Molecular Imaging of Tissue Sections by MALDI MS

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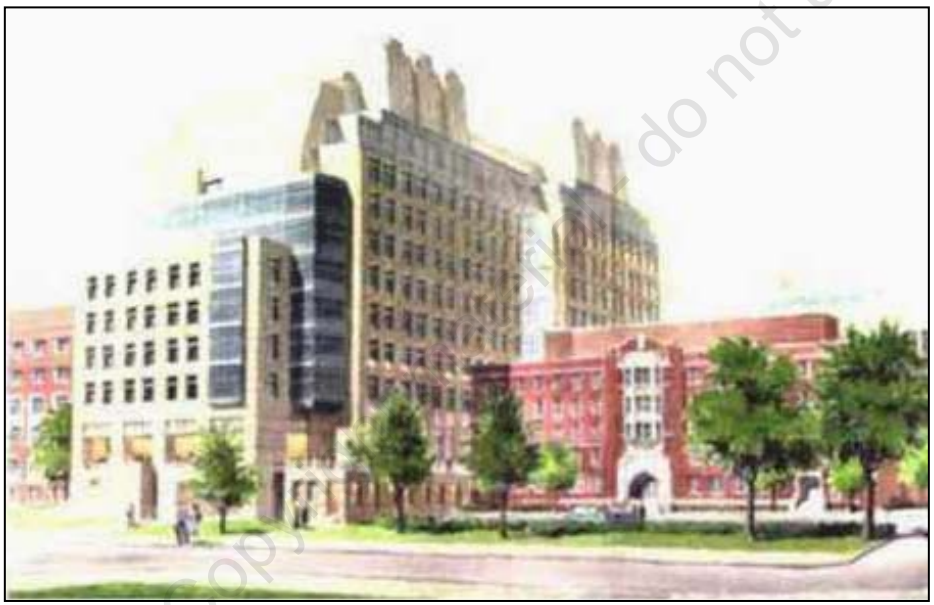

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Imaging Mass Spectrometry of Thin Tissue Sections

- **To obtain information on the local protein composition at any coordinate on the section**
- **To reconstruct 2-dimensional density maps (or images) for all of the signals detected**
- **To obtain molecular profiles and images indicative of health status**
- **Discovery tool to answer fundamental questions relevant to protein expression in normal and diseased tissues**

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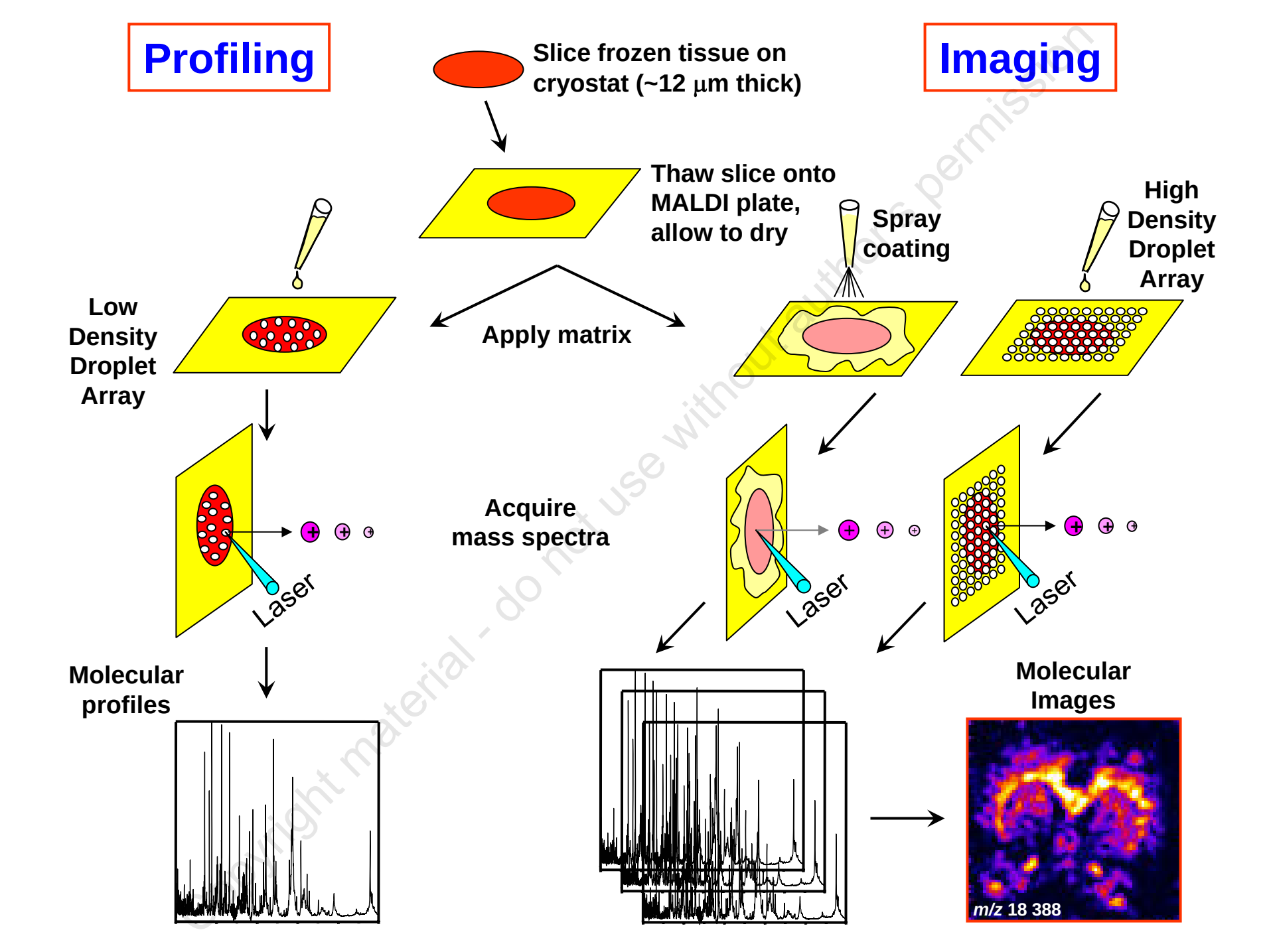

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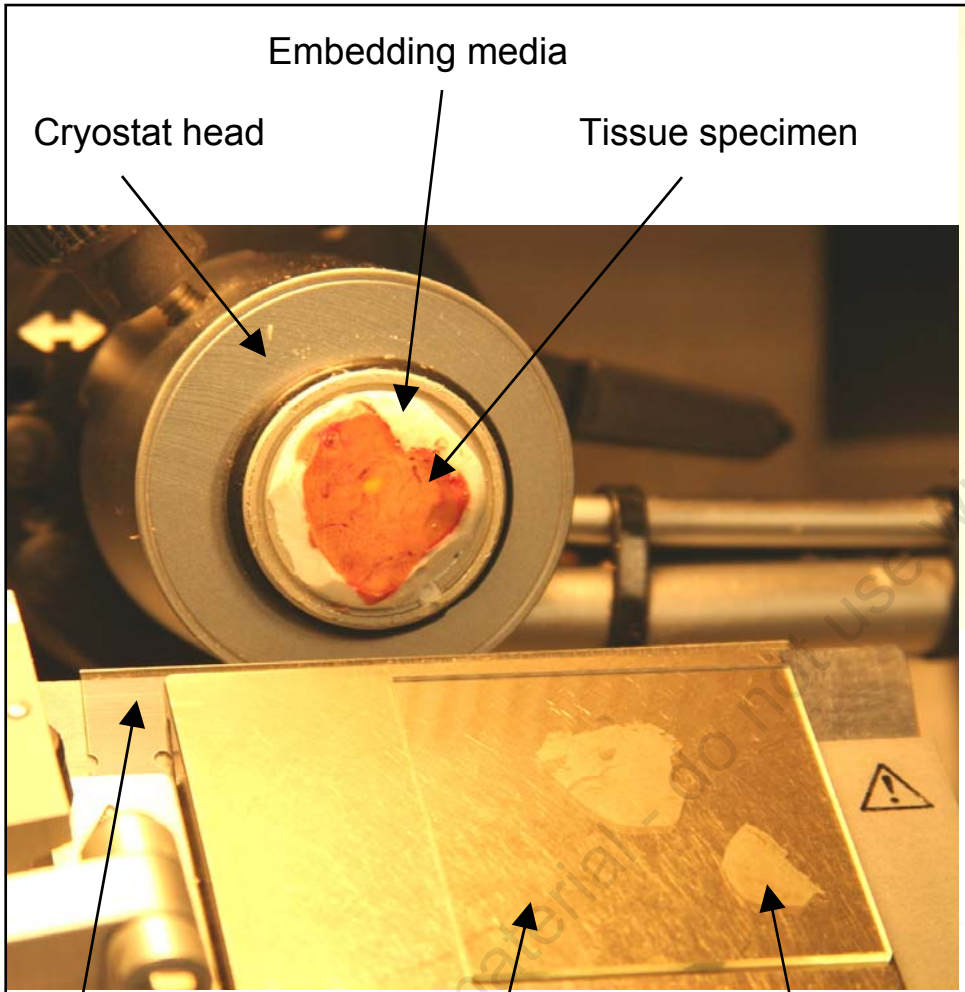
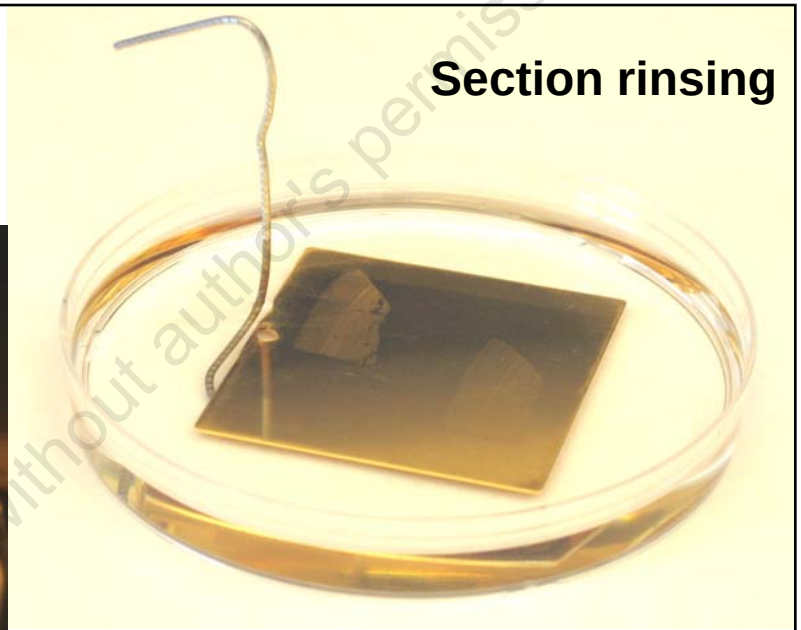
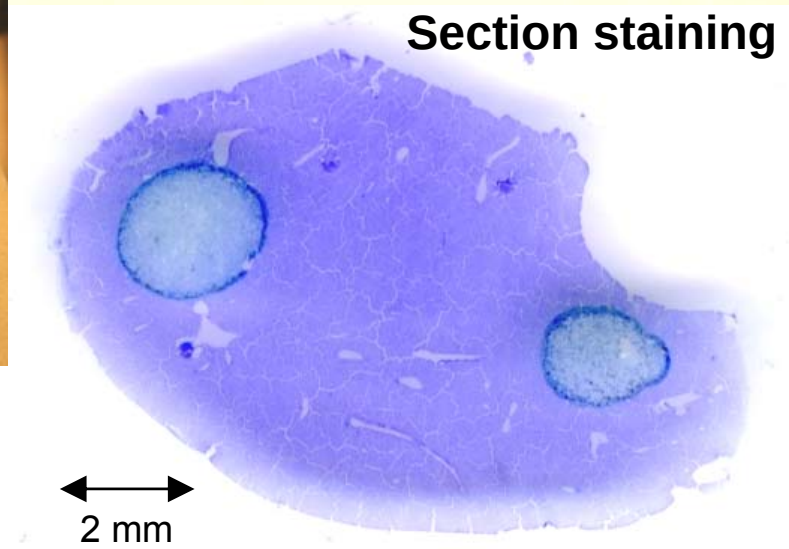
Optimizing tissue processing protocols







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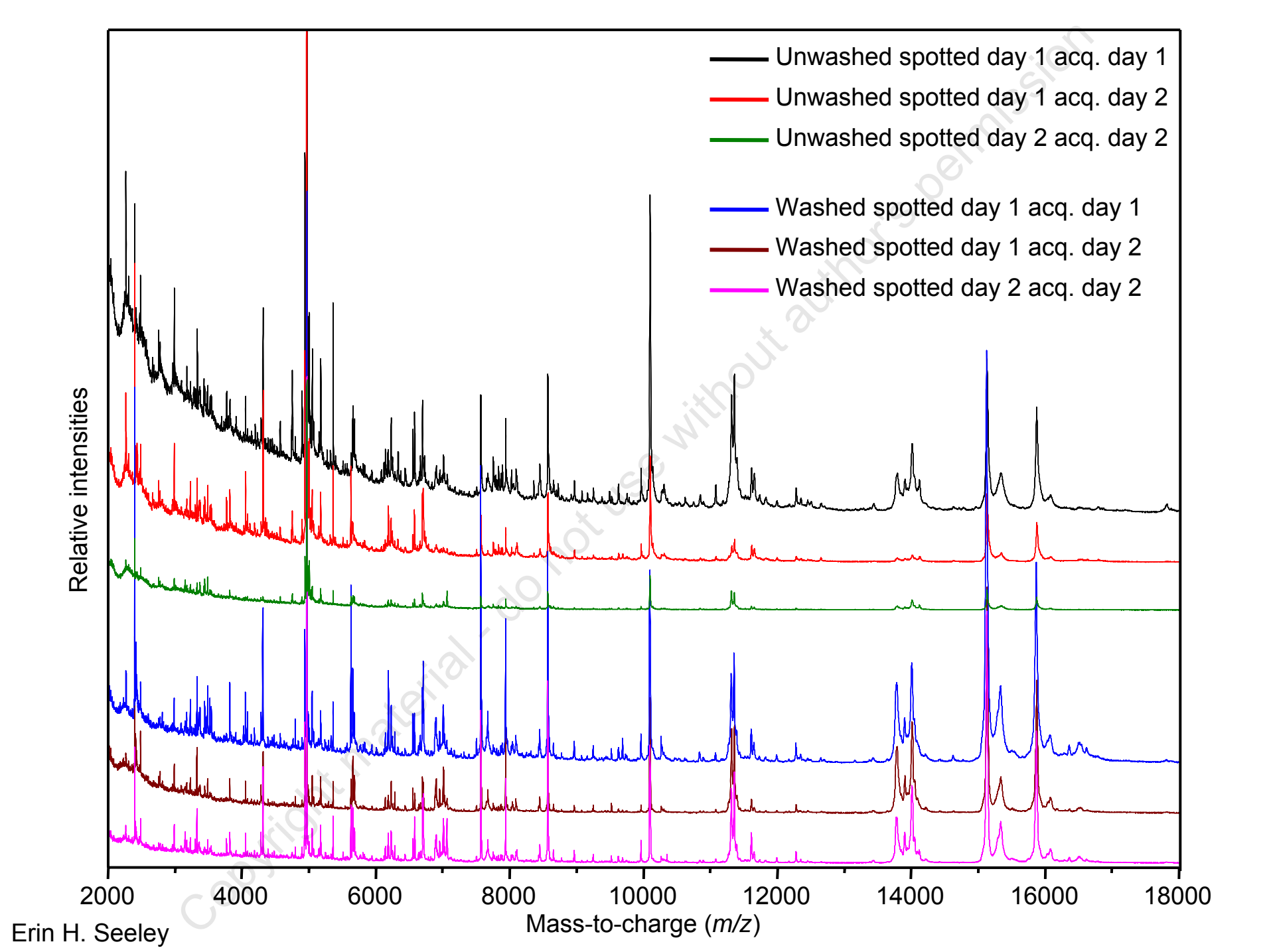
Chaurand *et al*. J. Proteome Res. 5, 2889-2900 (2006).



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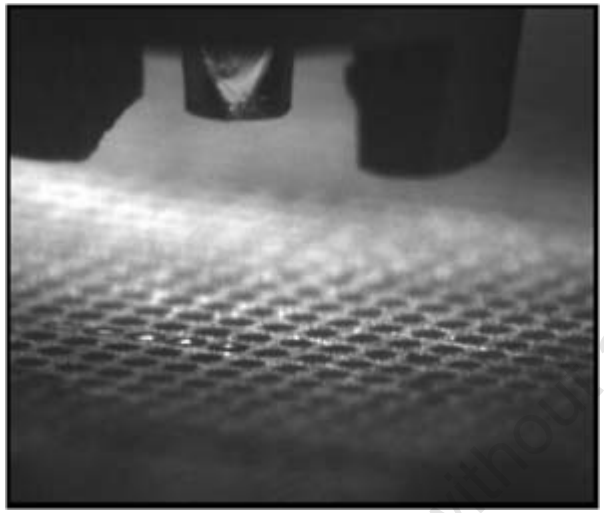
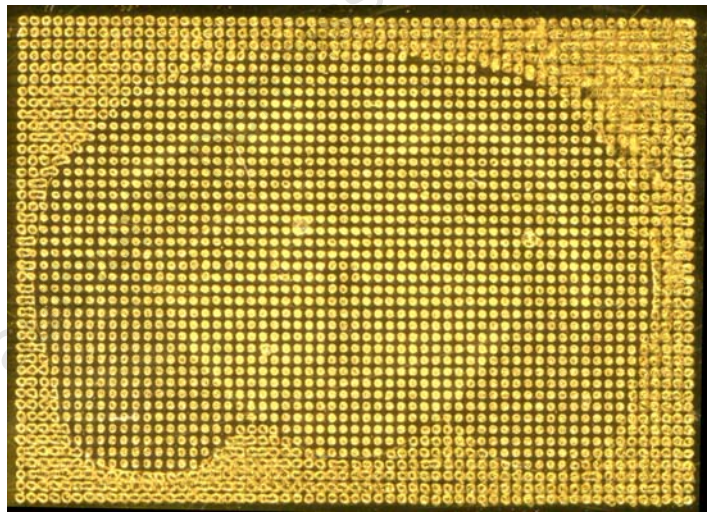
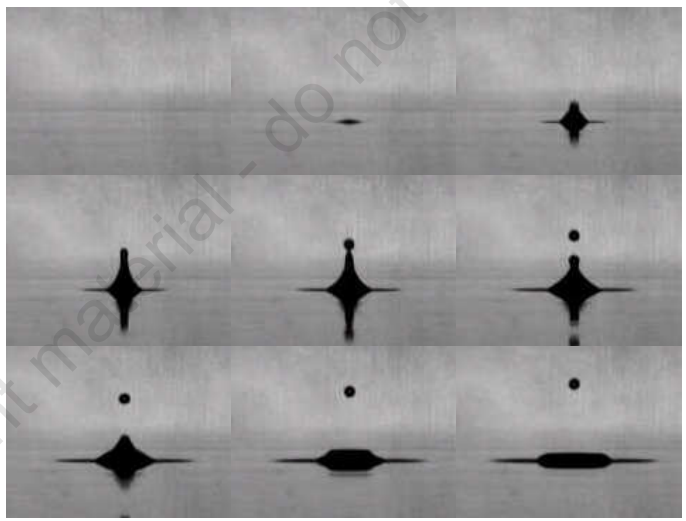
Erin H. Seeley

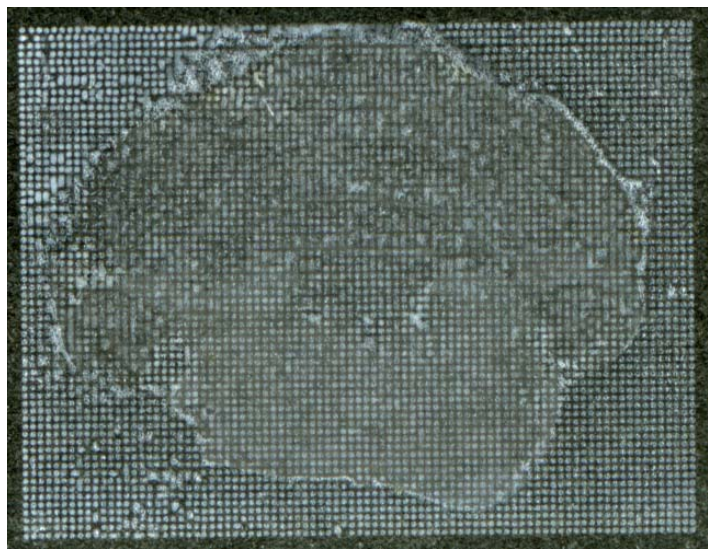
Shimadzu Chemical Inkjet Printer – Piezo print head





Labcyte Portrait 630 MALDI reagent multi-spotter – Acoustic ejection





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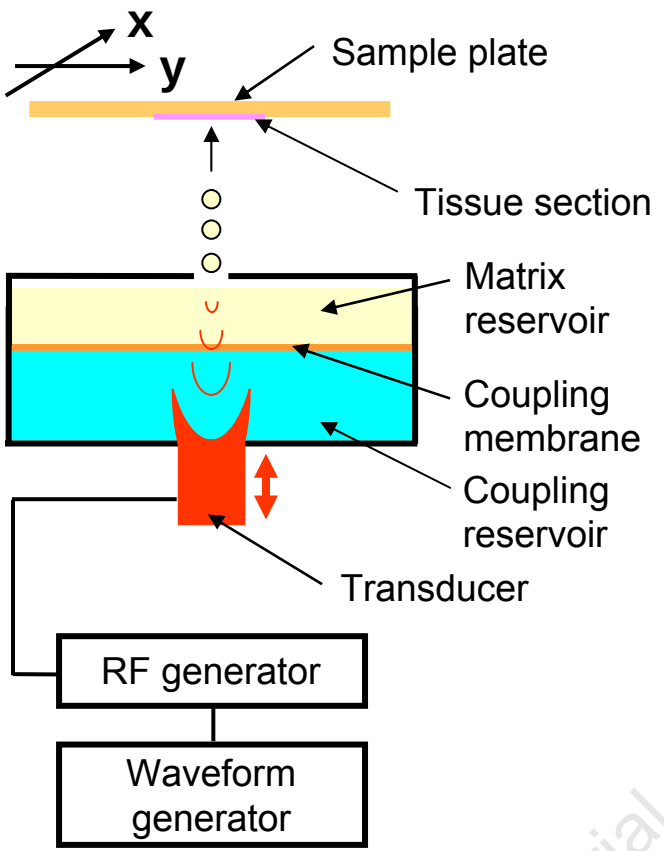
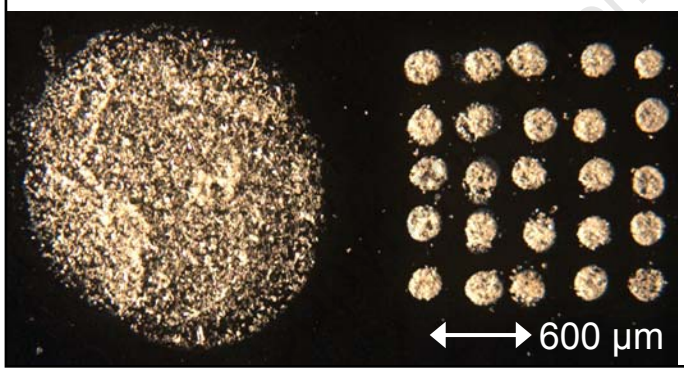
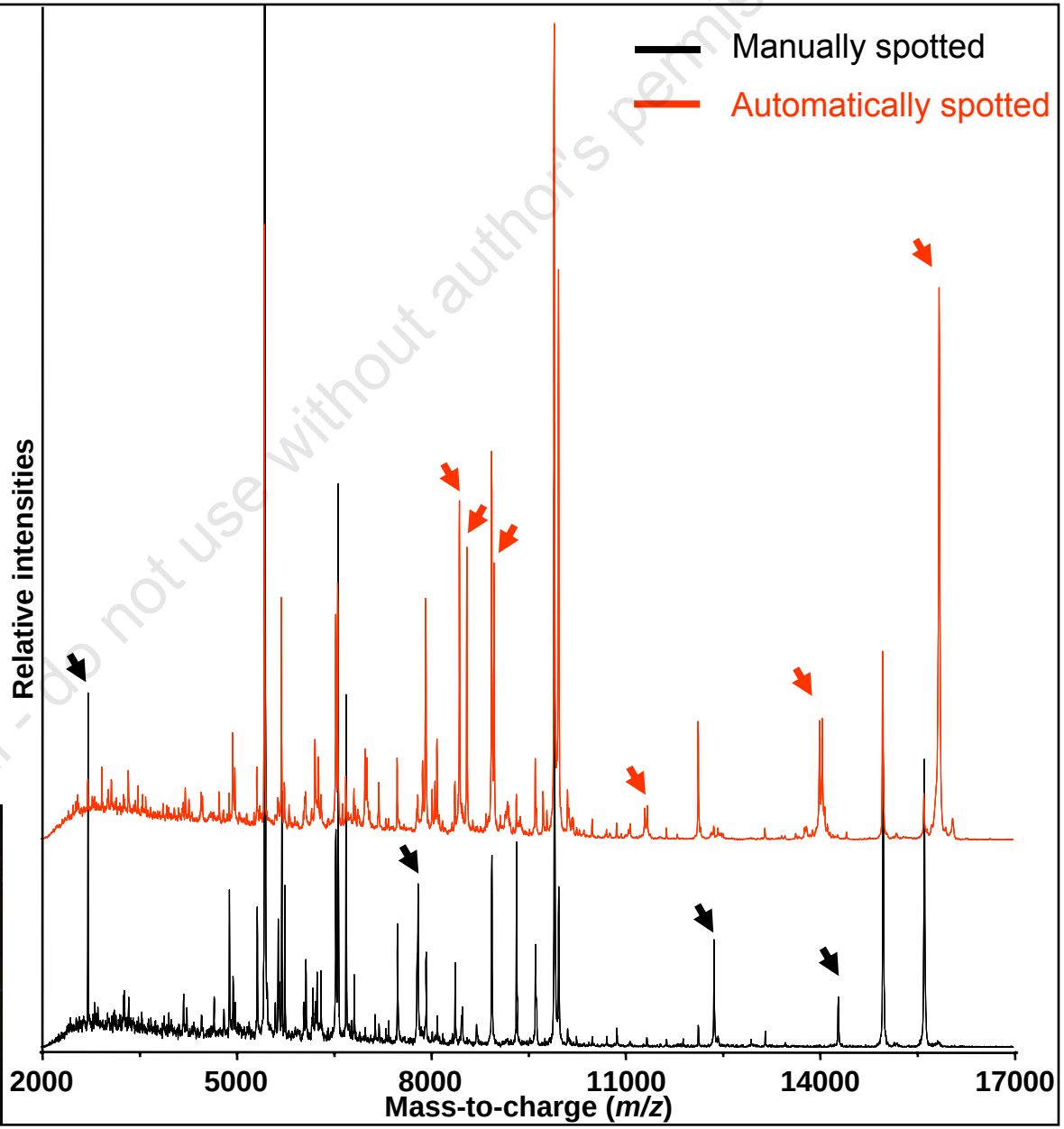
Automated Matrix Deposition







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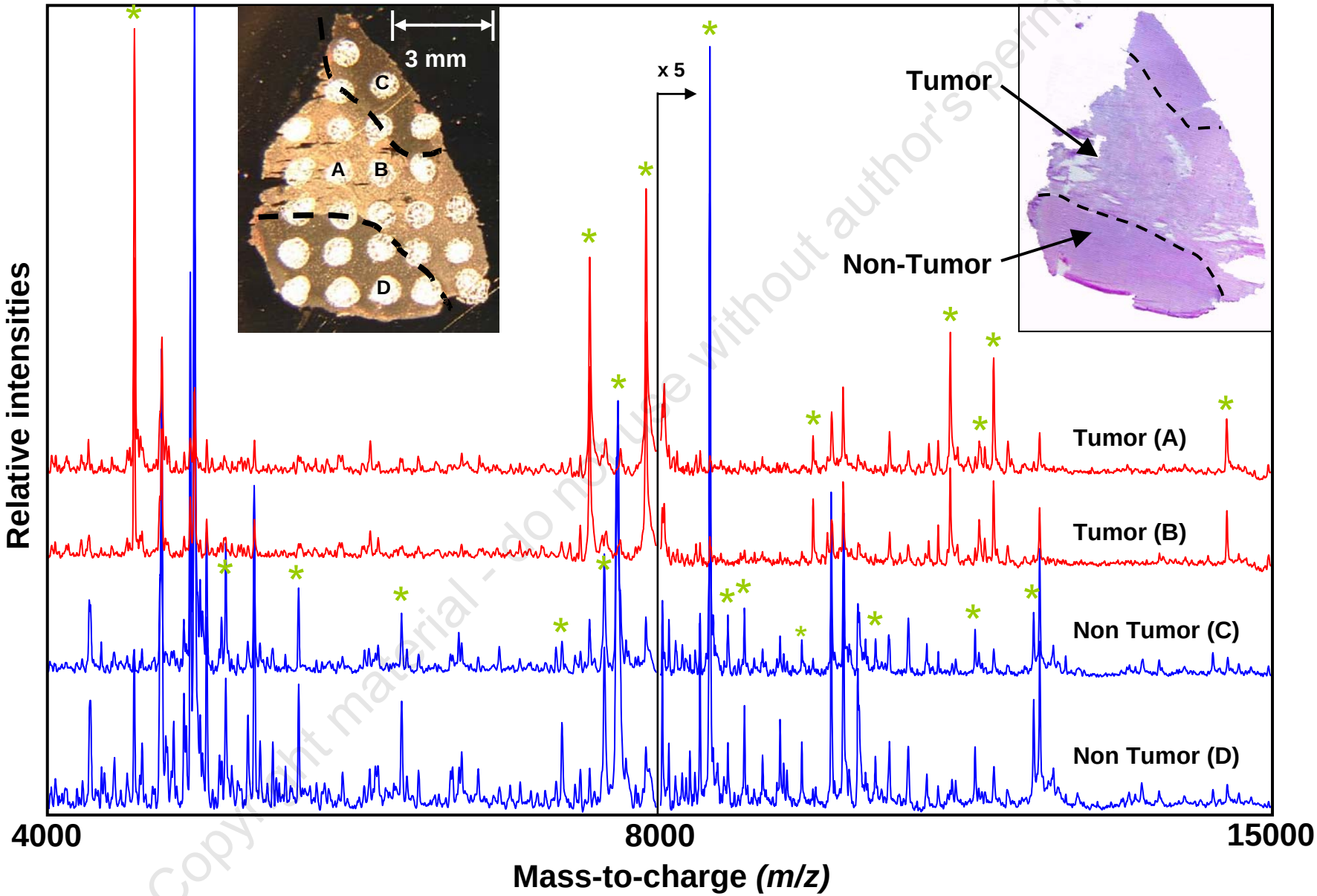
Profiling of a glioma biopsy



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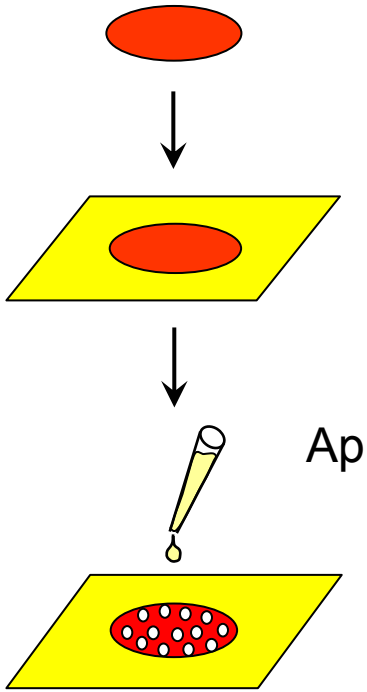
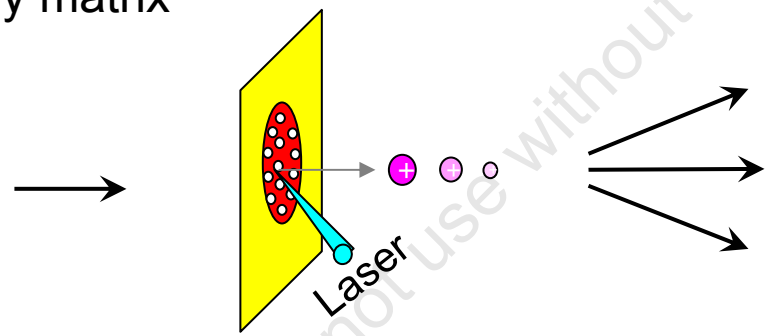
Workflow for MALDI MS Profiling of Clinical Biopsies

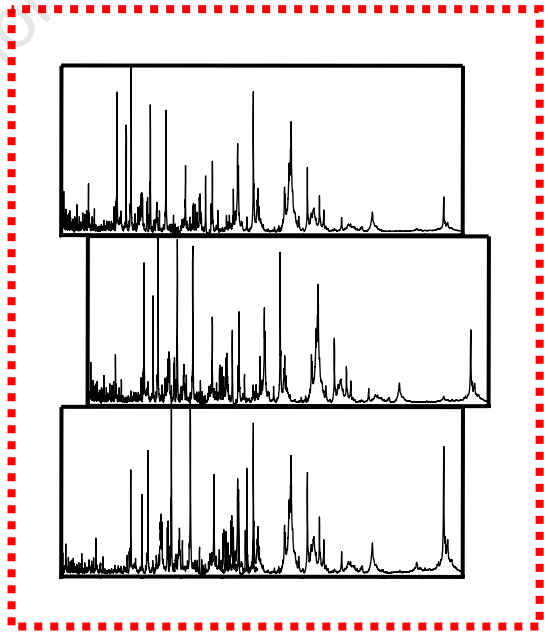


Cut and mount sections
onto MALDI plate. Rinse

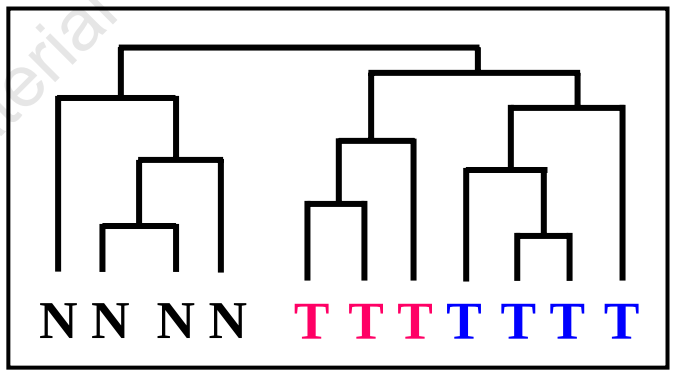
Apply matrix

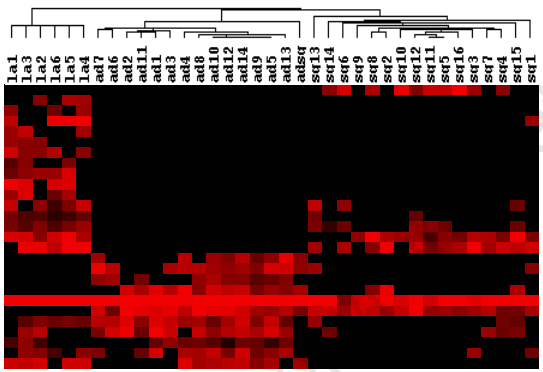


Acquire Protein profiles



Pre-processing





Data mining & Classification

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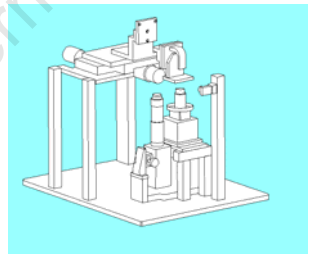
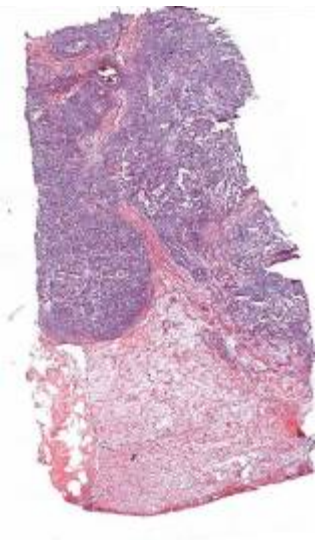
Integrated Strategy for Profiling Histo-pathologically Significant Sites

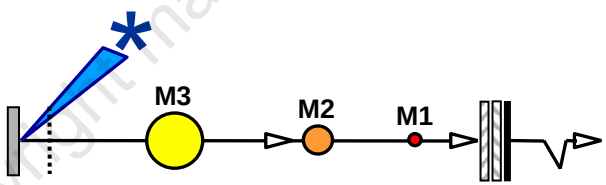
Histological Analysis

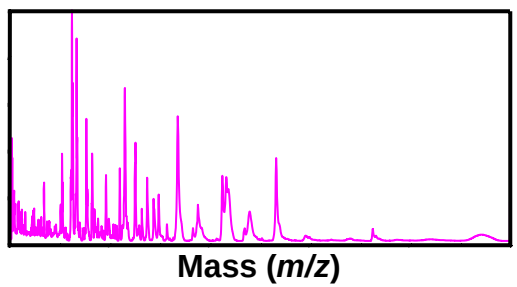
Matrix Spotting





Mass Spectrometry





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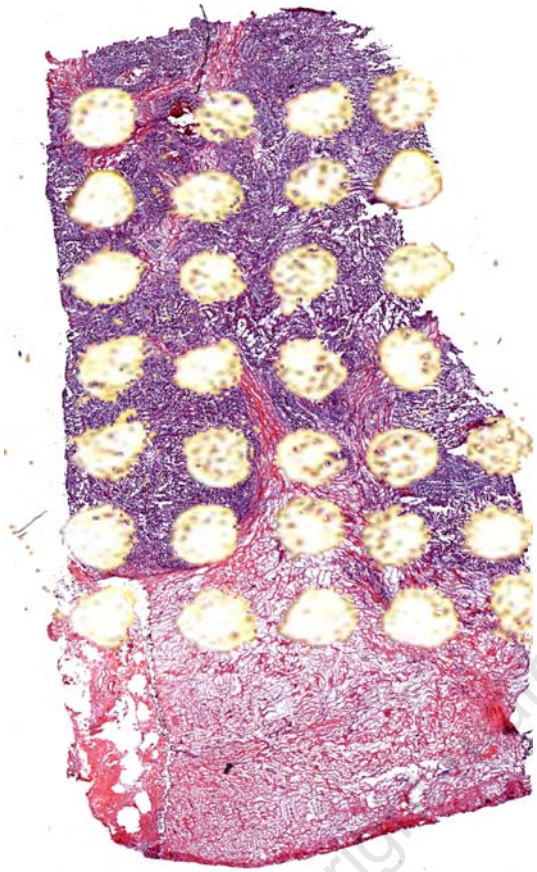
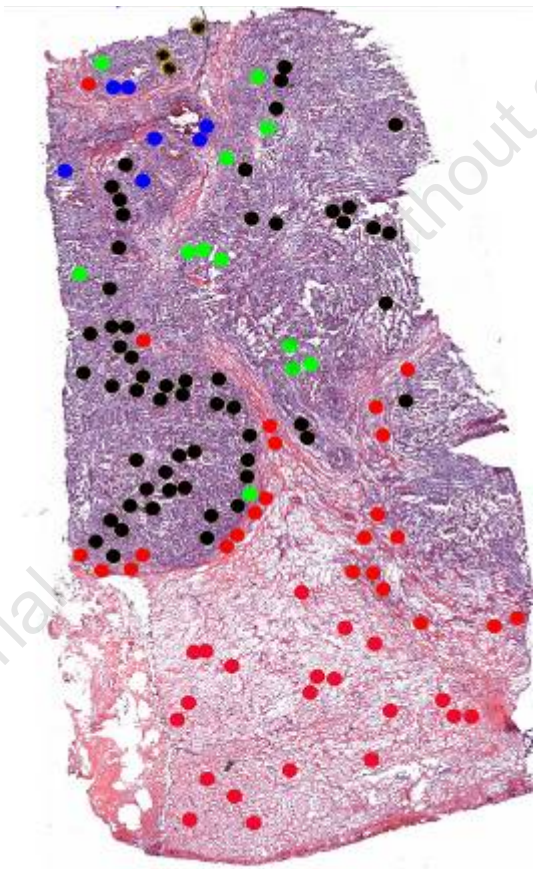
Cornett *et al*, Mol. Cell Proteomics, 5, 1975-1983 (2006).

Histology Directed Matrix Deposition

From this….
Arbitrary large spots



To this…pathologist-selected features



Breast tumor Biopsy:
Pathology Coded ID's

Blue -	DCIS
Red -	Stroma
Green -	Inflammatory Cells
Black -	IMC

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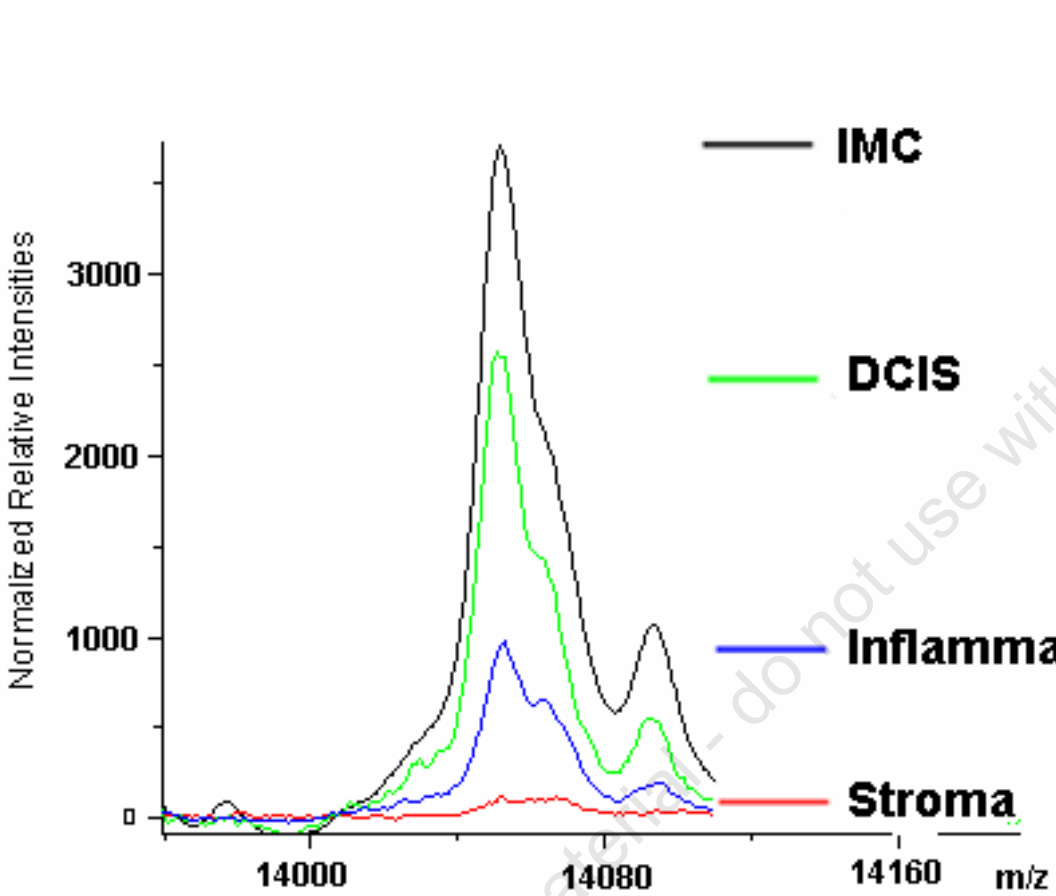
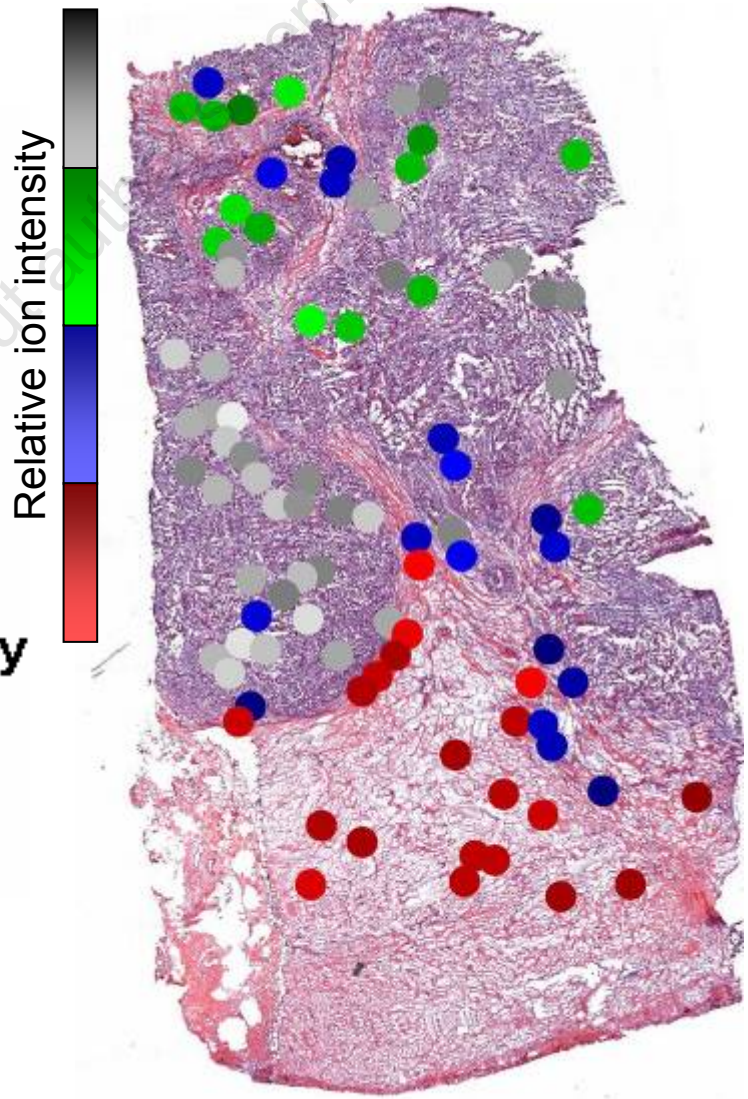
Cornett *et al*, Mol. Cell Proteomics, 5, 1975-1983 (2006).

DCIS and IMC Exhibit Higher Abundance of *m/z* 14,098





- Average spectrum representation for each annotated cell type

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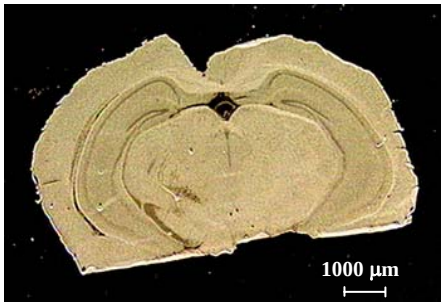
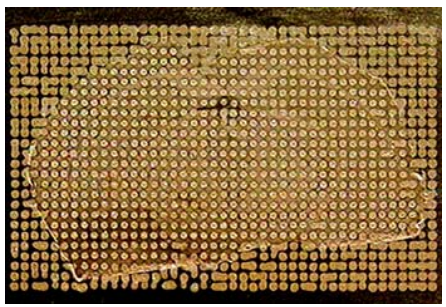
Imaging of a Mouse Brain Section

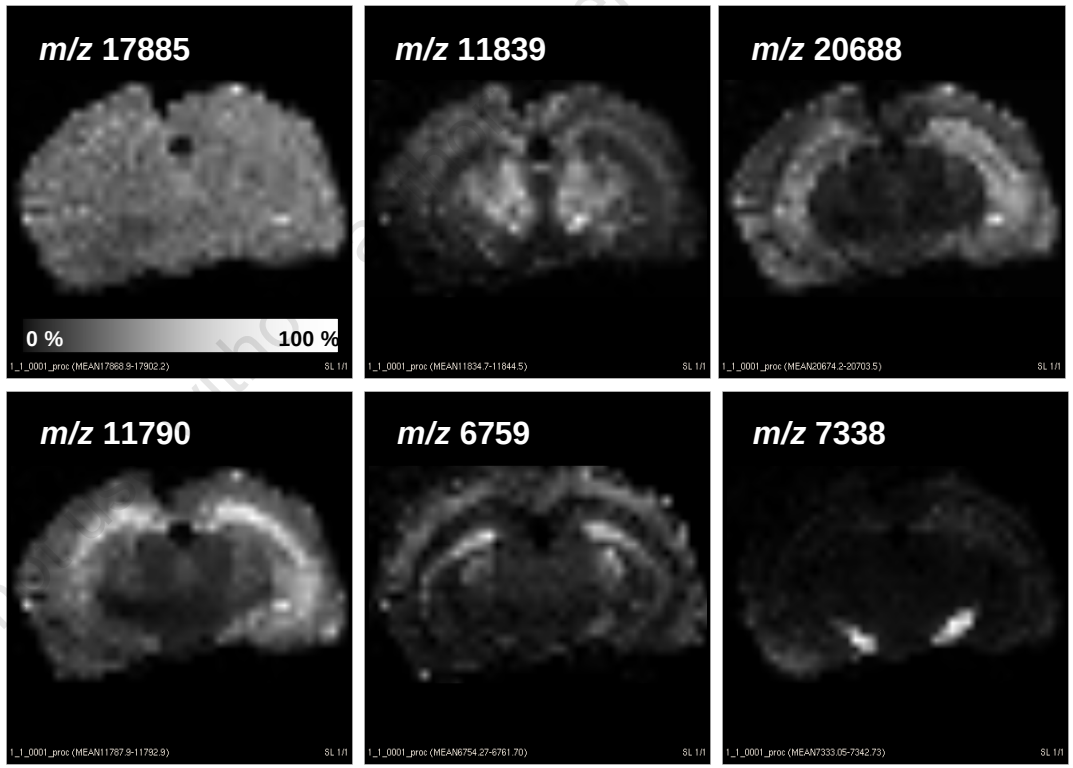


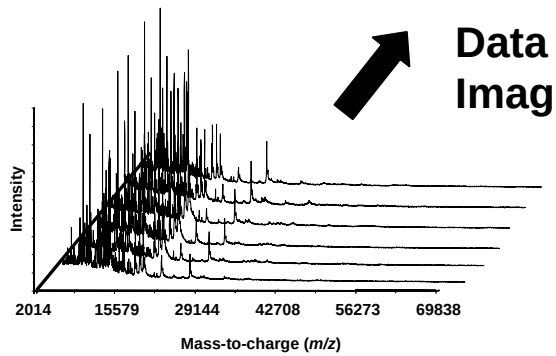
Matrix printing



Create custom plate file

Automated data acquisition





Data processing and Image reconstruction

Aerni *et al*., Anal. Chem. 78, 827-834 (2006).

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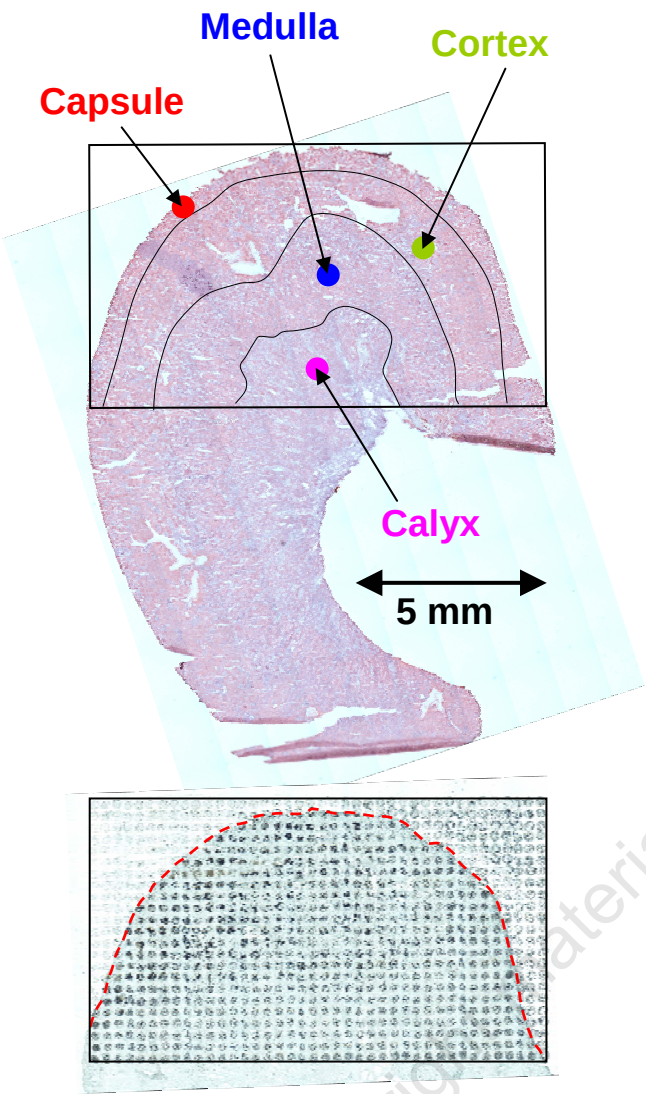
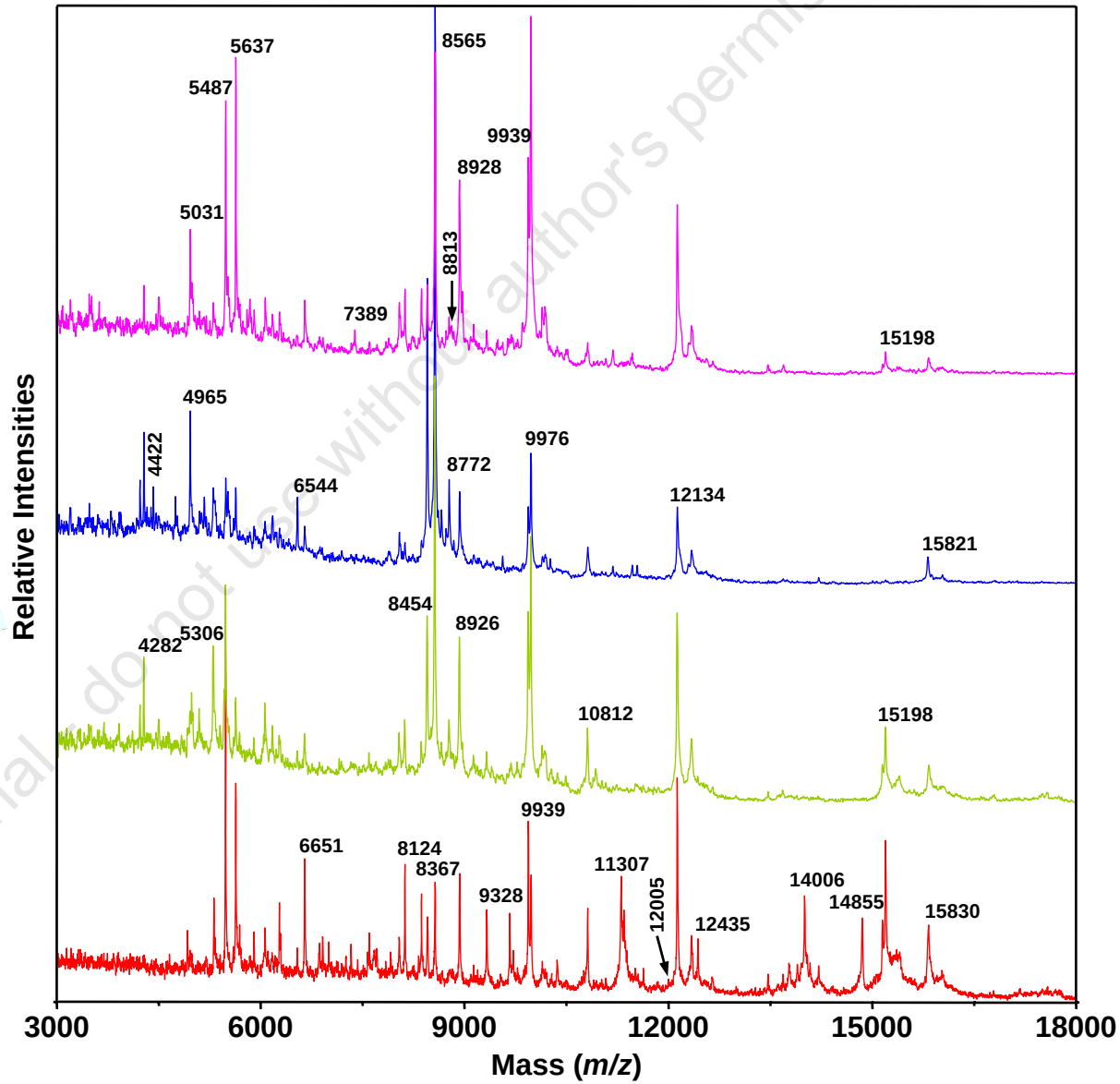
Rat Saggital Kidney Section



Printing resolution: 300 µm



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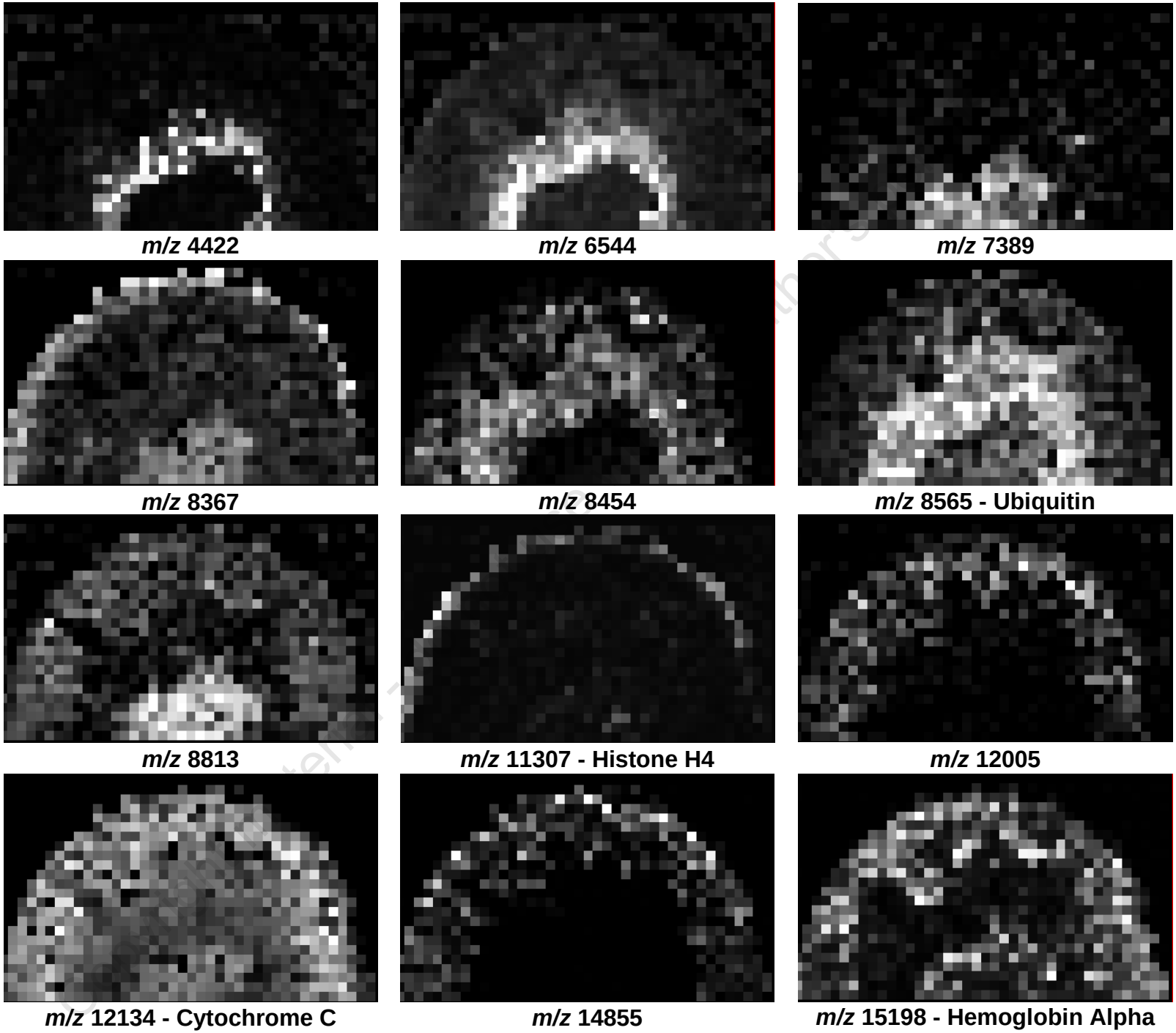
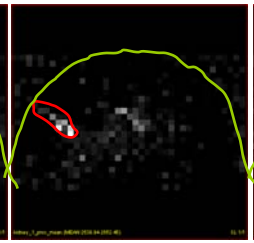
Chaurand *et al.*, Curr. Opin. Biotechnol. 17 (2006) 431-436.


100%
0
m/z 4422
m/z 6544
m/z 7389
m/z 8367
m/z 8454
m/z 8565 - Ubiquitin
m/z 8813
m/z 11307 - Histone H4
m/z 12005
m/z 12134 - Cytochrome C
m/z 14855
m/z 15198 - Hemoglobin Alpha

Biomarker discovery

m/z 2207.3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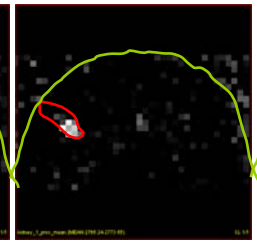
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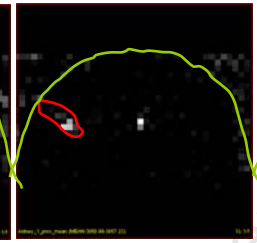
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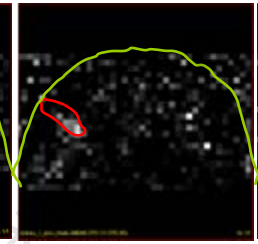
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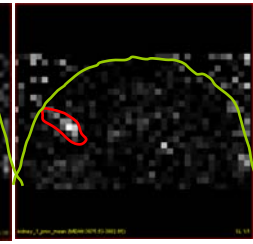
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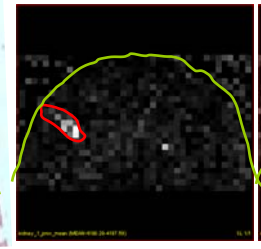
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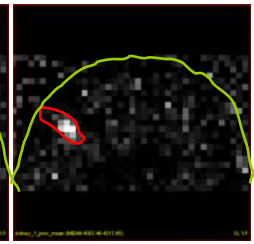
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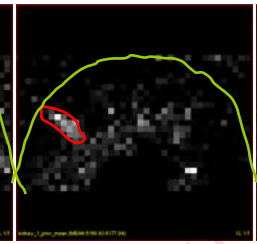
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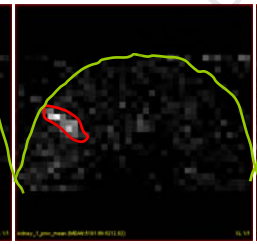
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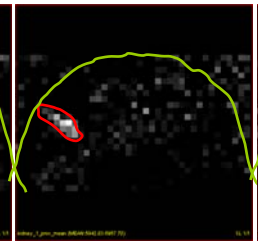
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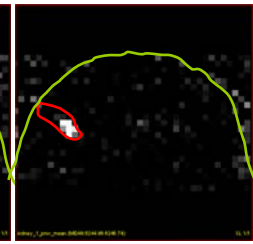
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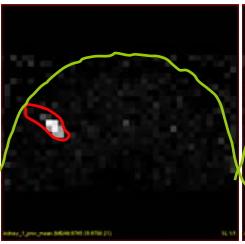
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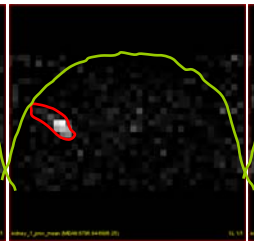
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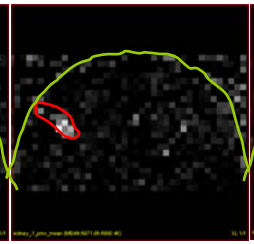
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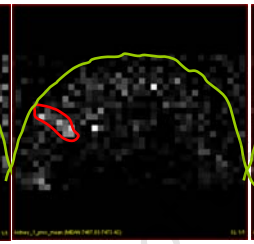
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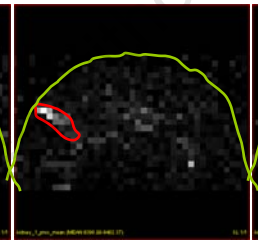
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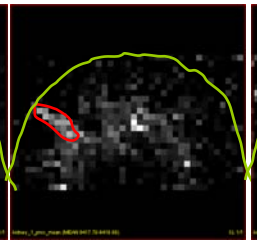
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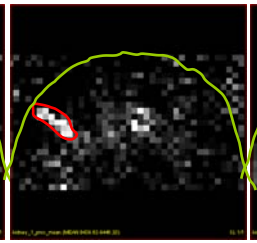
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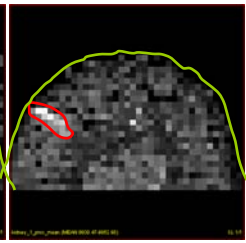
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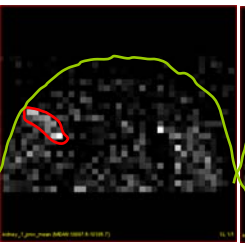
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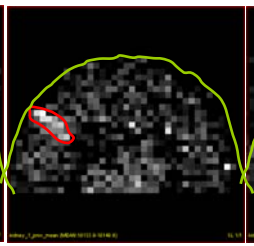
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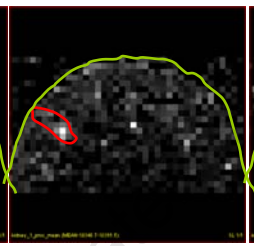
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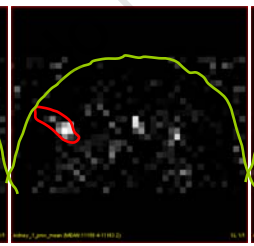
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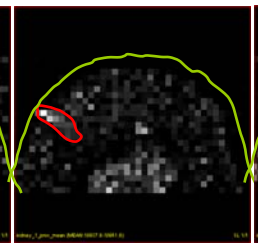
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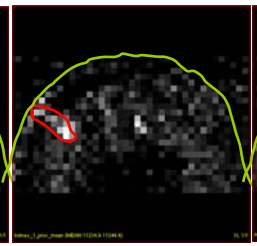
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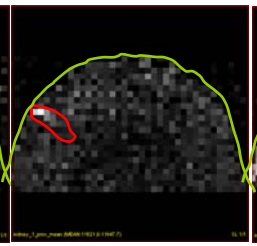
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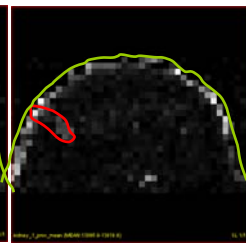
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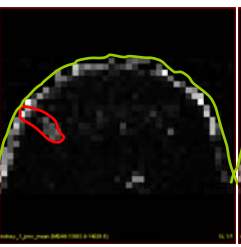
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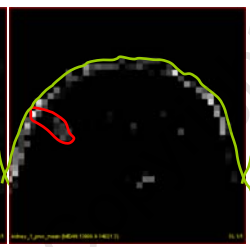
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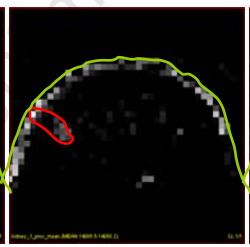
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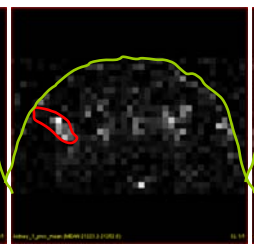
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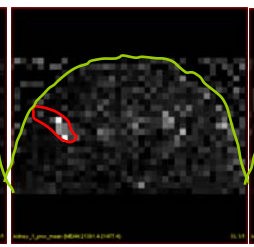
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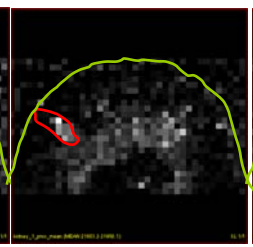
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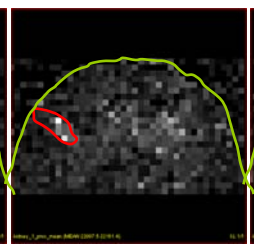
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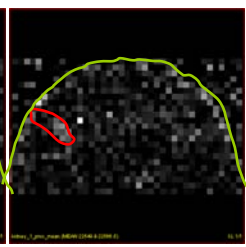
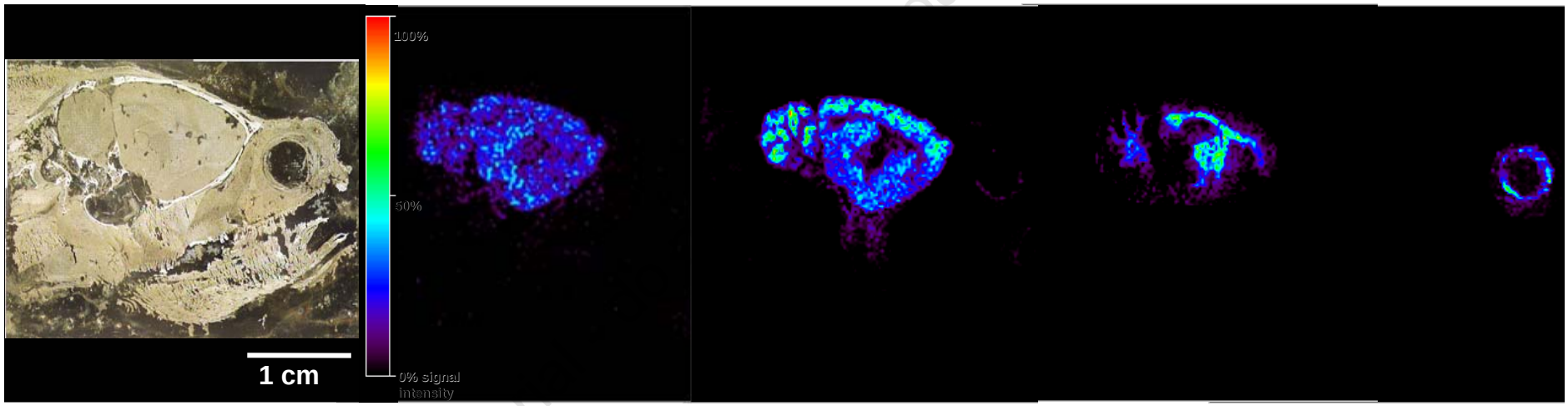

m/z 22561

Whole Rat Sagittal Section Imaging

Rat Head Subsection



Optical image | Brain *m/z* 20736 | White matter *m/z* 10271 | Corpus Callosum *m/z* 5978 | Eye socket *m/z* 6858

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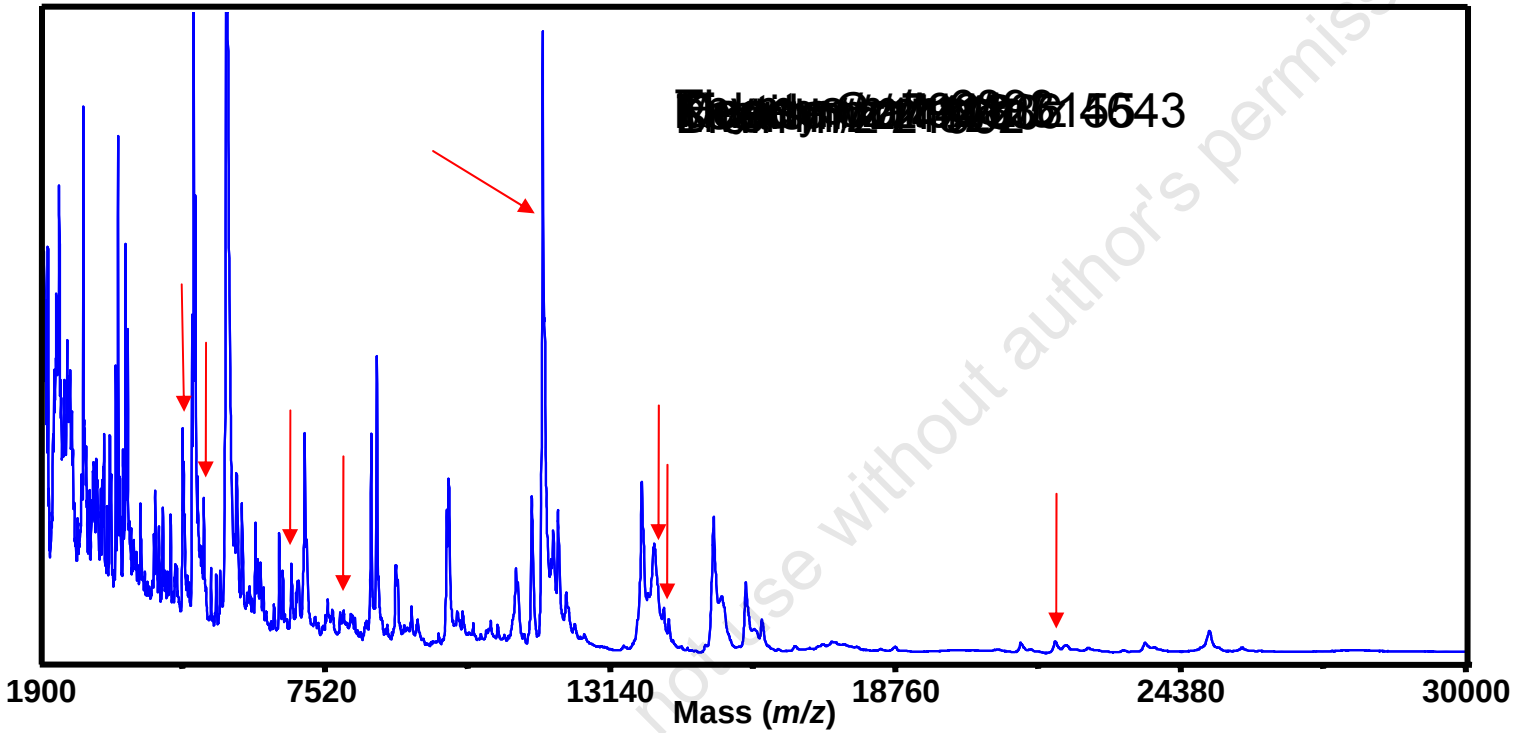
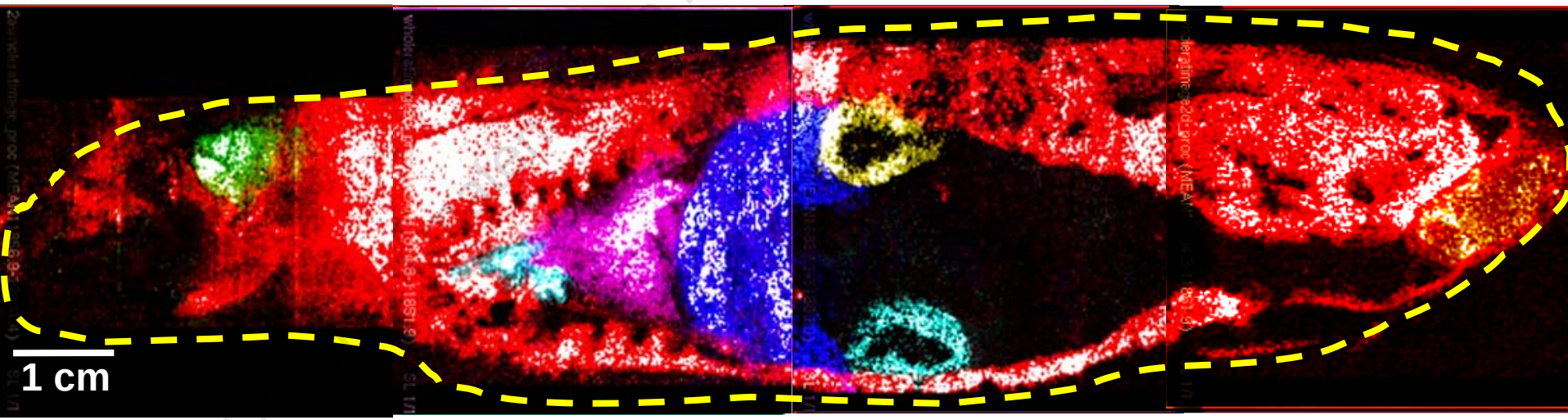
Khatib-Shahidi *et al*., Anal. Chem. 78: 6448-6456 (2006).

Whole Rat Imaging

Average Ion Spectrum for Whole Rat Sagittal Section





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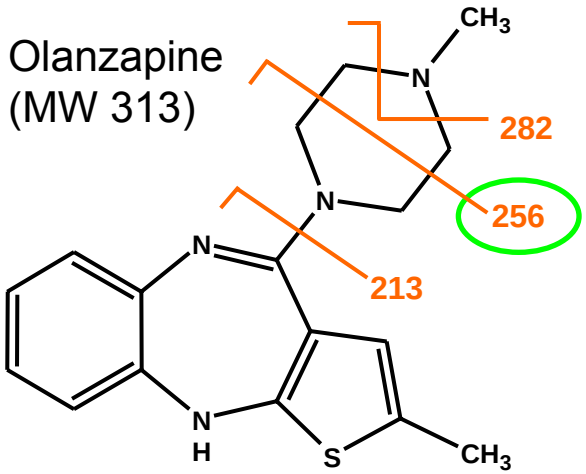
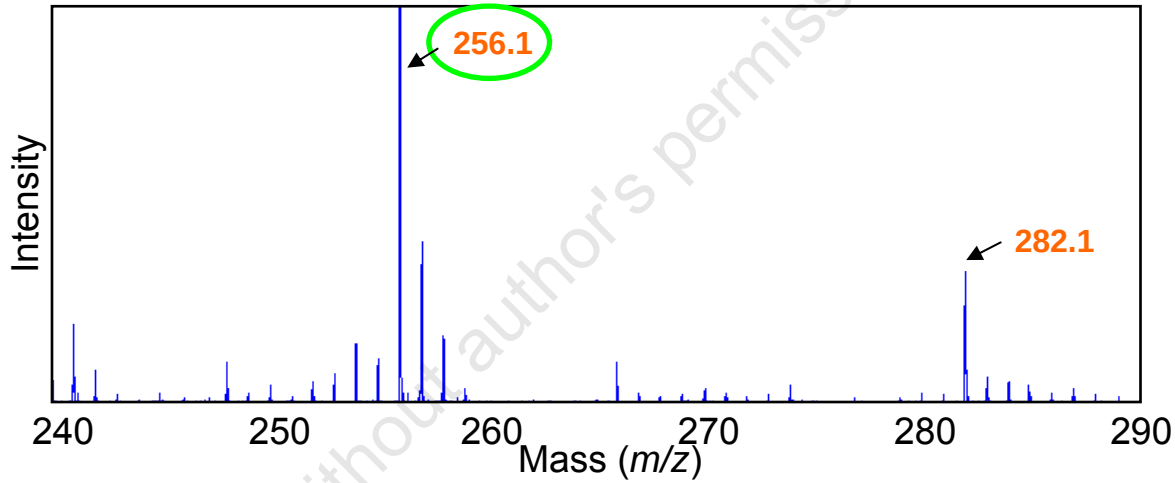
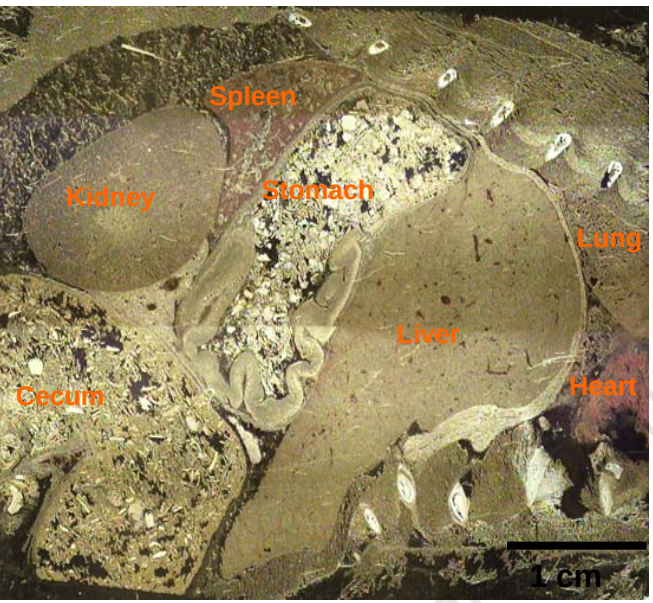
Khatib-Shahidi *et al*., Anal. Chem. 78: 6448-6456 (2006).

Drug Imaging: Olanzapine

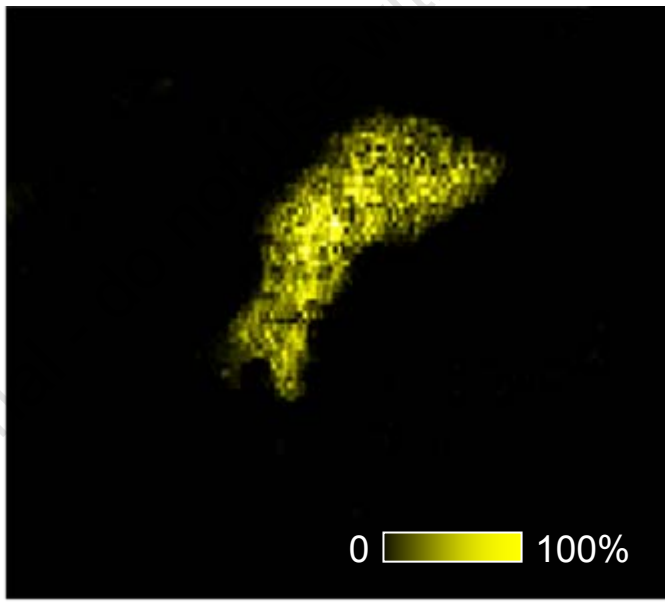
Olanzapine
(MW 313)



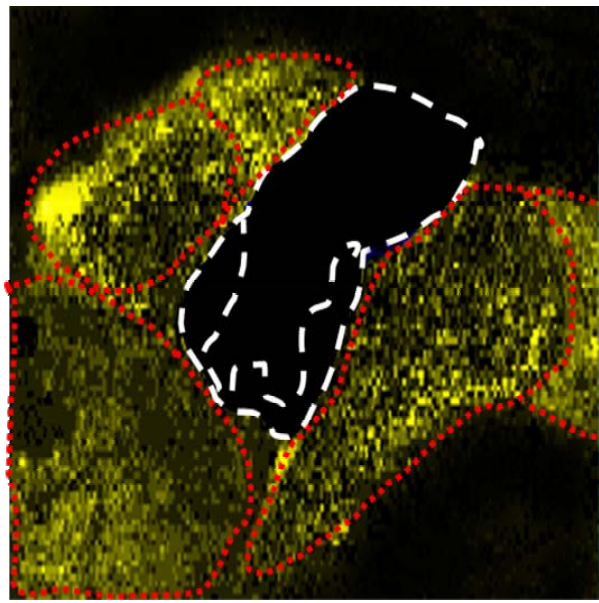




Optical image of orally dosed (8 mg/mL) rat abdomen



MALDI MS/MS Image of OLZ (*m/z* 256) in stomach 2 hours post-dose



MS/MS Image of OLZ, 10x signal intensity with stomach signals masked

Sheerin Khatib-Shahidi

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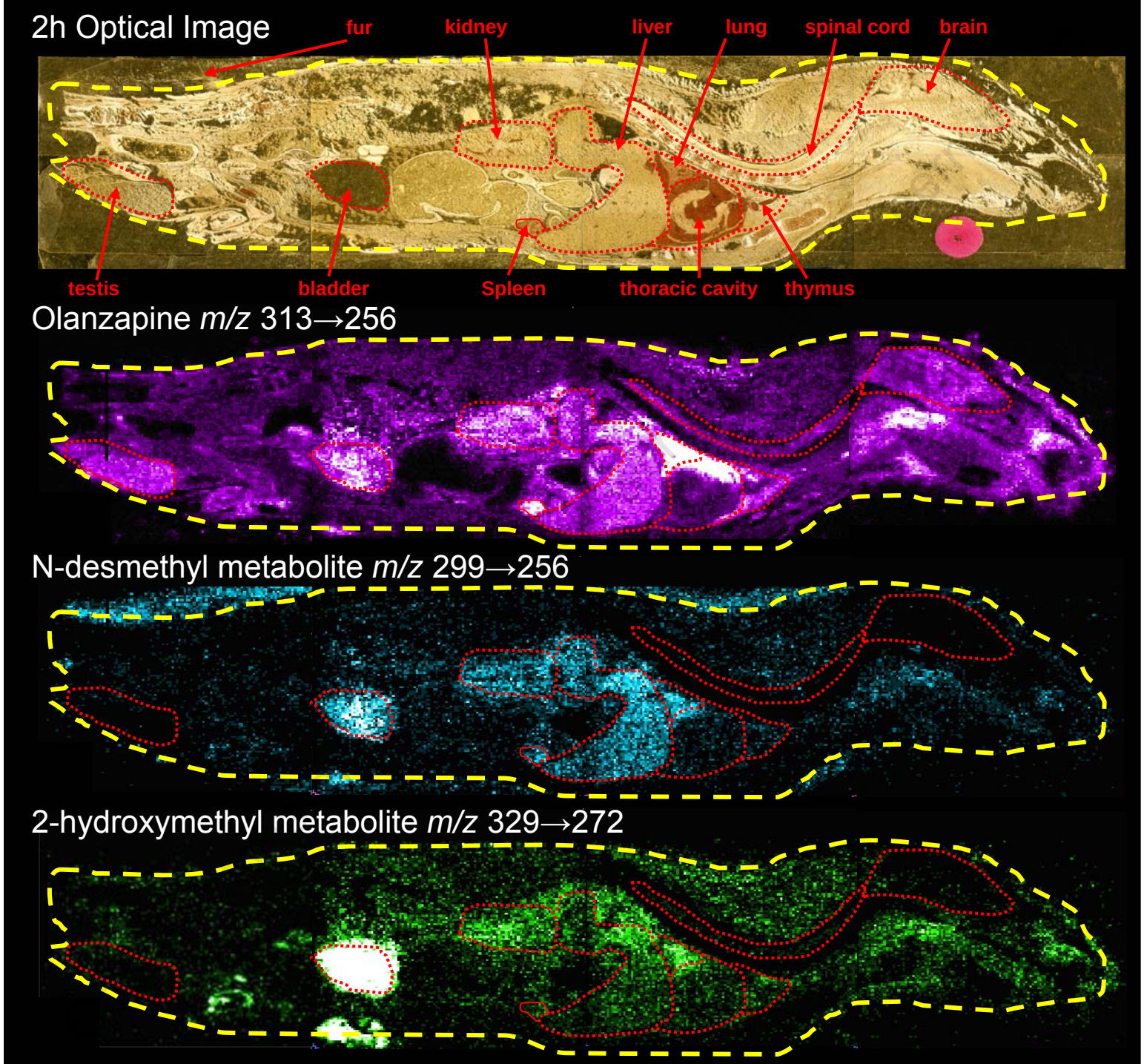
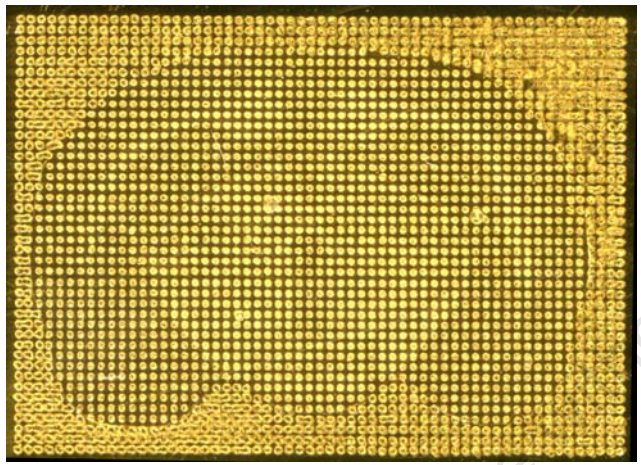

2h Optical Image
fur
kidney
liver
lung
spinal cord
brain
testis
bladder
Spleen
thoracic cavity
thymus
Olanzapine m/z 313→256
N-desmethyl metabolite m/z 299→256
2-hydroxymethyl metabolite m/z 329→272

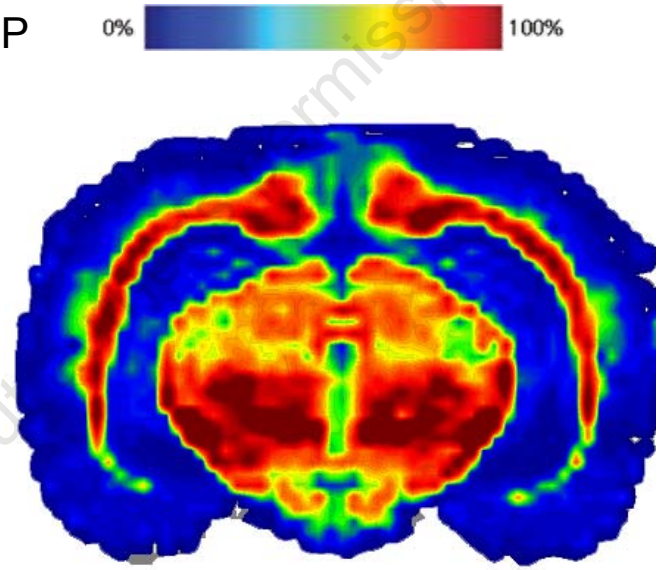
On-tissue trypsin digestion: Rat brain

Section 1: IMS of proteins

Matrix printing - SA - Shimadzu CHIP

Data acquisition - Bruker Ultraflex II





Myelin basic protein
(MBP, MW 14134)

?

Intensity

4964
5487
5637
6278
6651
6721
7539
8126
8369
8568
8931
9943
9983
10660
11315
12140
14135
15205
15863

3000
6000
9000
12000
15000
18000

Mass (*m/z*)

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Reid Groseclose et Al, J. Mass Spectrom. 42, 254-262 (2007).

On-tissue trypsin digestion: Rat brain

Section 2: IMS of tryptic peptides

Digestion with trypsin - Shimadzu CHIP

Matrix printing - 2,5-DHBA – Shimadzu CHIP

Data acquisition - Bruker Ultraflex II (rTOF)

MBP

m/z 699.47

m/z 726.47

m/z 1067.60

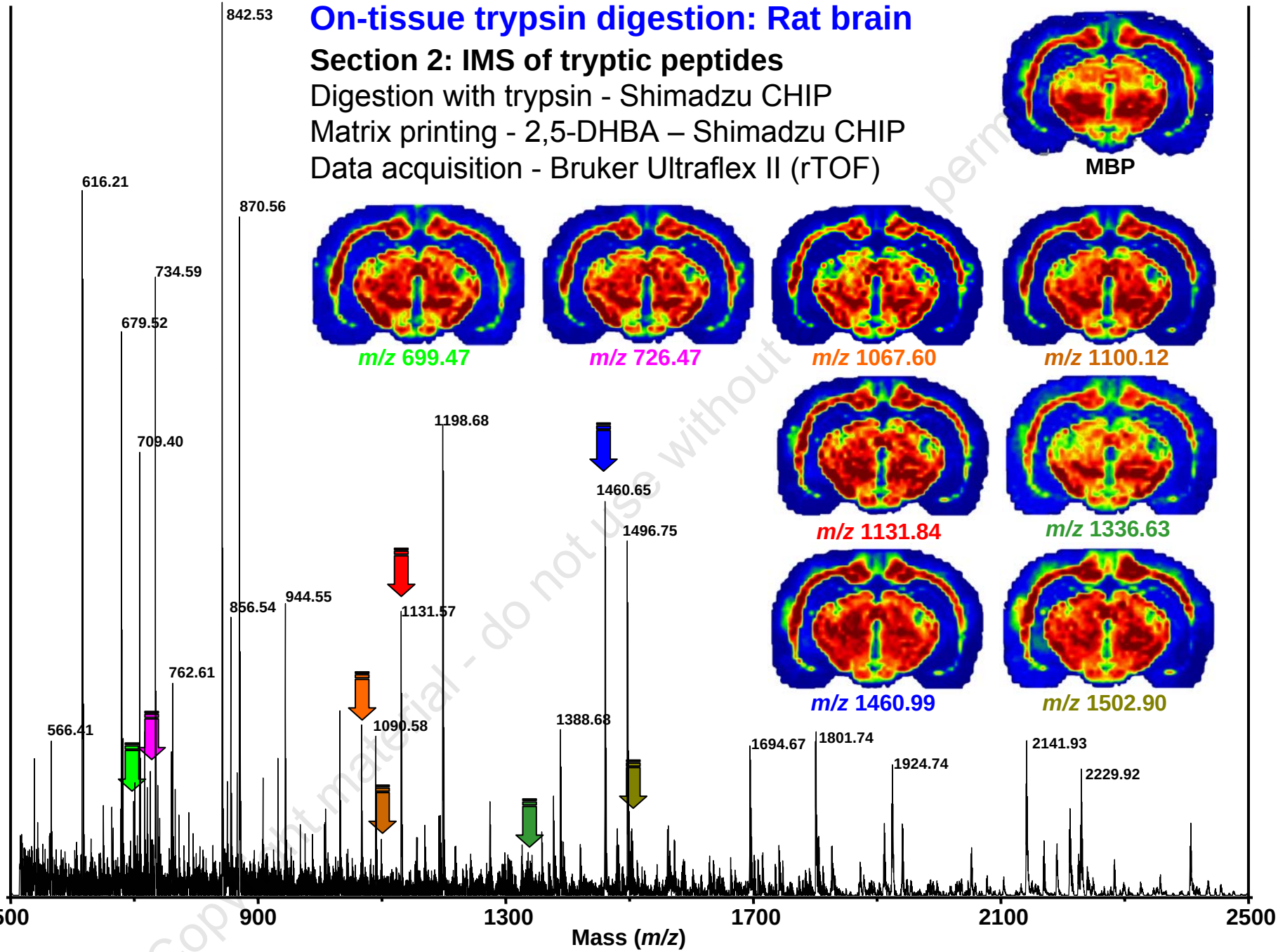
m/z 1100.12

m/z 1131.84

m/z 1336.63

m/z 1460.99

m/z 1502.90



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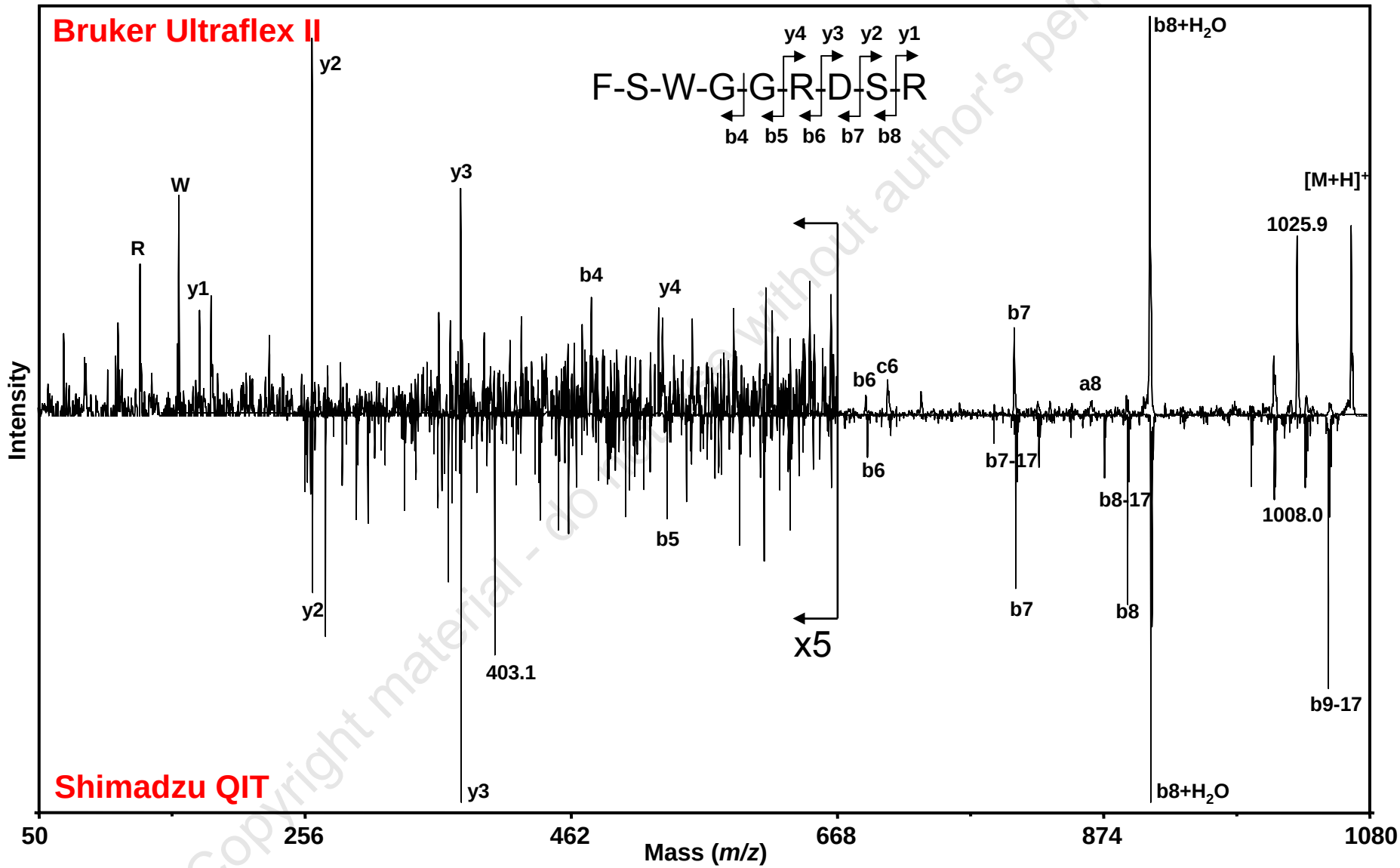
Reid Groseclose et Al, J. Mass Spectrom. 42, 254-262 (2007).

On-tissue trypsin digestion: Rat brain

Section 2: MALDI MS/MS of tryptic peptides (*m/z* 1067.60)



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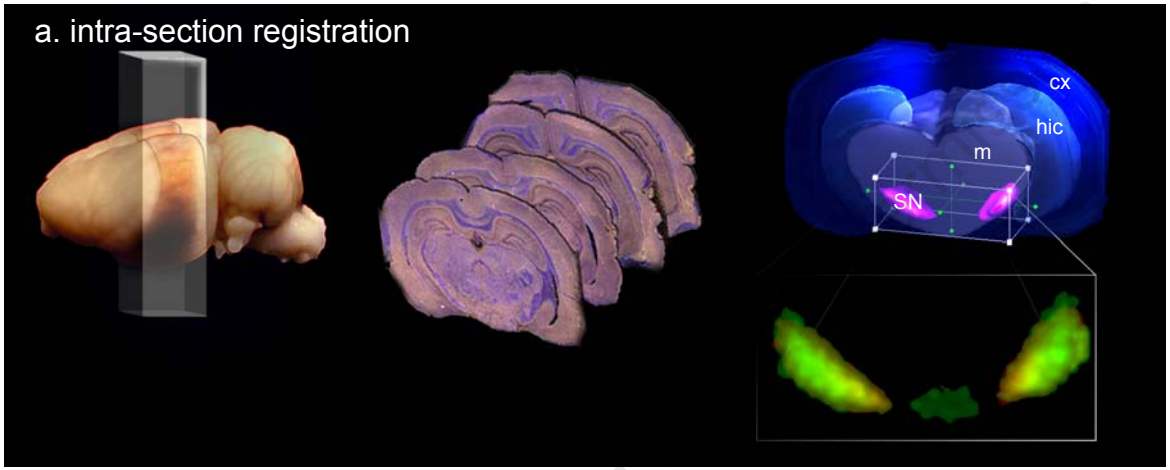
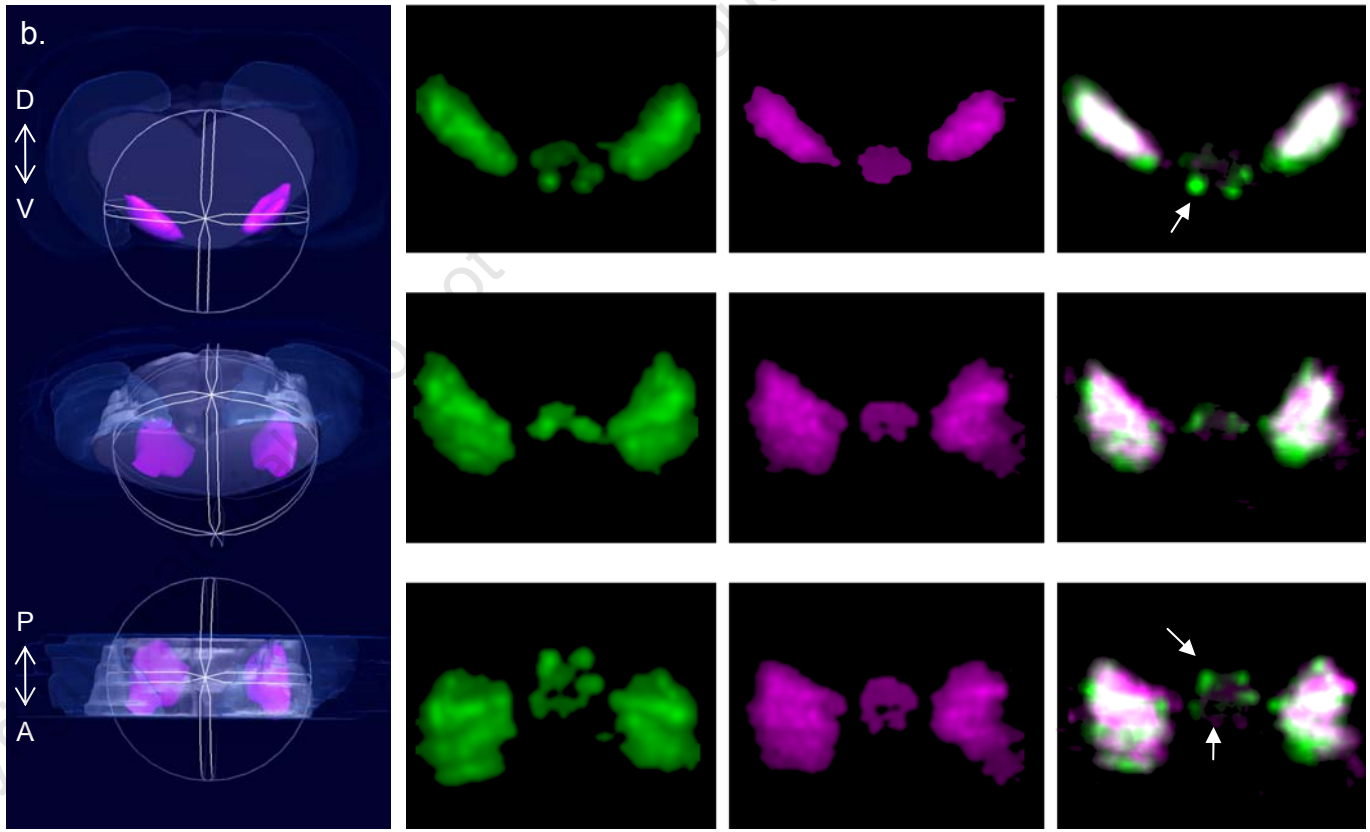
Reid Groseclose

3D-Imaging MS

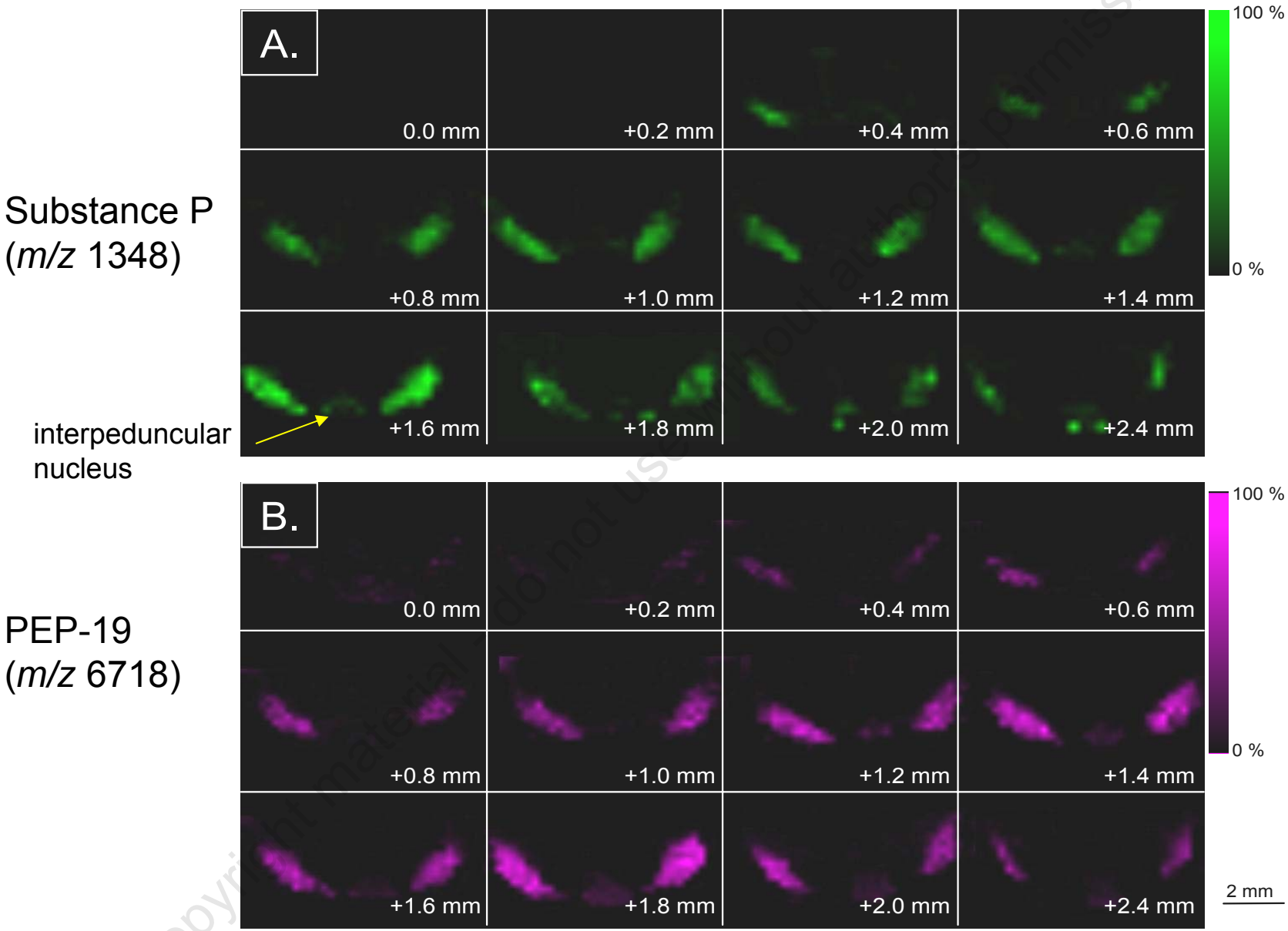
3D reconstruction of the substantia nigra in the mouse brain





M. Andersson et al, Accepted in *Nature Methods, 2007*

3D Imaging Mass Spectrometry



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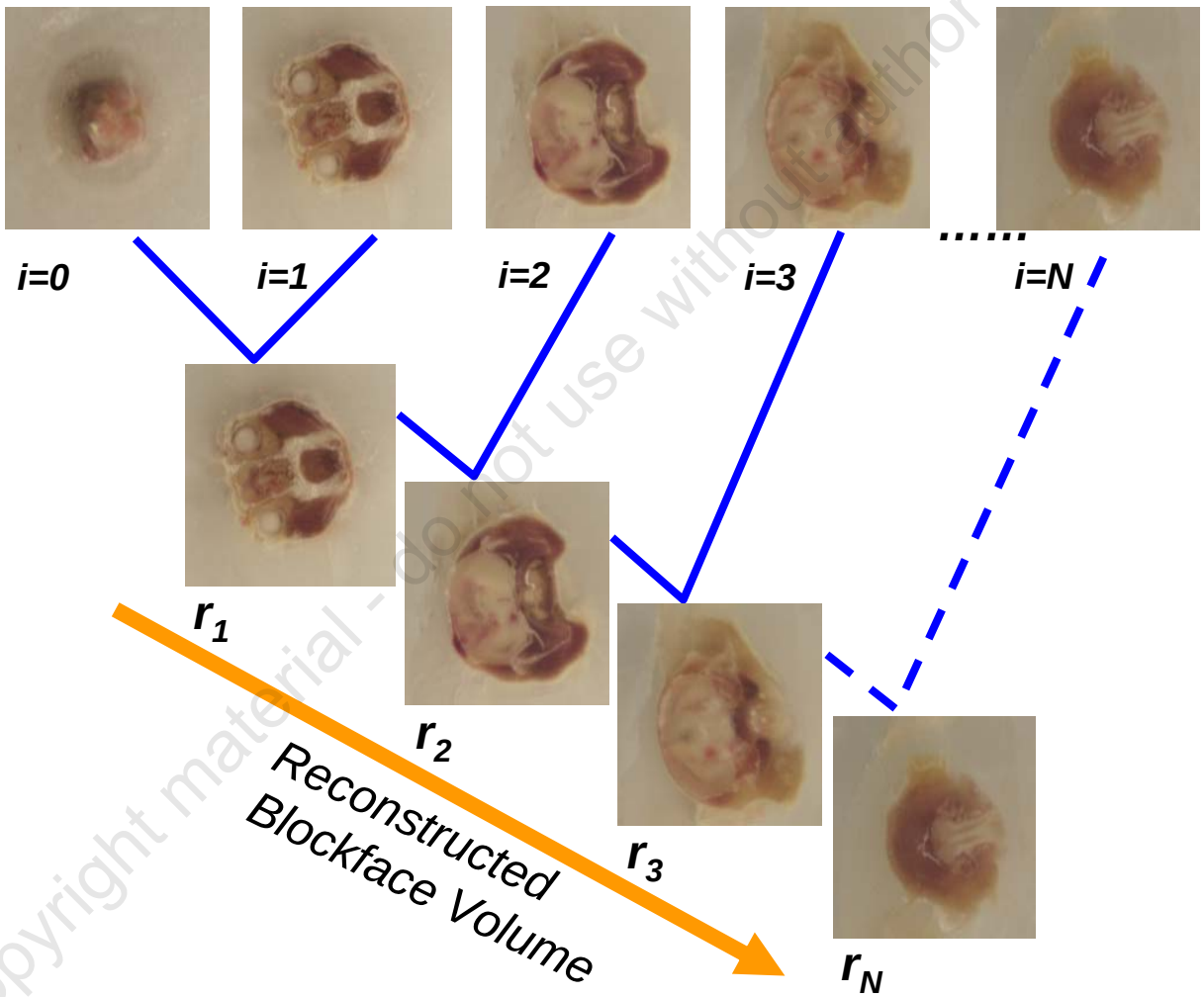
M. Andersson et al, Accepted in *Nature Methods, 2007*

Integrating IMS and MRI

Step 1: reconstruction of an optical blockface volume

Each image in the blockface volume is registered to a previously registered image in the volume



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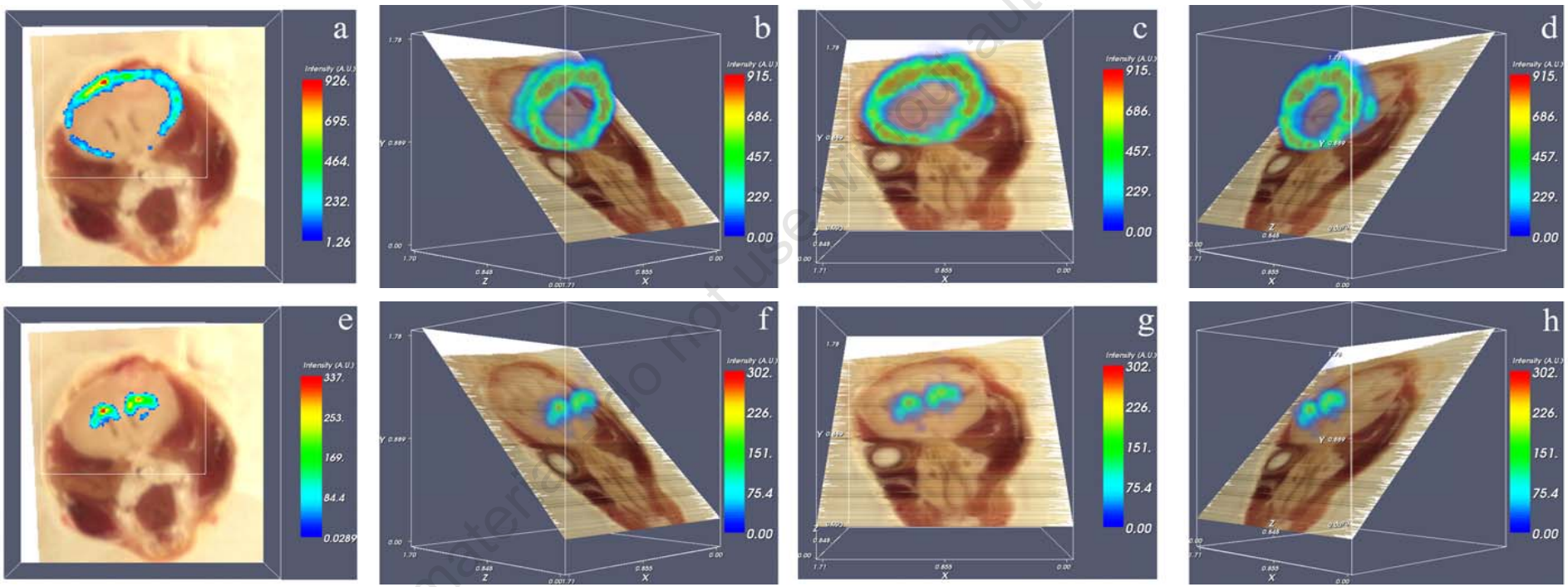
T. Sinha, S. Khatib-Shahidi et al. Accepted in *Nature Methods* (2007).

Integrating IMS and MRI

Step 2: Insertion of ion images into reconstructed blockface volume

m/z 5826



m/z 14182

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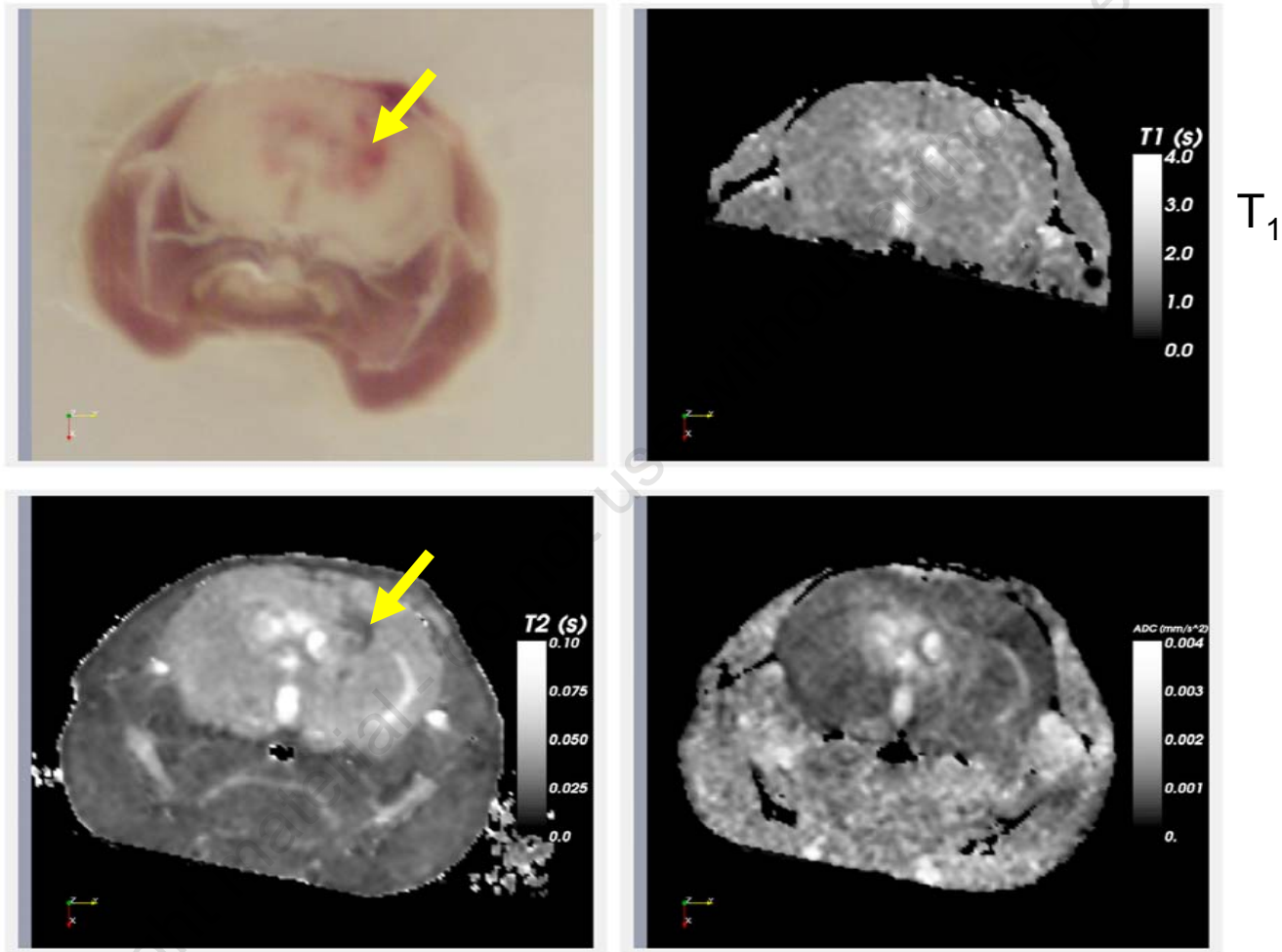
Integrating IMS and MRI

Step 3: Co-registration of MR to blockface images



T_1

T_2

ADC

T. Sinha, S. Khatib-Shahidi et al. Accepted in *Nature Methods* (2007).

Areas of Further Development

- **Increase number of proteins analyzed to >>1000**
  - Develop sample protocols to investigate peptides
  - Achieve high sensitivity above 50 kDa
  - Integrate detergents into tissue protocol for membrane associated proteins
- **Pixel-to-pixel cycle time of about 100 ms needed**
- **Integrate bioinformatic tools, image processing**
- **Improve protein identification protocols**
- **Develop sample protocols targeted at specific tissues/diseases**
- **Develop sample protocols to investigate FFPE biopsies**

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Tissue Profiling/Imaging MS

Conclusions

- **Excellent discovery tool**
- **MW annotated patterns keyed to tissue location**
- **Augments (not replaces) current molecular technologies**
- **Significant clinical potential (disease diagnosis, state and progression, prognosis, risk assessment?)**
- **Provides temporal proteomic profile that, with genomic profile, will be vital to personalized medicine**

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Acknowledgments

MSRC

Richard Caprioli (Director)
Shannon Cornett
Michelle Reyzer
Erin Seeley
Joey Latham
Stacey Oppenheimer
Sheerin Khatib-Shahid
Kristin Burnum
Kristen Herring
Satu Puolitaival
Hans Reudi Aerni
Reid Groseclose
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Jennifer Pietenpol
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Yu Shyr
Susan Kasper
Ken Schriver

Others

Per Andren, Uppsala University
Alain Brunelle, CNRS-Gif-sur-Yvette
Walter Korfmacher, Schering-Plough
Markus Stoeckli, Novartis

Funding

NIH (NCI, GMS, NIDA)
DOD
Vanderbilt University

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