MALDI Imaging Mass Spectrometry
Technical Advances and Clinical Problem Solving

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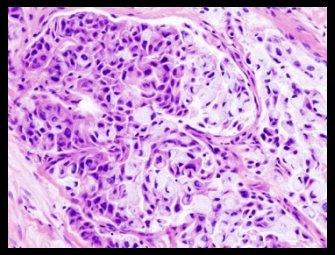
Tissue Pathology

The Current Approach

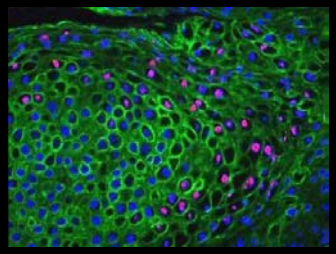
Tissue Prep



Analysis

Diagnosis

Reagents and Methods



Molecular Staining

Time and Money

The Facts

Fact: Historical approaches to tissue pathology are inadequate to answer many important clinical questions.

- Early detection
- Aggressiveness of disease
- Optimal treatment
- Drugs distribution/efficacy

Fact: Molecular testing has revolutionized the practice of medicine.

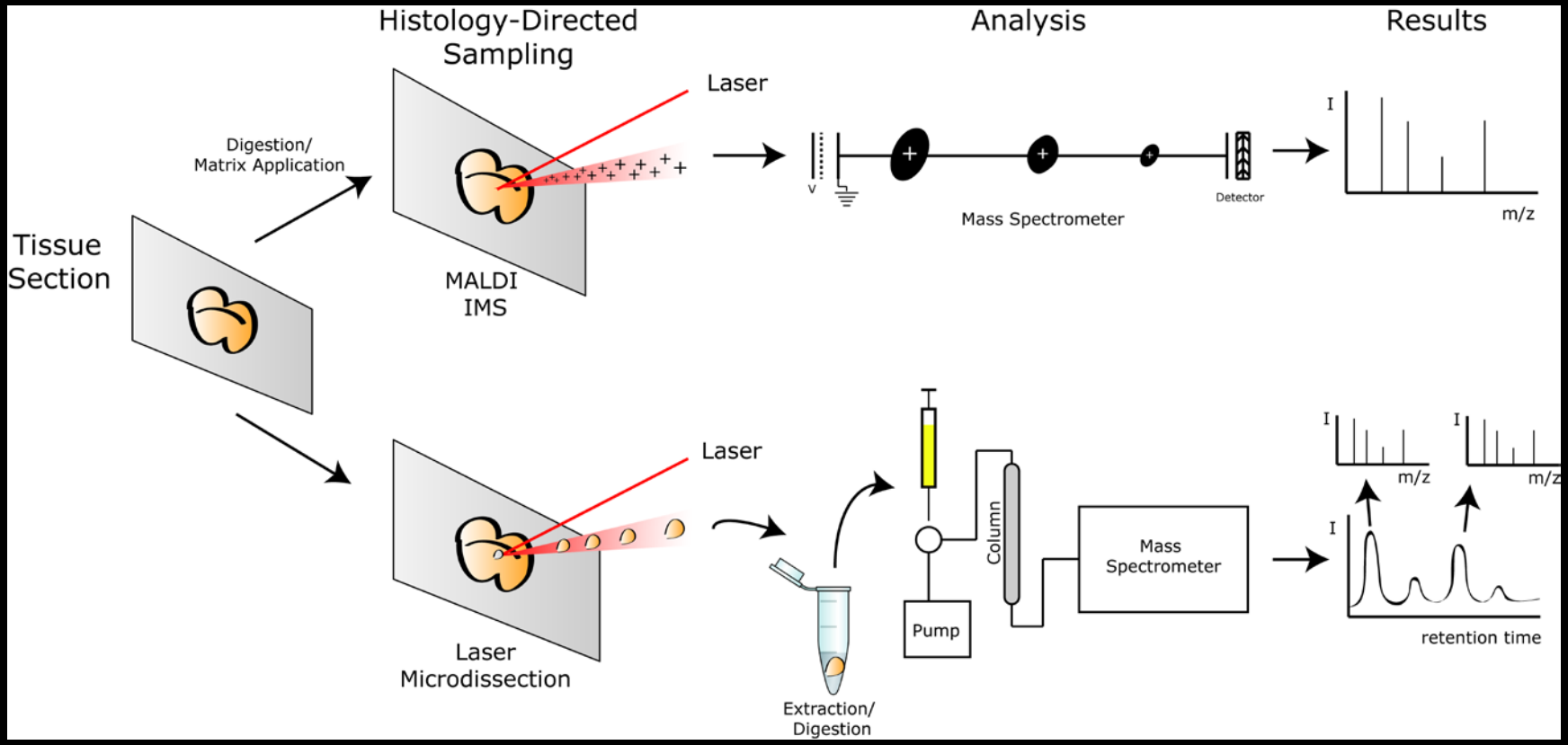
Fact: Relatively few molecular tests can be related to specific tissue pathology.

Anatomic Pathology

+ Mass Spectrometry

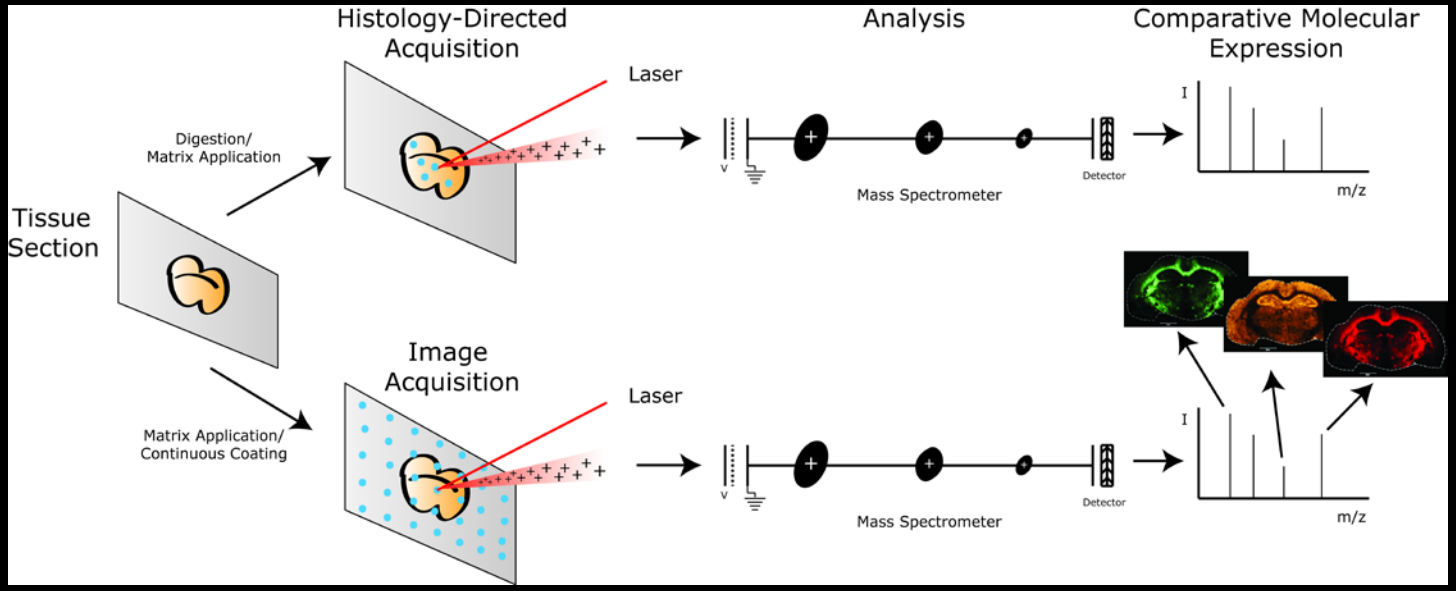
Clinical Value

Histology-Directed Analysis



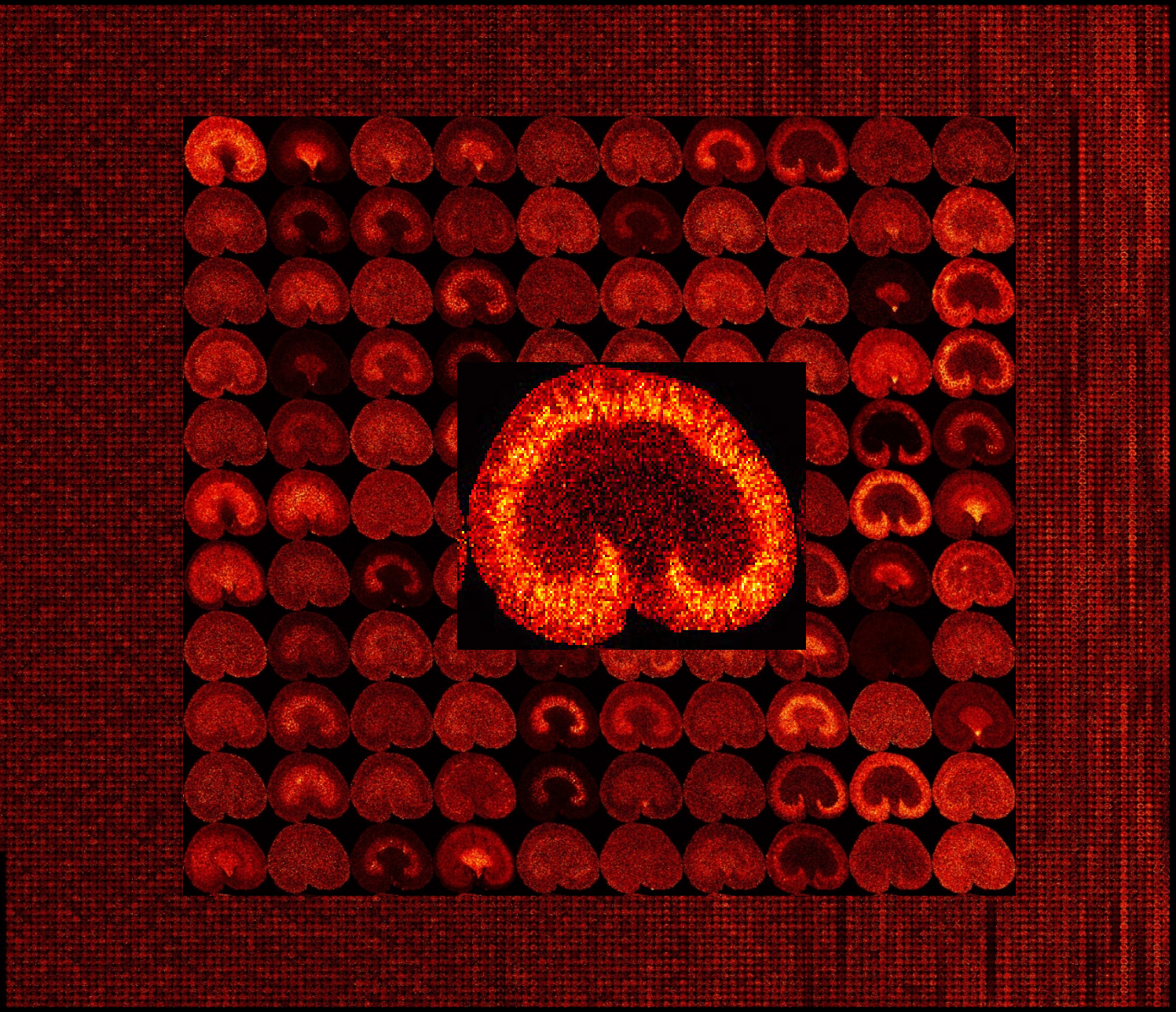
Proteomics Clin. Appl. 2013 Dec;7(11-12):733-8.

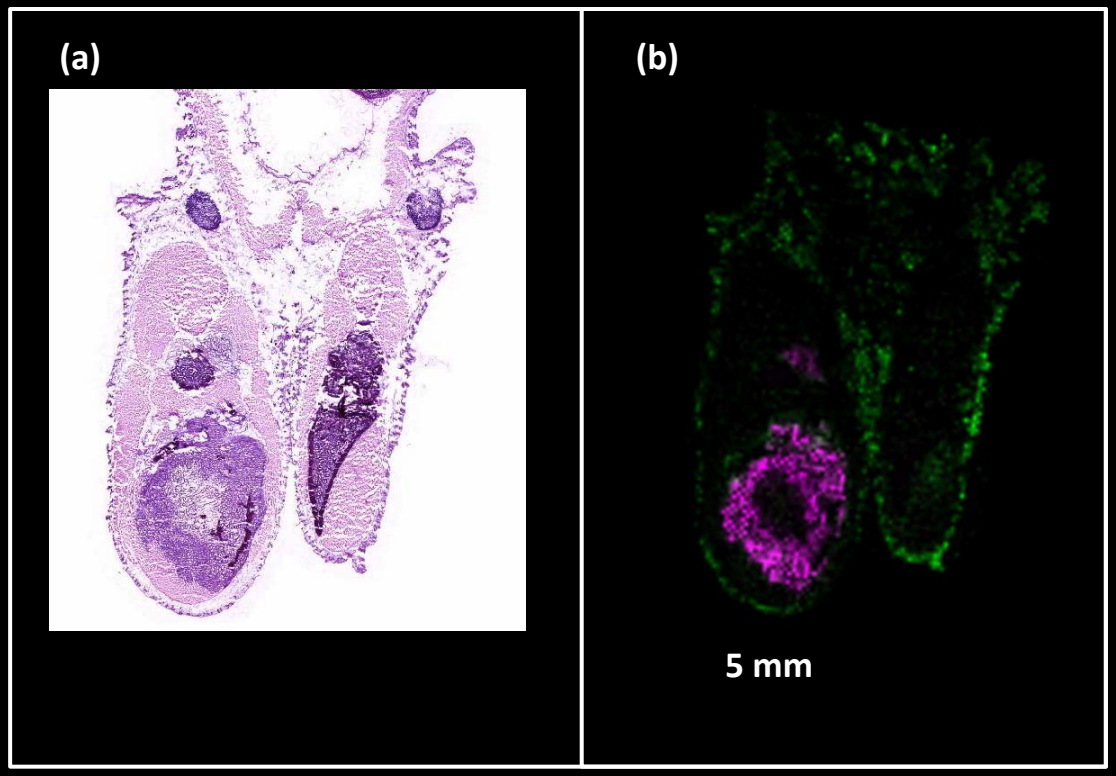
Imaging Mass Spectrometry



- Matrix applied to the tissue surface.
- Laser desorbs and ionizes molecules from the tissue surface.
- Mass spectrometer analyzes ionized molecules creating a molecular profile (fingerprint) at each position of the tissue.
- Molecular fingerprint is used for 1) disease classification and 2) analyzing molecular distribution of tissue.

Mouse Kidney – MALDI Imaging MS at 100 µm spatial resolution, 1 kHz rep rate laser, SA matrix



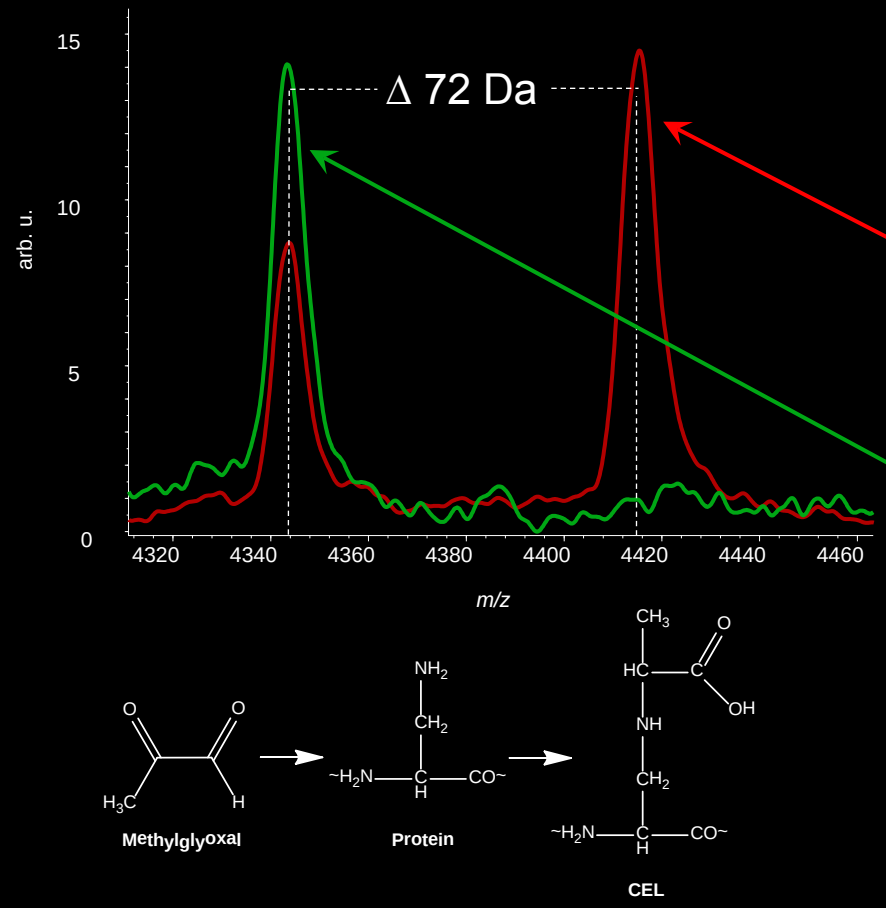


Human breast tumor cell line implanted into the tibia of a mouse.

Human calcyclin (m/z 10,090)

Mouse calcyclin (m/z 9960)

Erin Seeley, Lynn Matrisian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4420
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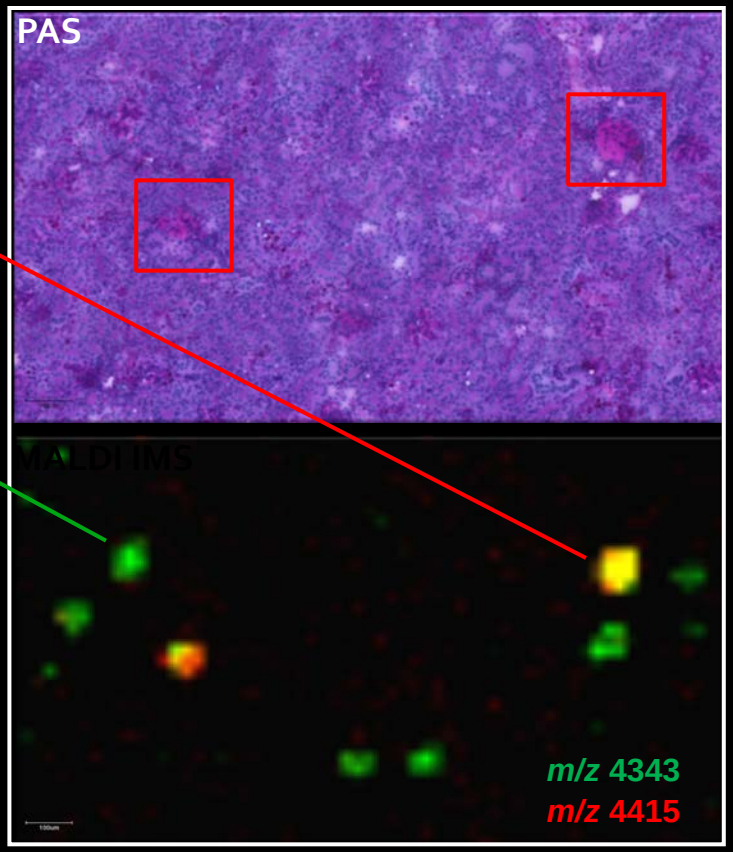
m/z
Methylglyoxal
Protein
CEL


PAS
MALDI IMS
m/z 4343
m/z 4415

IMS Performance Criteria

Traditional Considerations

Mass Resolving Power: defined as $m/\Delta m$

Mass Accuracy: the difference between the measured mass and the calculated exact mass.

Sensitivity: specifies the overall response of the instrument for a given analyte.

Dynamic Range: detection range for the instrument (most intense/smallest detectable signal)

MS^n capabilities: ability to perform fragmentation experiments for analyte identification.

IMS Special Considerations

Spatial Resolution: distance between two adjacent pixels (ablated spots) on the sample surface.

Throughput: the number of scans/spectra that can be acquired per unit time.

File Size/Data Storage: Considerations of storage costs and processing practicality

Instrumental / Methodology Challenges

Speed	-	**data collection too slow to be practical for routine analysis**
Sensitivity	-	**achieve more global coverage (fraction of analytes observed)**
Resolution	-	**better lateral resolution (single cell imaging)**
	-	**higher MS resolution (better resolve isoforms, PTMs**
Mass Range	-	**routinely beyond 100 kd**
Identification	-	***in situ* - fast, simple, accurate**
Quantitation	-	**reagents and methods - isotope based, relative and absolute**
Validation	-	**cross-lab, cross-platform reproducibility/standardization**
Availability	-	**single manufacturer provides entire technology 'solution'**
	-	**instruments and protocils are too complex for non-experts**

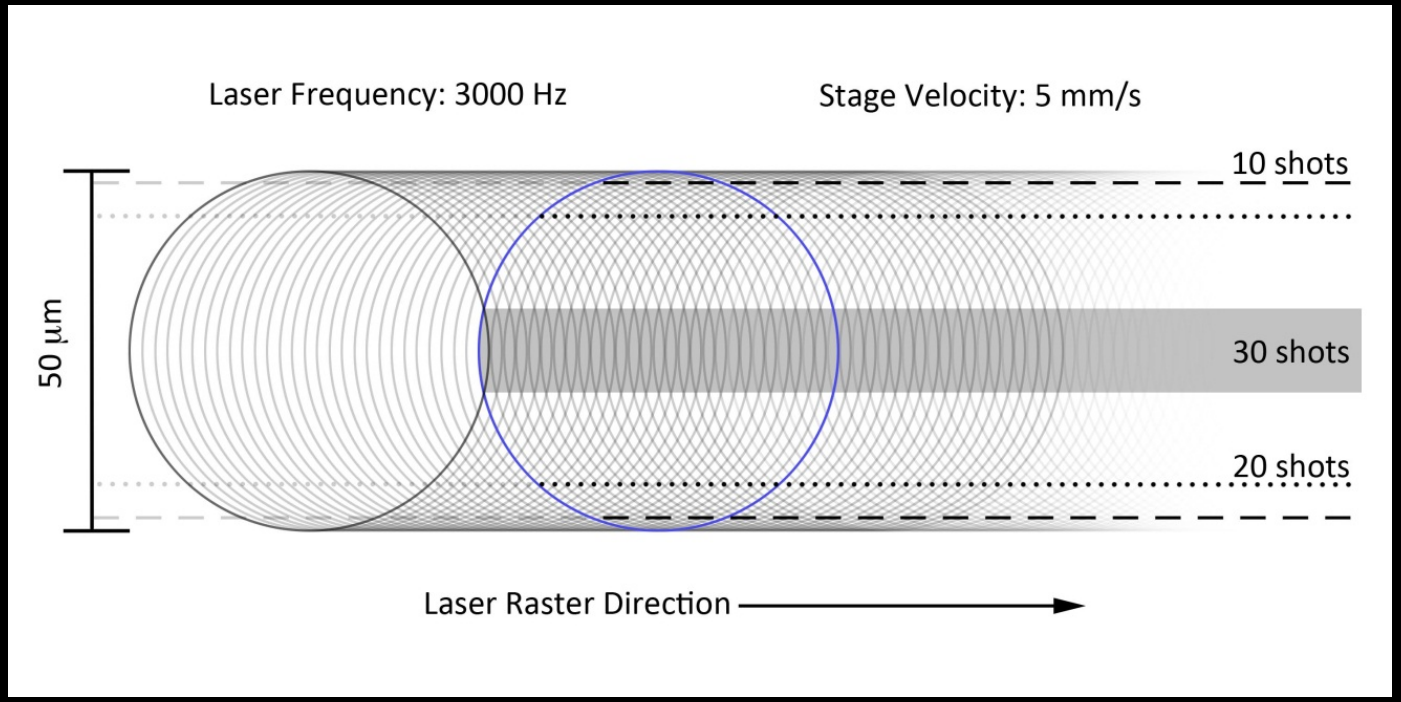
Increasing Throughput

Simultof Systems

High-Speed MALDI-TOF IMS
Continuous Laser Raster Sampling

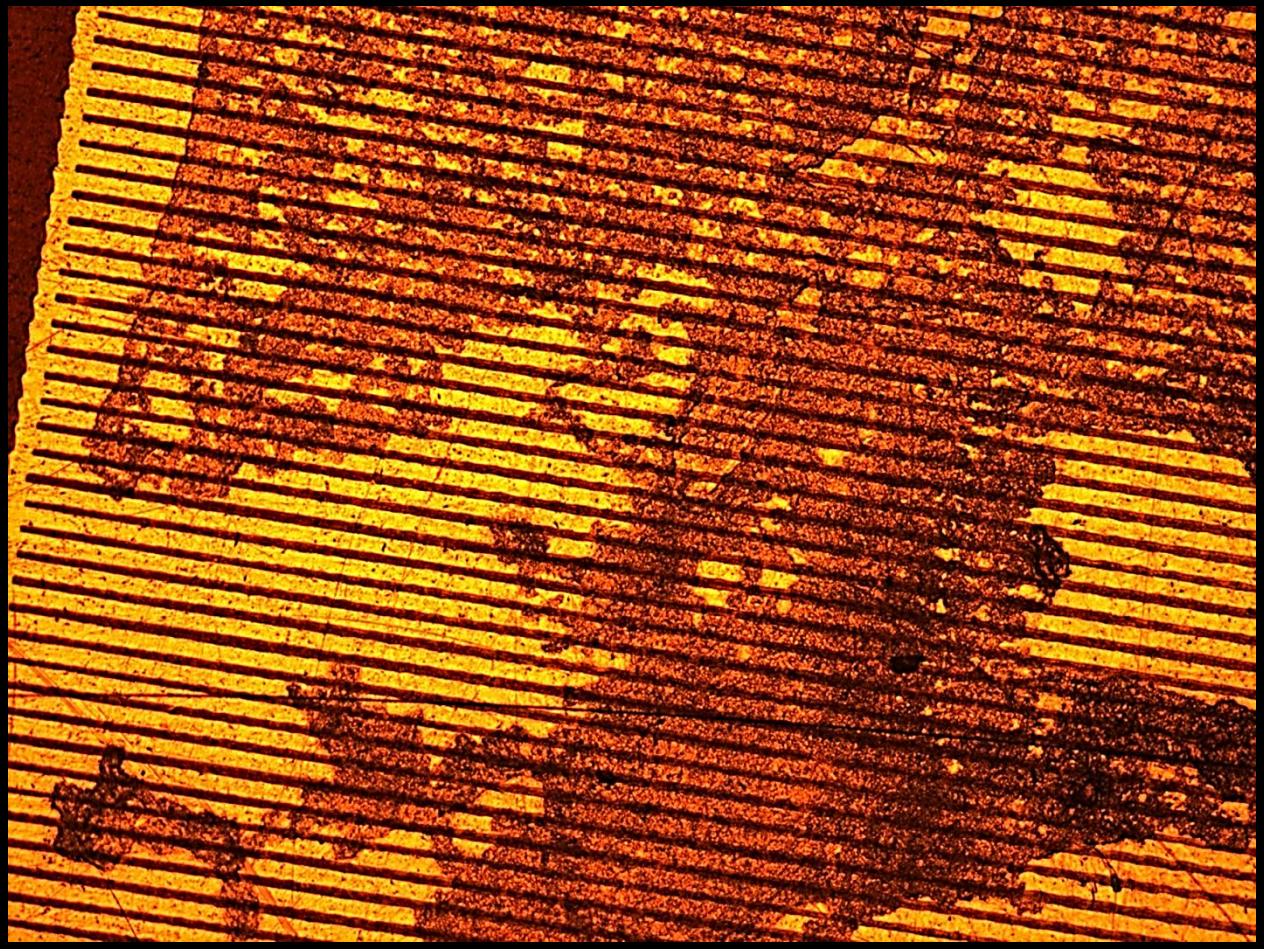
$$\text{Laser Shots/Unit Area} = f_{rep}\left(\frac{d}{v_{stage}}\right)$$

$$\text{Lateral Spatial Resolution} = H.A.\left(\frac{v_{stage}}{f_{rep}}\right)$$



J Am Soc Mass Spectrom. 2011 Jun;22(6):1022-31.

High-Speed MALDI-TOF IMS
Continuous Laser Raster Sampling



High Speed MALDI TOF
SimulTOF

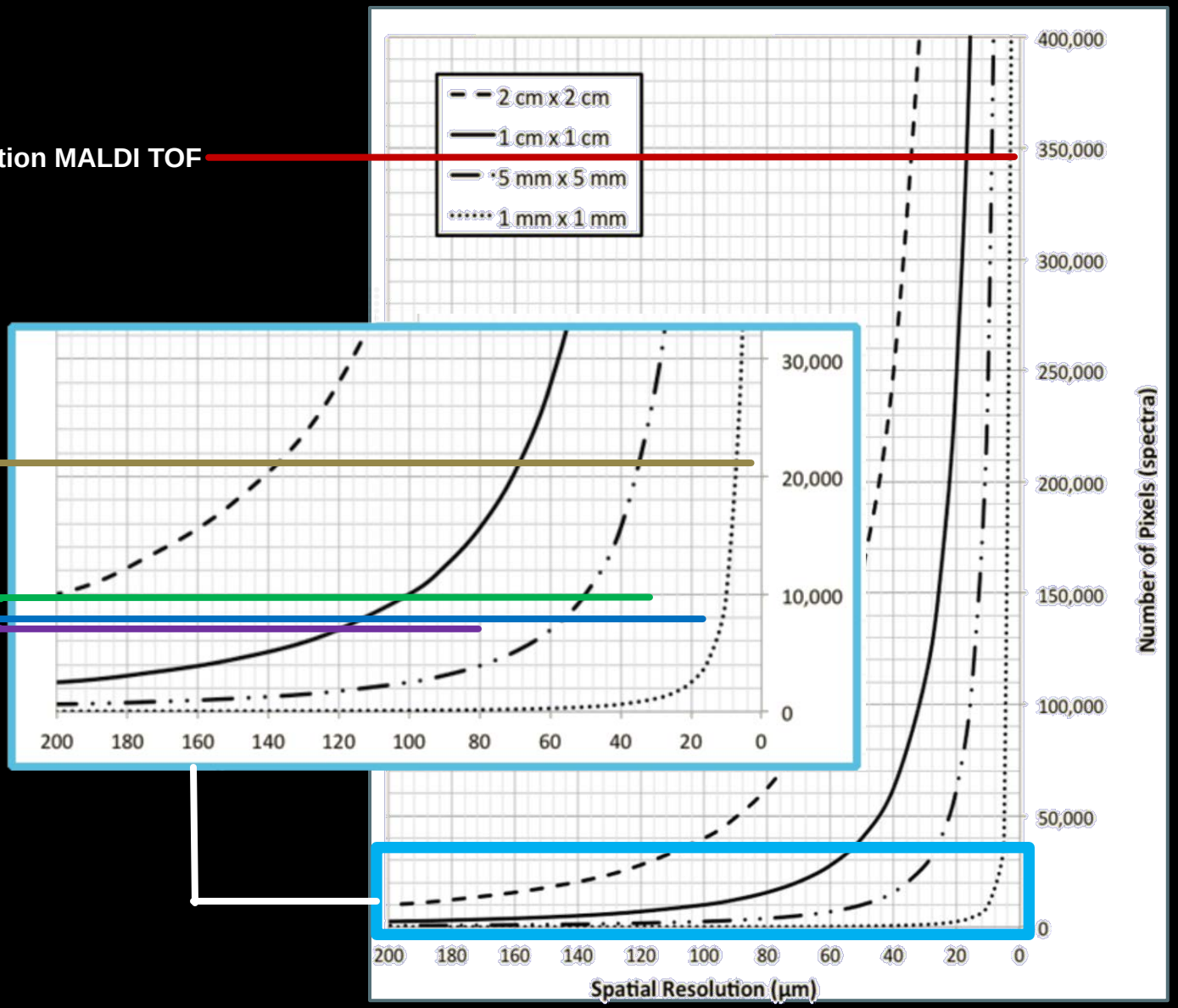
~345,000 pixels/3hr **Next Generation MALDI TOF**

~21,000 pixels/3hr **MALDI TOF**

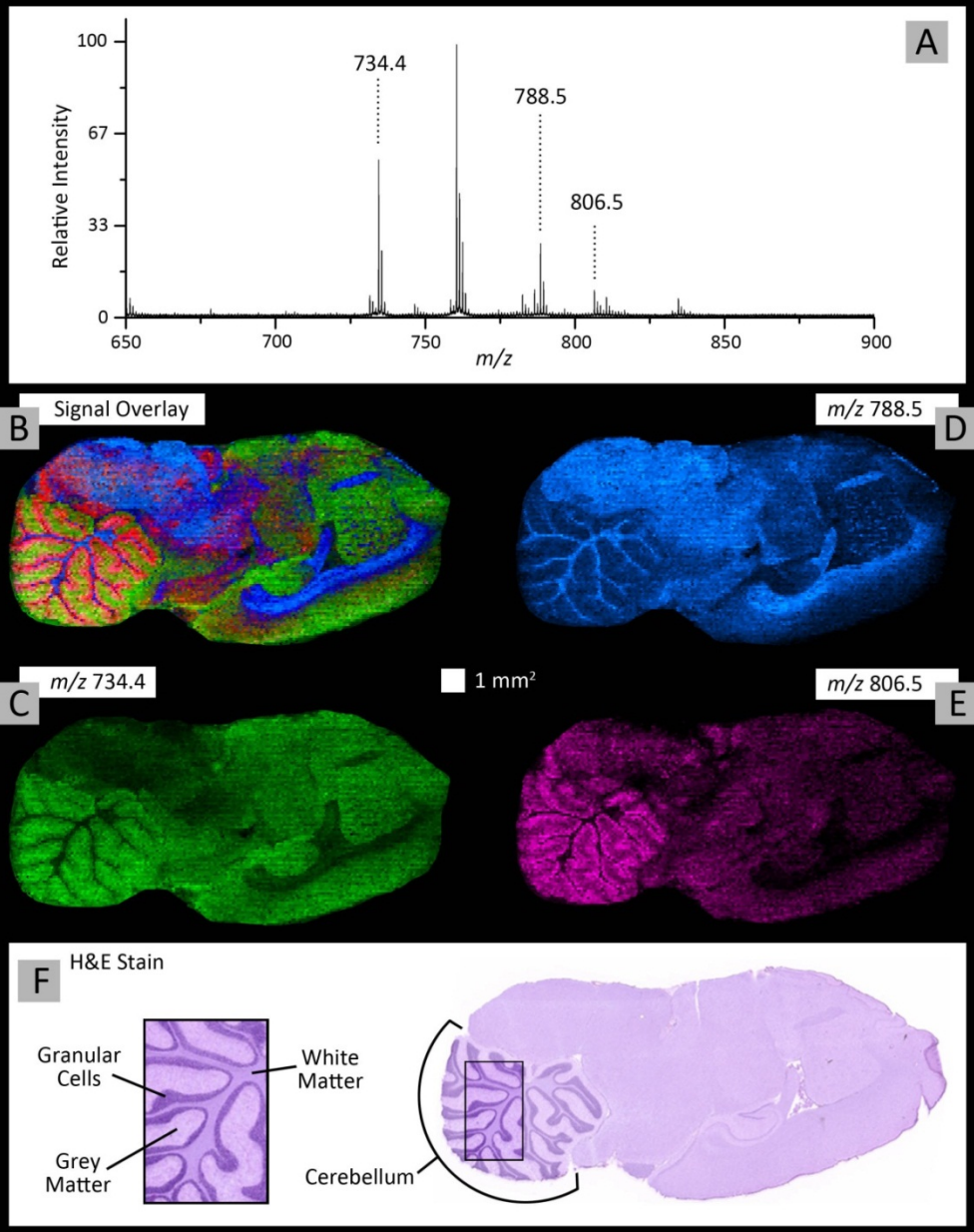
~9,800 pixels/3hr **LIT**

~7,700 pixels/3hr **FT-ICR**

~7,200 pixels/3hr **IM Q-TOF**



Lipid Imaging
SimulTOF



Experimental Details

- **Lipids – *m/z* 600-1200**
- **3 kHz Laser Rep Rate**
- **Velocity: 5 mm/s**
- **Lateral Resolution: 100µm**
- **Vertical Step: 100 µm**
- **Total Pixels: ~19,000**
- Effectively: ~30 Spectra/s
- Total File size: 4.33 GB

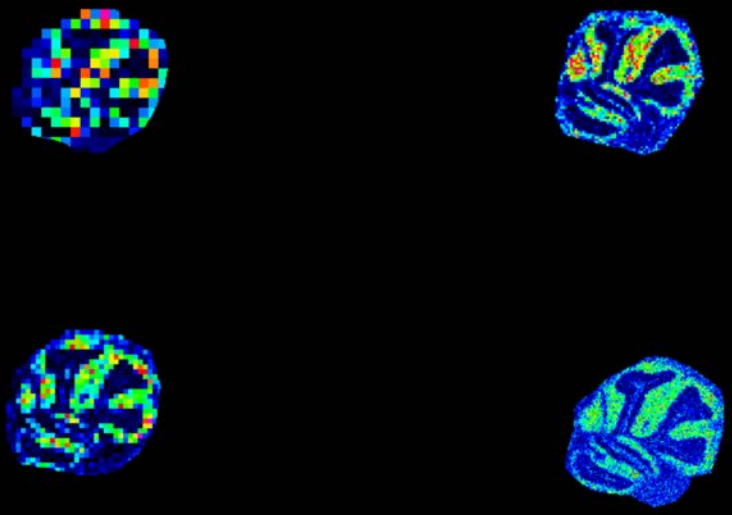
- **Time: ~10 min**

J Am Soc Mass Spectrom. 2011 Jun;22(6):1022-31.

Increasing Spatial Resolution

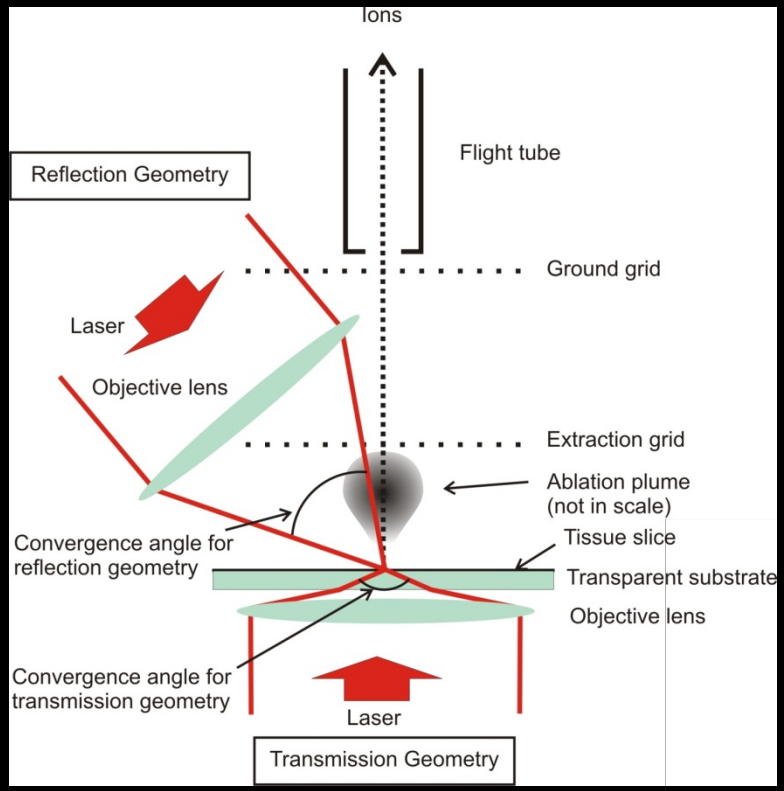
Spatial Resolution

- Image resolution defines the nature of molecular information that can be derived from an IMS experiment.
- The price of higher resolution can be significant time, effort, and money.
- Some biological questions can only be answered by high resolution imaging.



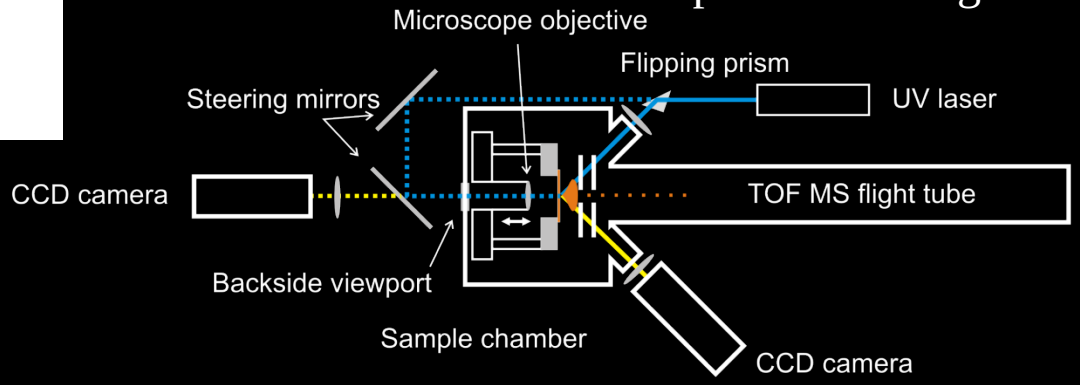
Ion image of mouse cerebellum (*m/z* 6765) at spatial resolutions of 200 µm, 100 µm, 50 µm, and 25 µm.

Transmission vs. reflection geometry



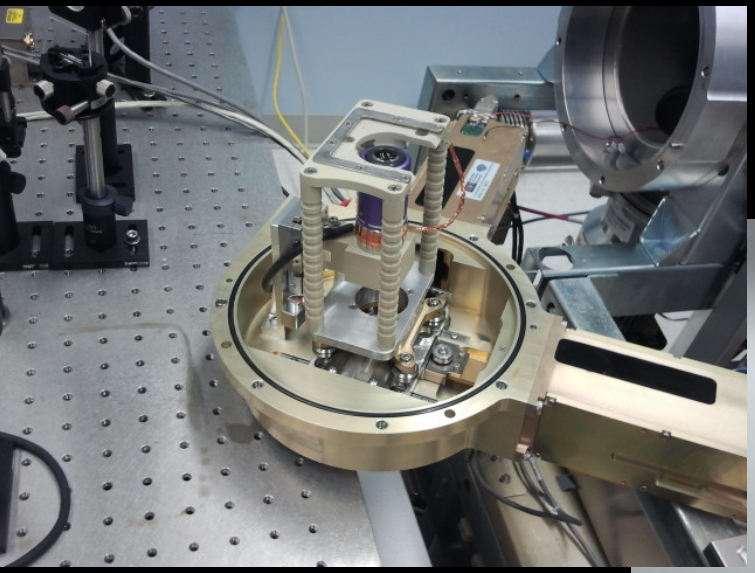
Why transmission geometry?

- Better access to target, permitting shorter working distance lenses.
- On-axis sample visualization permits better accuracy and better image quality at higher optical magnification in the instrument.
- Backside illumination may favor ion formation for matrix pre-coated targets.



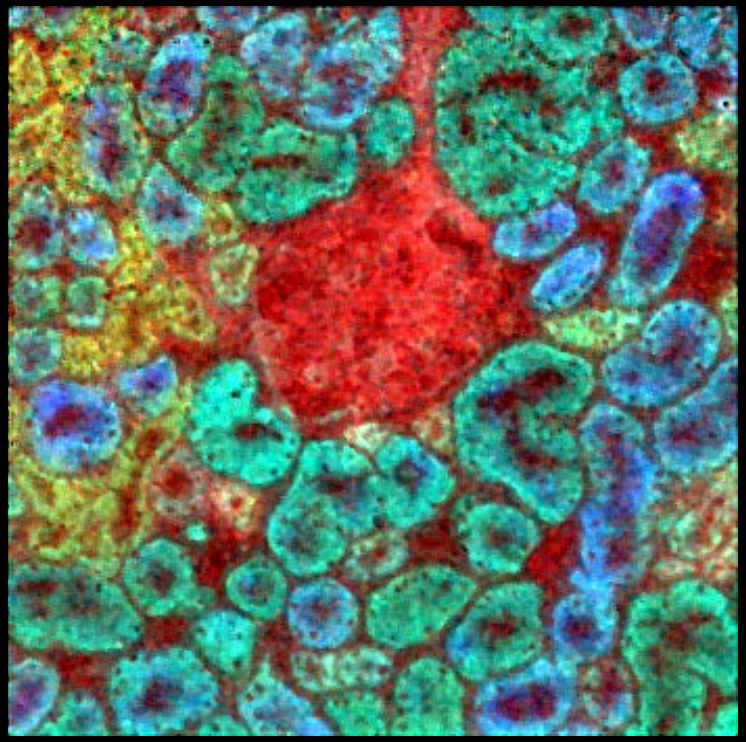
Transmission geometry Prototype

Modified AB 4700

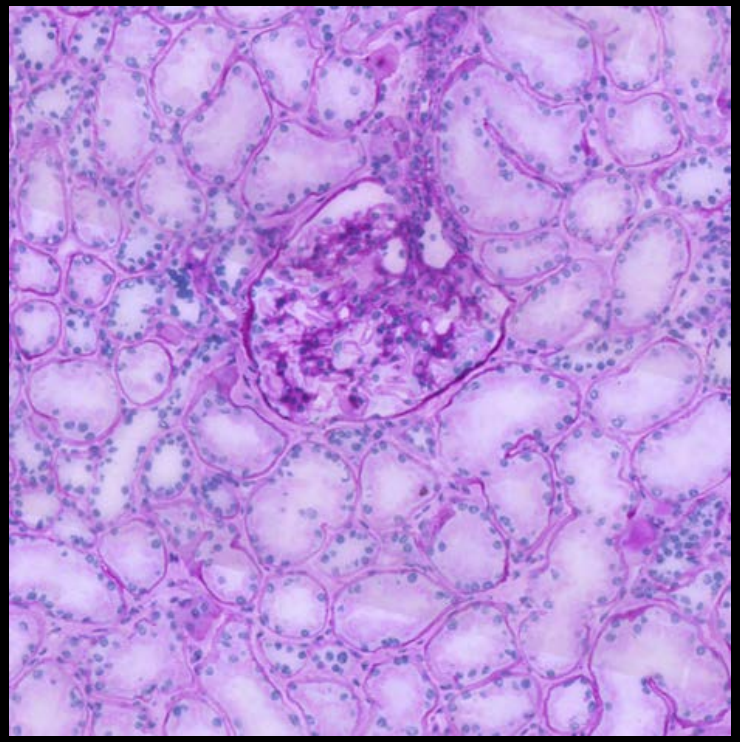


High Spatial Resolution Imaging using Transmission Geometry MALDI MS

Human Glomerulus



Imaging MS
(2 µm spatial resolution)



PAS Stain
(serial section)

Ion Overlay with Tentative IDs

Red = *m/z* 750; PE(P-38:4)

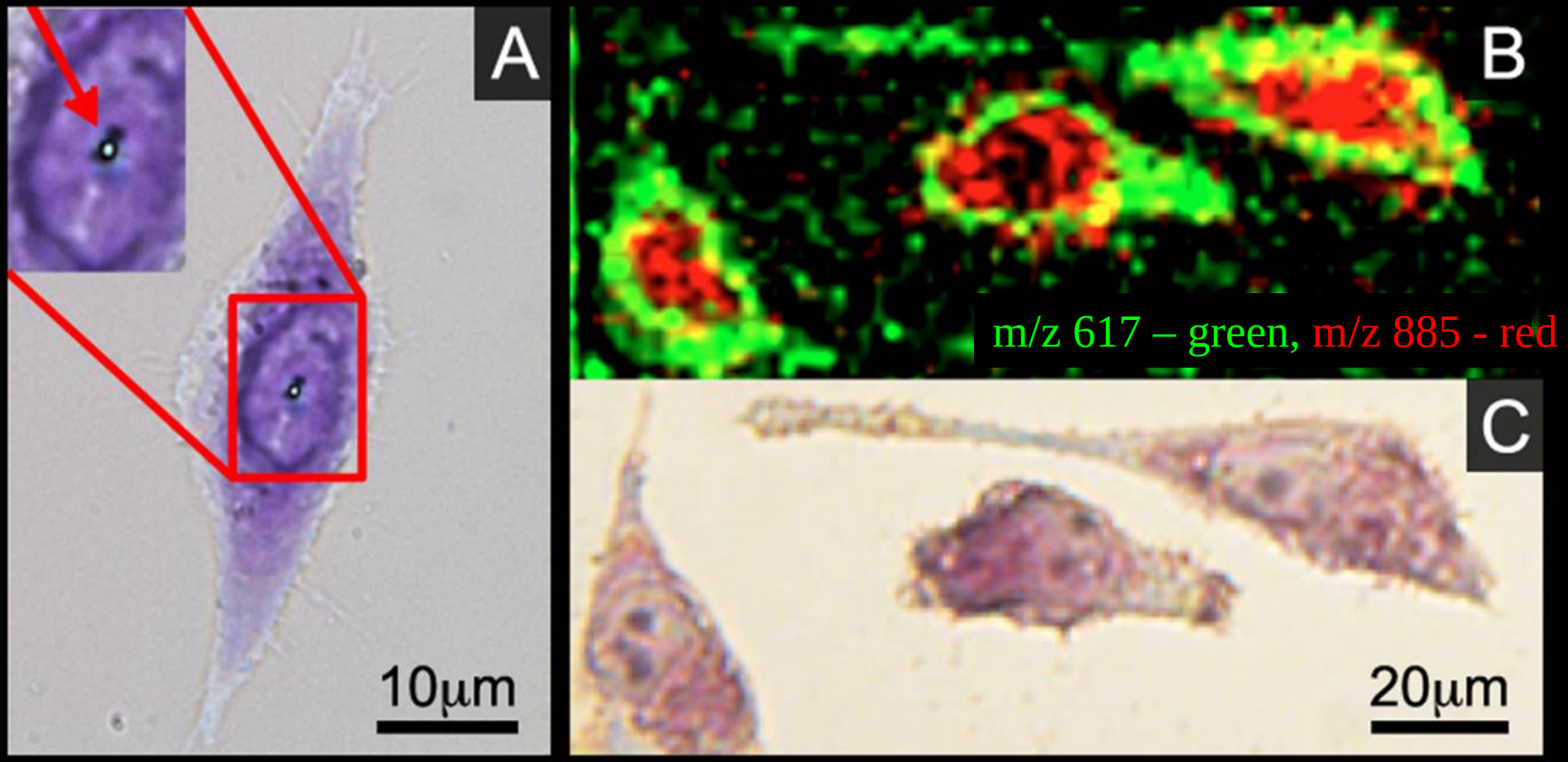
Yellow = *m/z* 863; PI(36:1)

Green = *m/z* 885; PI(38:4)

Blue = *m/z* 1052; SM3(d18:1/24:0)

Transmission Geometry AB4700 instrument

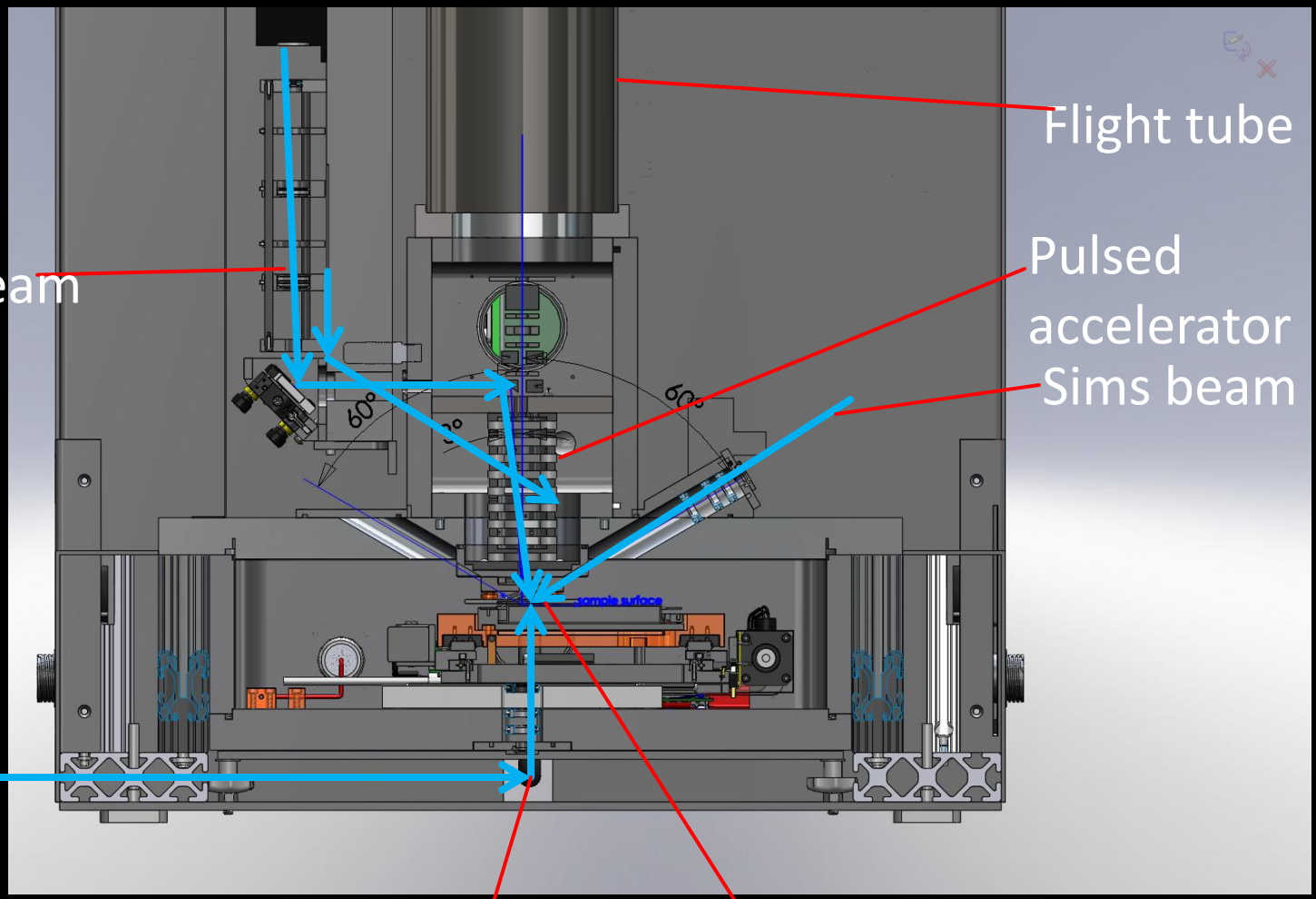
single cell imaging



A. Optical microscopy image of laser ablated spot in a nucleus area of a single HEK 293 cell in point-and-shoot mode
B. MS images of single HeLa cells at raster step 2 µm.
C. Optical microscopy image of the same HeLa cells before the MS imaging raster scan.

Angle	min spot(μm)
normal	25
3 deg	2.5
180 deg	1

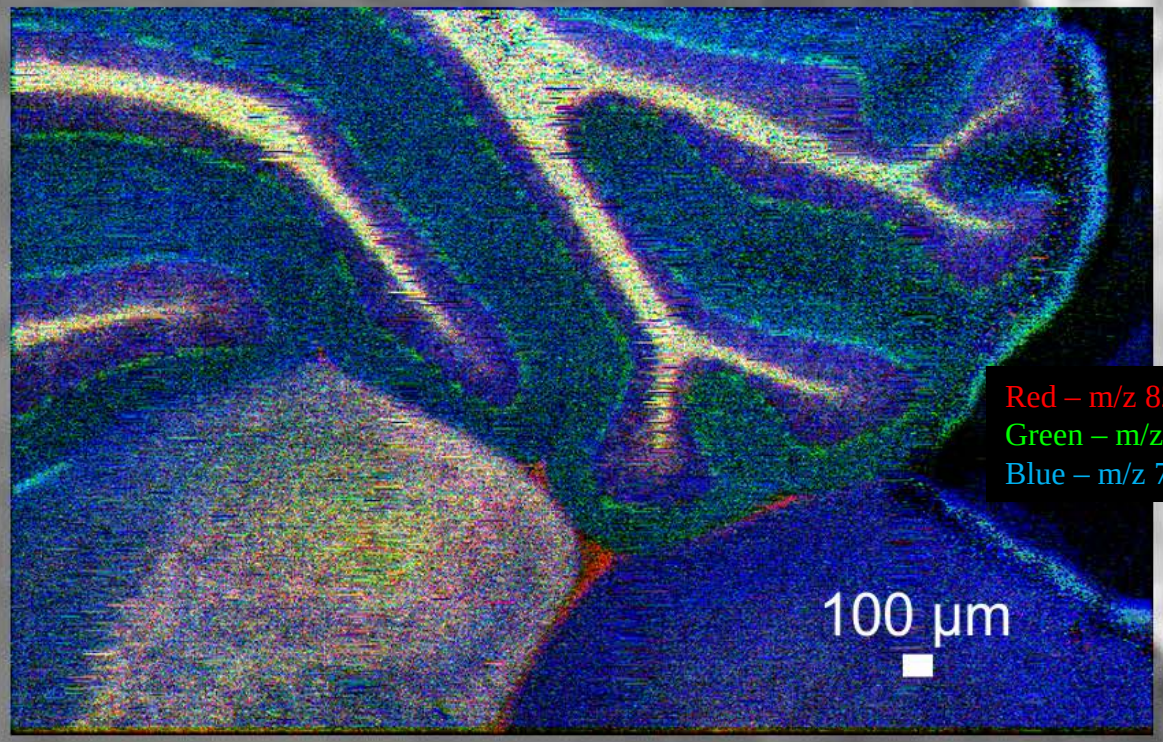
SimulTOF Systems
Multiport Sample Interrogation

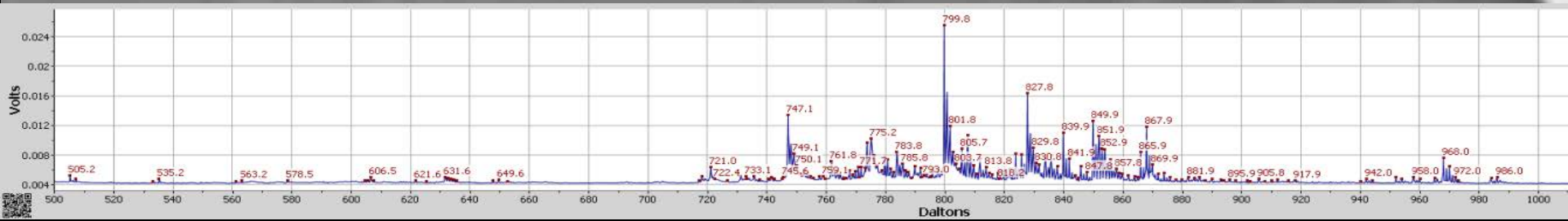


SimulTOF instrument in transmission geometry

5 µm pixel size, ~300,000 spectra

Mouse cerebellum, DHA matrix / positive mode





SimulTOF instrument in transmission geometry

2 µm pixel size, ~500,000 spectra

Mouse cerebellum, DHA matrix / positive mode

Red – m/z 827

Green – m/z 773

Blue – m/z 869

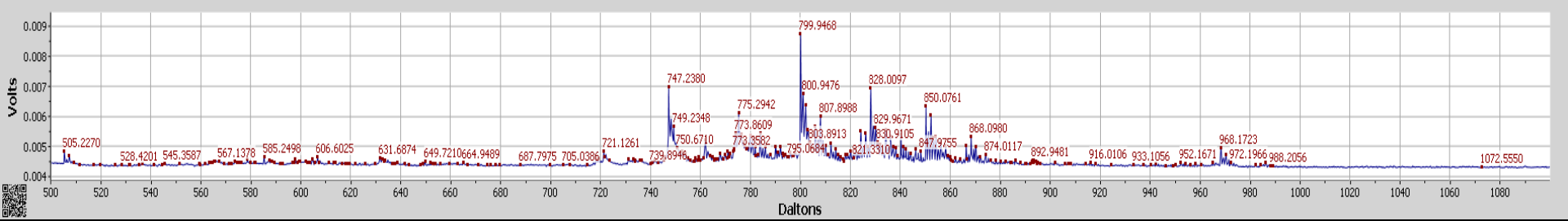


Image Fusion

What?

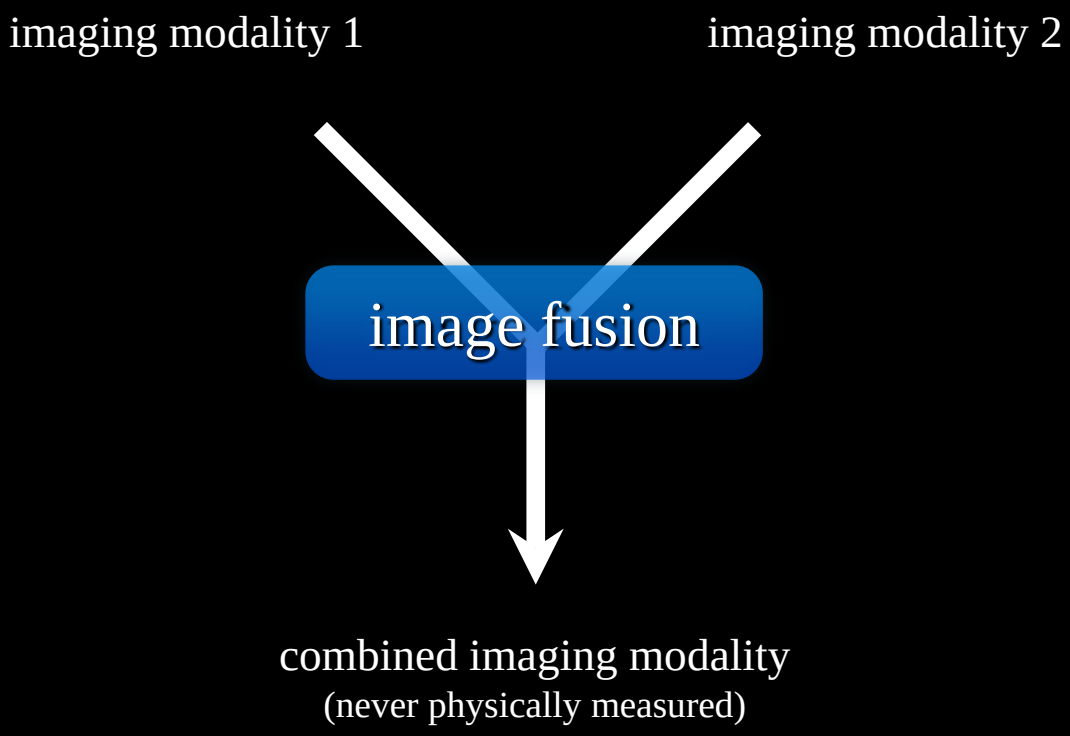


Image Fusion

Why?

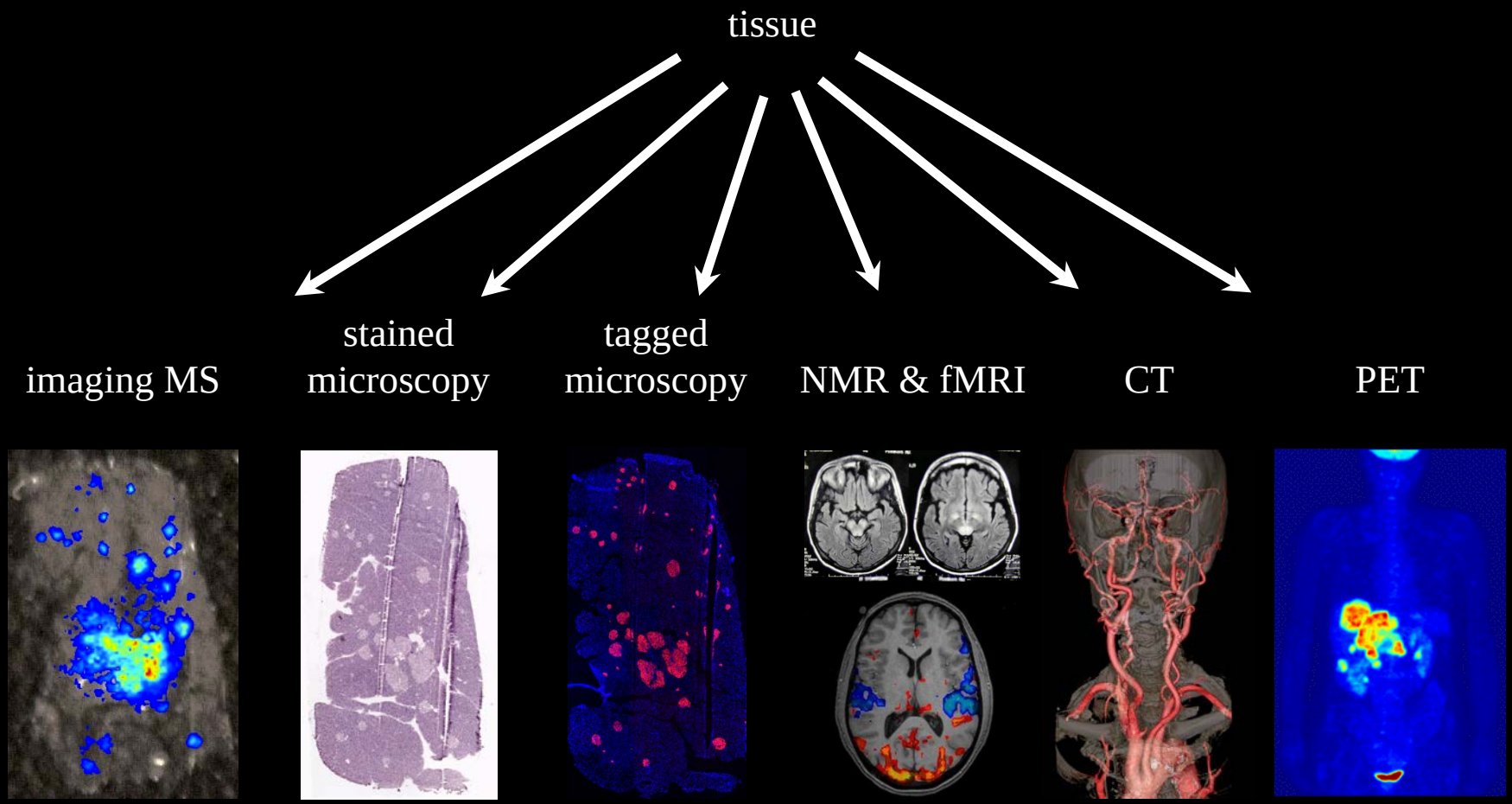


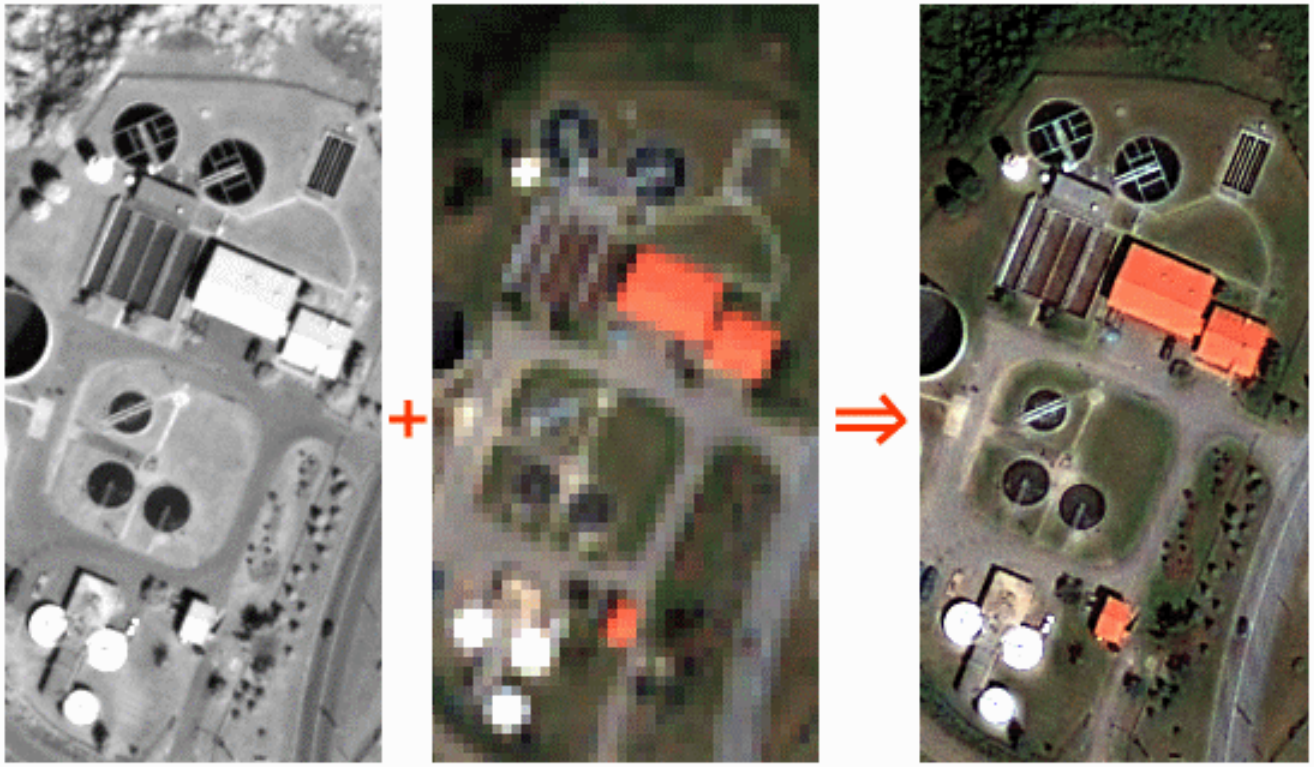
Image Fusion

Example of sharpening

pan-chromatic image
- high spatial resolution -
- little info per pixel -

multispectral image
-- low spatial resolution -
- a lot of info per pixel -

fused image
- high spatial resolution -
- a lot of info per pixel -

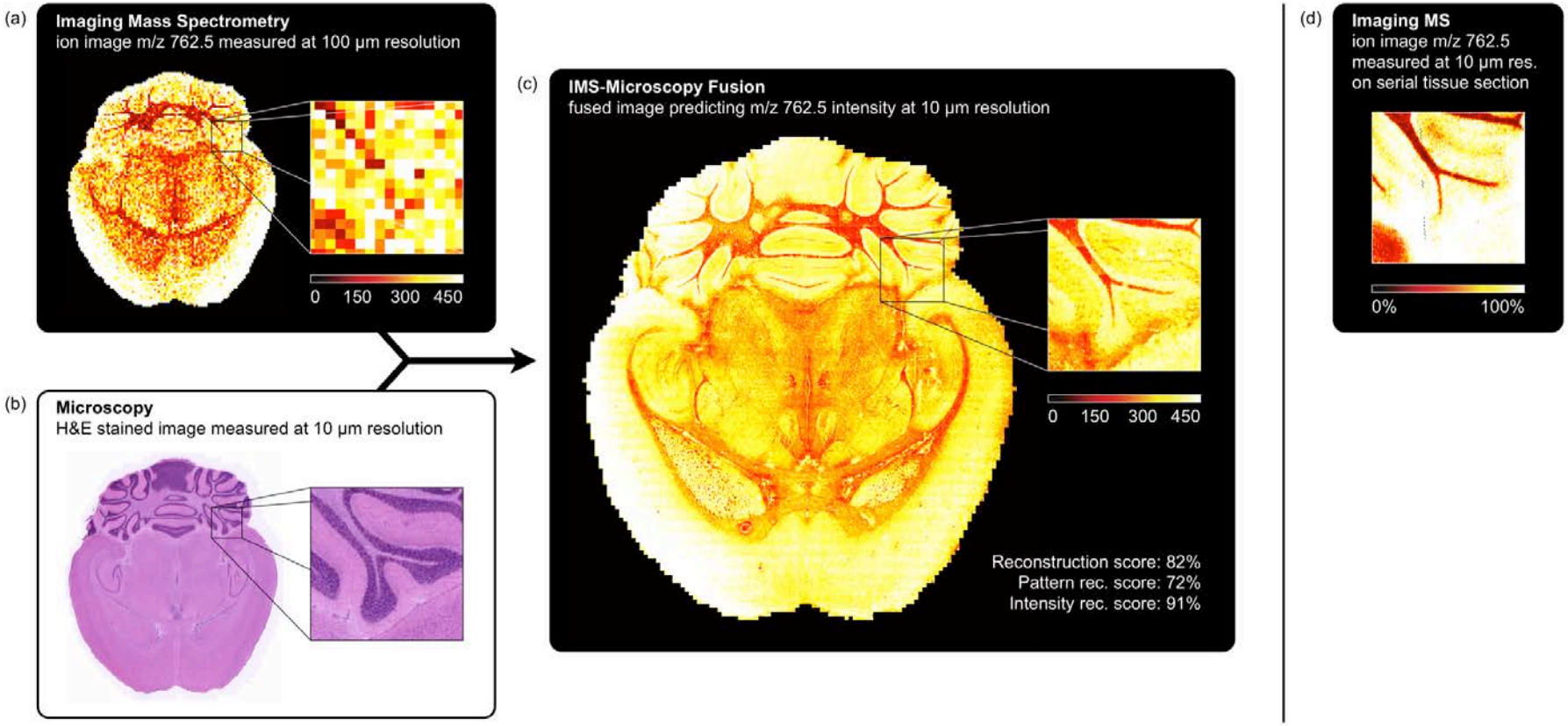


Satellite images from QuickBird

Image Fusion

Improved prediction and benchmarking

Prediction versus measurement benchmark.

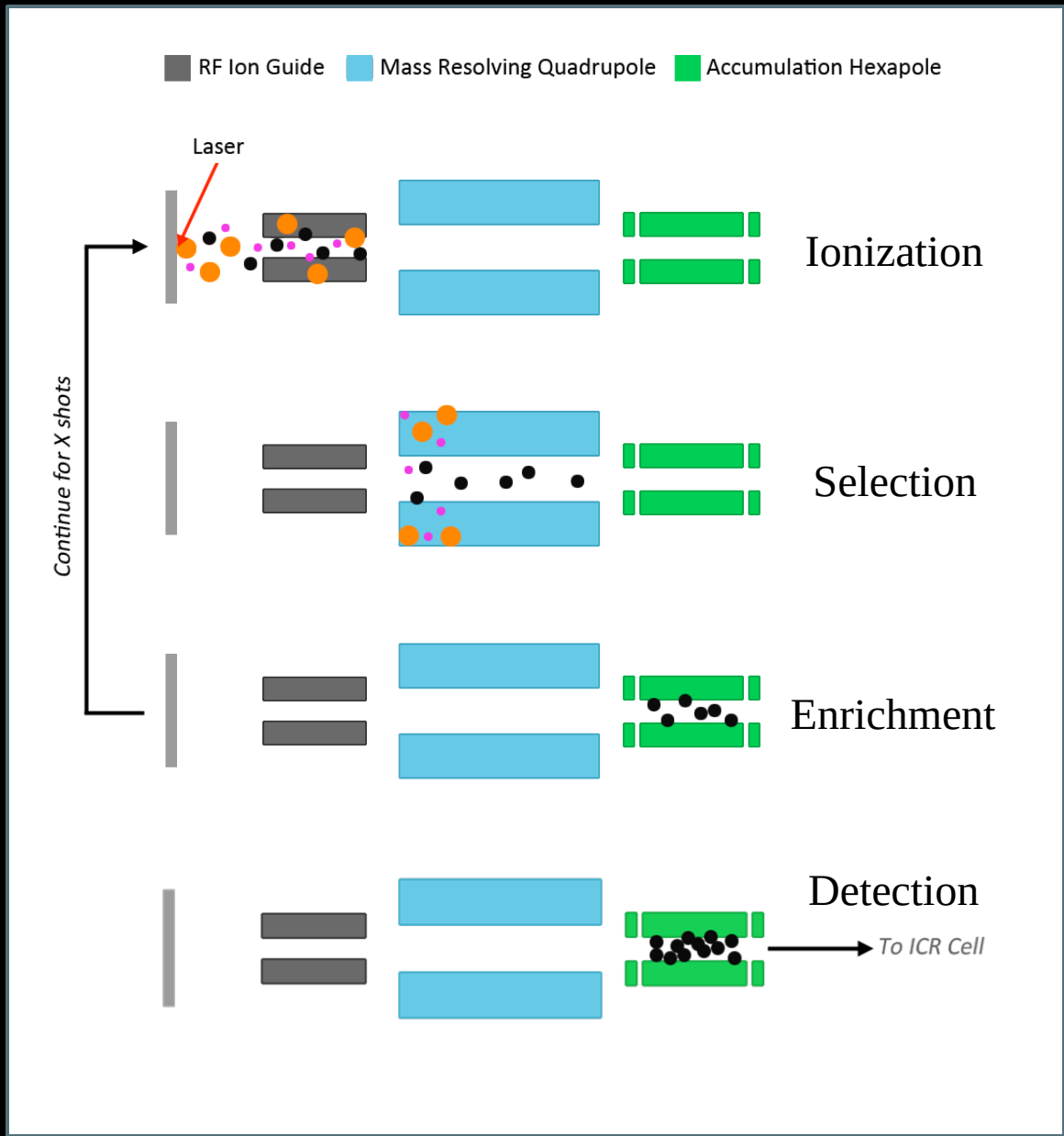


Increasing Sensitivity & Specificity

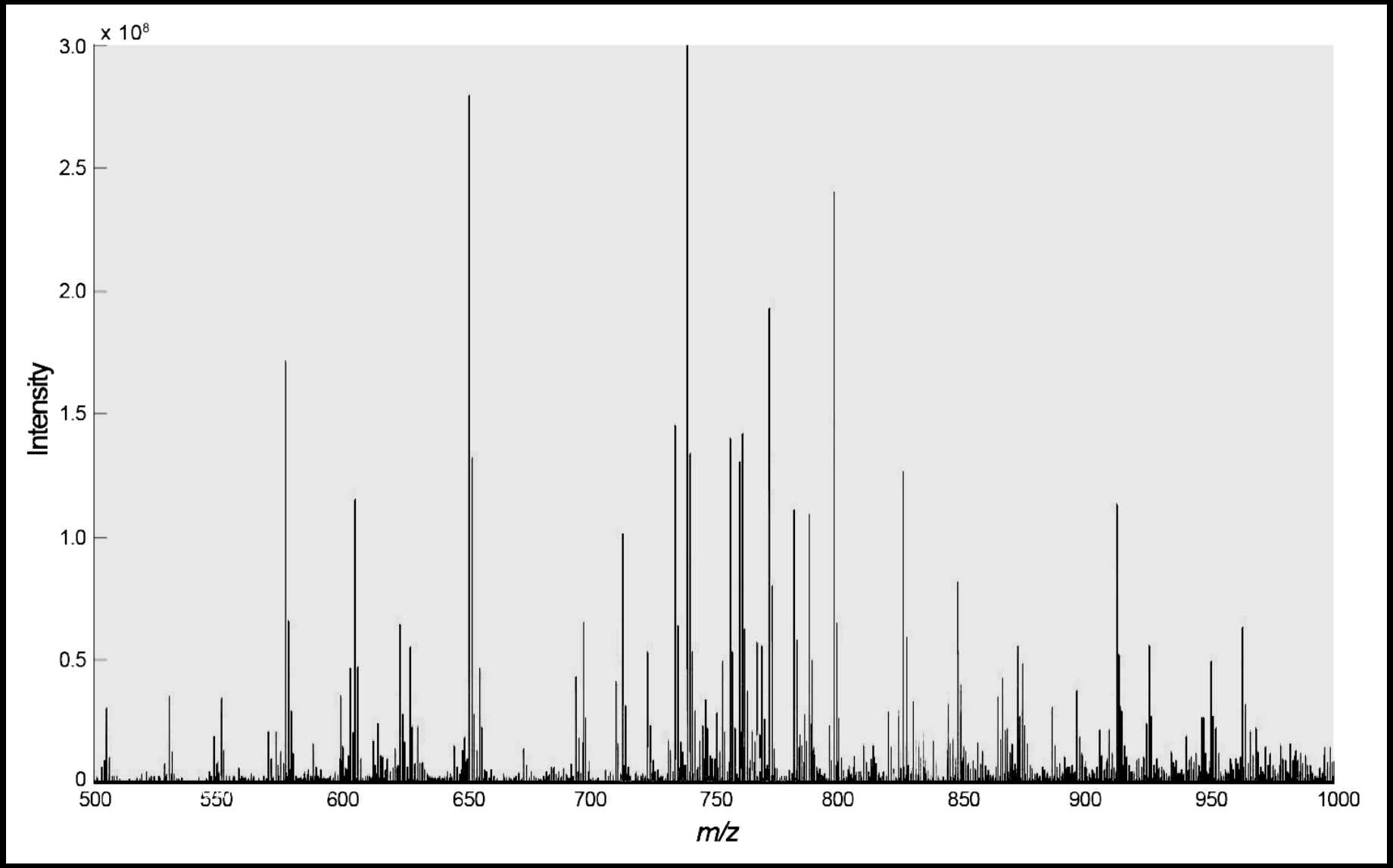
High Dynamic Range FT-ICR Imaging Mass Spectrometry

High Dynamic Range FT-ICR MS

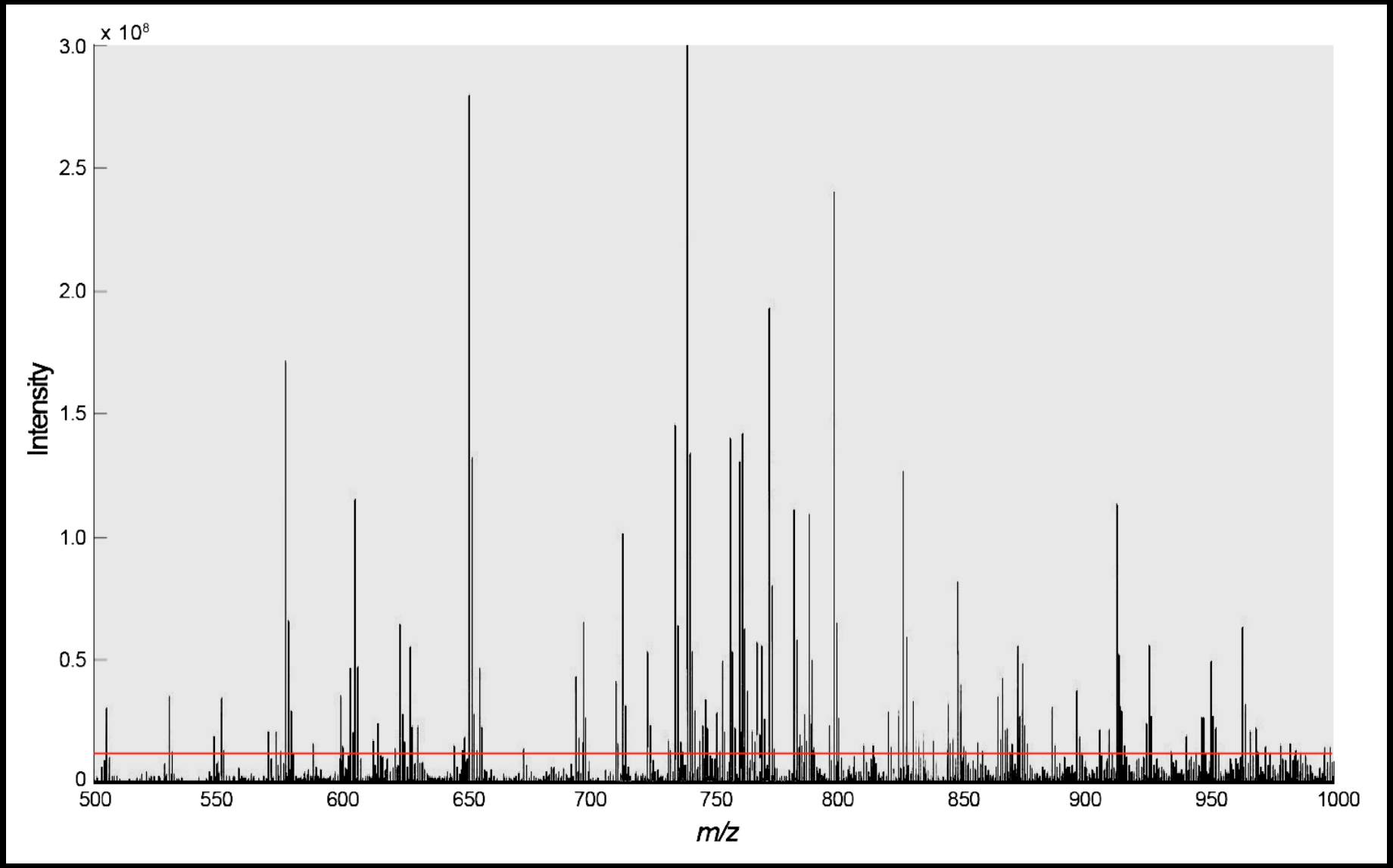
- High Dynamic Range MALDI FT-ICR Example
  - Continuous Accumulation of Selected Ions (CASI)
  - Result: 3 orders of magnitude increase in sensitivity.
  - Process repeated to cover entire mass range.



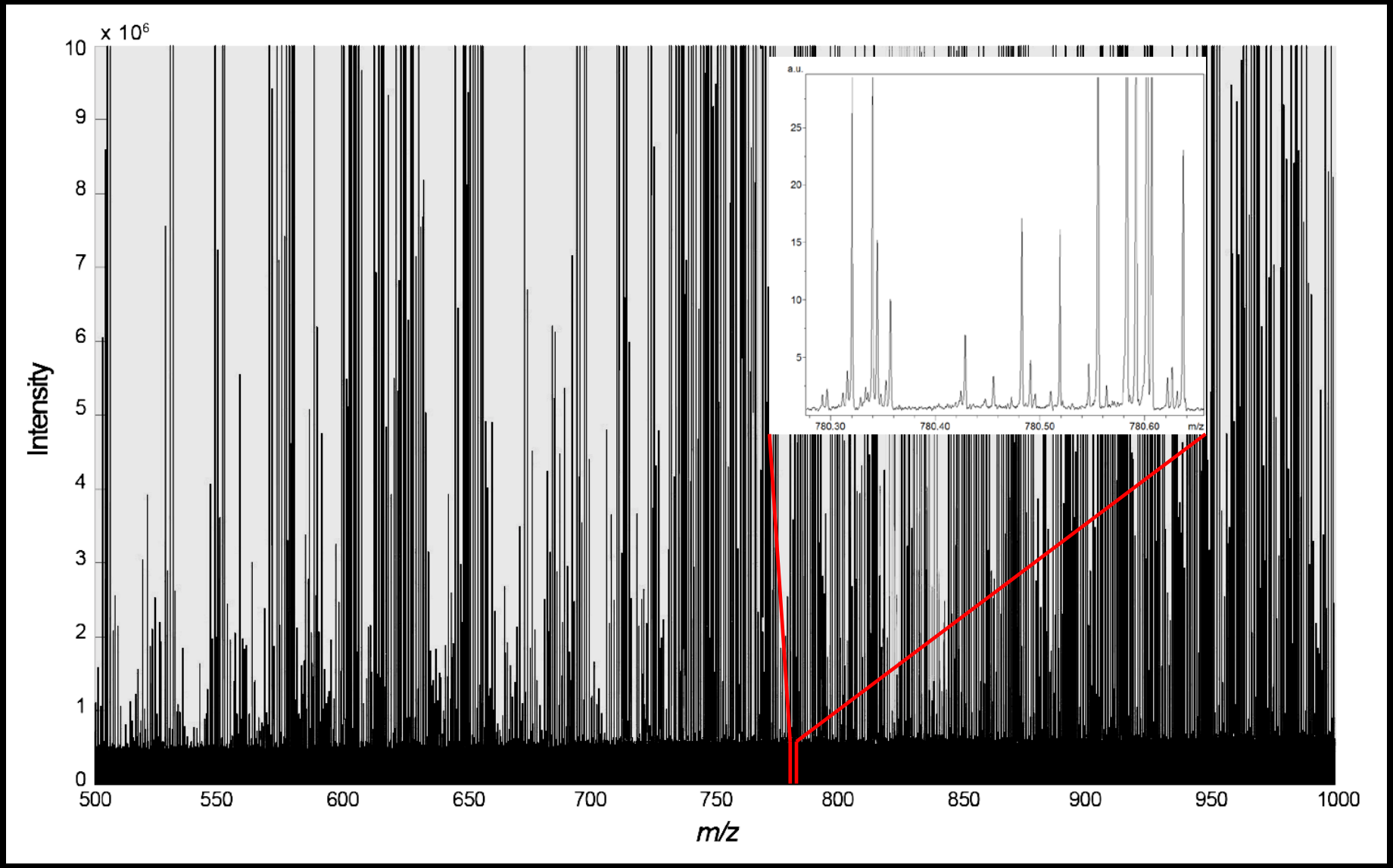
High Dynamic Range FT-ICR MS



High Dynamic Range FT-ICR MS



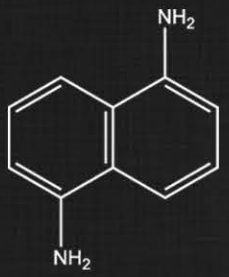
High Dynamic Range FT-ICR MS



Proof of Concept: MALDI FTICR MS lipid imaging

Sample Preparation

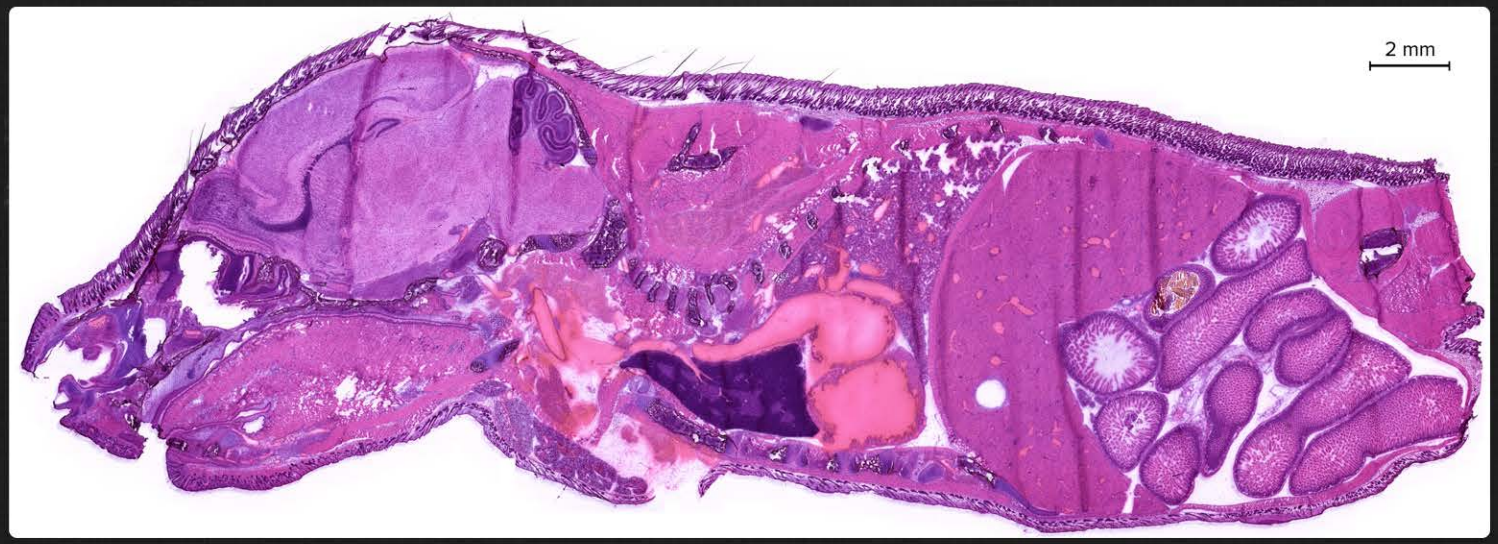
6 day old mouse pup
12 μm tissue section
Matrix: 1,5-Diaminonaphthalene
Matrix Application: Sublimation
(110 °C, 50 mTorr, 6.75 min)



1,5-Diaminonaphthalene (DAN)

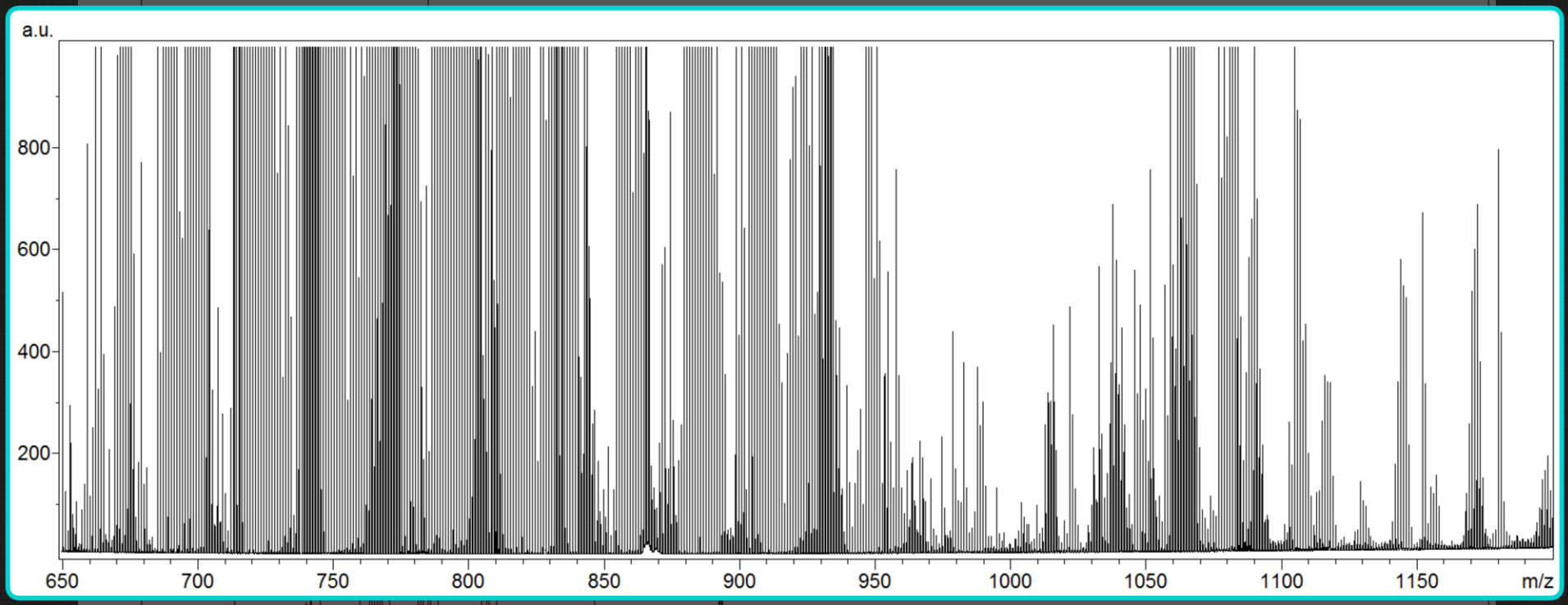
Imaging MS Details

Bruker SolariX 9.4T FTICR MS
m/z 600 - 1600
256k TD (~0.5 s transient length)
ADD acquisition mode
50 μm spatial resolution
Laser focus: Small I ~45 μm
50 shots/px
Laser Frequency: 2 kHz
122,204 px



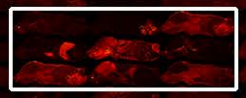
MALDI FTICR MS lipid imaging

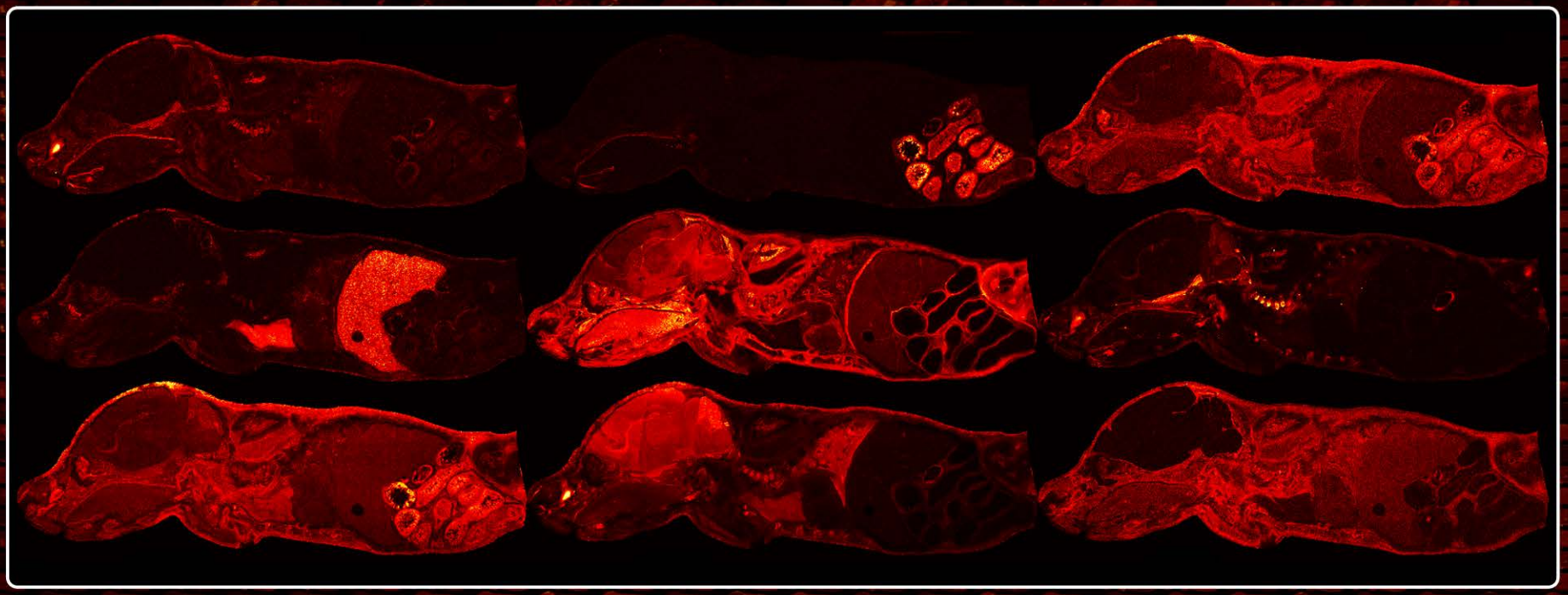
average over entire image



MALDI FTICR MS lipid imaging

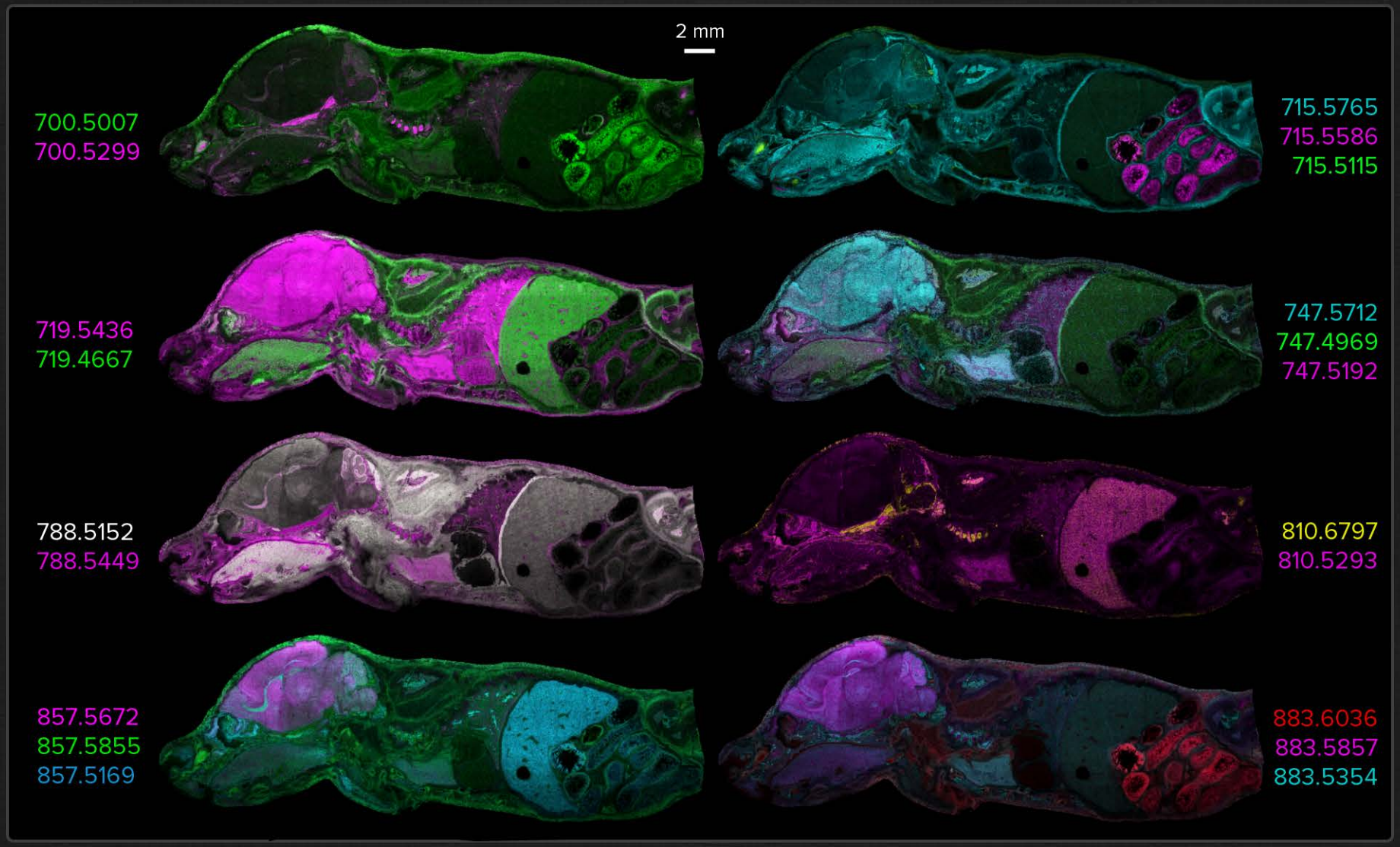
>2,000 ion images | 1,487 shown
122,204 total pixels
Spatial Resolution: 50 μm
Mass Resolving Power: 50,000 - 80,000
Acquisition speed: 2 Hz





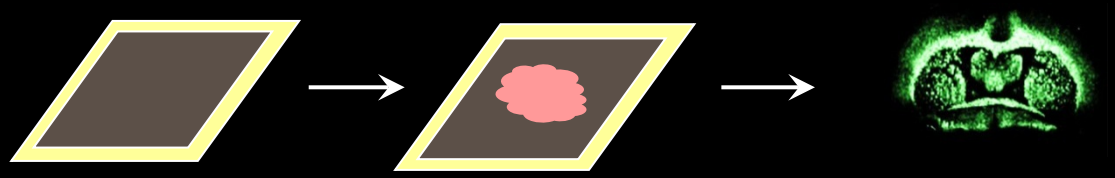
The Problem: IMS data complexity

Unique spatial distributions at each nominal mass



Improving Ease-of-Use

PRE-COATED TARGETS



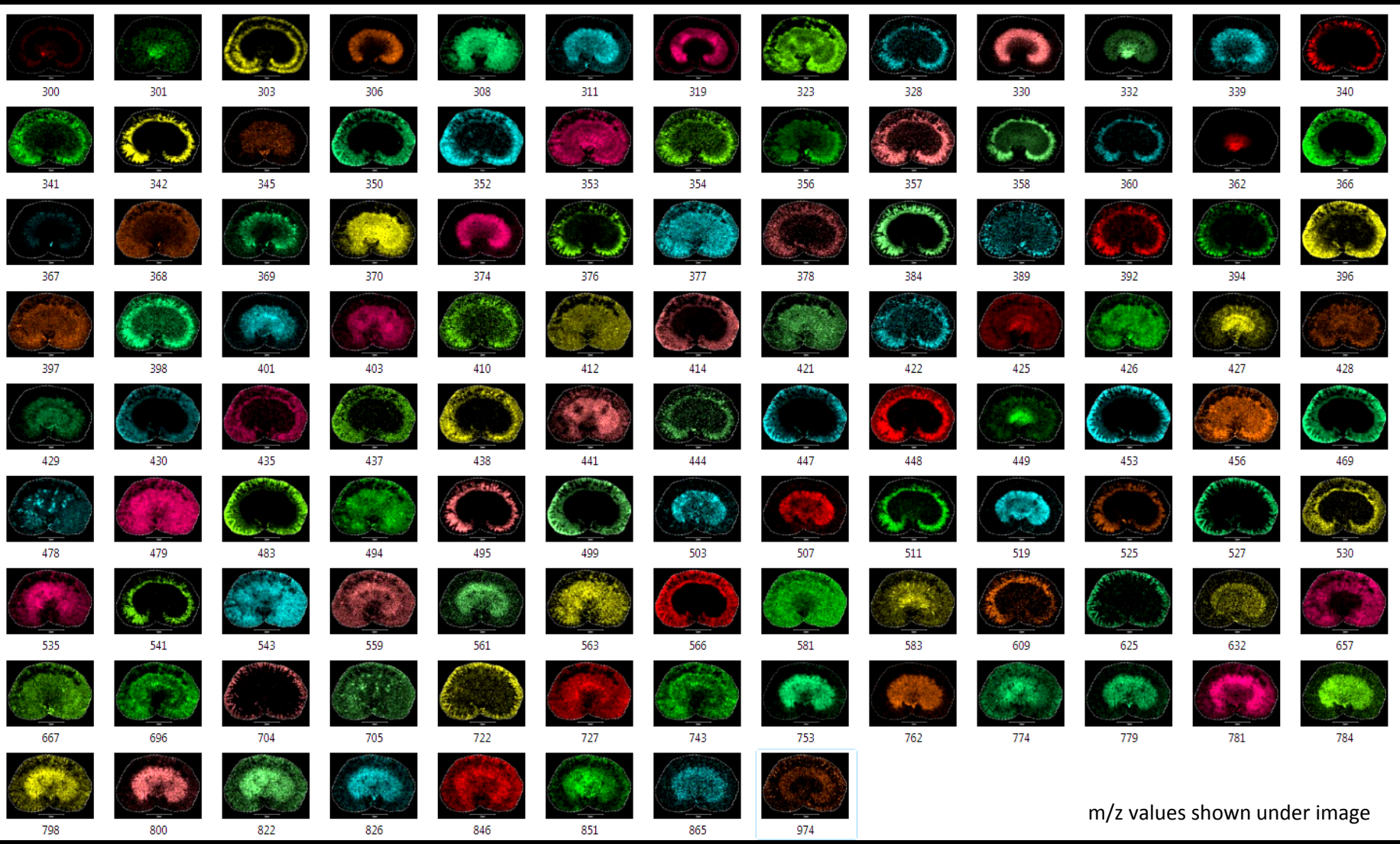
Matrix pre-coated target (on the shelf)

Apply tissue section

Acquire Images

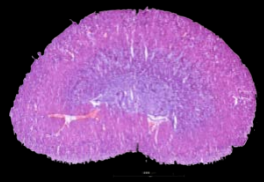
- Motivation:
  - Sample preparation is a perceived obstacle for the technology.
    - Users lack expertise
    - Time consuming
  - A matrix pre-coated target and an optimized protocol for use removes the burden of sample preparation from the end user.

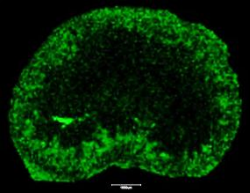
Pre-coated CHCA for imaging lipids (positive mode)



m/z values shown under image

Pre-coated sinapinic acid for imaging proteins (3k to 70k Da)

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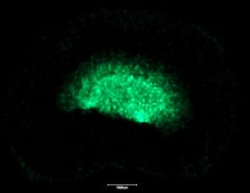
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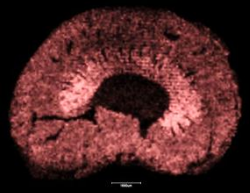
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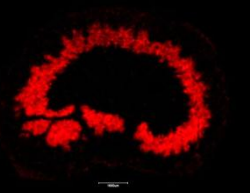
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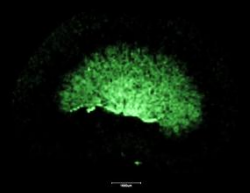
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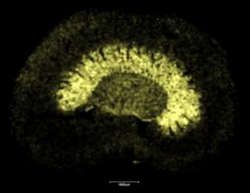
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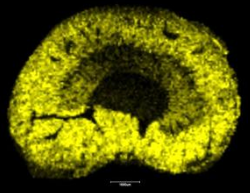
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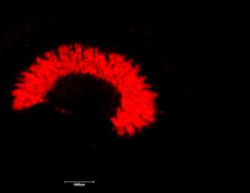
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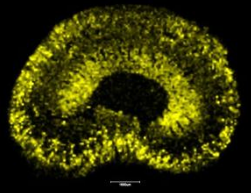
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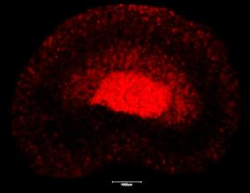
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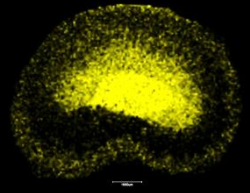
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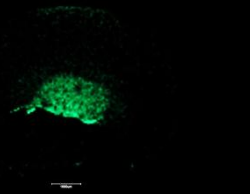
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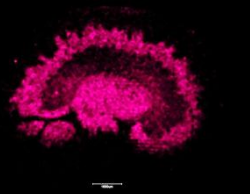
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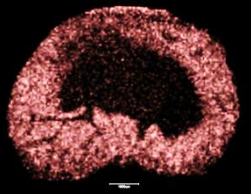
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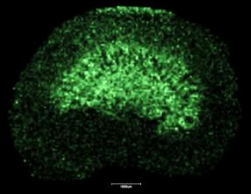
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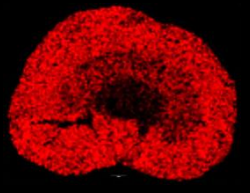
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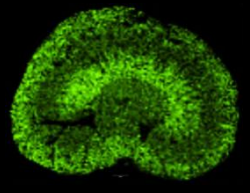
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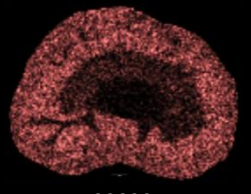
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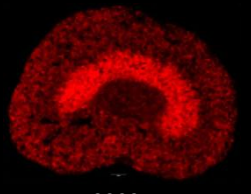
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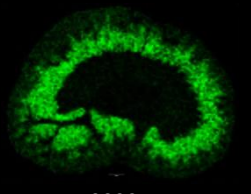
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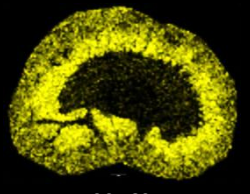
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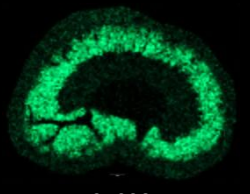
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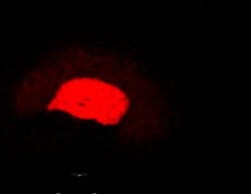
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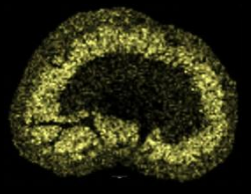
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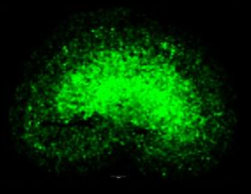
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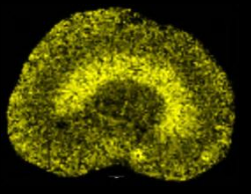
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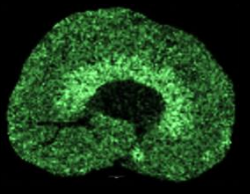
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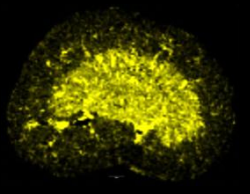
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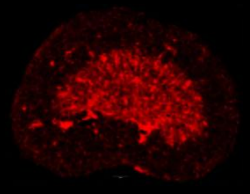
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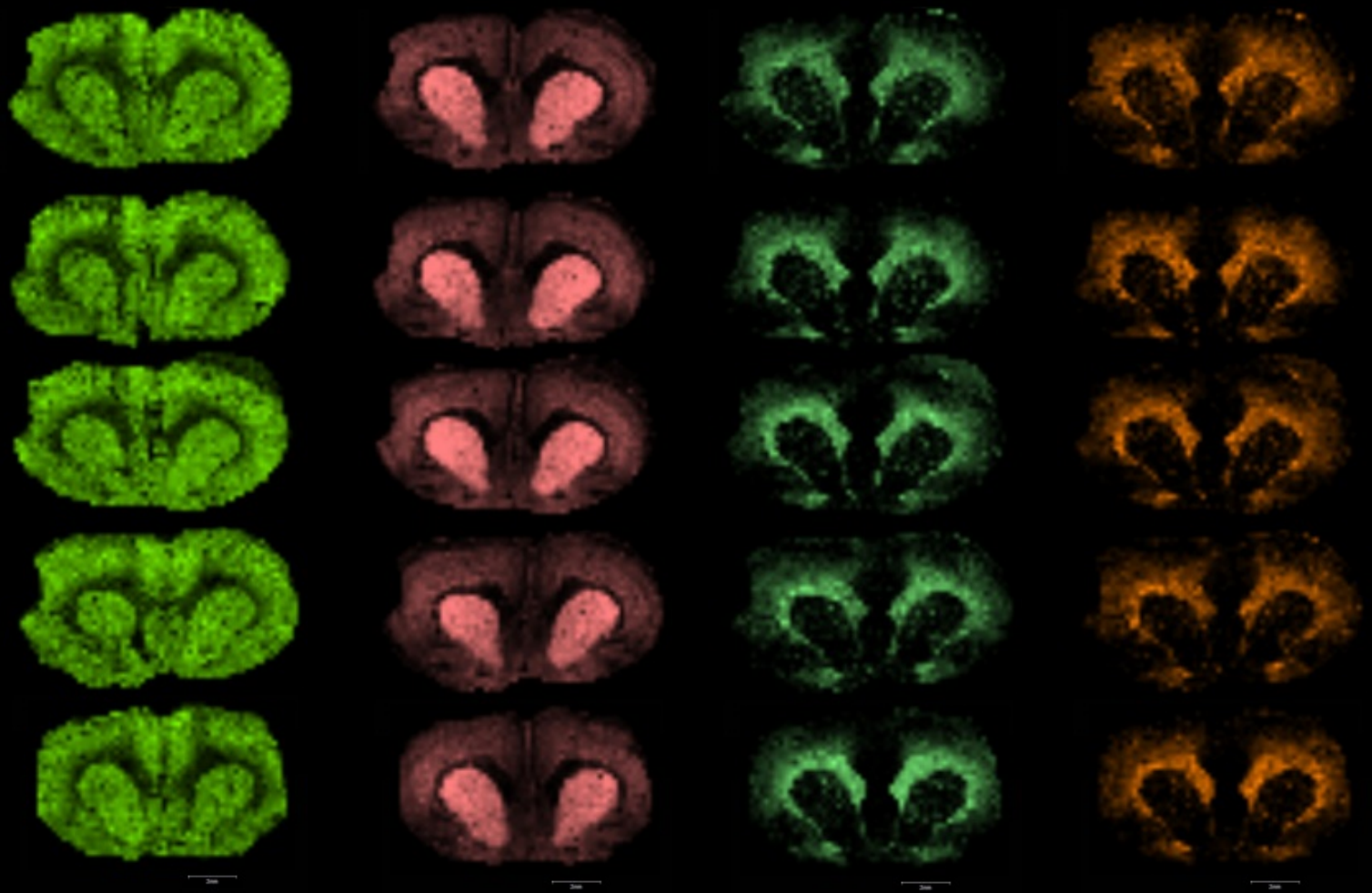
m/z values shown under image

MATRIX PRE-COATED TARGETS PRODUCE REPRODUCIBLE IMAGES

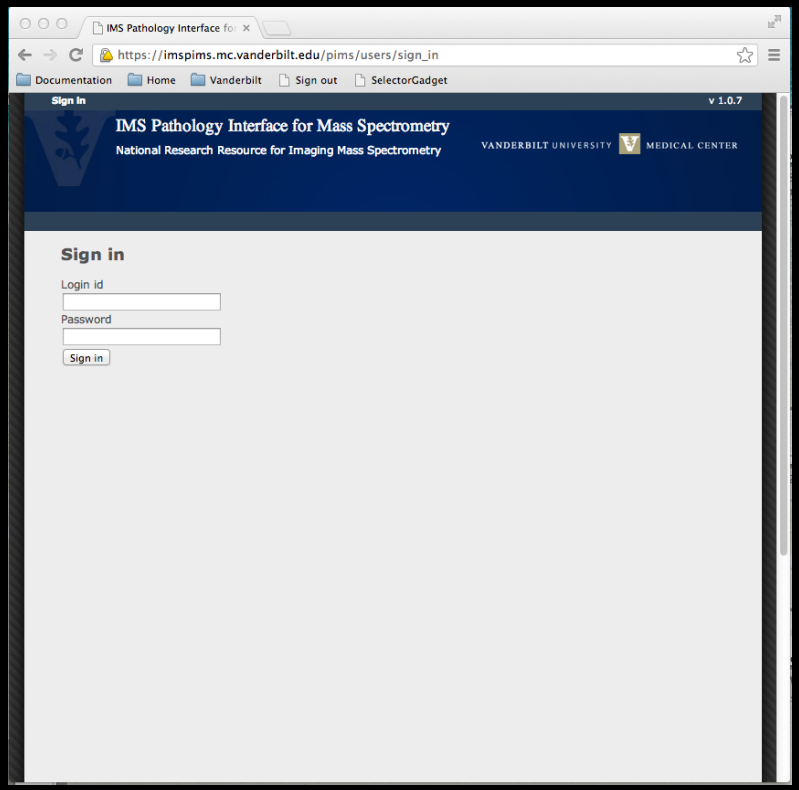
MOUSE BRAIN ANALYZED USING A PRE-COATED SINAPINIC ACID TARGET



m/z 5628 m/z 6710 m/z 7057 m/z 18,386

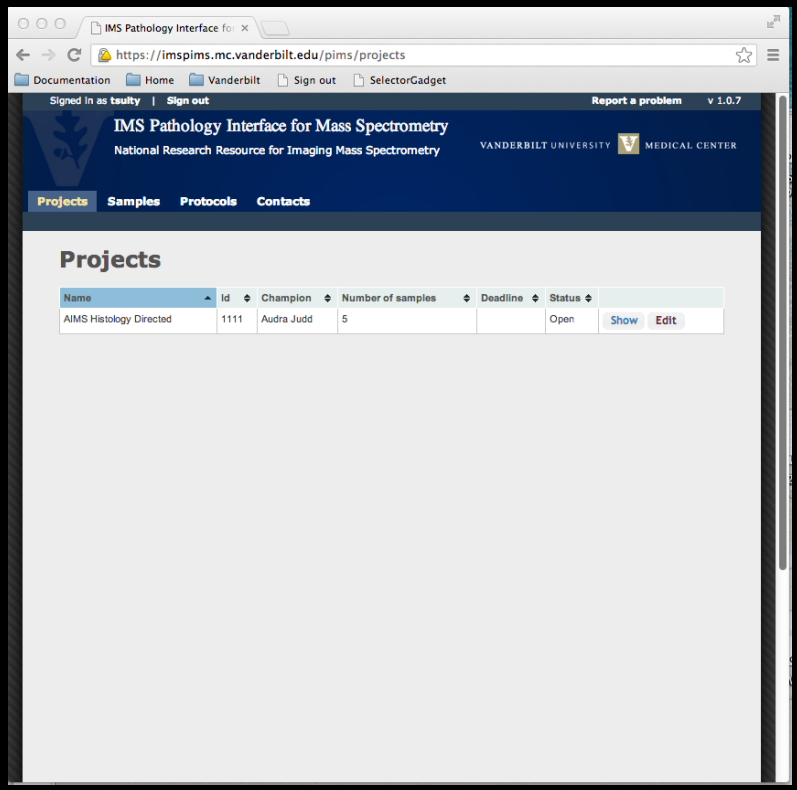
PATHOLOGY INTERFACE FOR MASS SPECTROMETRY (PIMS)

- Secure login provided to ensure access only to authorized users of the system.
- Access is controlled by the system administrator.
- Project level access is granted only to those collaborators involved in the project.
- Principal investigators and lab directors control access.



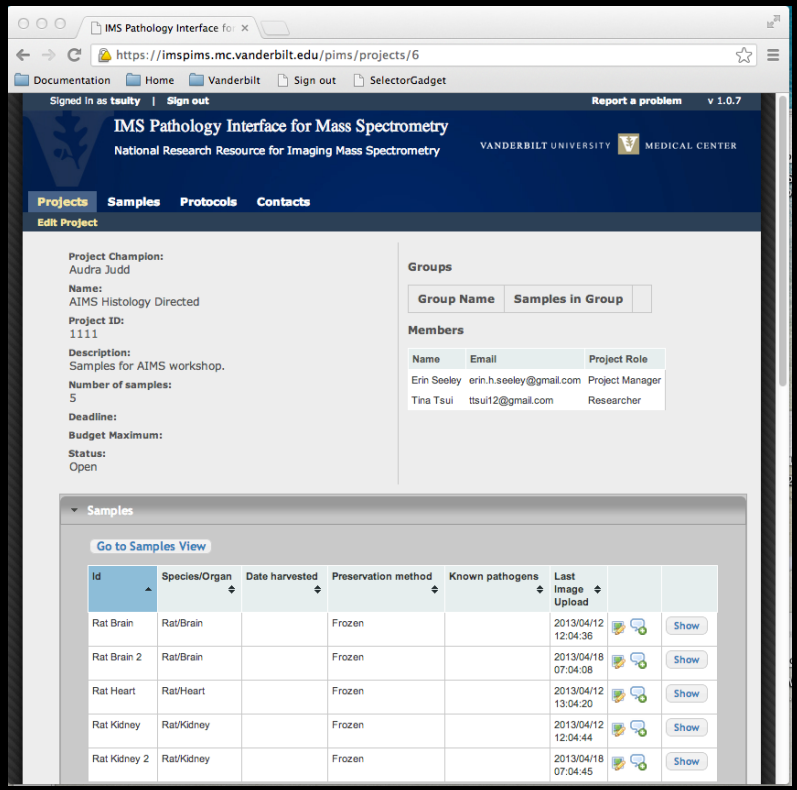
PATHOLOGY INTERFACE FOR MASS SPECTROMETRY (PIMS)

- Database is organized by projects.
- All projects are shown with basic information about that project.
- Investigators can only view projects to which they have been assigned.



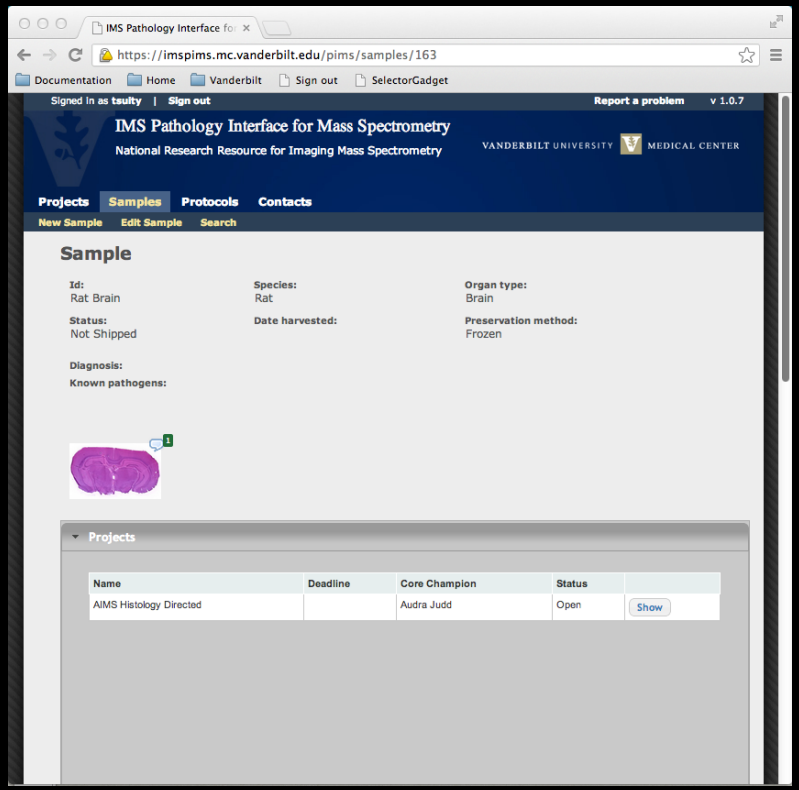
PATHOLOGY INTERFACE FOR MASS SPECTROMETRY (PIMS)

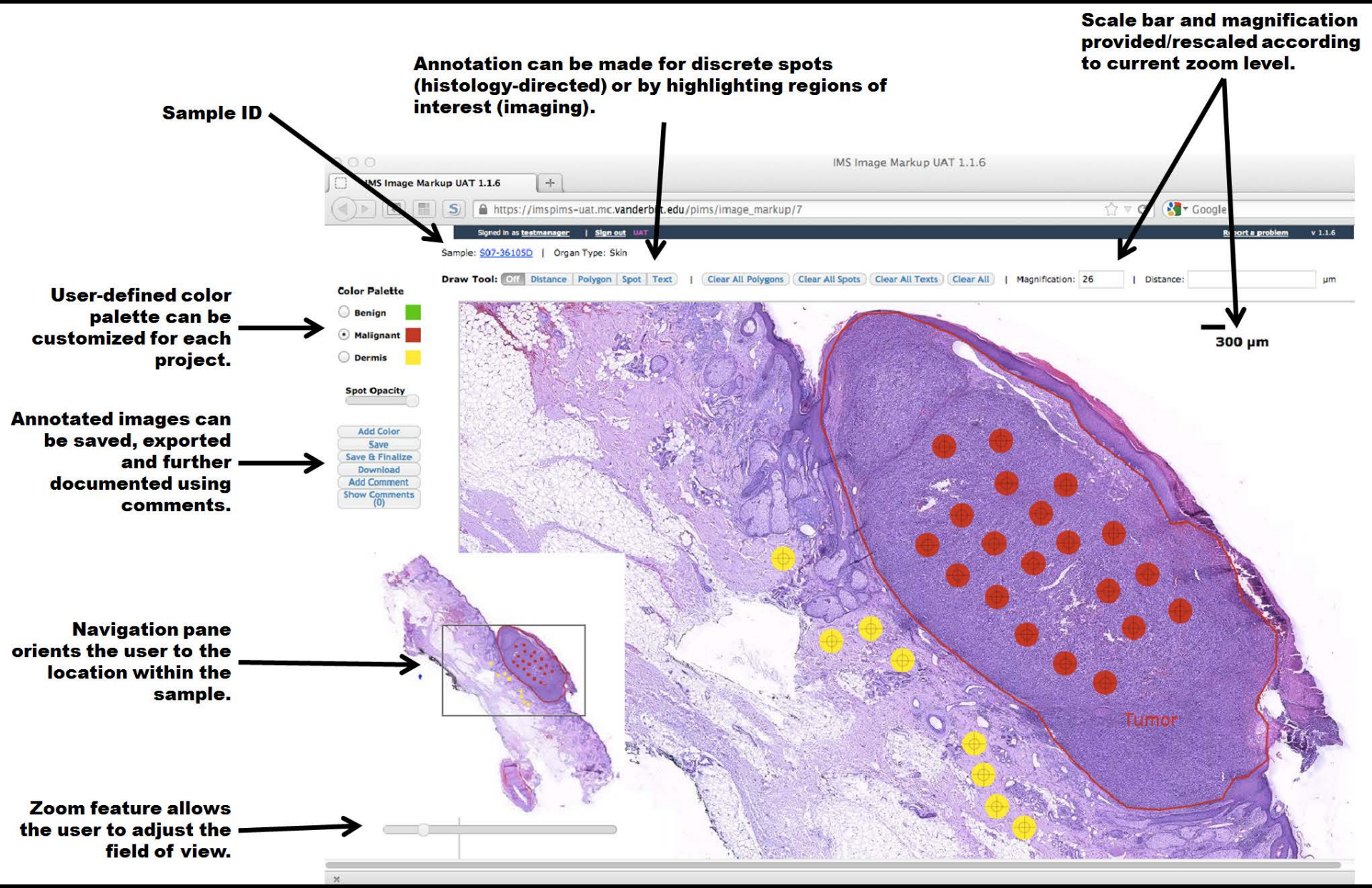
- Samples assigned to projects are shown in the Project View.
- Collaborators input sample information for the study.
- Micrograph files are automatically assigned to samples based on the filename at import.
- Samples may be assigned to more than one project.



PATHOLOGY INTERFACE FOR MASS SPECTROMETRY (PIMS)

- All sample information is displayed in Sample View along with thumb-nail images of the associated micrographs.
- All projects for which the sample has been assigned is shown in the dropdown below.
- Selection of thumbnails opens the image annotation window.




Scale bar and magnification provided/rescaled according to current zoom level.
Annotation can be made for discrete spots (histology-directed) or by highlighting regions of interest (imaging).
Sample ID
IMS Image Markup UAT 1.1.6
https://imspims-uat.mc.vanderbilt.edu/pims/image_markup/7
Signed in as testmanager | Sign out UAT
Report a problem
v 1.1.6
Sample: S07-36105D | Organ Type: Skin
Draw Tool: Off Distance Polygon Spot Text | Clear All Polygons Clear All Spots Clear All Texts Clear All | Magnification: 26 | Distance: µm
300 µm
User-defined color palette can be customized for each project.
Color Palette
Benign
Malignant
Dermis
Spot Opacity
Annotated images can be saved, exported and further documented using comments.
Add Color
Save
Save & Finalize
Download
Add Comment
Show Comments (0)
Navigation pane orients the user to the location within the sample.
Tumor
Zoom feature allows the user to adjust the field of view.

Case Study: Melanoma

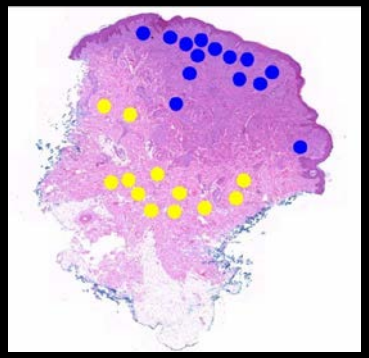
- In 2012, an estimated 76,250 new cases were diagnosed (annual increase of 3% since 2004).
  - Source: American Cancer Society.

- The number of biopsies performed in the US to rule out melanoma range between 1-2 M per year. Of these, 25% cannot be definitively classified using routine histopathology.
  - Source: *Am J Surg Pathol*, 33(8), 1146-56.

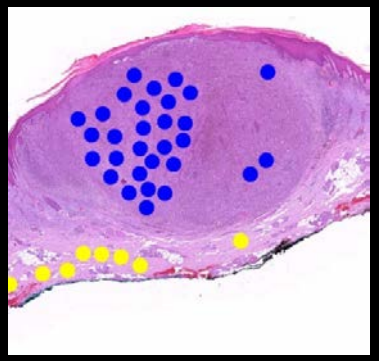
MS Analysis of Spitzoid Lesions in FFPE Biopsies

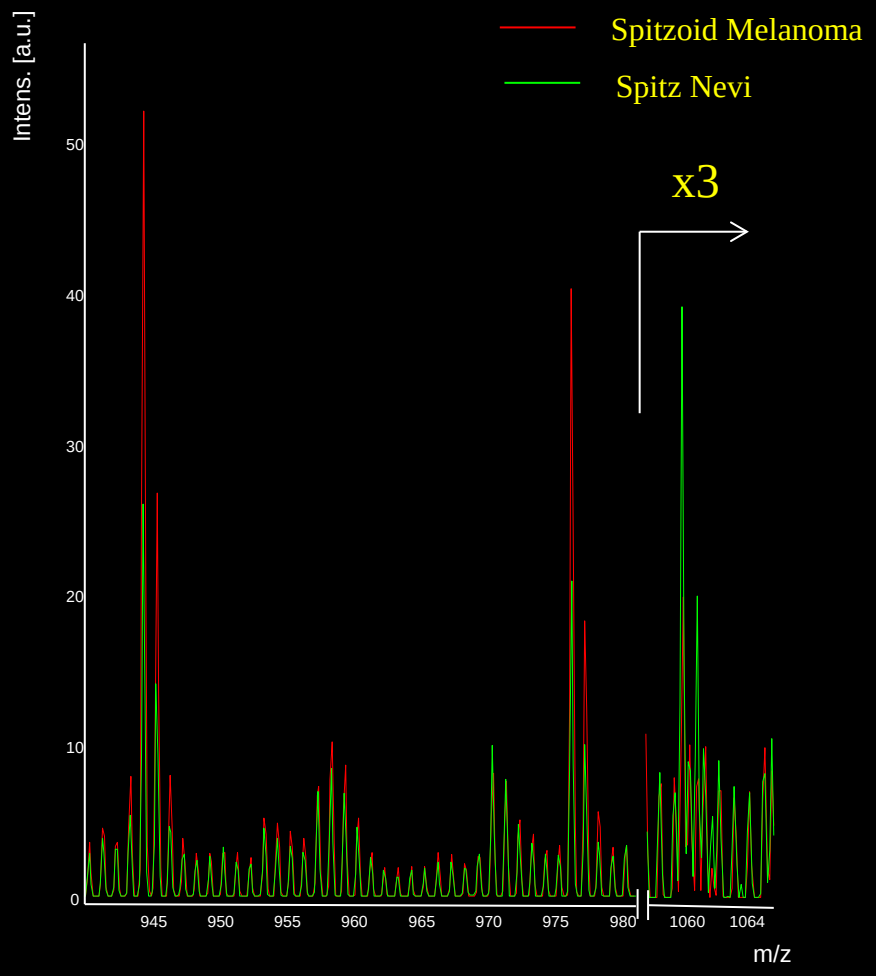
Lazova, R.; et al. Am J Dermatopathol. **34**, 82-90 (February 2012).

Spitz nevus



Spitzoid Melanoma





Classification of Spitzoid Lesions

56 SN and 54 SMM from Yale University Spitzoid Neoplasm Repository

Training set	# Patients	Classification Accuracy (%)
Spitz nevi (SN)	26	100
Spitzoid Malignant Melanoma (SMM)	25	96

Validation (test) set	# Patients	Classification Accuracy (%)
Spitz nevi (SN)	30	97
Spitzoid Malignant Melanoma (SMM)	29	90

International Spitzoid Neoplasm Study Group

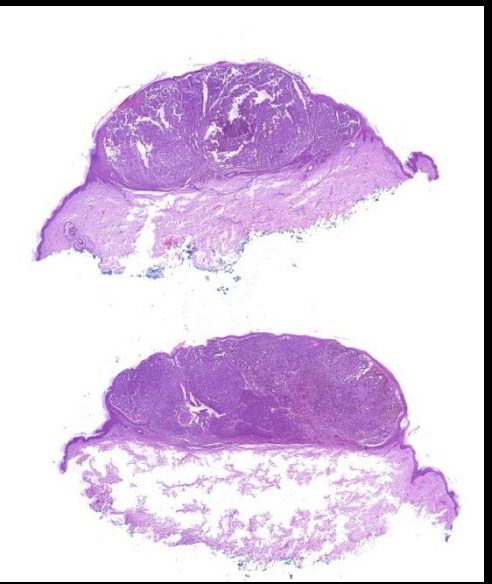
Atypical Spitzoid Neoplasms

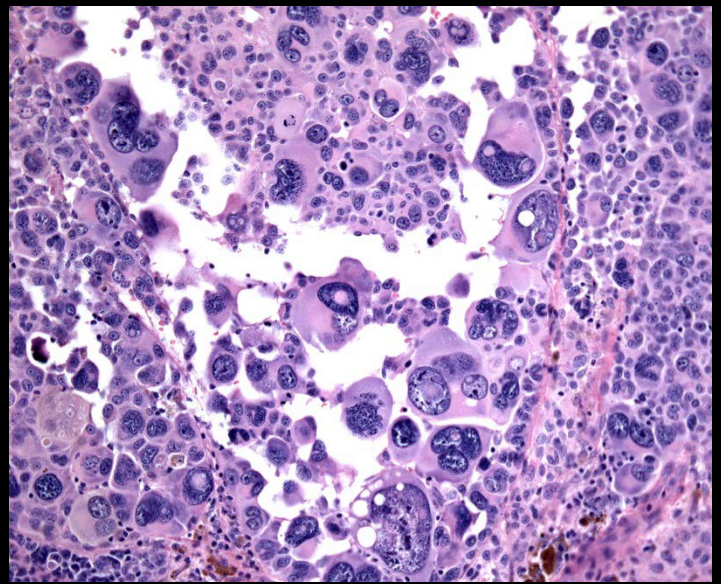
#	Age	Gender	Site	Histologic Dx	MS Dx	Follow up (y)	Clinical Status
1	43	M	Back	SMM	SMM	3.5	Negative LN; ANED
2	23	F	L calf	SMM	SMM	2	Positive LN; ANED
3	28	F	Thigh	SMM	SMM	12	Positive LN 8 years later; ANED
4	6	F	L neck	SMM	SMM	1.5	Positive LN; ANED
5	39	F	L post leg	SMM	SMM	1.5	Positive LN; ANED
6	5	F	Buttock	SMM	SMM	6	Positive LN; ANED
7	29	F	R upper back	SMM	SMM	14	Negative LN: Re-excision; ANED;
8	50	M	thorax	SMM	SMM	3	DOD with lung mets 3 years later
9	43	M	back	SMM	SMM	4	Negative LN; ANED
10	57	F	NK	SMM	SMM	3	Negative LN; ANED
11	15	F	L neck	SMM	SN	4	Negative LN; ANED
12	6	M	Abdomen	SMM	SN	1	ANED
13	44	F	R upper arm	SMM	SN	7	ANED; 2 other ASN favor SN
14	16	M	Back	SMM	SN	10	Negative LN; ANED
15	55	M	R mid back	SMM	SN	2	ANED
16	40	F	R upper arm	SMM	SN	11	Negative LN; ANED
17	9	M	R upper arm	SMM	SN	14	Negative LN; ANED
18	17	M	Chest	SMM	SN	1	Negative LN; ANED
19	54	F	R upper arm	SMM	SN	8	Negative LN; ANED
			R buttock	SMM	SN	9	Negative LN; ANED
20	44	F	R upper arm	SMM	SN	8	Negative LN; ANED
21	30	F	R shin	SMM	SN	14	ANED
22	57	M	R thigh	SMM	SN	12	ANED
23	46	M	R arm	SMM	SN	4	1 Positive LN-1 cell; ANED
24	54	F	R upper arm	SMM	SN	8	Negative LN; ANED

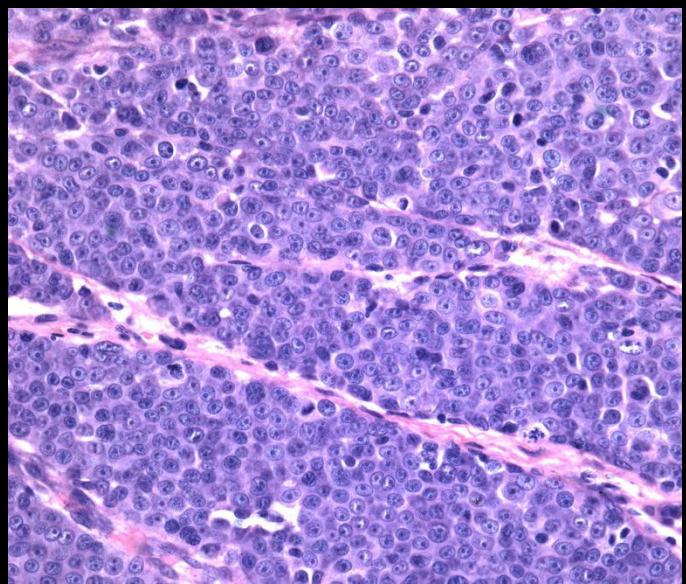
LN – Lymph Node; ANED – Alive, No Evidence of Disease; DOD – Dead of Disease

Case Study

- 36 year old pregnant woman presents with lesion on upper arm
- Excisional biopsy performed and determined to be malignant
- Insufficient margins taken for size of lesion
- No further treatment during pregnancy





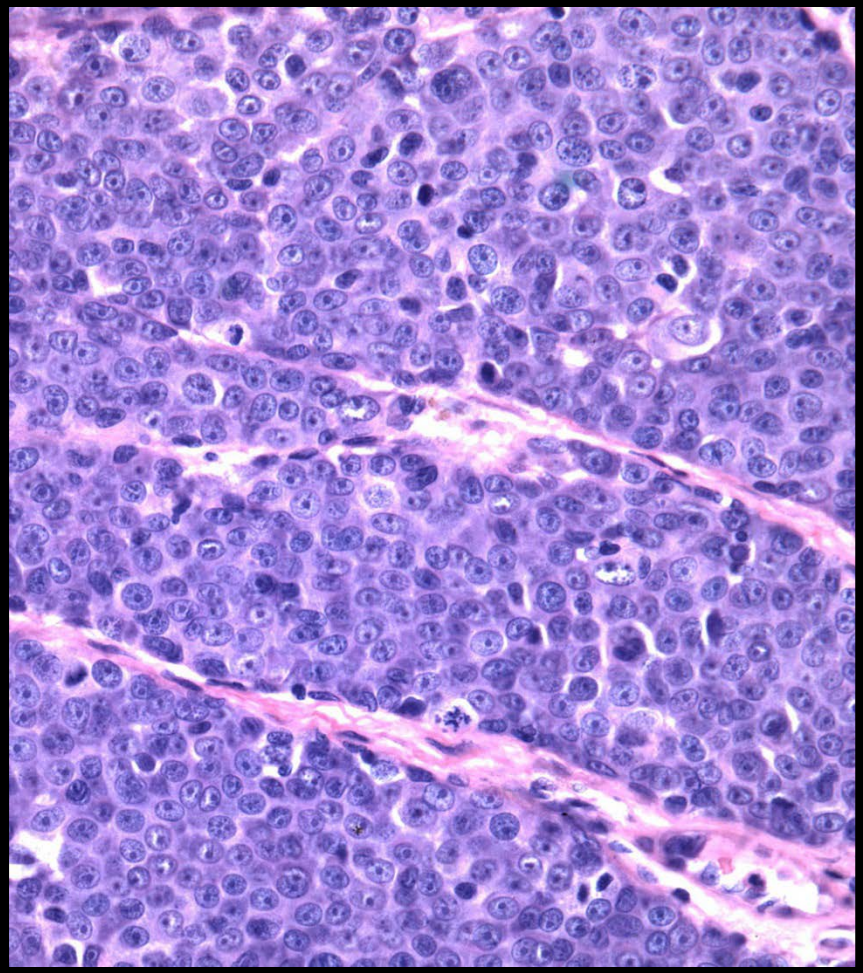


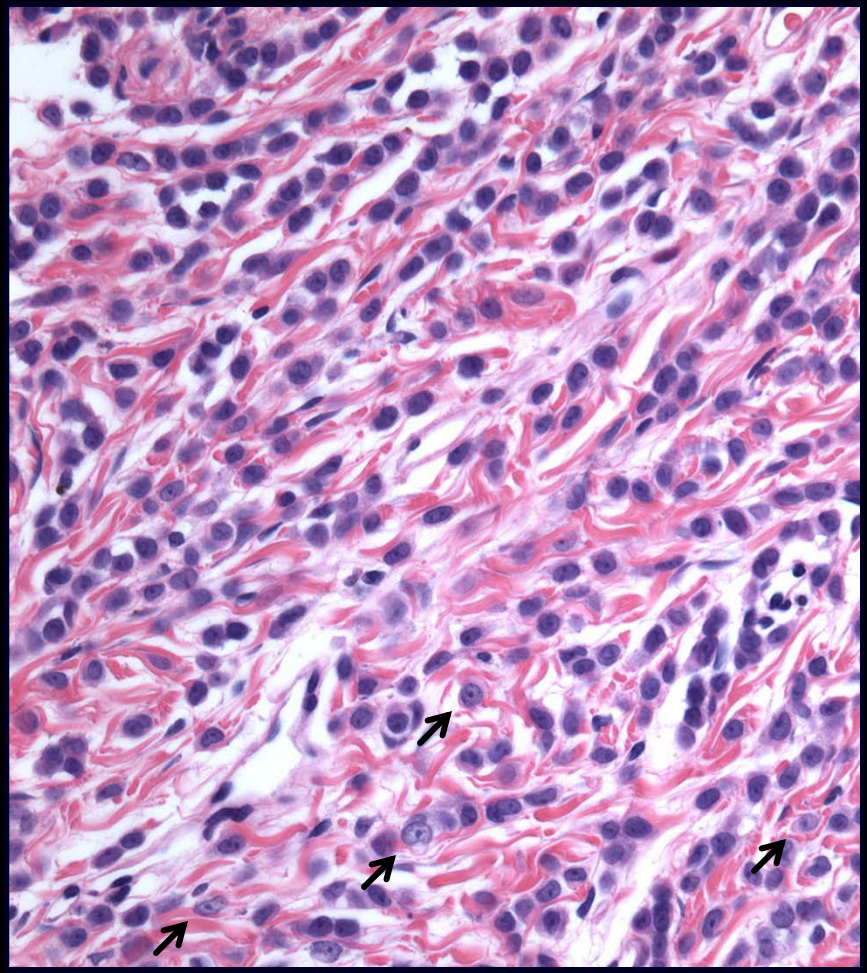
Case Study

Two months later, male baby born with multiple nevi

Mother

Baby





Metastases or Congenital Nevi?

Mass Spectrometry Analysis

Mother	Mass Spectrometry
Clinical Diagnosis, Malignant Melanoma	29/29 regions, Malignant Melanoma

Skin lesions Baby	Mass Spectrometry
Sample A: Indeterminate	9/9 regions, Spitz nevus
Sample B: Indeterminate	23/23 regions, Spitz nevus

Cells within lesions on baby contained *y* chromosome

Conclusions

- Evidence supports MALDI-based profiling as a useful clinical tool.
- Next-generation instrumentation is moving us closer to clinical applications of Imaging Mass Spectrometry.
  - Throughput
  - Spatial resolution
  - Expertise
- What’s needed?
  - A more elegant solution.
  - Continue form build clinical case for tissue based classification.

Mass Spectrometry Research Center

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