample (12 samples in total). A total of 8 rings were mapped (96 ROIs) and used for multivariate analysis to study the differences in the chemical composition of the rings by principal component analysis.
- Food additives (tetrahydrofurfuryl butyrate and propyl levulinate) were among the molecules found mainly in the center of the kidney stone sample.

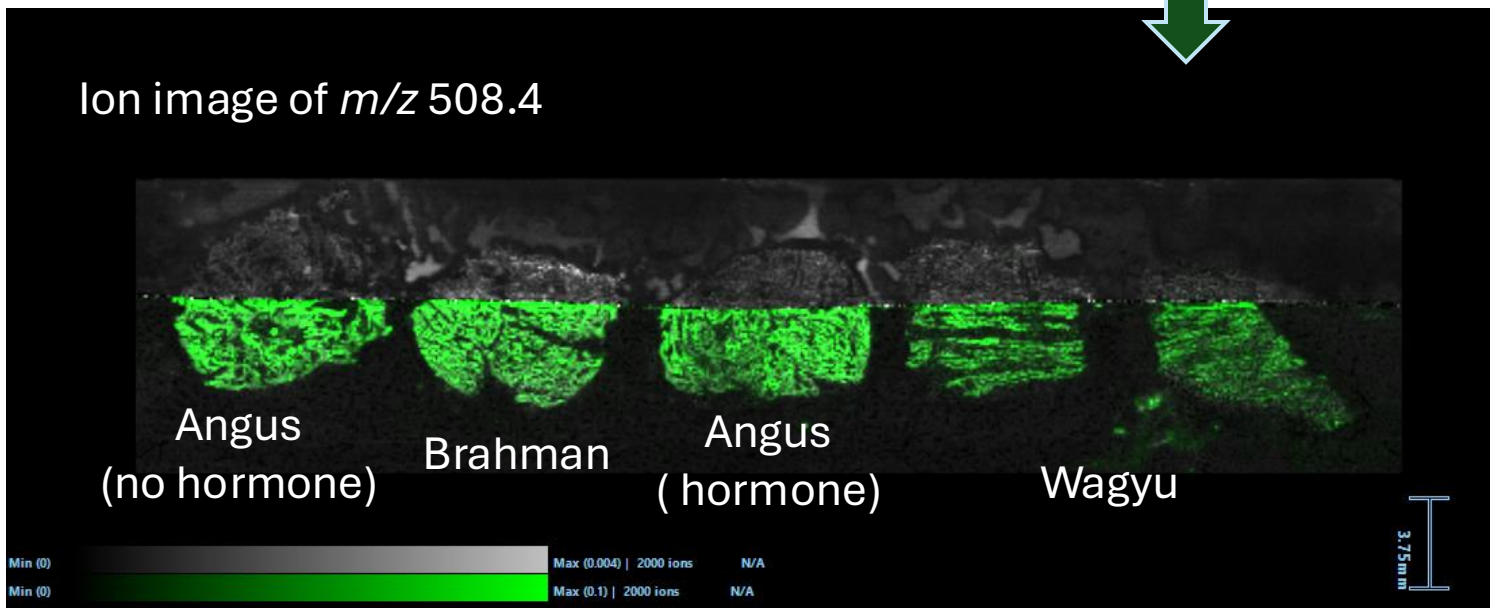
Unpublished data

REACTIVE DESI-MSI FOR IN SITU CHOLESTEROL DETECTION

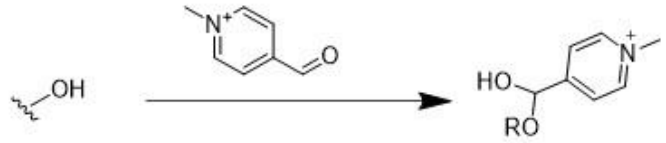
The top half was used as a control (no reagent)

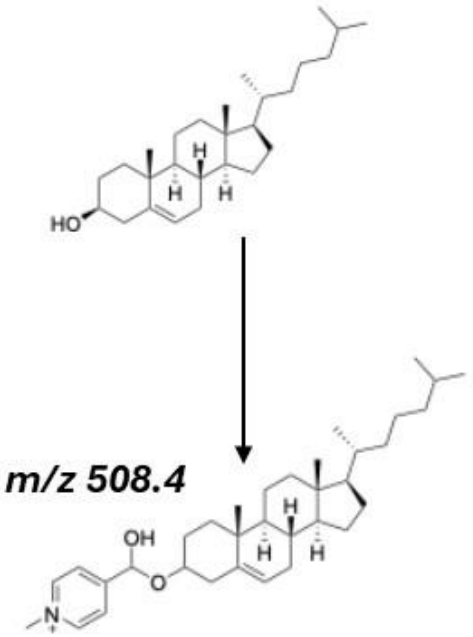
The bottom half was sprayed with 4-formyl-1-methyilpiridinium benzenefulfonate





Py-OH reaction





Cholesterol does not ionize well. Reactive DESI-MSI allow spatial mapping of fast cholesterol detection in food products

Angew Chem Int Ed Engl. 2023 Nov 20;62(47):e202310884

Unpublished data

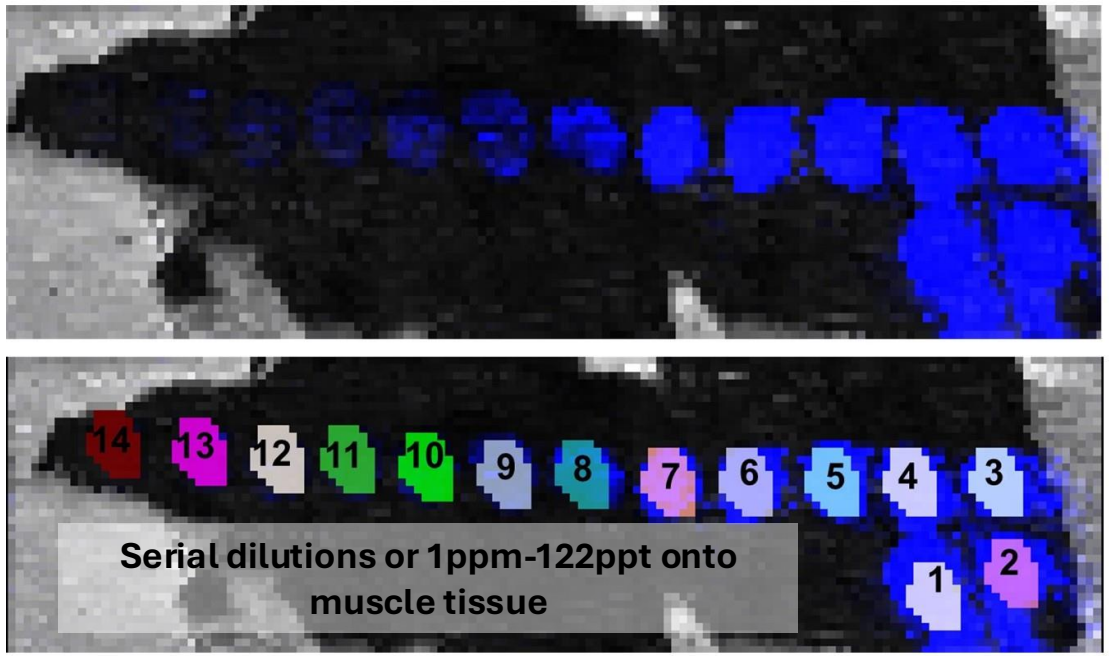
DESI-MSI for targeted analysis of PFAS in tissues

List of MRMs and settings used to monitor selected PFAS

No.	Compound Name	Parent m/z	Daughter m/z	Cone (V)	Collision (eV)
1	GenX-HFPO-DA-n	285	119	5	35
2	GenX-HFPO-DA-nq	285	169	5	7
3	Arachidonic acid	303.2	303.2	10	10
4	PFHxA-nq	312.9	119	5	20
5	PFHxA-nq	312.9	269	5	10
6	ADONA-nq	376.9	85	10	25
7	ADONA-n	376.9	251	10	10
8	PFHxS-nq	398.9	80.1	10	35
9	PFHxS-n	398.9	99.1	10	30
10	PFOA-nq	412.9	369	10	10
11	PFOA-n	412.9	169	10	15
12	PFNA-n	462.9	219	10	15
13	PFNA-nq	462.9	418.9	10	10
14	Leu-Enk-Neg	554.26	554.26	20	4
15	PFTriDA-PFTrDA-n	662.9	169	5	30
16	PFTriDA-PFTrDA-nq	662.9	618.9	5	20
17	PFTreDA-PFTeDA-nq	712.9	668.9	10	25
18	PFTreDA-PFTeDA-n	712.9	169	10	15

No.	Compound Name	Parent m/z	Daughter m/z	Cone (V)	Collision (eV)
1	PFBS-n	298.9	99.1	15	30
2	PFBS-nq	298.9	80.1	15	30
3	Arachidonic acid	303.2	303.2	10	10
4	PFHpA-nq	362.9	319	15	15
5	PFHpA-n	362.9	169	15	15
6	PFOS-n	498.9	99.1	15	40
7	PFOS-nq	498.9	80.1	15	40
8	PFDA-nq	512.9	468.9	15	15
9	PFDA-n	512.9	219	15	15
10	9Cl-PF3ONS-n	530.9	82.9	15	25
11	9Cl-PF3ONS-n	530.9	350.9	15	25
12	Leu-Enk-Neg	554.26	554.26	20	4
13	PFUnDA-nq	562.9	518.9	25	10
14	PFUnDA-n	562.9	269	25	20
15	N-MeFOSAA-nq	569.9	418.9	35	20
16	N-MeFOSAA-n	569.9	219.1	35	25
17	N-EtFOSAA-nq	584	418.9	25	20
18	N-EtFOSAA-n	584	525.9	25	10
19	11Cl-PF3OUdS-nq	630.9	450.8	30	25
20	11Cl-PF3OUdS-n	630.9	82.9	30	25

ROI selection on bovine longissimus dorsi tissue



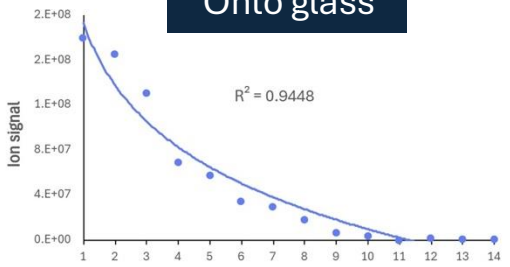
Data analysis: Selection of regions of interest (ROIs) and using the total ion signal for building calibration curves

ROIS of 50 pixels, Pixel dimensions: 300 x 300 um

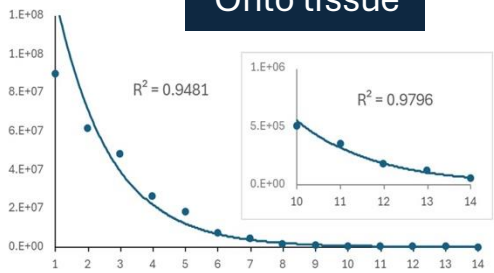
Instrumentation allows detection sensitivity compatible with biomagnification levels

Unpublished data

Onto glass

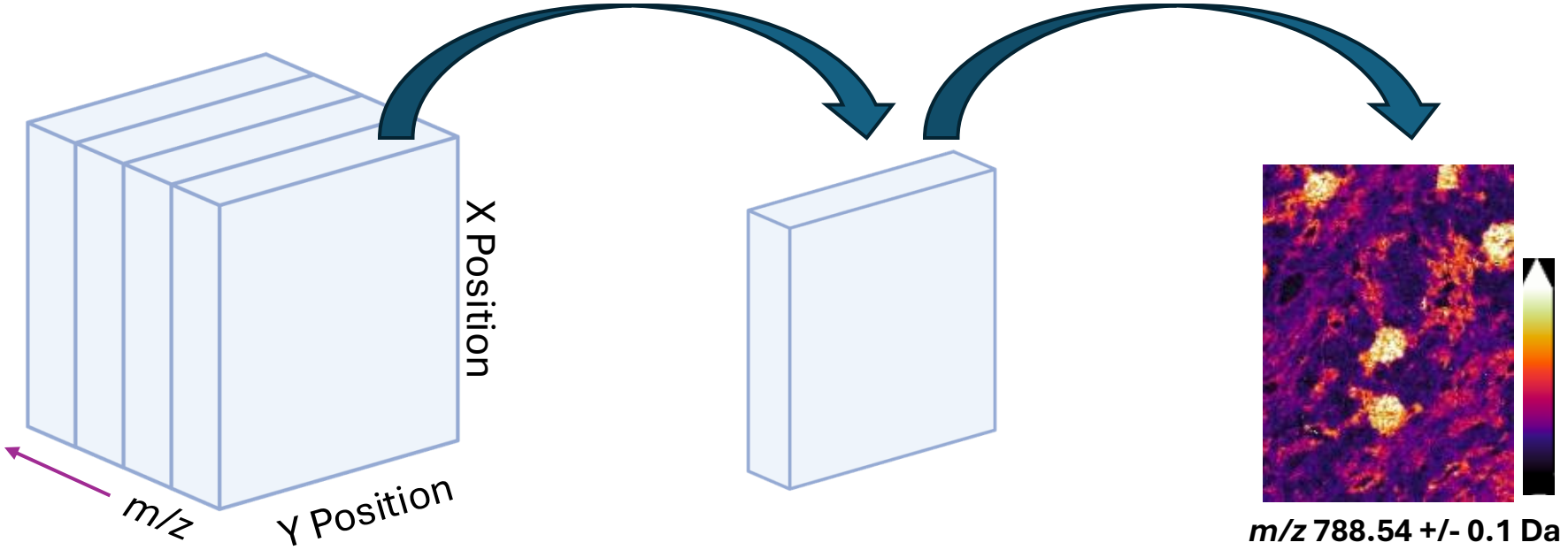


Onto tissue



Linear range response 1ppm-122ppt

Software overview of MS imaging data analysis



MSI data is a multi-dimensional array of individual mass spectra at a single (x,y) coordinate on a 2-dimensional plane

An “ion image” is a heat map representation of a defined width in *m/z* space where the intensities of that width are plotted at each coordinate

ALL RAW DATA WILL EXIST SOMEWHERE IN THIS STATE!

Ask your instrument vendor to provide you with an SDK or API to access the raw data format for writing custom software

Available Software (as of 11/4/2025)

Commerical (Vendor Specific)

- HDImaging (Waters)*
- FlexImaging&SCiLS Lab (Bruker+vendor neutral)*
- Bruker TDF/SDK (raw data access)*
- ImageQuest (Thermo)
- TissueView (AB SCIEX)
- IMAGEREVEAL MS (Shimadzu)
- SpectroSwiss (ICR and Orbi)
- MSiReader (vendor neutral)
- LipostarMSI (vendor neutral)/Pyxis MSI
- ----

* Compatible with ion mobility

Open Source

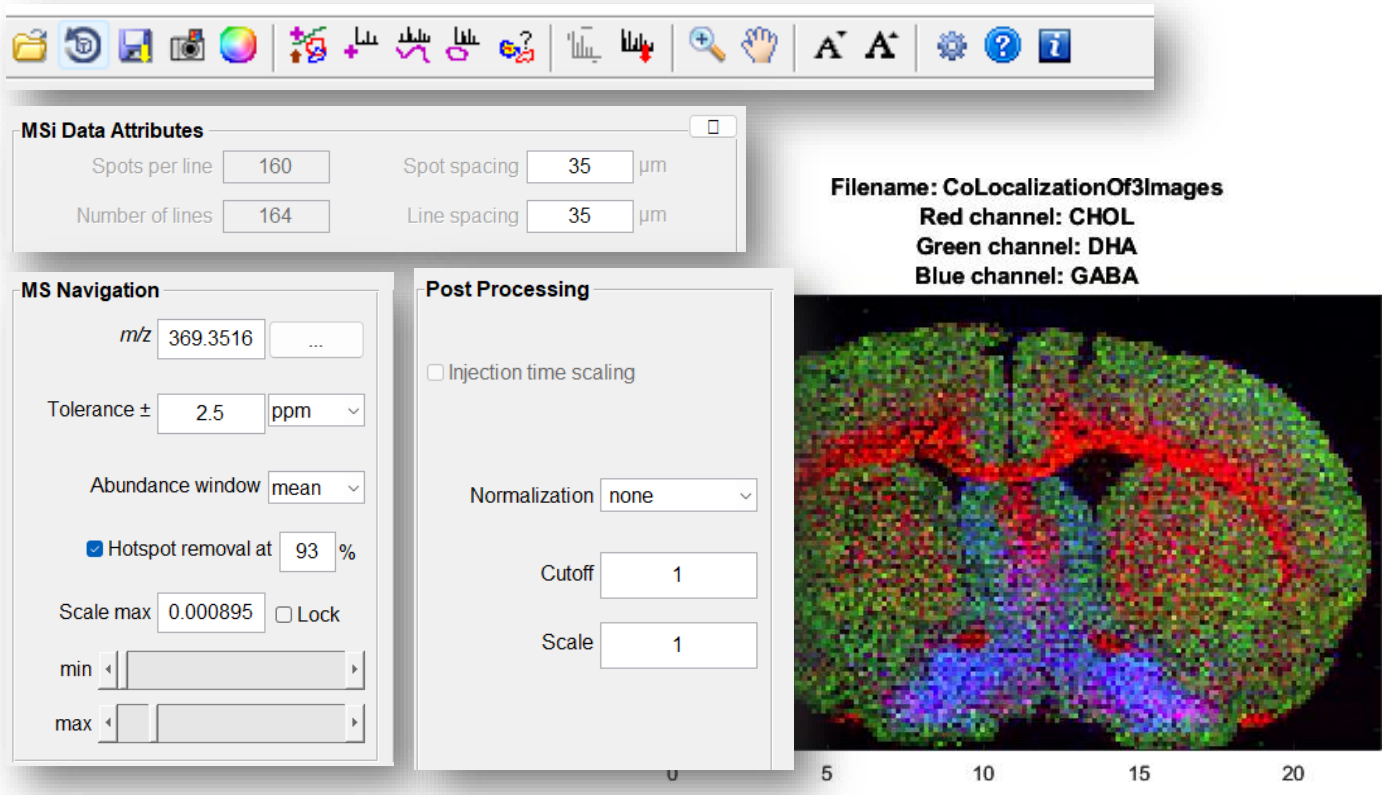
- Cardinal MSI/Shiny Cardinal (R)
- MZmine (GUI)
- msiFlow (GUI → Runs in docker container)
- MSI-explorer (GUI→ Napari → Python)
- METASPACE (online)
- Galaxy Workbench for MSI (GUI → Runs in docker)
- MSIGen (GUI →python thru Conda)
- MassVision (User responsible for data formatting to H5)
- Vandeplaslab/image2image (GUI → image registration)
- MSIr (registration → R)
- MSIreg (registration →R)
- ---- (yours missing? Let us know!)

Processing Mass Spectrometry Imaging Data

Analysis, Visualization, Statistical Methods, Annotation, Multimodal Integration

Session 1: Processing Mass Spectrometry Imaging Data

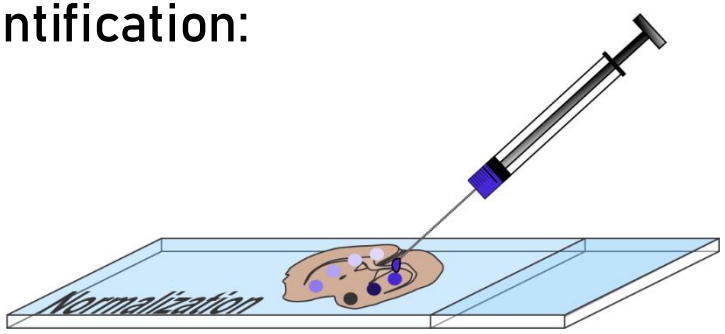
1. Customization of MSiReader for End-User
2. Importing Data and Defining MMA
3. Navigating the Main GUI
   - Hot Spot Removal
   - Normalization
   - Interpolation and SPC
   - Color Maps
4. Data Pre-Processing and Analysis
   - Mass Correction
   - Ion Classification Tool
   - Profile to centroid conversion / filter
   - Scan Scrubber
   - Viewing Mass Spectra
   - Molecular Adduct Search
   - Colocalization Plots

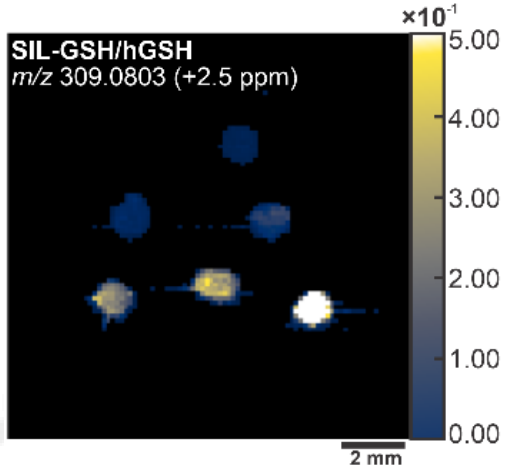


Session 2: Statistical Methods and Machine Learning

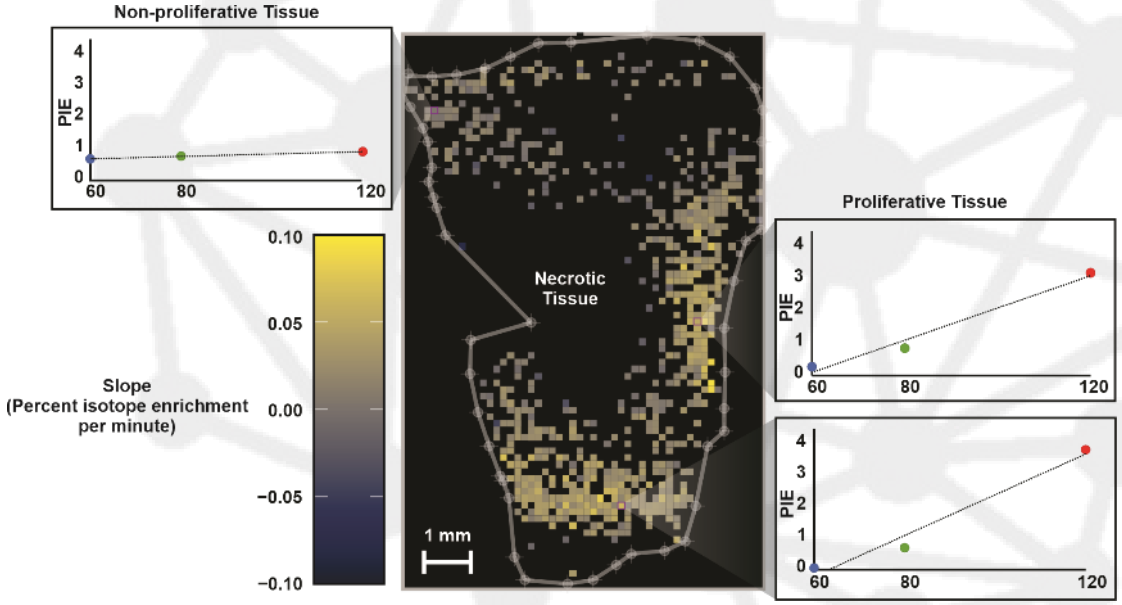
1. Exporting Data and Relative Quantification: Volcano Plots

2. Absolute Quantification
   - Spatial Calibration Curve
   - V×V Quantification
   - Machine Learning





3. Functional Mass Spectrometry Imaging: The PIE Tool



4. Machine Learning
   - Quality Control
   - Supervised vs. Unsupervised Classification

Session 3: Data Annotation and Multimodal Integration

1. Data Annotation: METASPACE and MSiReader Custom DB

2. Multimodal Integration
   - Optical Images
   - Spectroscopy

3. Biological Interpretation
   - SSIM
   - Biological Pathways

4. Summary

Annotation	m/z	MSM	Isomers	Isobars
$[C_{11}H_{10}O_2 + H]^+$	175.0754	0.800	5	0
$[C_{12}H_{14}O + H]^+$	175.1117	0.366	2	0
$[C_6H_{14}N_4O_2 + H]^+$	175.1190	0.093	1	0
$[C_9H_{18}O_3 + H]^+$	175.1329	0.722	2	0
$[C_{13}H_{18} + H]^+$	175.1481	0.220	1	0
$[C_{10}H_9NO_2 + H]^+$	176.0706	0.031	7	0
$[C_6H_{13}N_3O_3 + H]^+$	176.1030	0.067	1	0
$[C_{10}H_8O_3 + H]^+$	177.0546	0.783	5	0
$[C_{11}H_{12}O_2 + H]^+$	177.0910	0.792	5	0
$[C_{10}H_{12}N_2O + H]^+$	177.1022	0.073	4	0
$[C_{12}H_{16}O + H]^+$	177.1274	0.070	5	0
$[C_{13}H_{20} + H]^+$	177.1638	0.640	4	0
$[C_{10}H_{11}NO_2 + H]^+$	178.0863	0.125	3	0
$[C_{10}H_{15}N_3 + H]^+$	178.1339	0.895	1	0
$[C_9H_6O_4 + H]^+$	179.0339	0.462	6	0

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