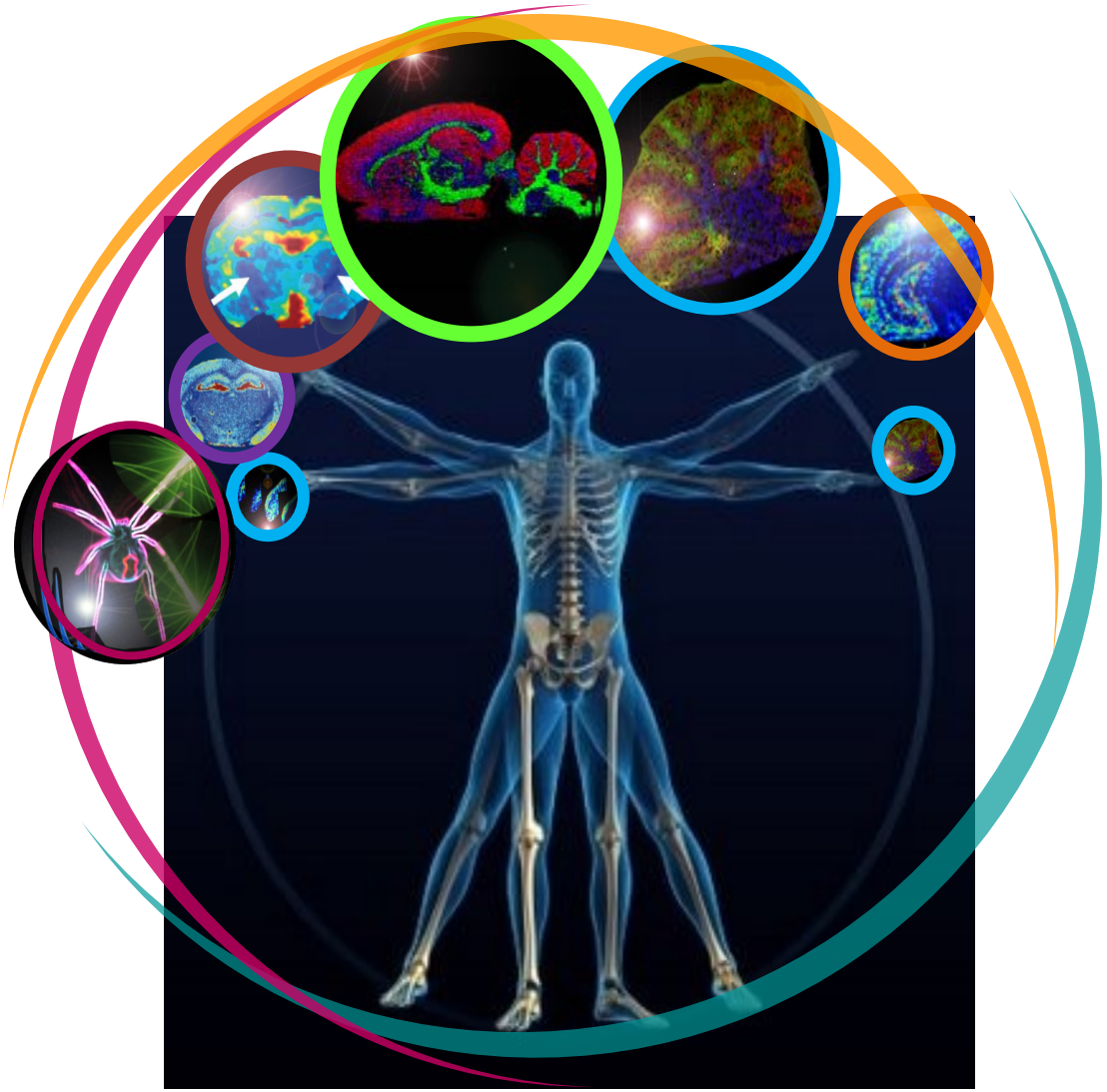
MALDI
Mass Spectrometry Imaging

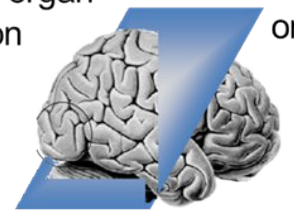
5/09/2022



Mass Spectrometry Imaging

General workflow

I. Sacrifice & organ dissection

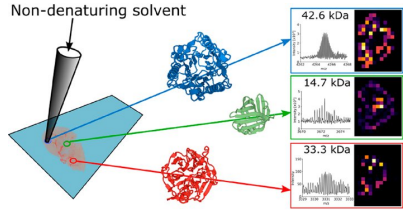


(Cryostat, Microtome)

II. Sectioning & transfer on conductive transparent glass slides

III. Washing step (optional)

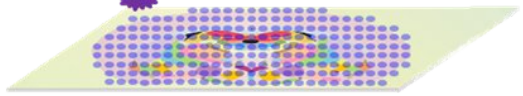
Ambient sources



Laser diameter ~ 50-80µm

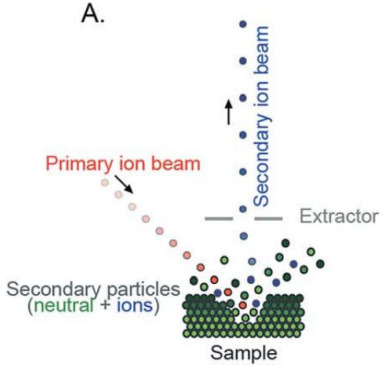
IV. Homogenous matrix deposition

Micro spotting or spray coating

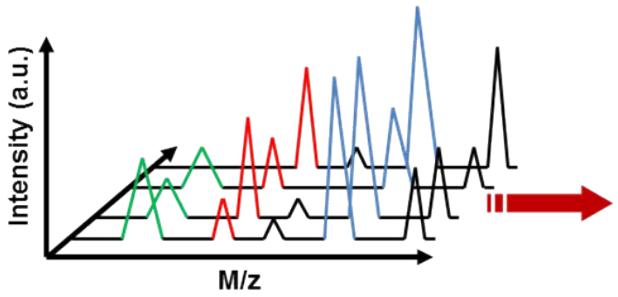


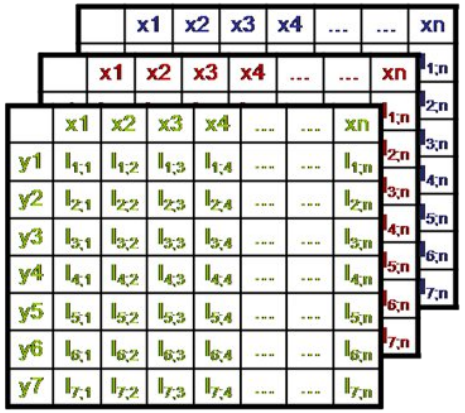
V. MALDI analysis

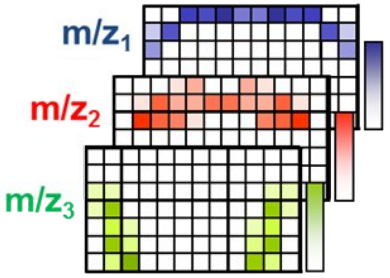
A.

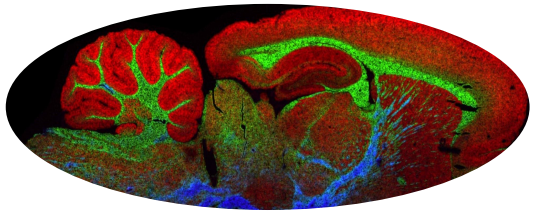


VI. Data processing & images reconstruction

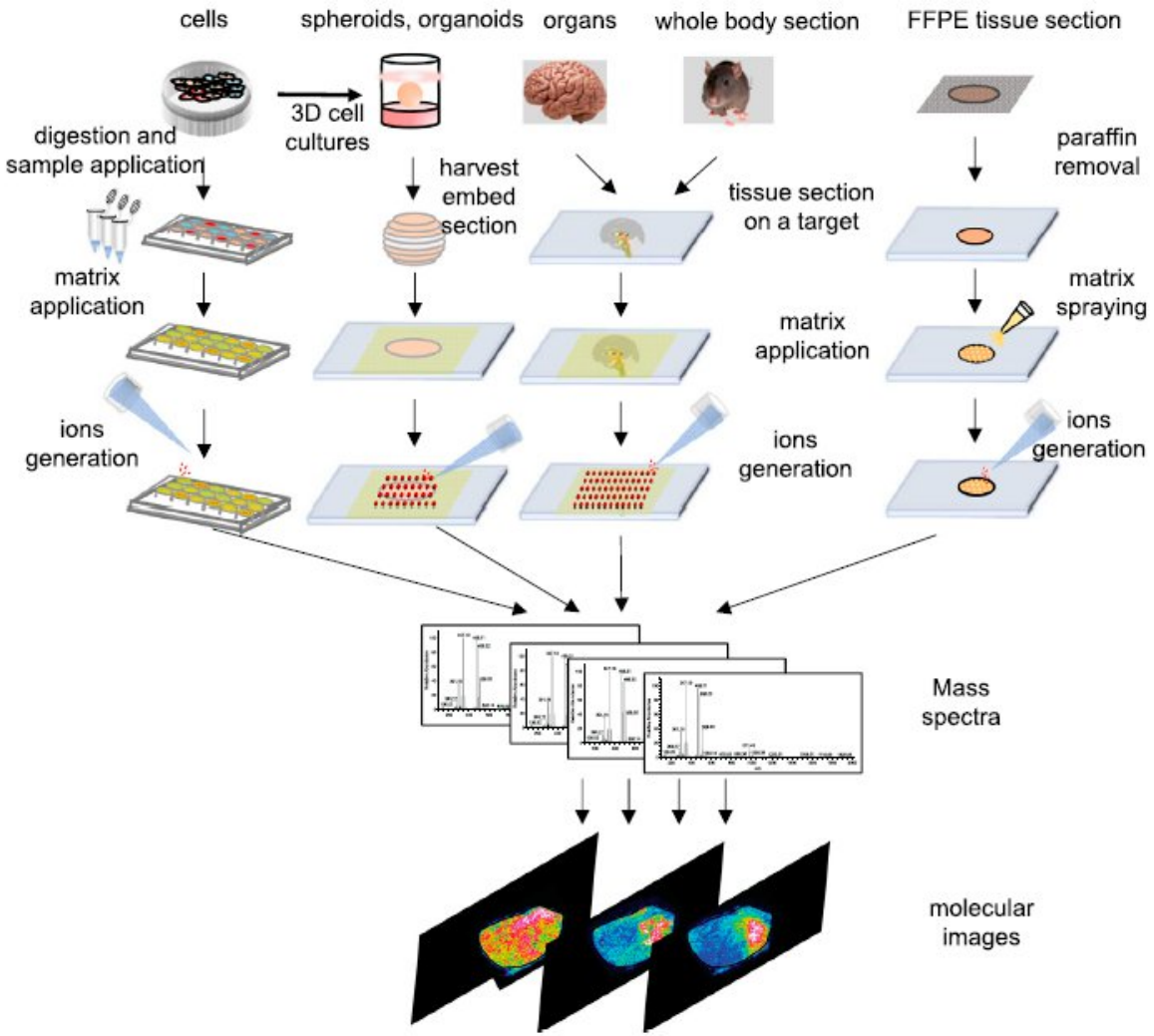




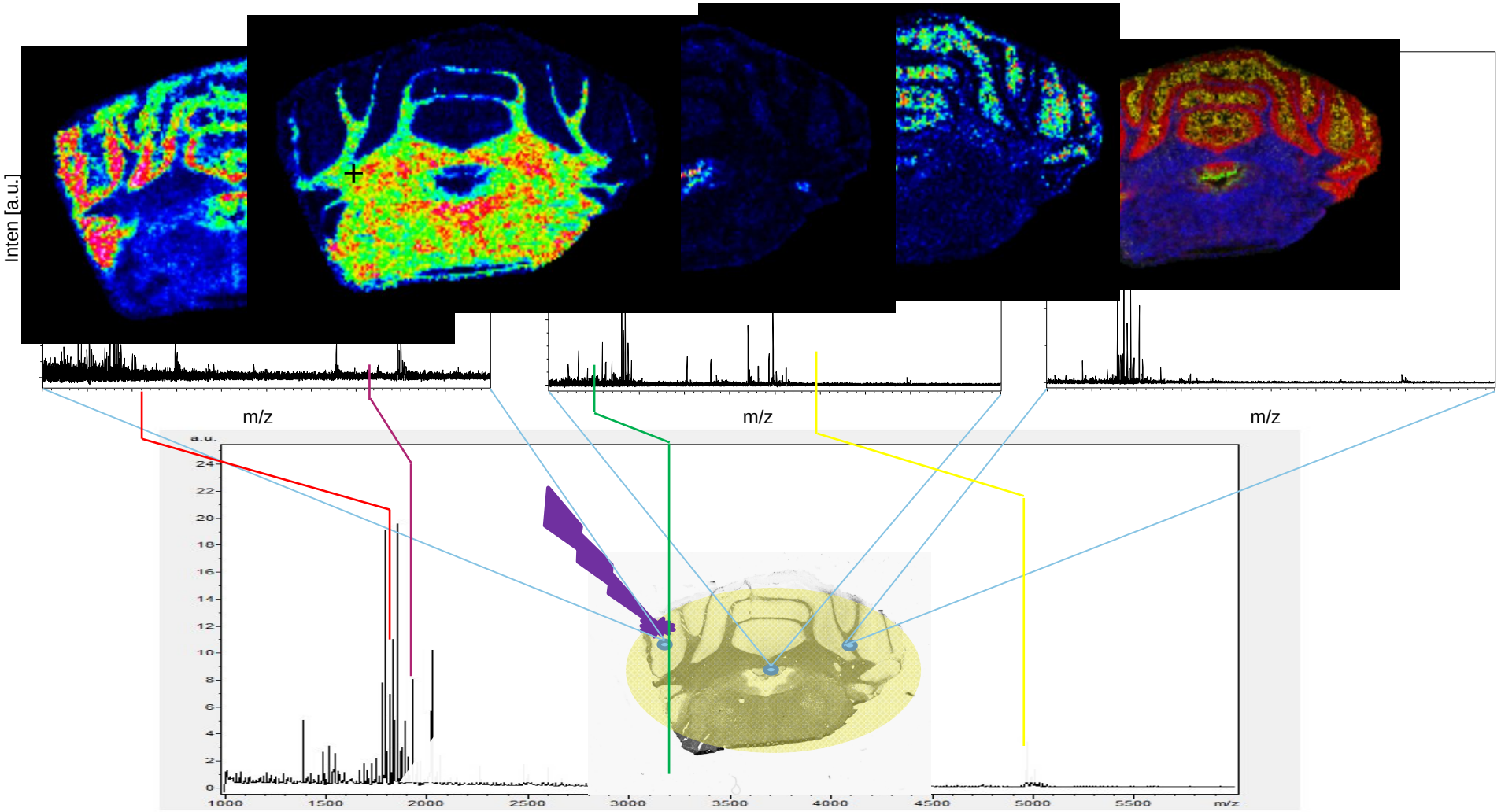




Schematic outline of workflow

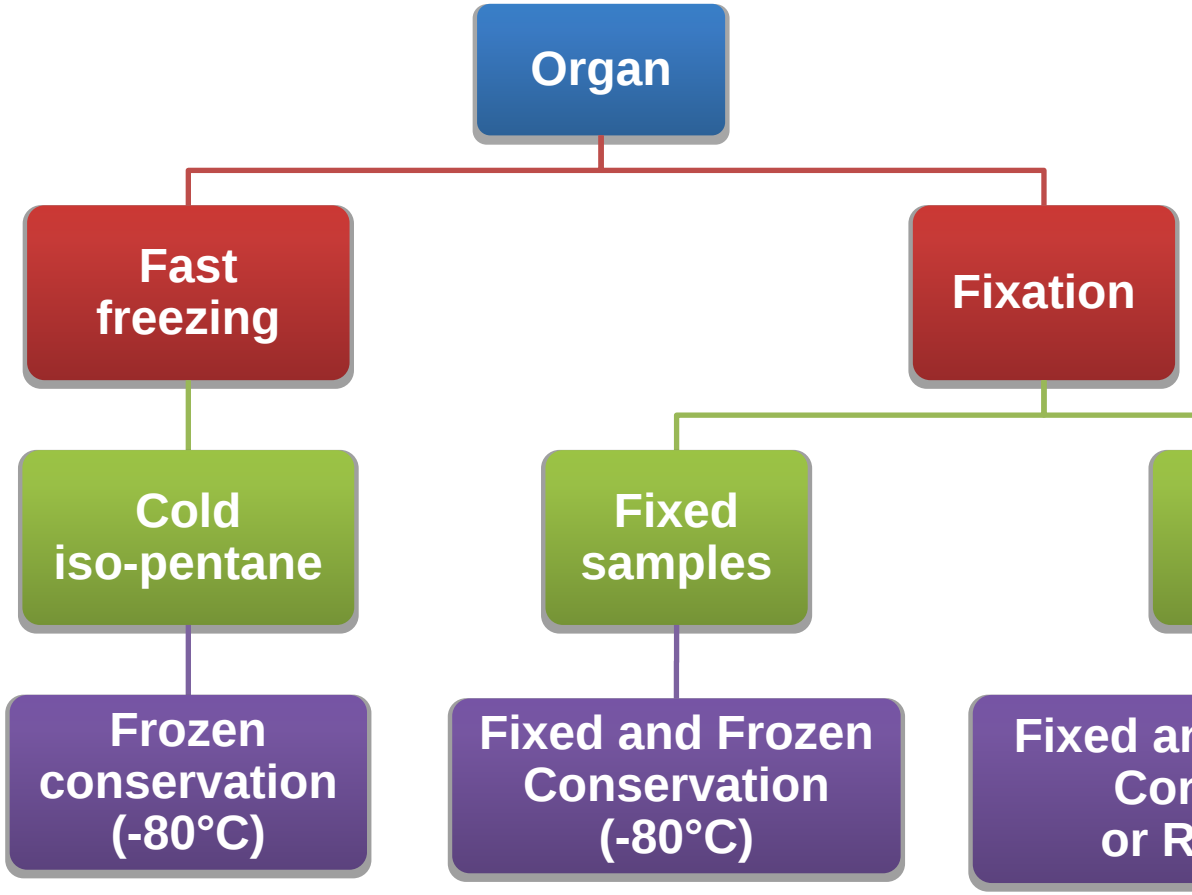


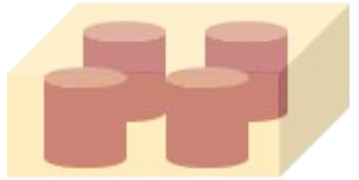
MALDI toward MALDI-MSI

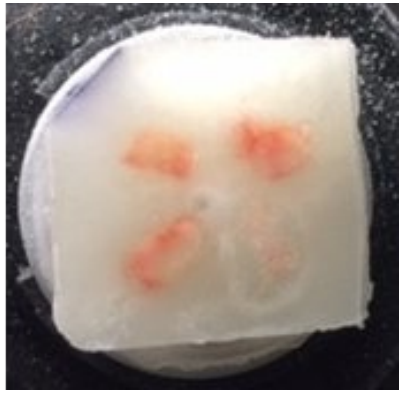


Tissue Storage

Organ storage







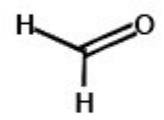
Stability Vs. Time?

Very high stability along the time

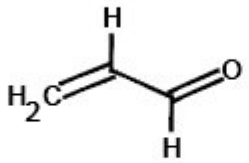
Reactivity with Fixative Agents

Fixateur	Groupements réactifs							
	Amines $-NH_2$	Hydroxyl -OH	Thiol -SH	Carboxyl –COOH	Protéines	Polysaccharides	Lipides C=C	Acides nucléiques
Chlorure mercurique		+	+	+	+			+
Tétroxyde d'osmium			+				+	
Aldéhydes	+				+			
Périodates						+		
Carbodiimides	+			+	+			
Acide tannique					+	+		
Diéthylpyrocarbonate	+			+	+			
Benzoquinone	+	+			+			

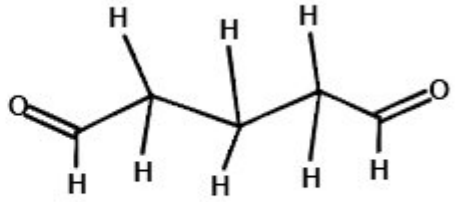
Aldehydes



Formaldéhyde



Acroléine



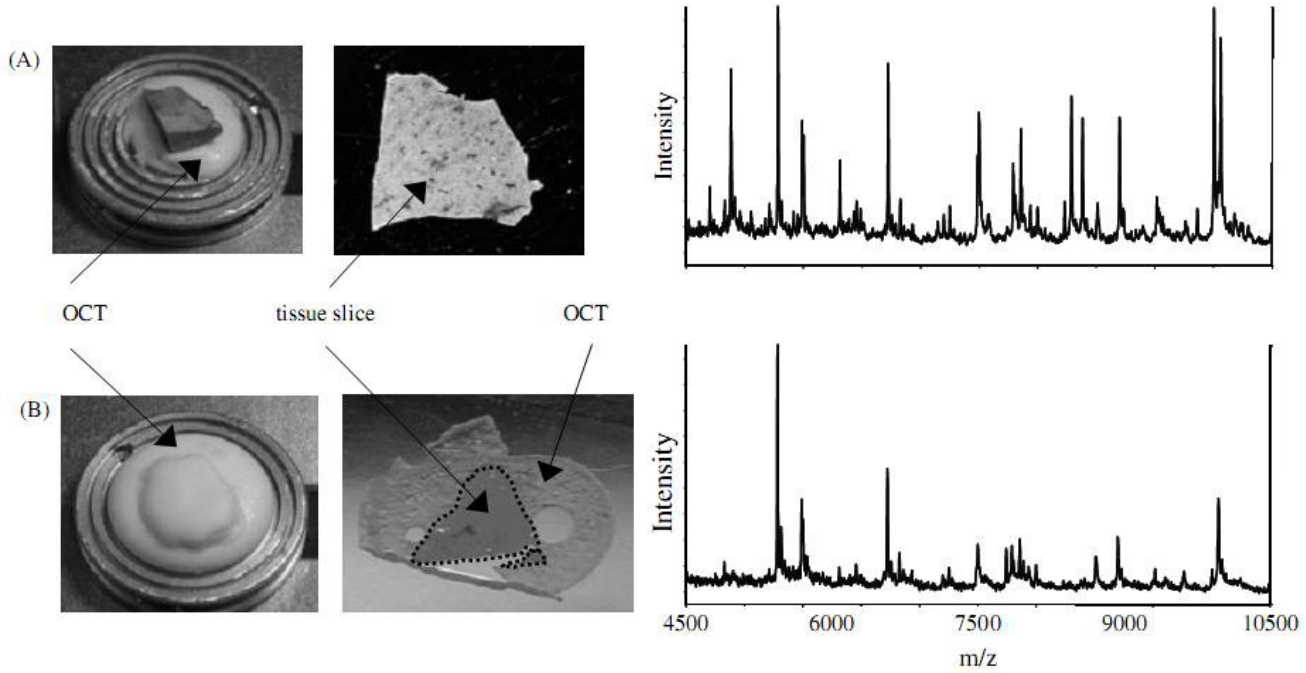
Glutaraldéhyde

Tissue embedding

Type	Nom	Hydrophilie	Température d'imprégnation habituelle	Durcissement ou polymérisation	Application	
					MO	ME
Paraffine	Paraplast	0	60°C	t. ambiante	+	0
Protéines	Gélatine	+++	37°C	Sous vide	+	+
Acryliques	Glycol méthacrylate	+++	0°C à t. ambiante	UV, ~ 0°C	+	+
	Hydroxyéthylméthacrylate	+	0°C à t. ambiante	60°C	+	+
	LR White	+	-20°C à t. ambiante	UV, ~ 0°C ou 60°C	+	+
	Lowicryl(s) K4M K11M HM20 HM23	 +++ +++ + +	 ≤ -30°C ≤ -50°C -20 à –50°C -50 à –80°C	UV à basse ou très basse température	+	+
	Unicryl	+	0°C à ~ -25°C	UV (0 à ~ -25° C) ou 50-60°C	+	+
Epoxy	Araldite	0	30-35°C	60°C	+	+
	Épon	0	t. ambiante	60°C	+	+
	Spurr	0	0°C ou t. ambiante	60-70°C	+	+

Tissue cutting

Optimal Cutting Temperature (OCT)



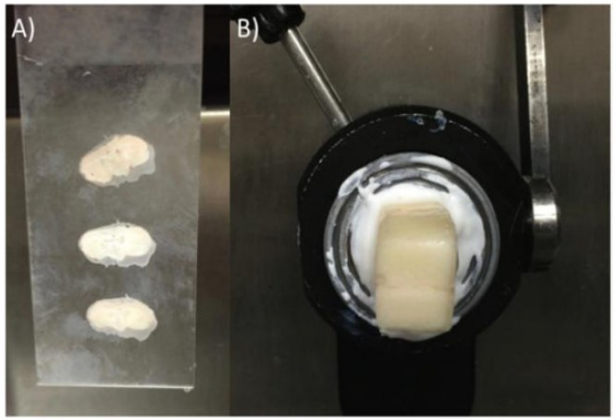
(A) OCT used to adhere the tissue to the sample stage but does not come into contact with the sliced tissue.
(B) The tissue was embedded in OCT and attached to the sample stage.

- ***Tissue embedding into OCT => poor S/N***
- ***Never embed tissue in OCT***

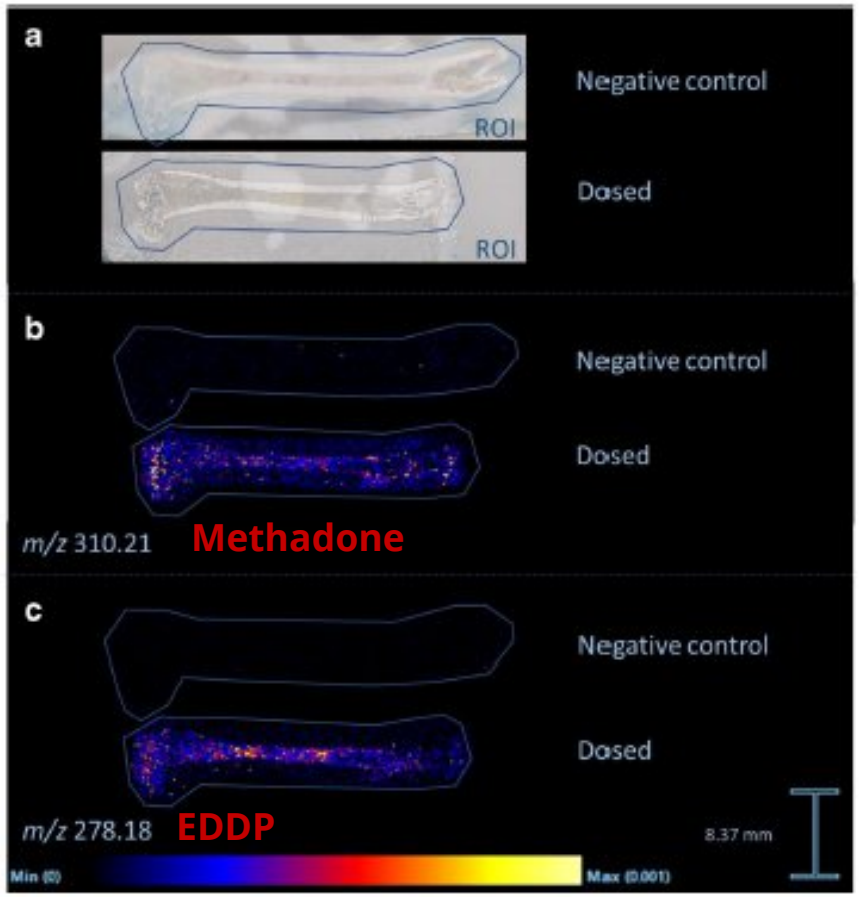
Schwartz et al., J. Mass Spectrom., 2003, 38, 699-708

Gelatin/CMC based tissue embedding

Gelatin embedding

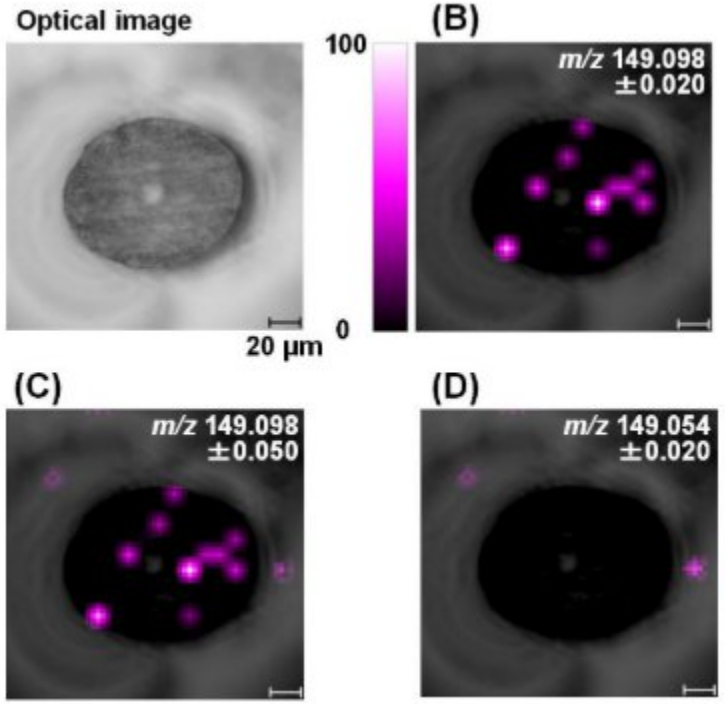


Rat bone sections, solution 20% gelatin (w/v) and 7.5% CMC, (w/v)



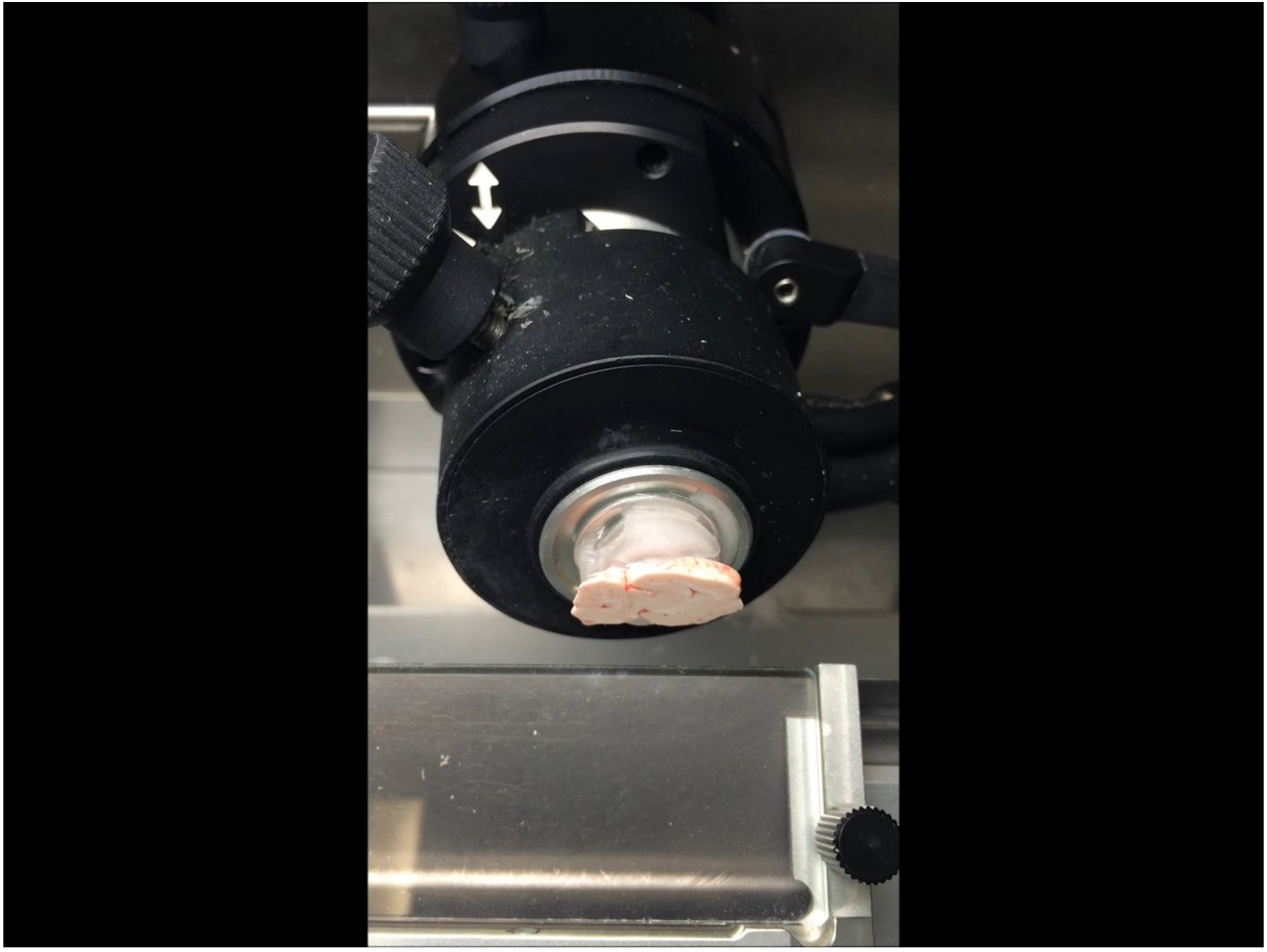
10.1007/s00216-020-02920-1

Human hair in 10% gelatin



10.1021/acs.analchem.9b05401

Tissue Sectionning & Mounting



Provided by
Gregory Hamm

Recommended Temperatures for Cutting Unfixed Frozen Tissues

tissue type	working temp., °C
brain	−12
liver	−14
lymph node	−14
kidney	−16
spleen	−16
muscle	−20
thyroid	−20
skin	−25
breast	−25
breast with fat	−30 or below
adipose tissue	−30 or below
fixed tissue	−12 to −17

Whole body Autoradiography vs MSI

Image Optique

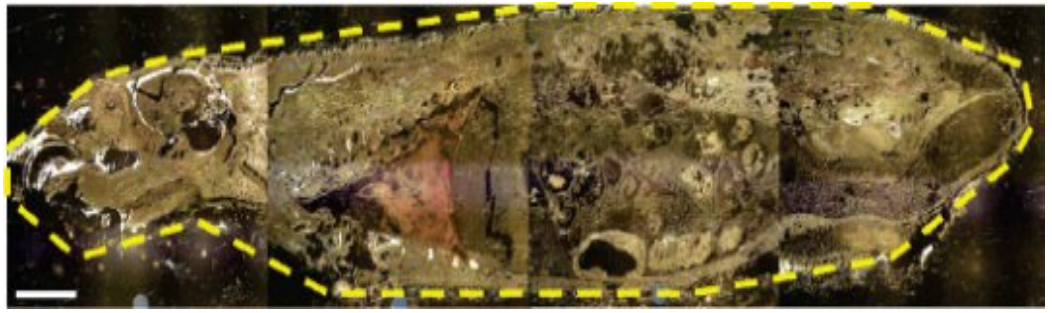
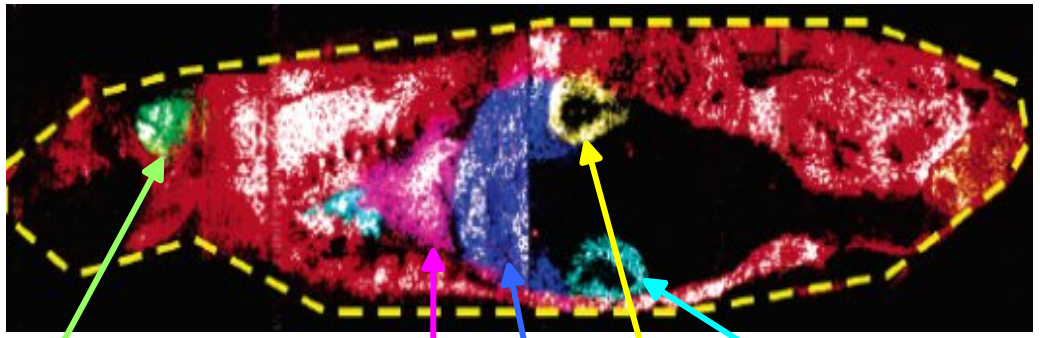
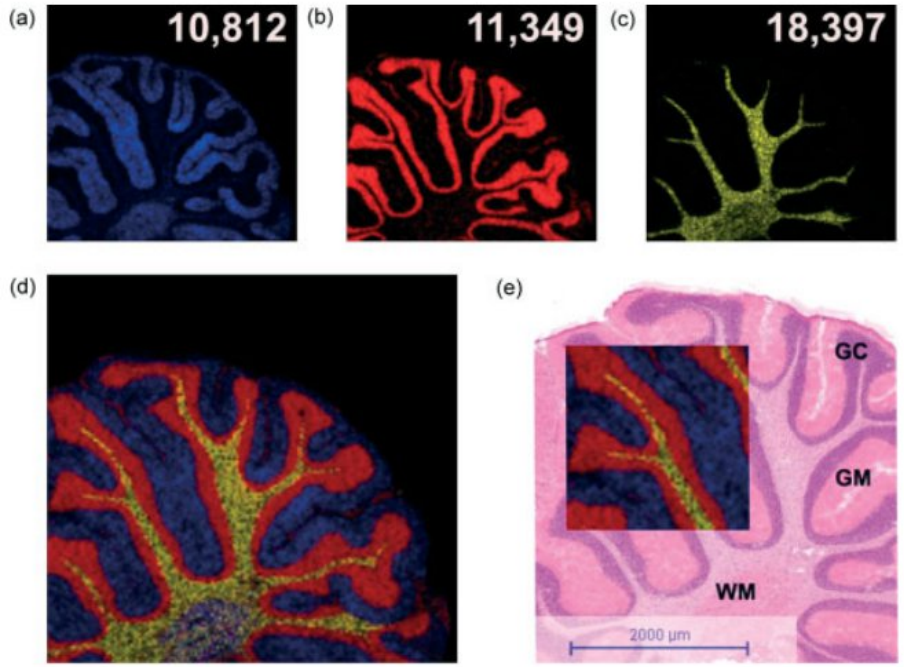


Image MALDI



STAINING

High spatial resolution and classical histology on a single tissue section

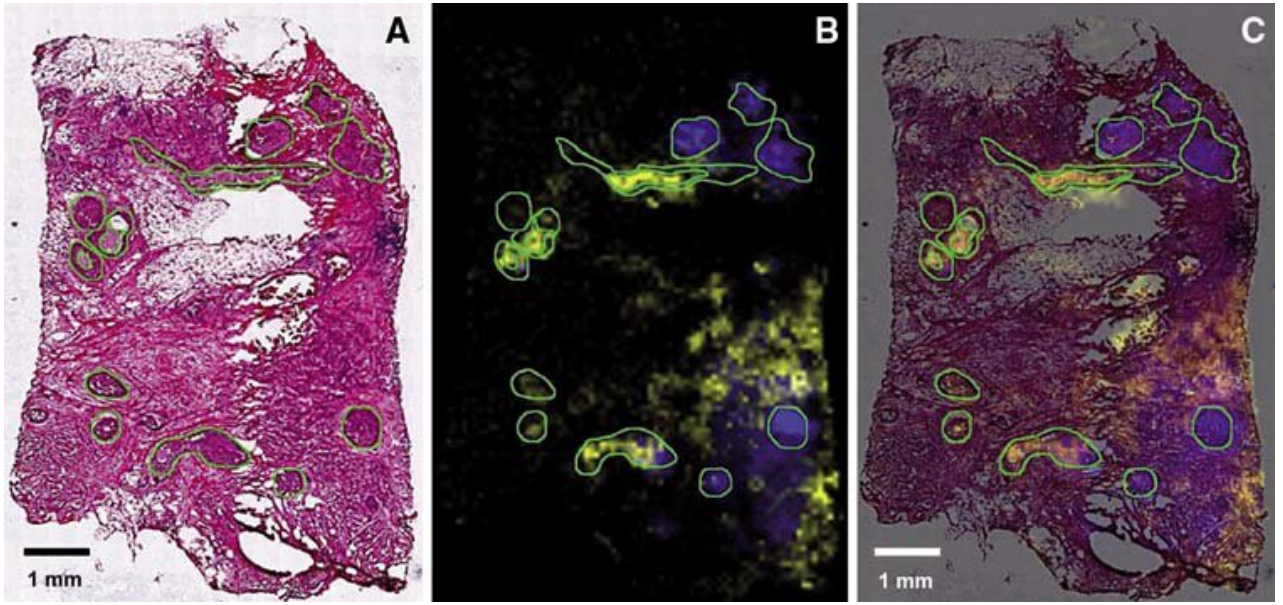


H&E staining of the tissue
After the MALDI measurement, the remaining matrix was washed off the tissue by gently shaking it in a Petri dish in 95% ethanol for 20 s.

(a)–(c) Protein markers for different regions of the cerebellum
(d) Overlay of these proteins in a single image
(e) Overlay of the MALDI IMS image and the H&E stain (WM, whitematter; GM, gray matter; GC, granule cells)

10.1002/jms.1926

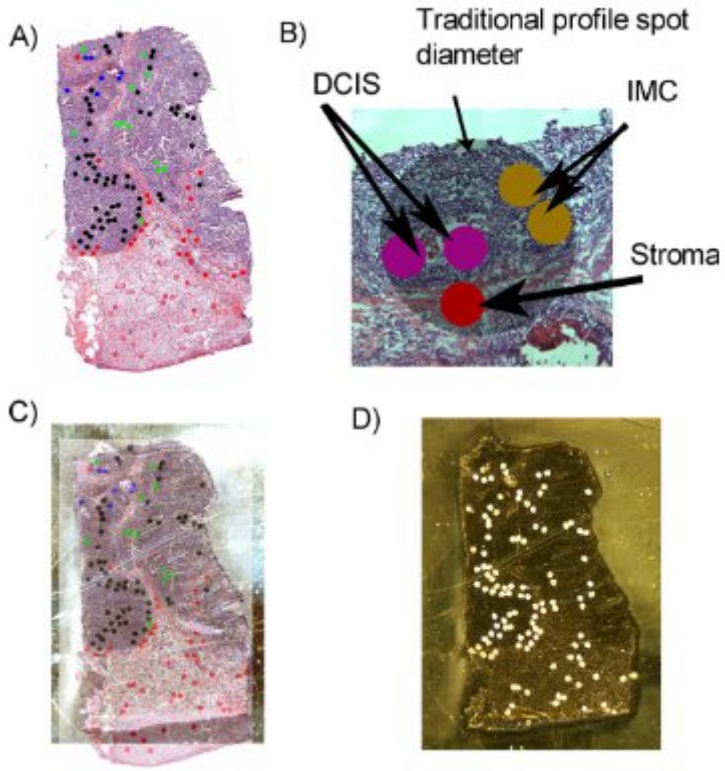
MALDI MSI and classical histology on a single tissue section



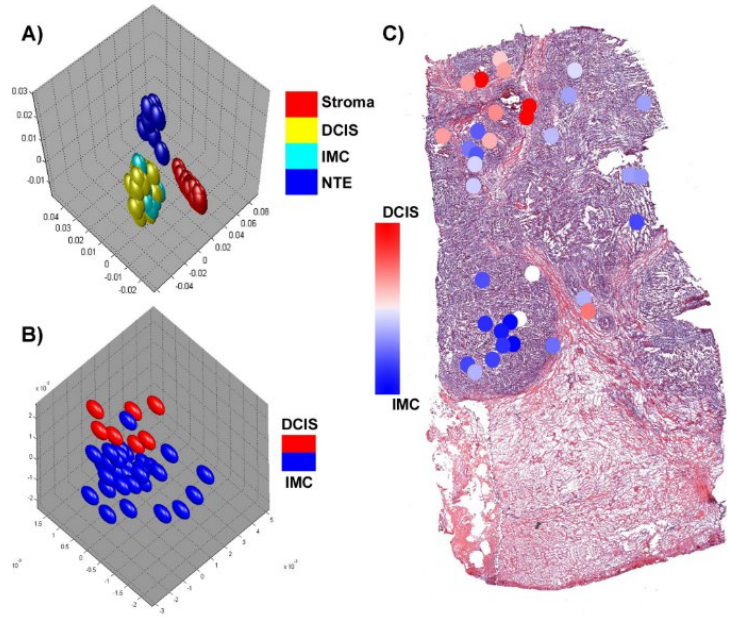
A. Optical image of a H&E stained tissues section showing several carcinomata *in situ* regions Molecular images of m/z 9,750 (Yellow) & m/z 4,519 (blue)
B. Overlay of H&E staining and molecular images

Walch et al., Histochem. Cell. Biol., 2008, 130, 421-34

Histology-directed Tissue Profiling



A. H&E section with circular marks placed at sites to be profiled and colored according to histopathology, **red**, peritumoral stroma; **black**, IMC; **blue**, DCIS; and **green**, NTE
B. illustration of the different surface areas profiled by the histology directed strategy (colored spots) and traditional profiling (100 nL of matrix deposited with mechanical pipette, shaded area)
C. overlay of aligned H&E image and MALDI image
D. MALDI section after robotic deposition of matrix onto designated sites

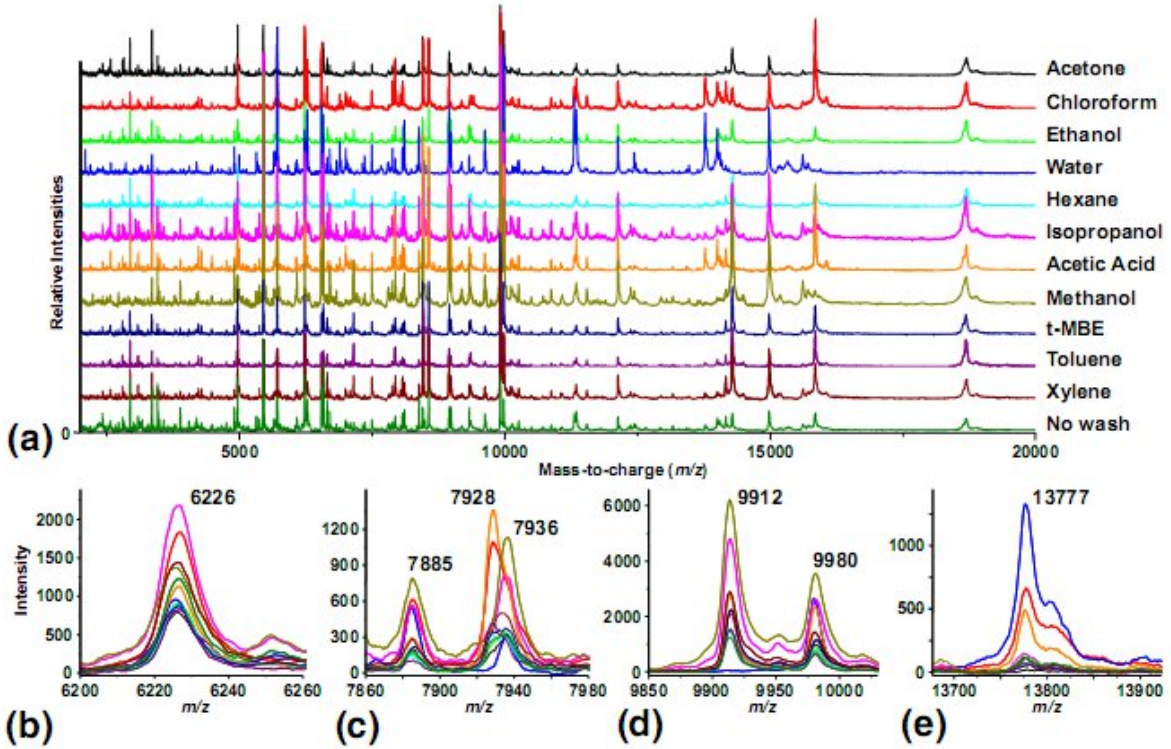


A. unsupervised classification of profiles of specific cell types acquired from one breast tumor section
B. spatial plot representing profile similarity of DCIS versus IMC as determined by multidimensional scaling of the top ranked markers identified by supervised classification
C. H&E section with annotation marks colored to represent results of classification analysis

10.1074/mcp.M600119-MCP200

Washing

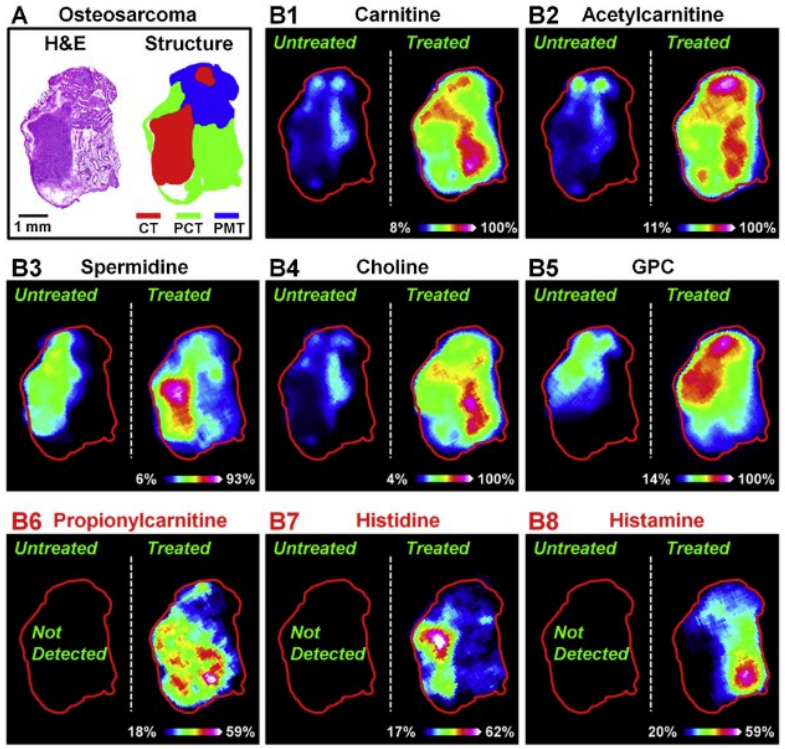
Washing



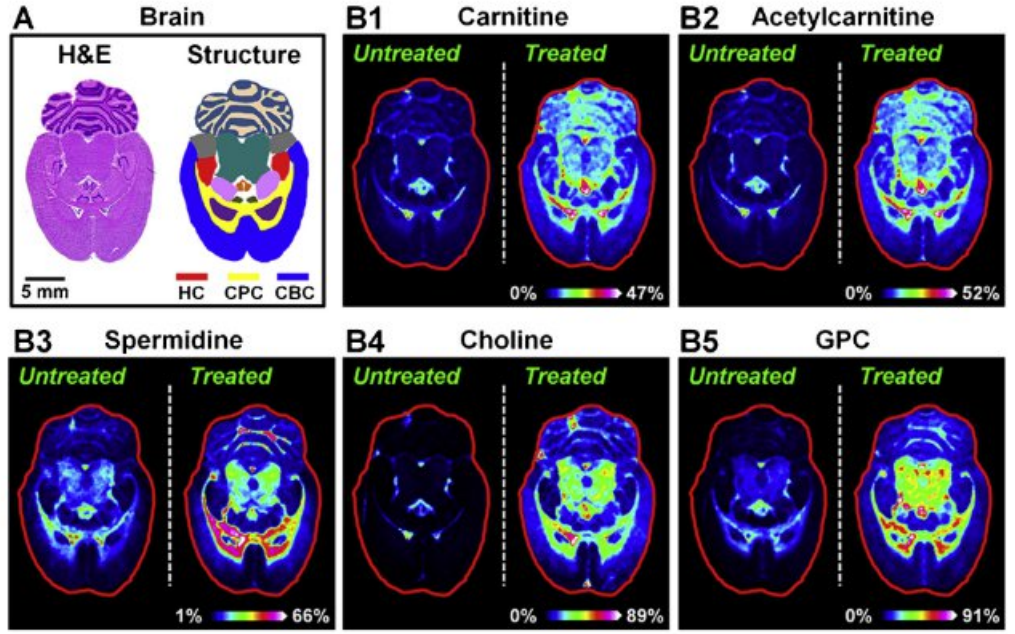
(a) Comparison of rat brain submitted to different organic solvent
(b-e) Intensity of ions after those washing step

Seeley et al., J. Am. Soc. Mass Spectrom., 2008, 19, 1069-1077

Acetone immersion enhanced small molecule metabolites (SMMs)



MS images of representative SMMs which presented enhanced ion intensities after immersing with acetone in osteosarcoma section. CT, cancer tissue; PCT, para-cancerous connective tissue; PMT, paracancerous muscular tissue.



MS images of representative SMMs which presented enhanced ion intensities after immersing with acetone in rat brain section. HC, hippocampus; CPC, corpus callosum; CBC, cerebral cortex.

10.1016/j.jpba.2019.112797

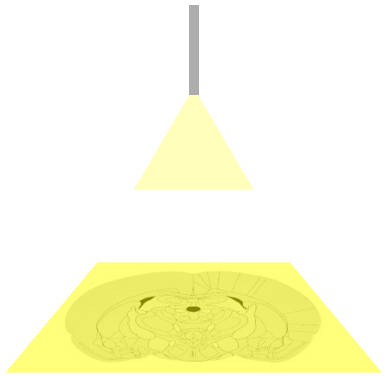
Tissue Washing Procedures

analyte	protocol
proteins	70% ethanol, 90% ethanol, 95% ethanol, 3 wash steps, 30 s each step
proteins[65,71]	70% ethanol, 100% ethanol, Carnoy's fluid (2 min), 100% ethanol, H_2O, 100% ethanol; six wash steps, 30 s each step unless noted; Carnoy's fluid is composed of 6:3:1 (v:v:v) ethanol/chloroform/glacial acetic acid
proteins/in situ trypsin digest[37,73]	70% ethanol, 95% ethanol (2×), final wash in solution of 90% ethanol, 9% glacial acetic acid, 1% H_2O, 4 wash steps, 30 s each step
peptides[73−75]	70% ethanol, 95% ethanol (2×), 3 wash steps, 10 s each step
lipids[76]	50 mM ammonium formate (pH 6.4, 4 °C) for 15 s

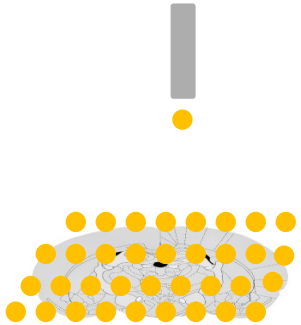
MALDI Imaging

Matrix application

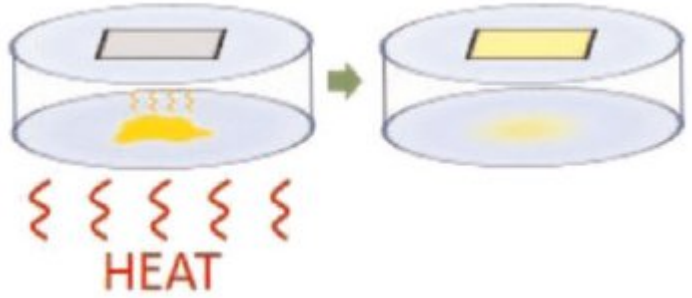
Spray coating



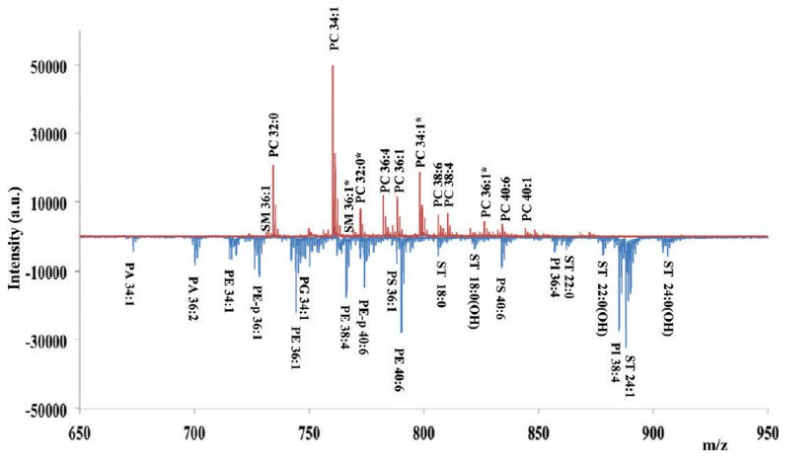
Microspotting



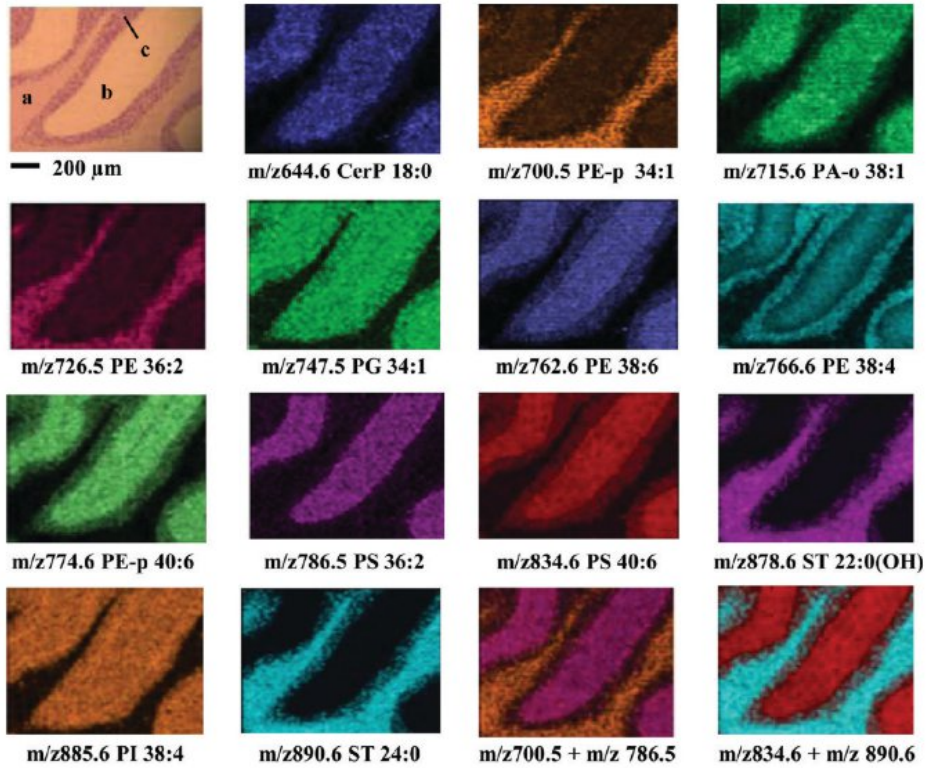
Sublimation



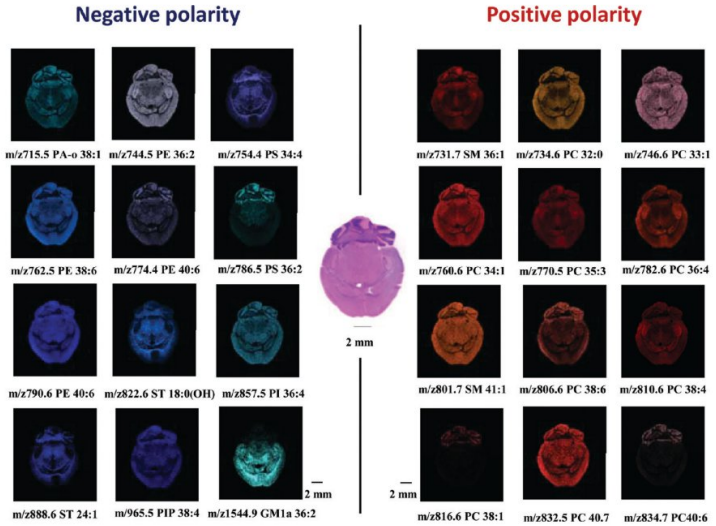
Sublimation of 1,5-DAN



MALDI MS spectra acquired from a mouse brain section coated with DAN by sublimation in the positive (red) and negative (blue) ionization modes. A∗ indicates potassium adducts.



Consecutive IMS of lipids in the negative and positive ionization modes from a transversal mouse brain section after DAN sublimation. Ion images are correlated to a serial section after H&E staining. Imaging MS data were acquired with a lateral resolution of 100 µm with a 50 µm offset between the positive and negative grid arrays.



High spatial resolution IMS of lipids in the negative ionization mode from a transversal mouse cerebellum region coated with DAN by sublimation and acquired with a lateral resolution of 10 µm. In the H&E staining, a, b, and c represent white matter and the molecular and granular layers, respectively

10.1021/ac2033547

Sublimation

matrix	positive polarity	negative polarity	sublimation temperature (°C)	sublimation time (min)	deposited amount ($\mu g/cm^2$)
CHCA	**	*	180	20	50
DAN	****	****	140	5	110
DHA	–	–	140	2.45	120
DIT	**	**	140	2.5	100
DHB	***	**	140	4.25	120
DMAN	–	–	140	2	150
MBT	****	**	140	3.5	80
NIT	–	–	100	3	110
SA	*	*	165	10	150
THAP	****	**	160	2	180
3-HPA	**	*	160	5	100
9-AA	**	*	190	15	110

[a]The number of stars indicates the observed performance of the matrix according to the polarity with *, **, ***, **** representing low, medium, high, and very high, respectively. Matrices unstable under vacuum are not evaluated (–).

10.1021/ac2033547

MATRIX deposition devices

SHIMADZU
Excellence in Science

iMatrixSpray

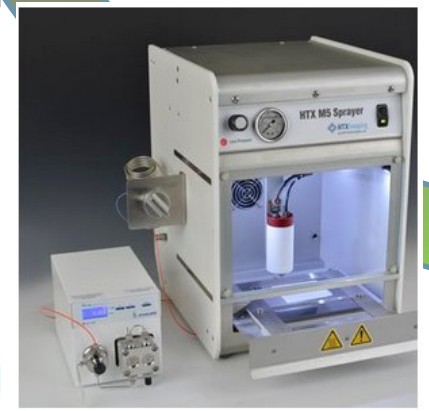
© TransMIT GmbH
SMALDIPrep

SunChrom
Wissenschaftliche Geräte GmbH

SHIMADZU
Excellence in Science

iMLayer Matrix Vapor Deposition System

HTX Sublimator™

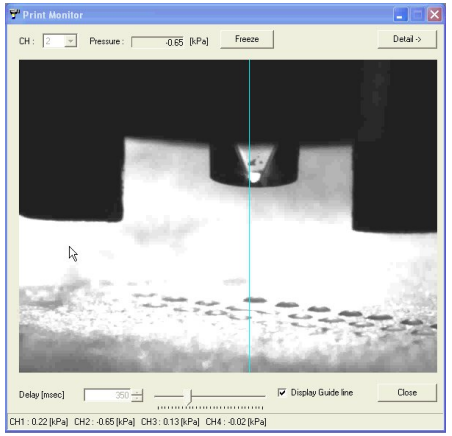


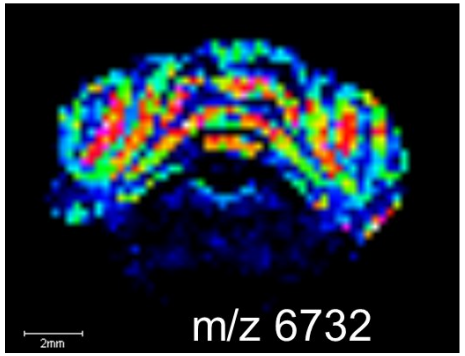
HTX M5 Sprayer™

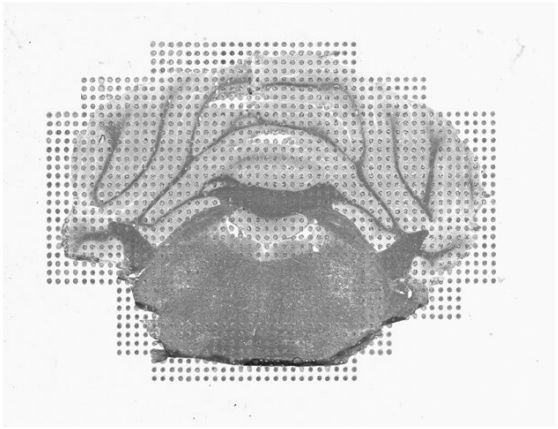
Microspotter

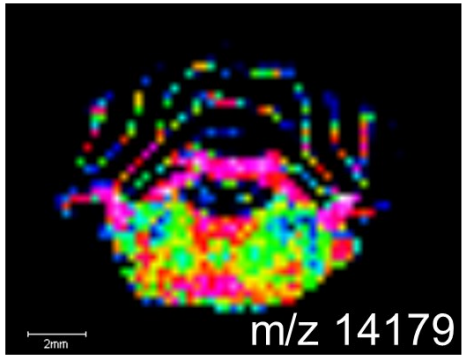
SHIMADZU BIOTECH

Chemical Inkjet Printing
CHIP-1000

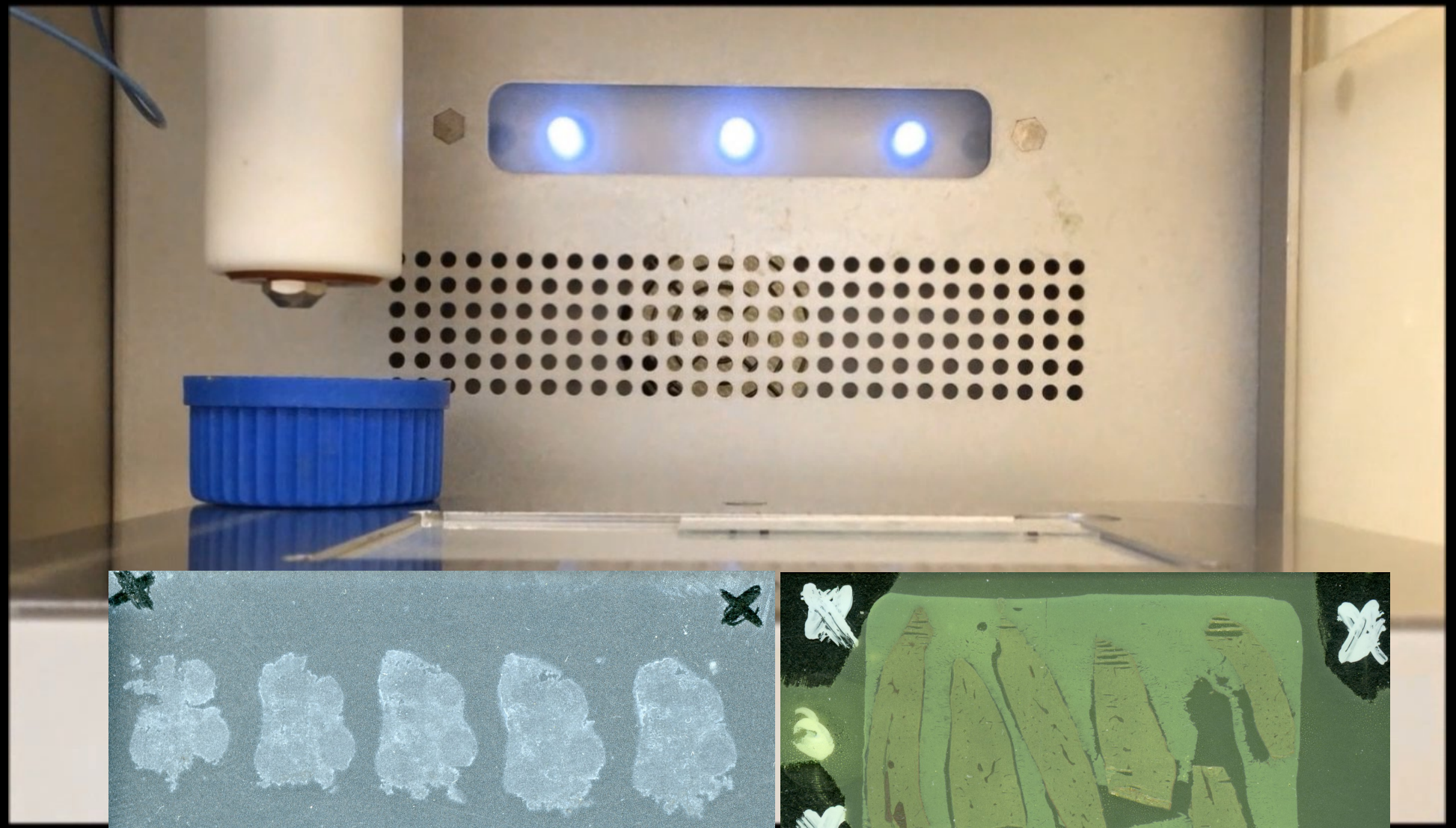








***Spatial Resolution*: Low**
***Applications*: Drugs, Lipids, peptides/proteins**



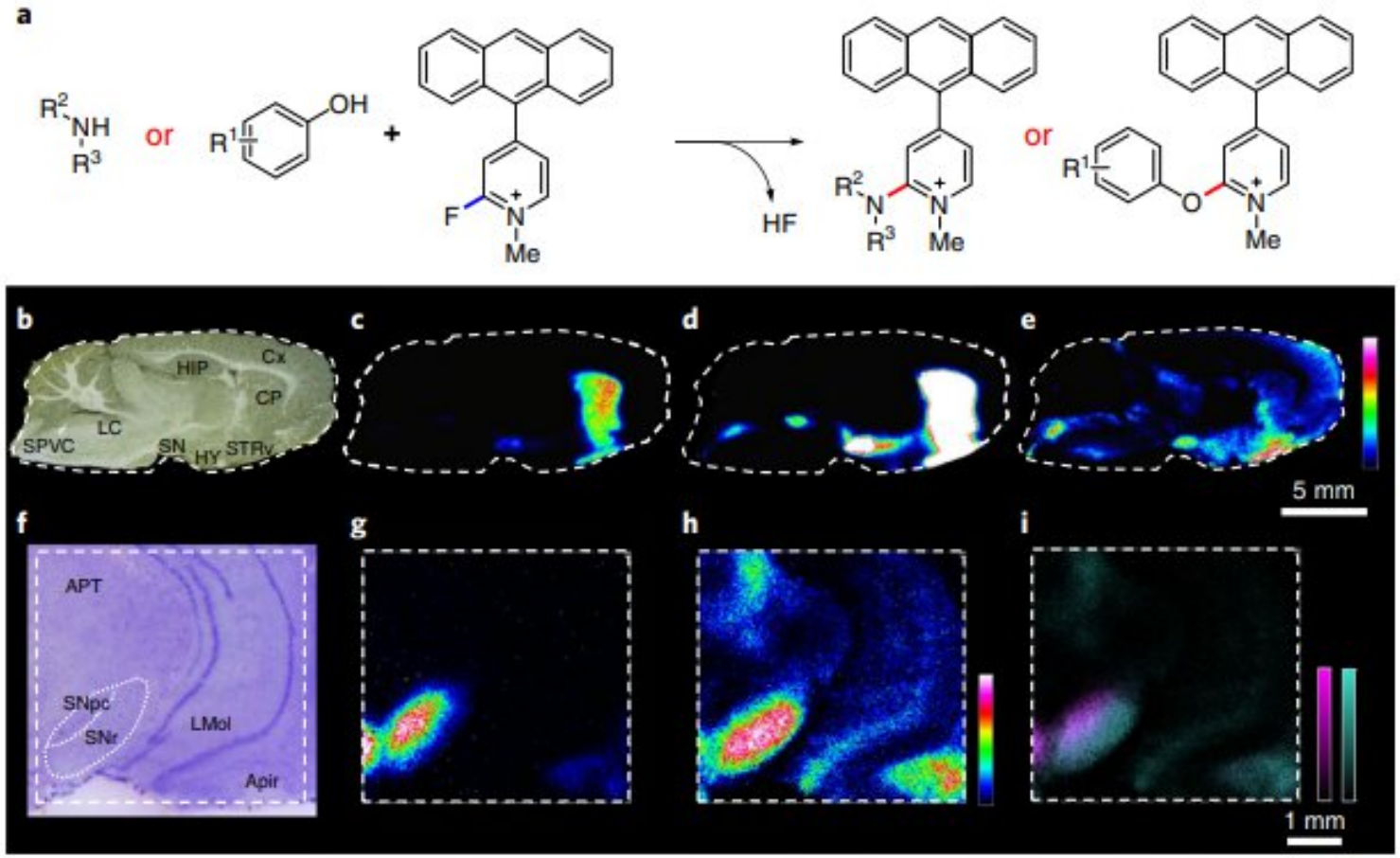
DHB

9AA

Different matrices for different applications

Matrix class	Matrix names	Targets
Classical organics	2,5-Dihydroxybenzoic acid (DHB)	Lipids, peptides, neuropeptides, drugs, small proteins
	α-Cyano-4-hydroxy cinnamic acid (CHCA/CCA)	Proteins, peptides, N-glycans, lipids
	Sinapinic acid (SA)	Proteins and peptides
	4-Chloro-α-cyanocinnamic acid (ClCCA)	Proteins and peptides
	2,5-Dihydroxyacetophenone (2,5-DHAP)	Phospholipids, proteins
	9-Aminoacridine (9-AA)	Free fatty acids, lipids
	1,5-Diaminonaphthalene (1,5-DAN)	Glycolipids, metabolites
	2-(2-Aminoethylamino)-5-nitropyridine	Phospholipids
	2-Mercaptobenzothiazole	Phospholipids
	4-Nitroaniline (PNA)	Phosphatidylethanolamine
	Norhamane	Bile acids, lipids
	Dithranol	Di-and triacylglycerols
	1,6-Diphenyl-1,3,5-hexatriene (DPH)	Free fatty acids
	1,8-Bis(dimethylamino) naphthalene (DMAN)	Free fatty acids
	N1,N4-Dihbenzylidenebene-1,4-diamine (DBDA)	Fatty acids
	Meso-tetratkis (pentafluorophenyl)-porphyrin	Free fatty acids
	2,4-Dihydroxyacetophenone (DHAP)	Glycoproteins
	2.4,6-Trihydroxyacetophenone (THAP)	Lipids
	Picolinic acid	Oligonucleotides
	Succinic acid	Oligonucleotides
Reactive matrices	2.4-Diphenyl-pyranylium tetrafluoroborate (DPP-TFB)	Small molecule amines, neurotransmitters
	2.4,6-Trimethyl-pyranylium tetrafluoroborate (TMP-TFB)	Dopamine
	p-N,N,N-Trimethy lammonioanilyl N-hydroxysuccinimidyl carbamate iodide (TAHS)	Steroids and catecholamine
	4-Hydroxy-3-methoxycinnamaldehyde (CA)	
	2,3,4,5-Tetrakis (31,4-dihydroxylphenyl)thiophene (DHPT)	
	2-Fluoro-1-methyl pyridinium (FMP) derivatives	Neurotransmitters
Inorganic nanomaterials	Metal based (e.g., gold, silver, titanium oxide)	Small molecules
	Silicon based (e.g., nanopost arrays, nanowires, nanopillars)	Small molecules
Room-temperature ionic liquids	DHB-Py, DHB-MI (1-methylimidazole), DHB-TBA, SA-TBA	Small molecules
	CCA-DEA (N,N-diethylaniline), CCA-ANI (Aniline)	Peptides
	SA-TBA, SA- Et_3N (triethylamine)	Proteins
	9-AA-NEDC	Lipids
	DHB-BuA (n-butylamine), CCA-MI, DHB-Py	Carbohydrates
	CCA-Py, CCA-MI, CCA-BuA	Phospholipids
	HPA (hydroxypicolinic acid)-DEA, CCA-ANI, CCA-MI	Oligonucleotides

Polyphenylated fluoromethylpyridinium reactive matrix

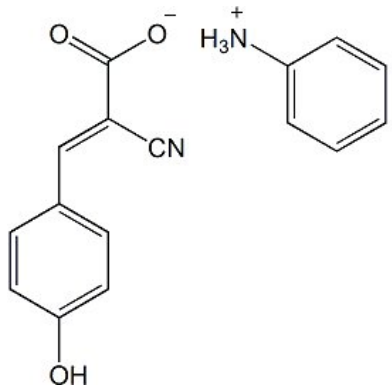


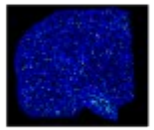
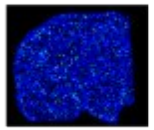
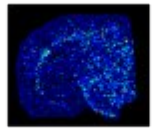
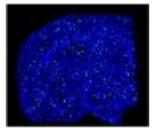
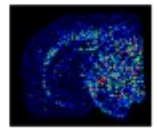
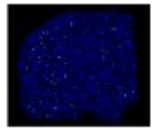
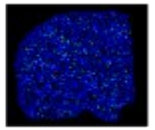
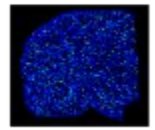
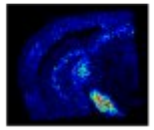
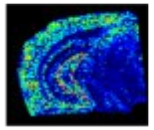
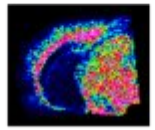
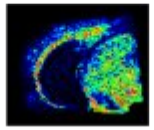
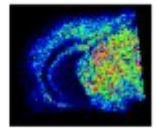
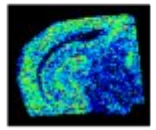
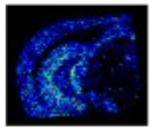
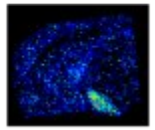
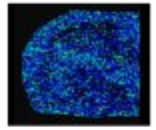
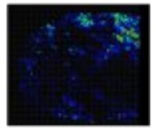
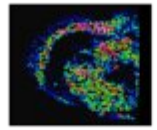
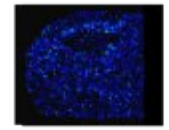
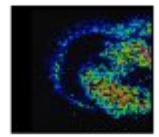
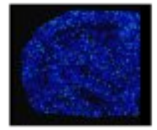
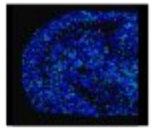
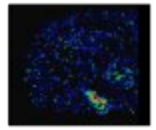
10.1038/s41592-019-0551-3

Specific matrices for MSI

Solid Ionic Matrices (SIM)

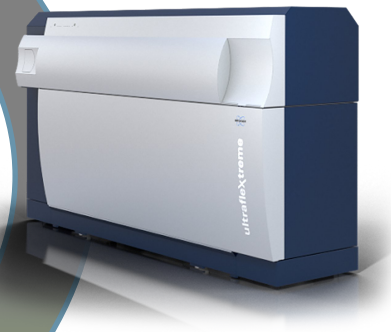
HCCA/ANI



Matrices \ m/z	1393,6	1839,2	1851,4	1931,4	2028,3	2914,0	3464,7	4982,2
HCCA	 Intens: 213,9 S/N: 26,9	 Intens: 550,3 S/N: 38,7	 Intens: 163,0 S/N: 20,6	 Intens: 33,2 S/N: 4,2	 Intens: 715,2 S/N: 77,3	 Intens: 15,2 S/N: 6,1	 Intens: 24,8 S/N: 4,4	 Intens: 32,4 S/N: 4,3
HCCA/ANI	 Intens: 1064,7 S/N: 88,8	 Intens: 11407,9 S/N: 345,0	 Intens: 27460,4 S/N: 650,6	 Intens: 12853,9 S/N: 368,3	 Intens: 2912,1 S/N: 119,5	 Intens: 1163,9 S/N: 31,9	 Intens: 139,9 S/N: 6,3	 Intens: 184,8 S/N: 6,5
HCCA/DANI	 Intens: 179,7 S/N: 4,9	 Intens: 3066,2 S/N: 82,3	 Intens: 18414,6 S/N: 512,2	 Intens: 1384,1 S/N: 15,9	 Intens: 24657,9 S/N: 348,5	 Intens: 181,0 S/N: 4,6	 Intens: 159,7 S/N: 8,3	 Intens: 2216,5 S/N: 30,0

- ***SIM provide better intense signal of peptides, better extraction?***

Mass spectrometers

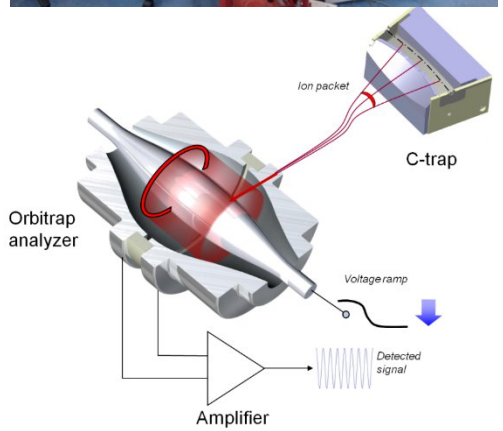


SELECT SERIES MRT

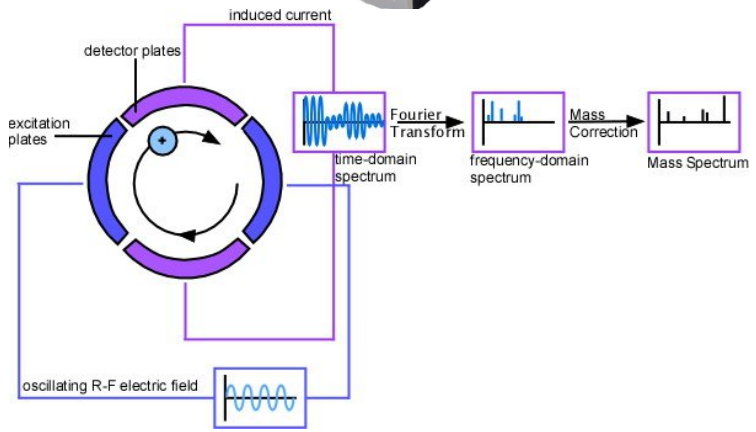
Most Common Mass Analyzer for MALDI-MSI

ToF=
Time-of-Flight

Orbitrap

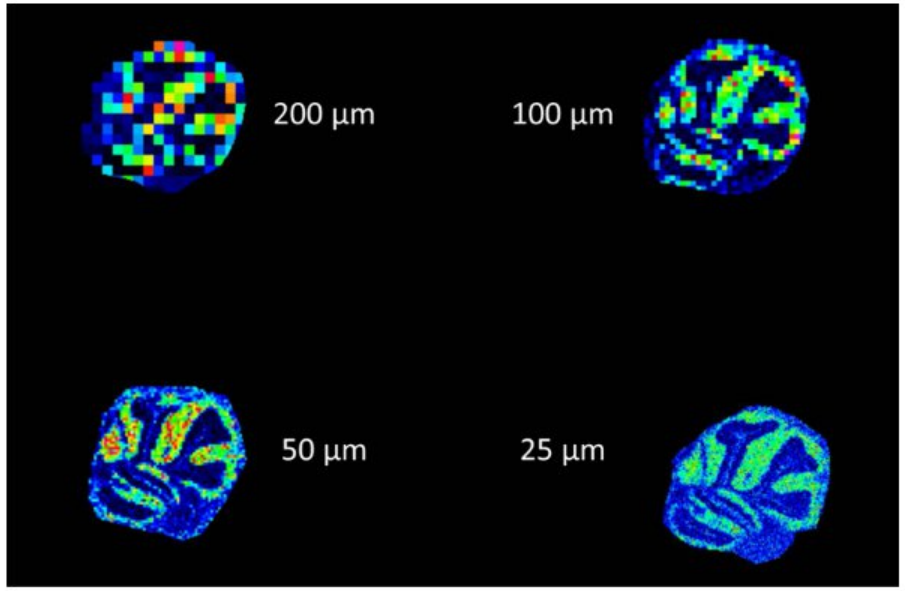


FTICR= Fourier Transform
Ion Cyclotron Resonance

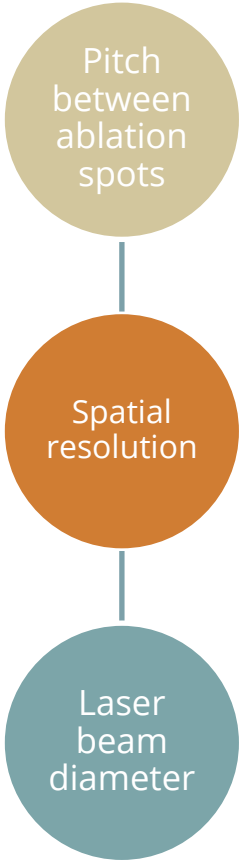


Speed	**+++**	**++**	**+**
Resolving power	**+**	**++**	**+++**
Mass accuracy	**+**	**++**	**+++**

Effect of resolution of the resulting image

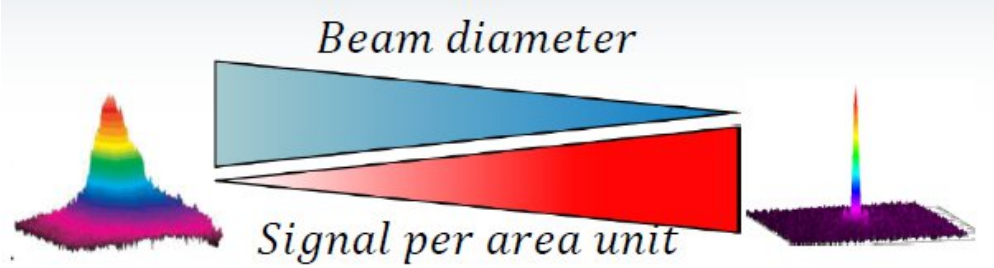


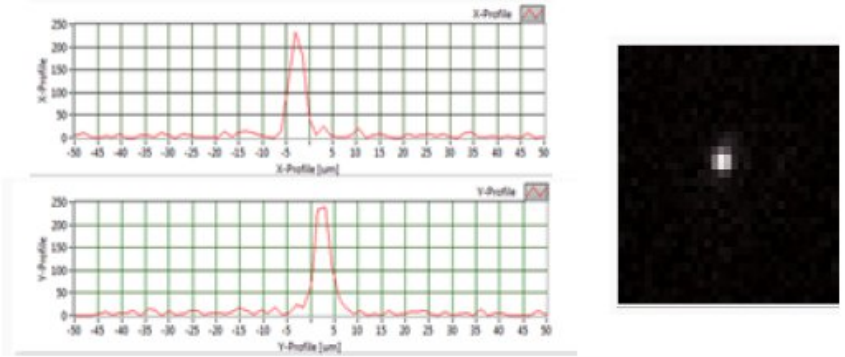
Comparison of serial mouse brain acquired at different spatial resolutions.
m/z 6755 of mouse cerebellum @ 200, 100, 50 & 25µm

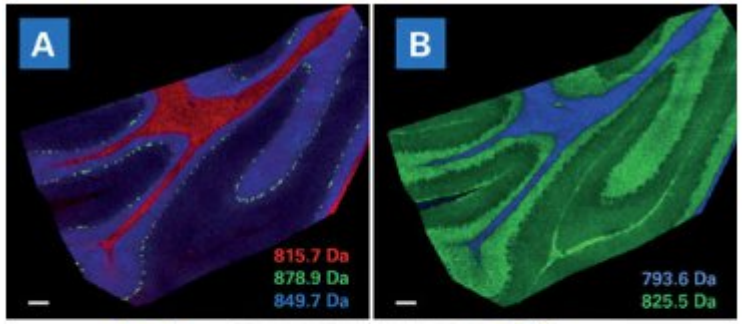


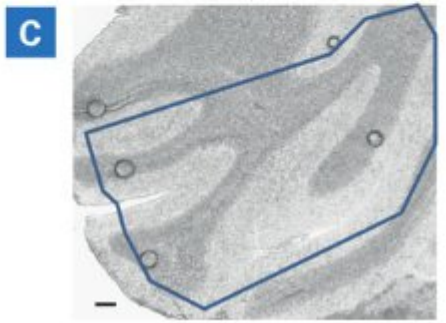
Lateral resolution: laser focusing

Smartbeam 3D: 5µm /10kHz

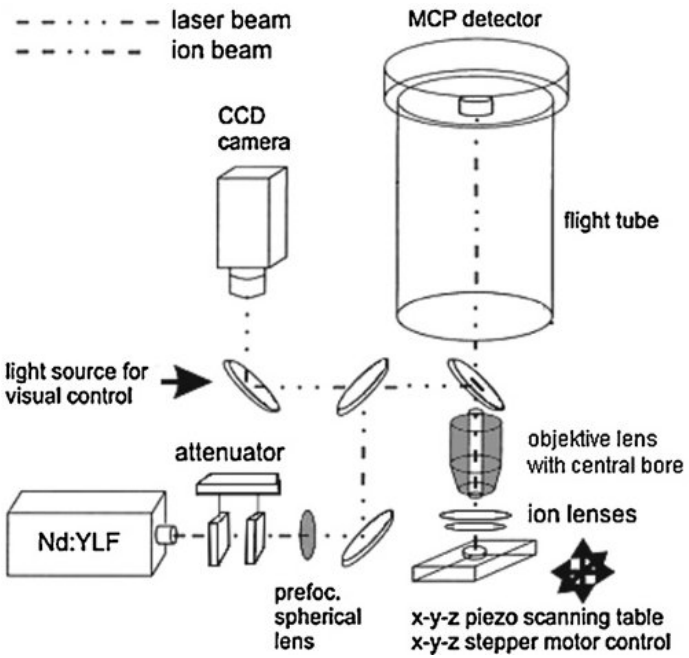


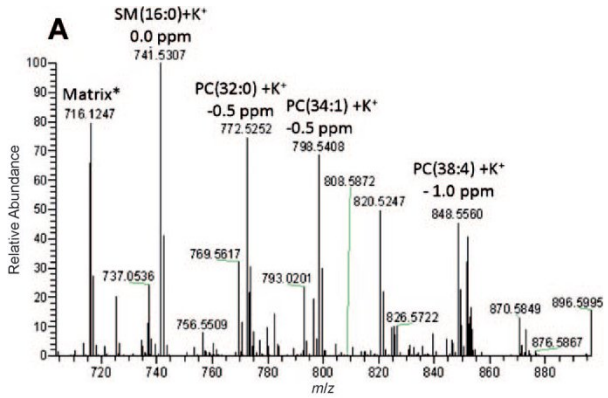


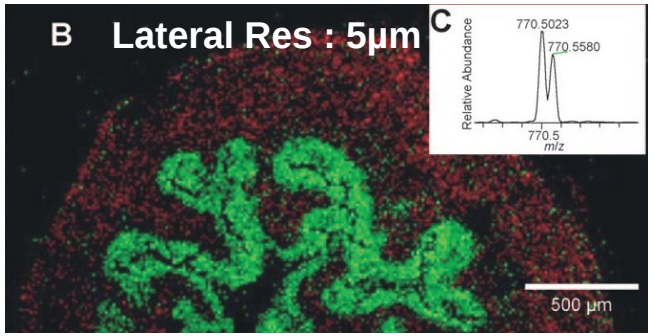




AP-SMALDI





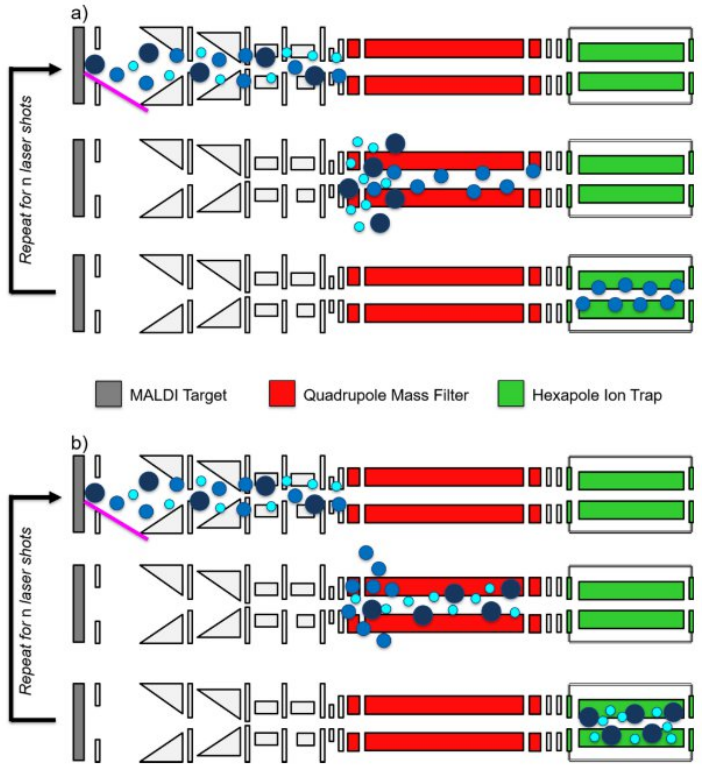


A) Orbitrap full-scan spectrum. B) Overlay of ion images: m/z 770.5097 (red) and m/z 770.5580 (green). C) Averaged orbitrap spectrum showing both separated peaks

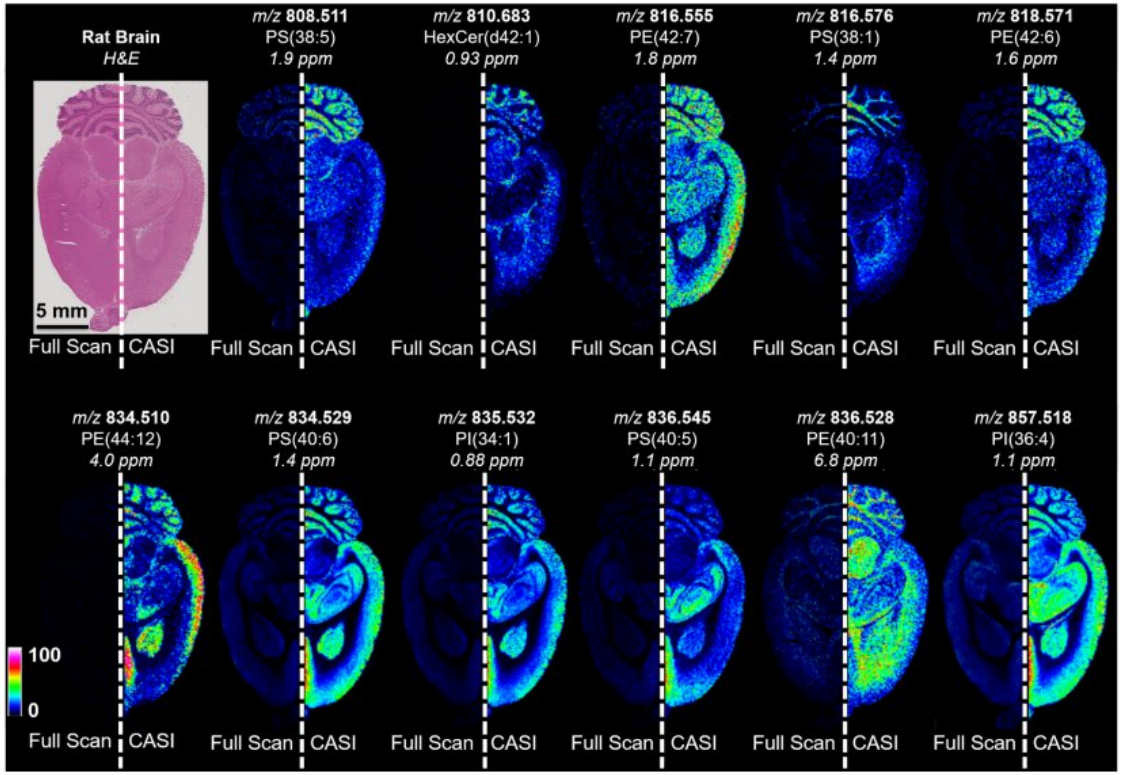
- ✓ ***SMALDI-MS-Orbitrap => sub-cellular resolution (0.5–10µm)***
- ✓ ***Mass accuracy of 2 ppm.***
- ✓ ***Applications : small molecules***

Römpp et al., Angew Chem Int Ed Engl., 2010, 49, 3834-3838

Continuous Accumulation of Selected Ions (CASI)



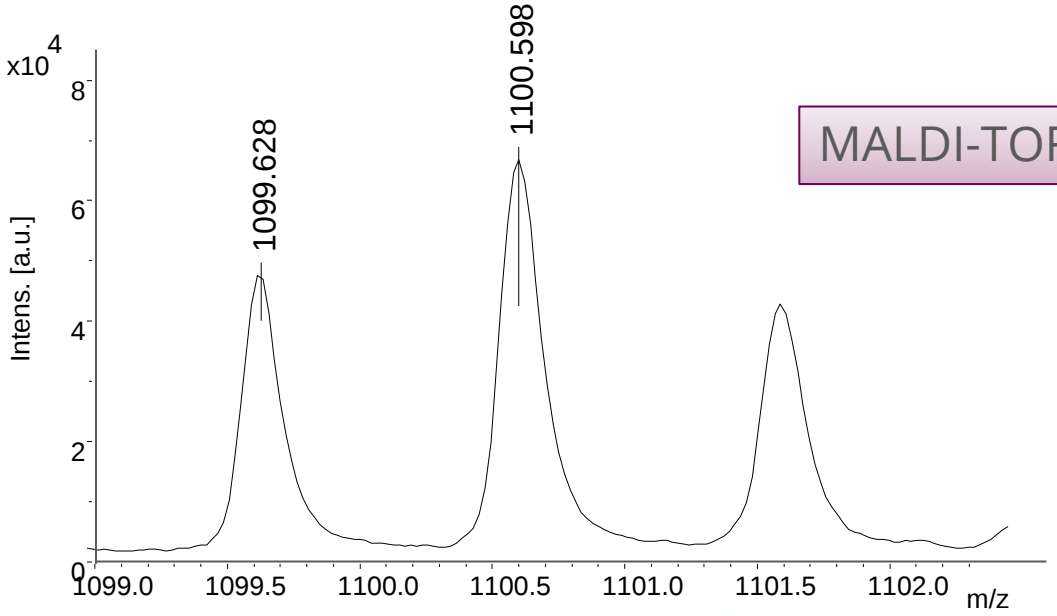
(a) isolation of a small m/z window by CASI
(b) selected ion ejection of a small m/z range



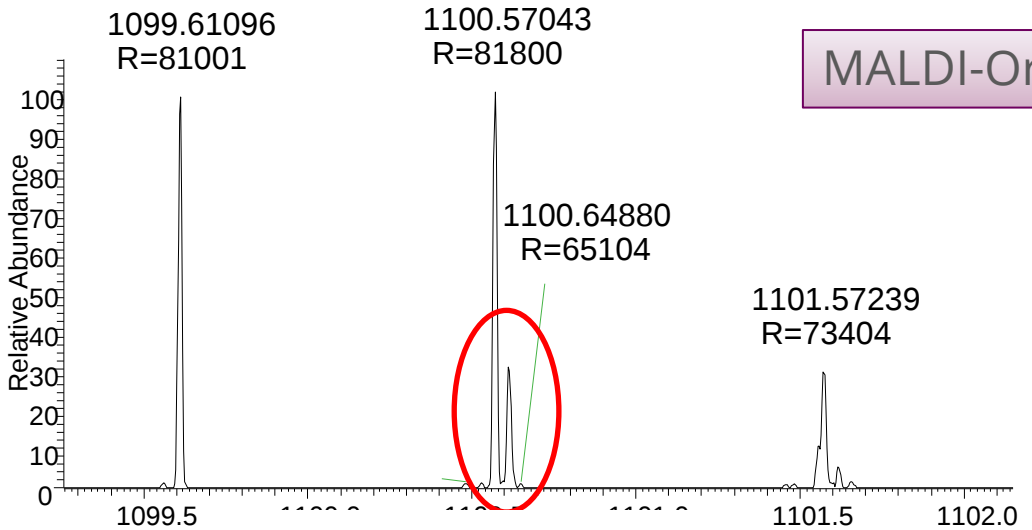
Imaging mass spectrometry analysis of a rat brain
(left hemisphere) full-scan acquisition mode
(right hemisphere) using a 75 Da CASI window centered at m/z 845
Ion images for a range of lipids within the CASI window show improved brightness (i.e., sensitivity) and contrast (i.e., dynamic range).

10.1021/acs.analchem.0c02121

Contribution of high spectral resolution

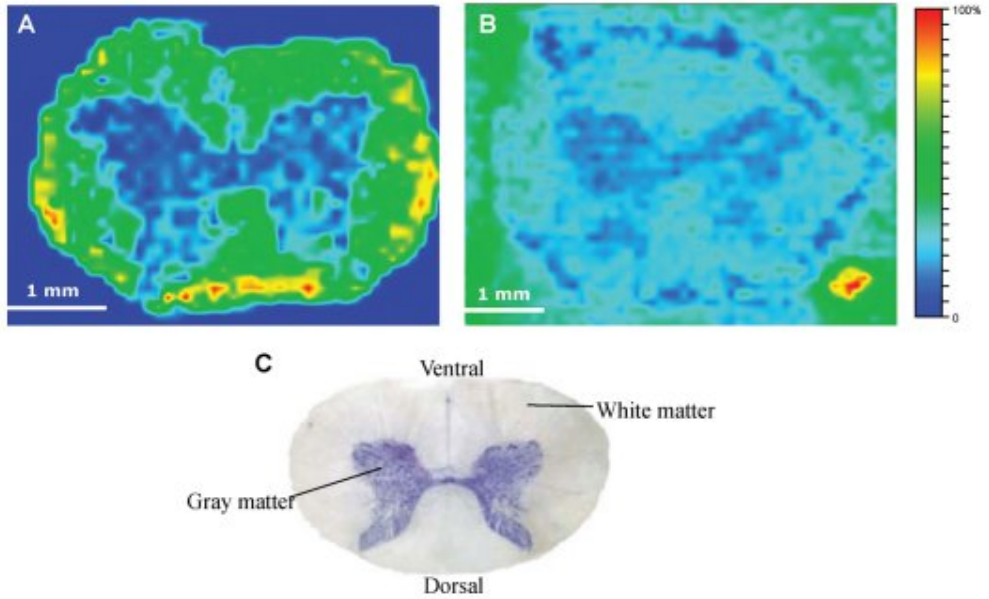


MALDI-TOF

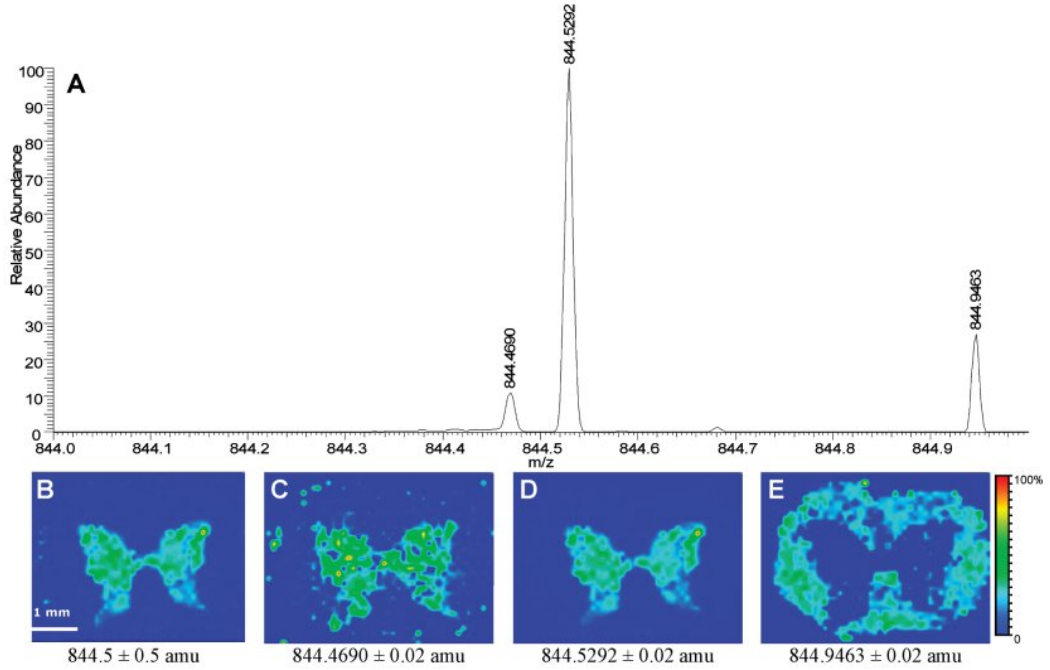


MALDI-Orbitrap

MALDI Orbitrap



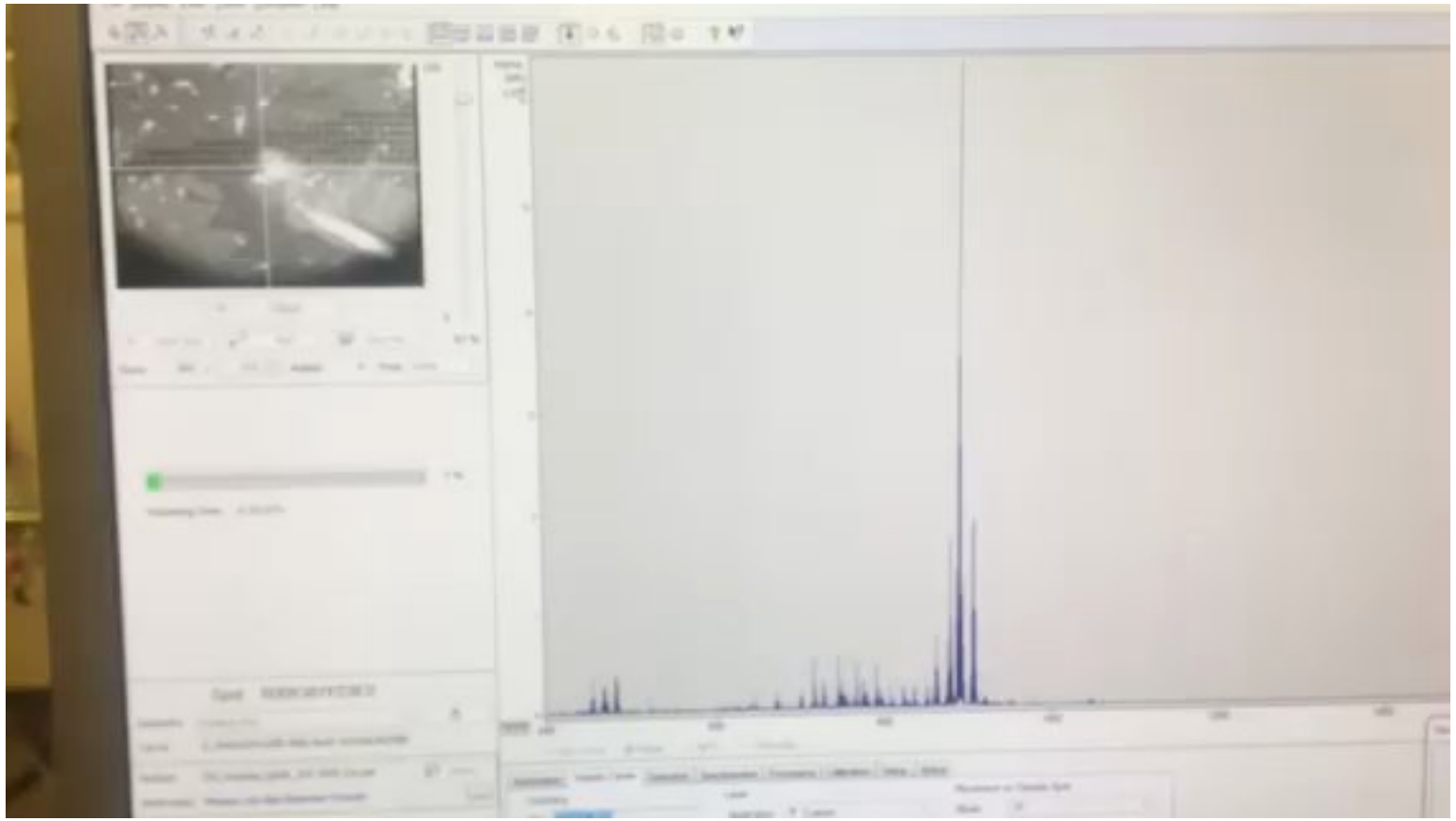
Mass spectrometric images of the total ion current of serial rat spinal cord sections analyzed by
(A) an Orbitrap analyzer
(B) a linear ion trap



(A) Mass spectrum of m/z region 844-845 showing at least that 5 peaks are detected. The mass spectrometric images correspond to (B) the 1 amu mass range and the peaks at (C) 844.4690, (D) 844.5292, and (E) 844.9463

10.1021/ac901387u

High-speed MALDI Imaging



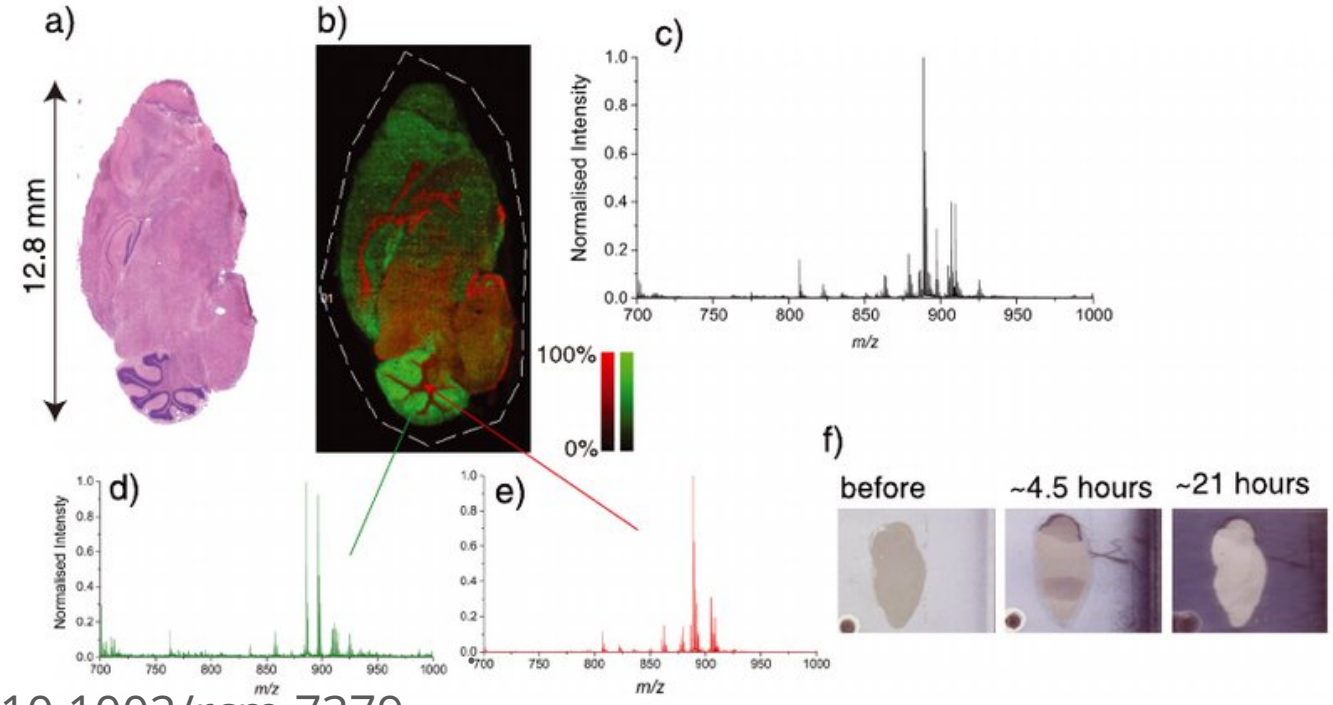
Bruker rapiflex Tissuetyper

Ogrinc Potočnik et al., Rapid Commun. Mass Spectrom., 2015.

High-speed MALDI Imaging

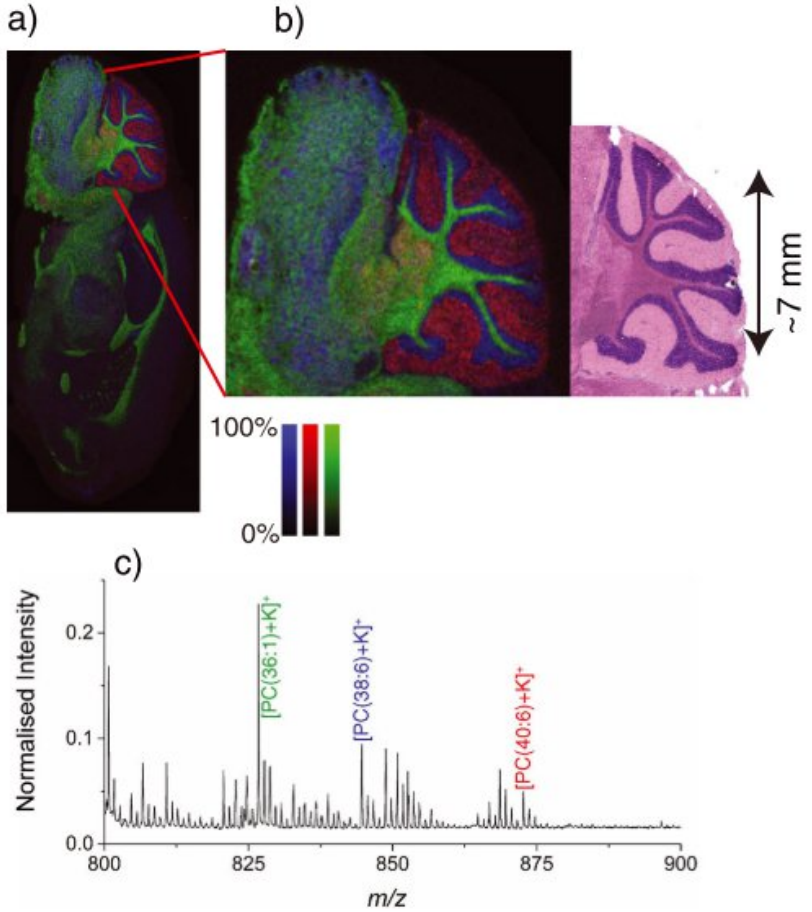
- 33,934 pixels in ~17 min, ~33 pixels/s
- 1 pixel/s would take over 9 h for a single image

Negative ion imaging of mouse brain
50×50 µm raster



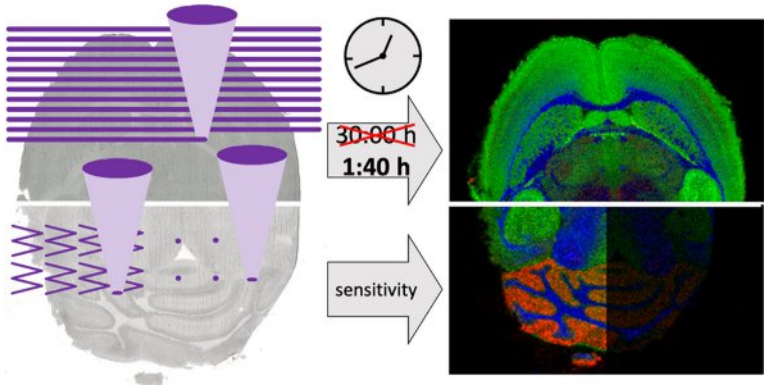
10.1002/rcm.7379

20×20 µm raster



10.1021/jasms.0c00368

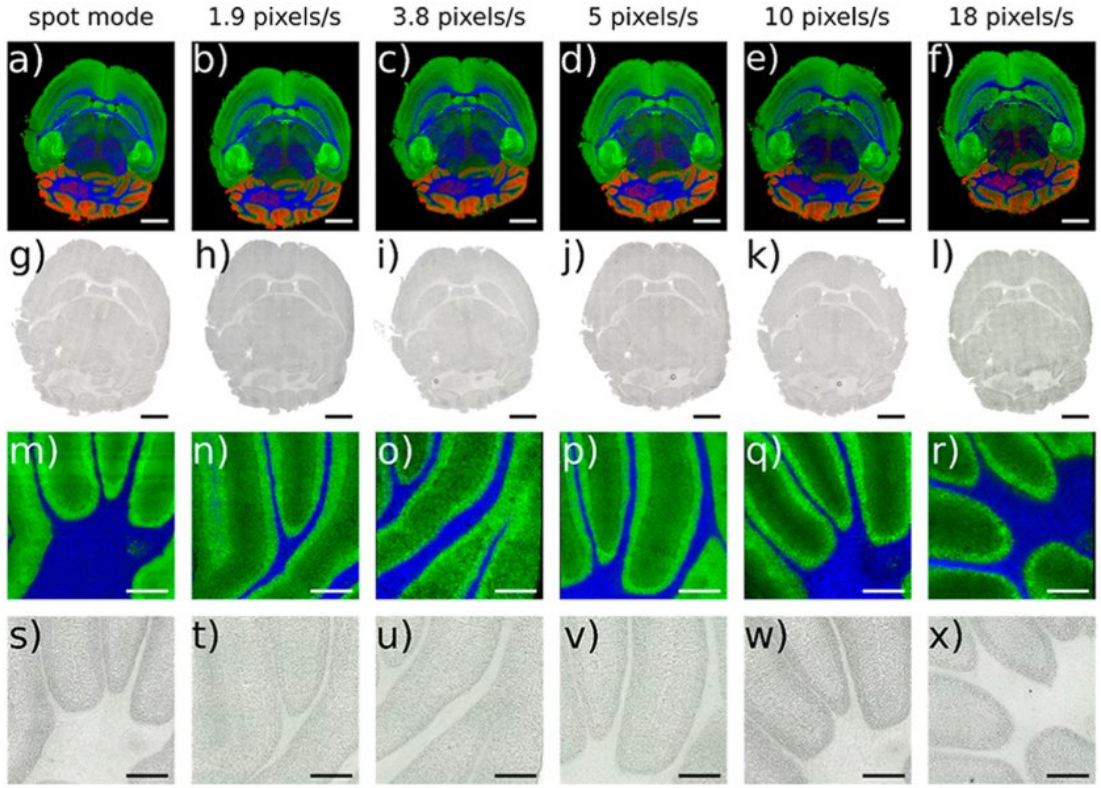
High-Repetition-Rate Laser in an AP-SMALDI

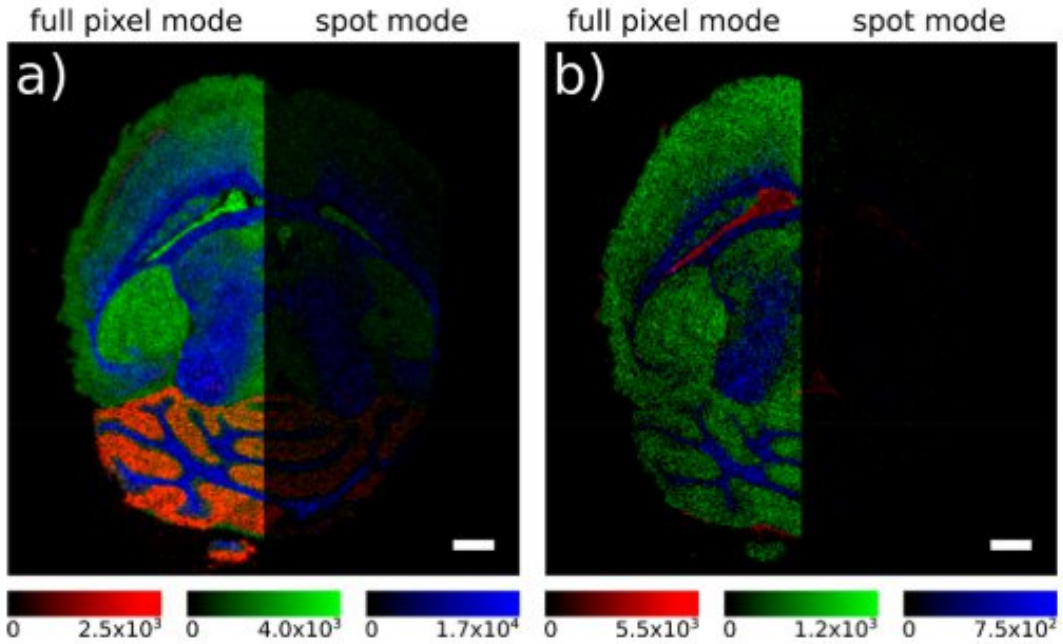


Pixelated scan		Line scan	
Spot mode	Full pixel mode (pixel size ≥ 25 um)	Continuous mode (pixel size ≤ 20 µm)	Burst mode (pixel size ≥ 20 µm)

10.1021/jasms.0c00368

High-Repetition-Rate Laser in an AP-SMALDI

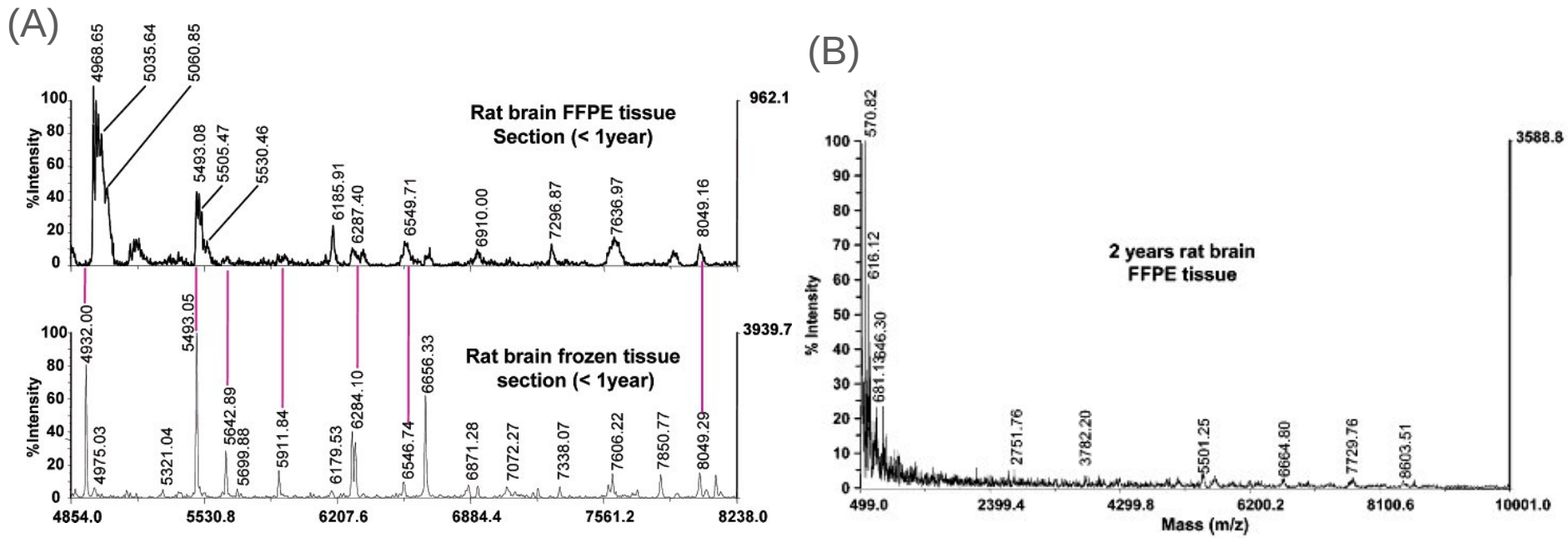




10.1021/jasms.0c00368

Antigen retrieval & FFPE tissue

MALDI analysis of FFPE tissue



(A) Comparison of MALDI mass spectra in the linear positive mode of the direct analysis of a <1 year old FFPE and fresh frozen rat brain tissues recorded in the same region with sinapinic acid as matrix

(B) MALDI mass spectrum in the linear positive mode of the direct analysis of a >1 year old FFPE tissue

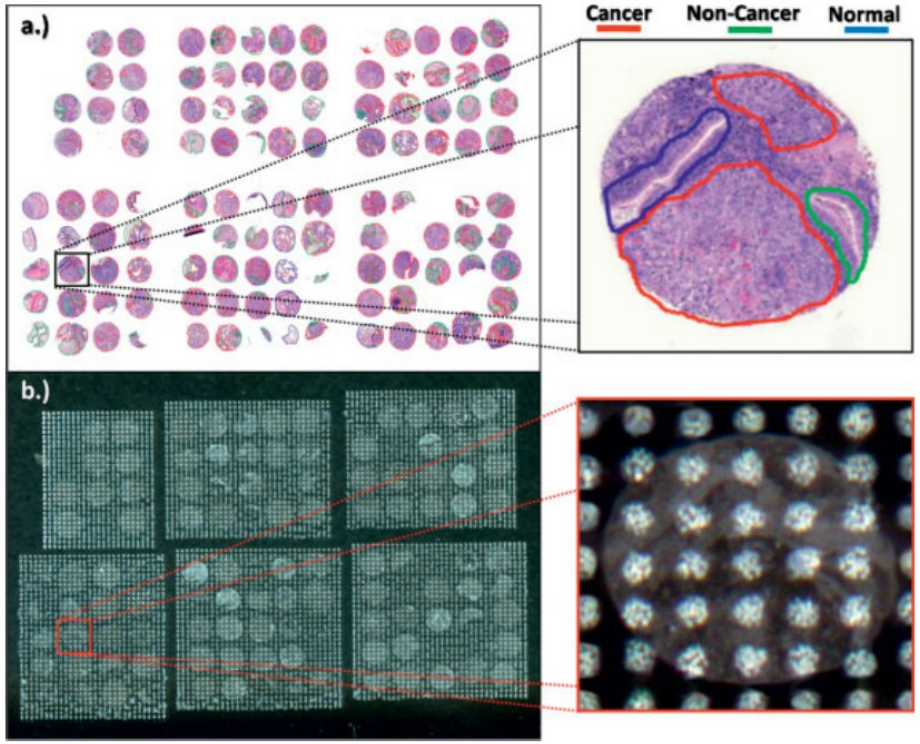
- ***Adducts observed (+12Da) => formation of a Protein-N=CH2***
- ***After 1 year, sample are difficult to analyze => crosslink***

Antigen Retrieval Strategies

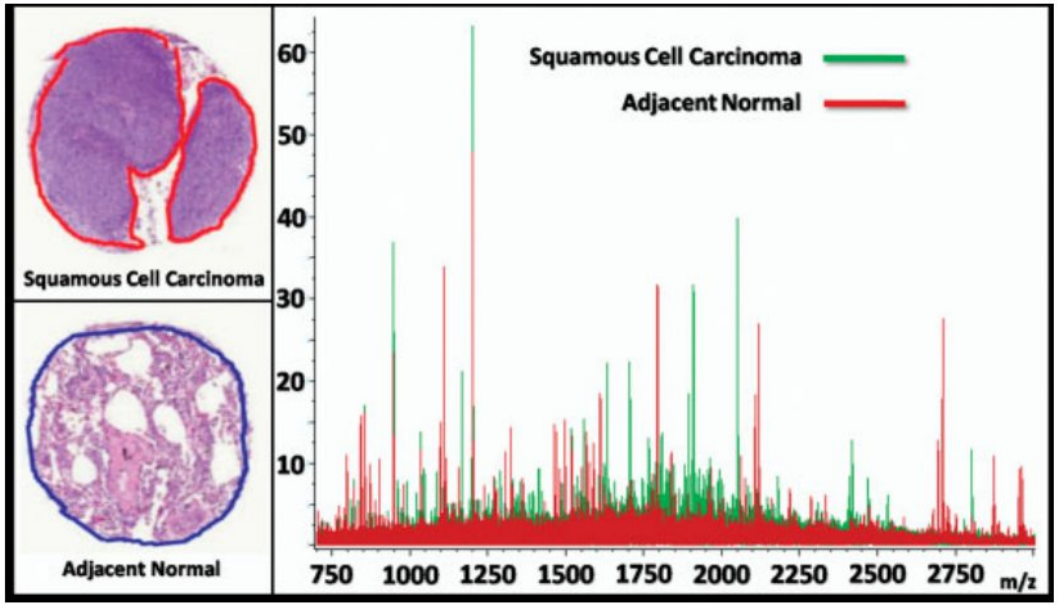
Chemical approach	
Enzymatic digestion	Proteinase K, trypsin, chymotrypsin, pronase, pepsin, N-glycanase F, hyaluronidase
Denaturant and chaotropic treatments	Formic acid, guanidine hydrochloride, guanidine thiocyanate, urea, boric acid, acetic acid SkipDewax™, sodium dodecyl sulfate, citraconic acid
Bleaching (oxidizing treatment)	Periodic acid, hydrogen peroxide, sodium meta periodate
Etching	Sodium (potassium) hydroxide in (m)ethanol
Detergent treatment	Triton X-100
Physical approach	
Heat treatment	Source: microwave, autoclave, pressure cooker, steamer, water bath. In solution of: distilled water, sucrose, EDTA, EGTA, TBS, aluminum chloride, zinc sulfate, lead thiocyanate, citrate buffer, borate.
Ultrasound treatment	

D'Amico et al., J. Immuno. Methods., 2009, 341, 1-18

High-throughput proteomic analysis of FFPE tissue



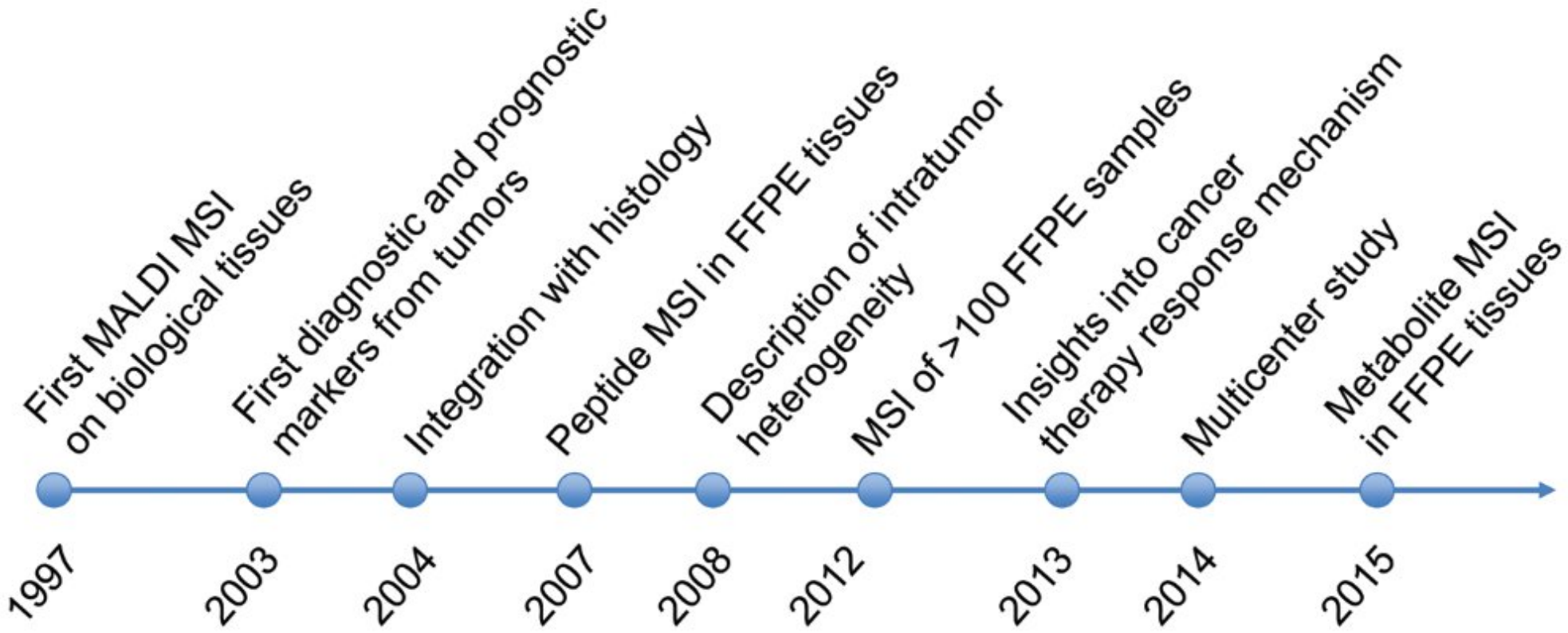
(a) TMA H&E with histological regions marked
(b) TMA spotted with trypsin/matrix for MS analysis



Overlay of average spectra from a squamous cell carcinoma needle core biopsy and an adjacent normal tissue needle core biopsy taken from the same patient

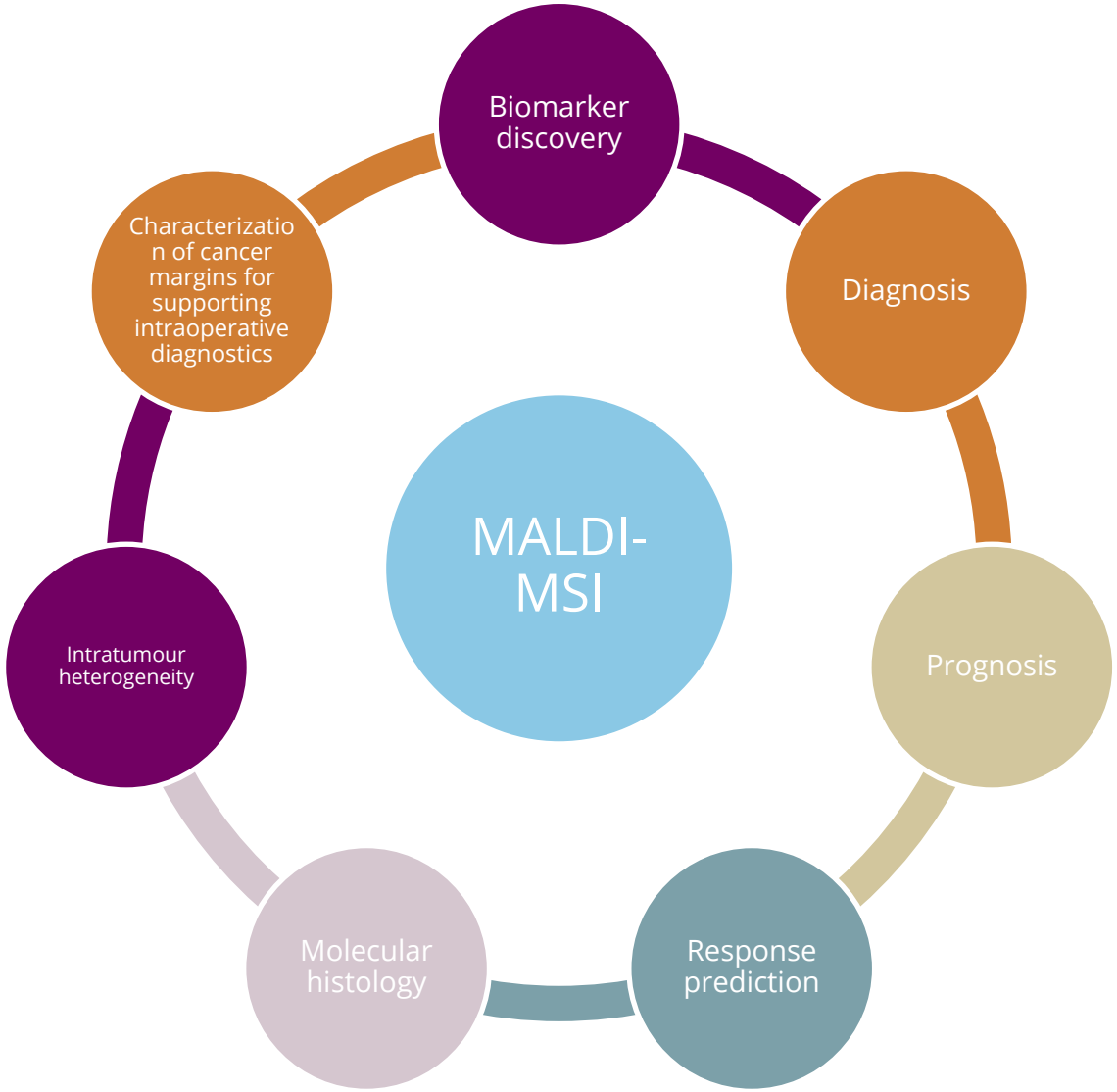
10.1002/pmic.200800495

Highlights and breakthroughs of MALDI-MSI in oncology

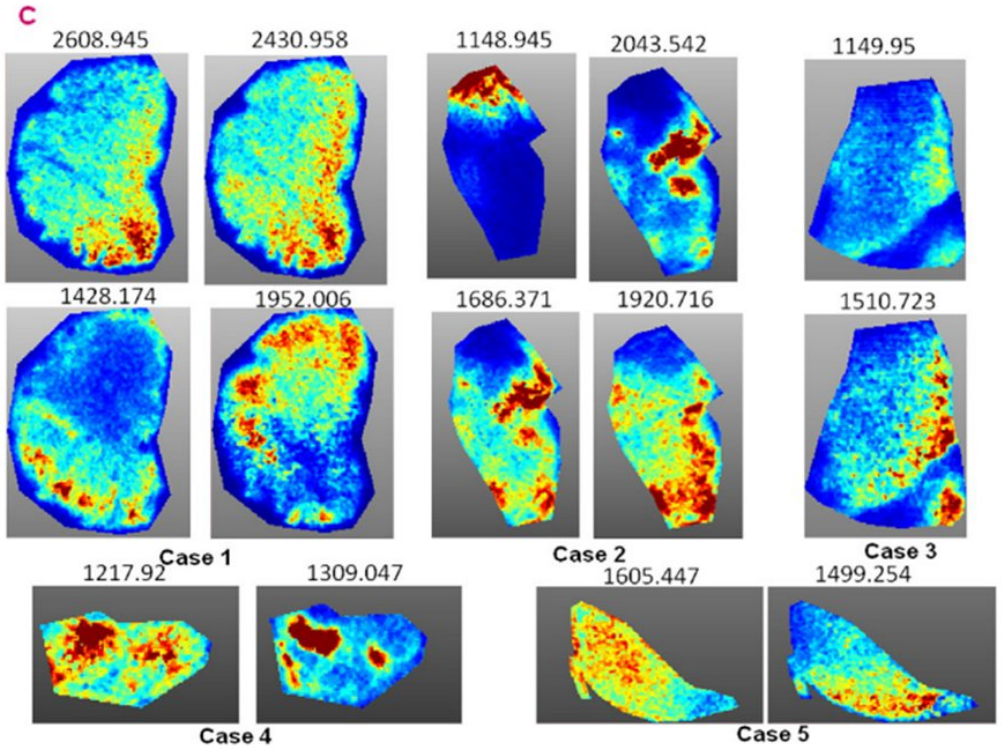


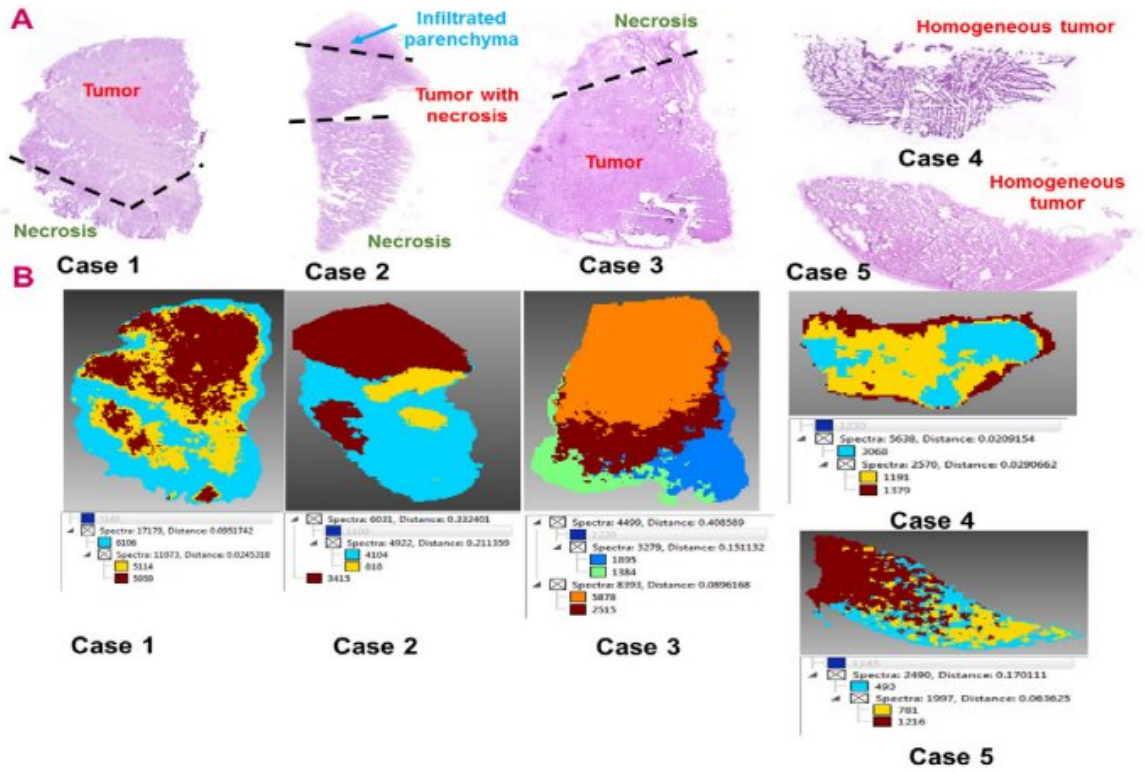
10.1039/c7an00565b

MALDI-MSI in oncology



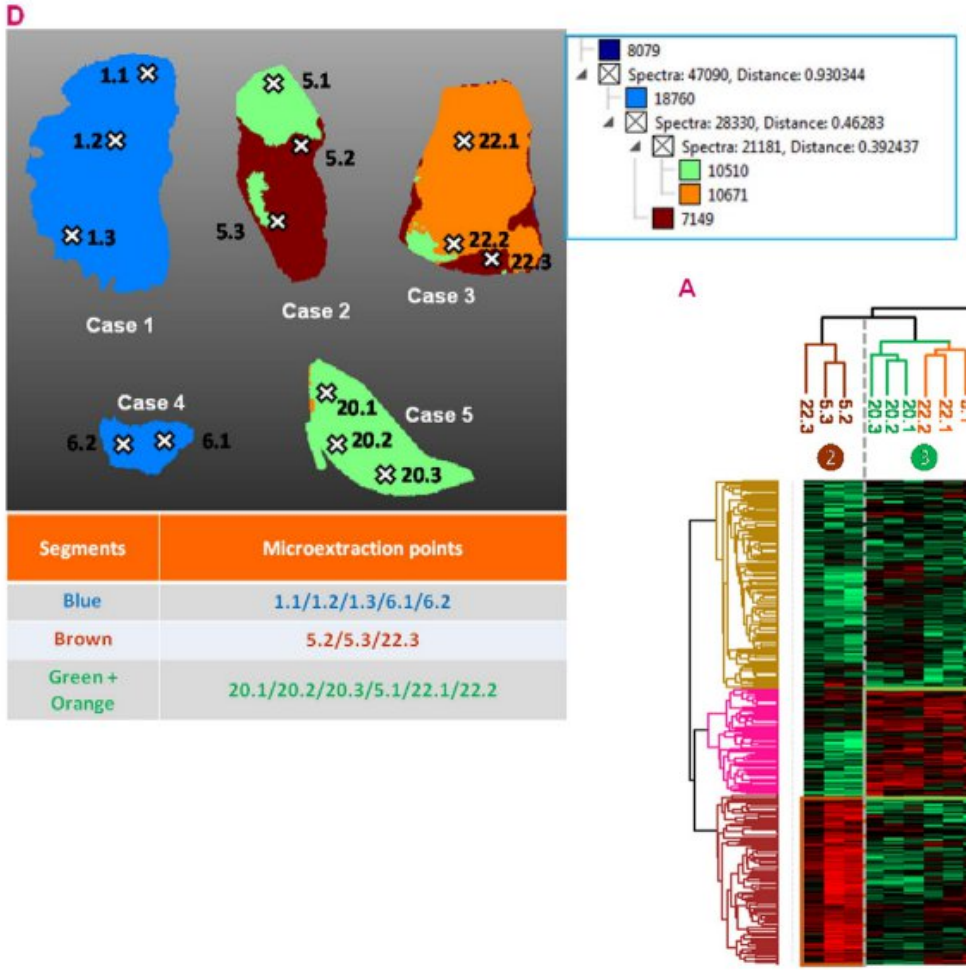
Glioma grade III classification



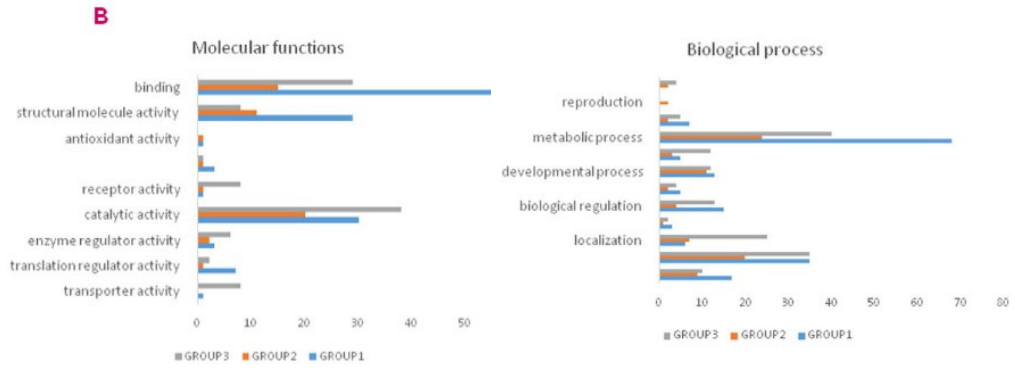


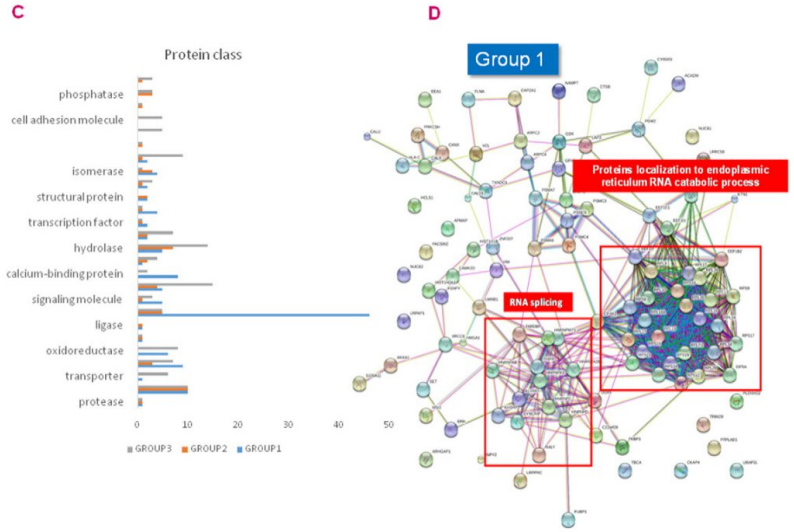
10.1016/j.bbapap.2016.11.012

Glioma grade III classification



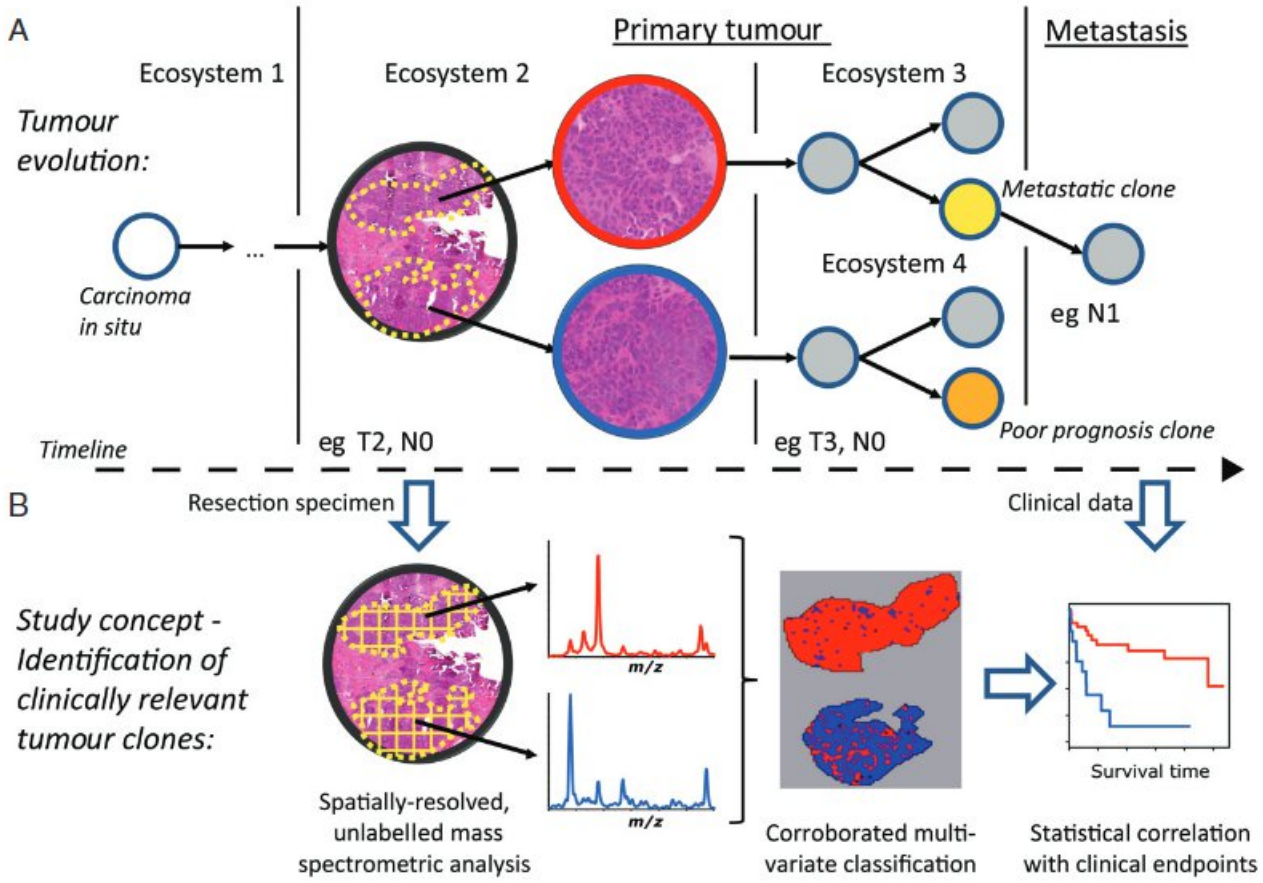
Segments	Microextraction points
Blue	1.1/1.2/1.3/6.1/6.2
Brown	5.2/5.3/22.3
Green + Orange	20.1/20.2/20.3/5.1/22.1/22.2

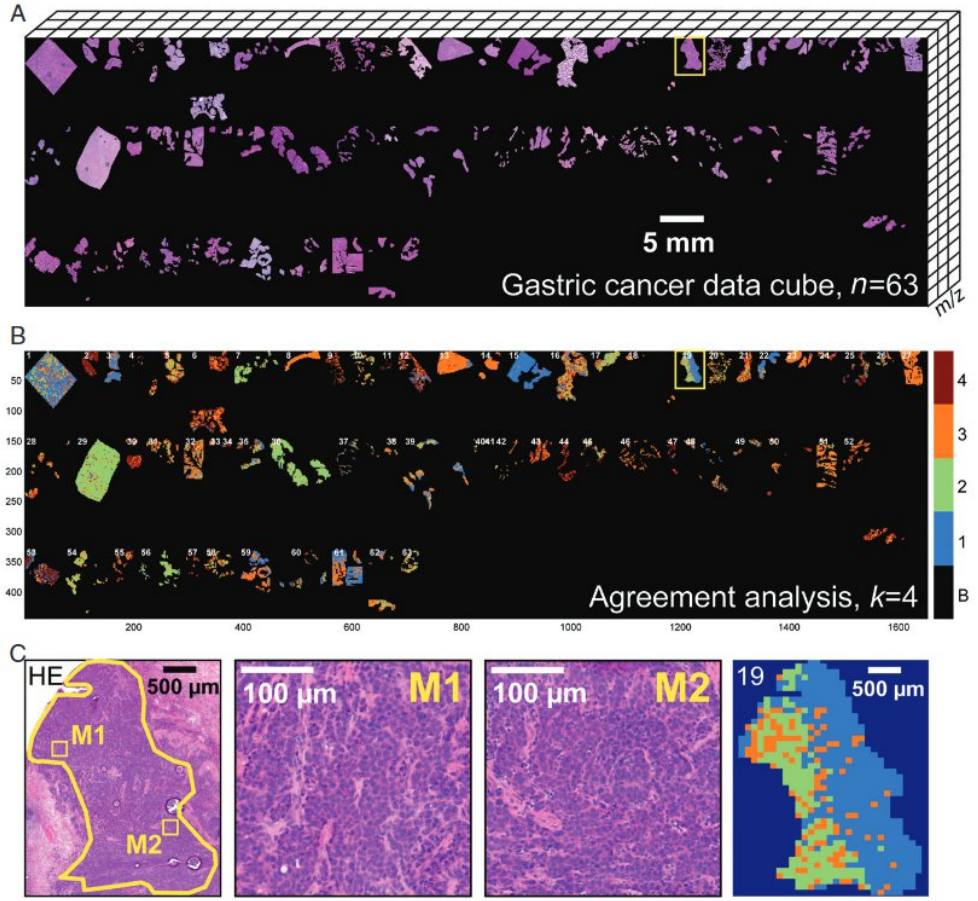




10.1016/j.bbapap.2016.11.012

Intratumour heterogeneity intestinal-type gastric cancer





10.1002/path.4436

Spatially Resolved Mass Spectrometry at the Single Cell

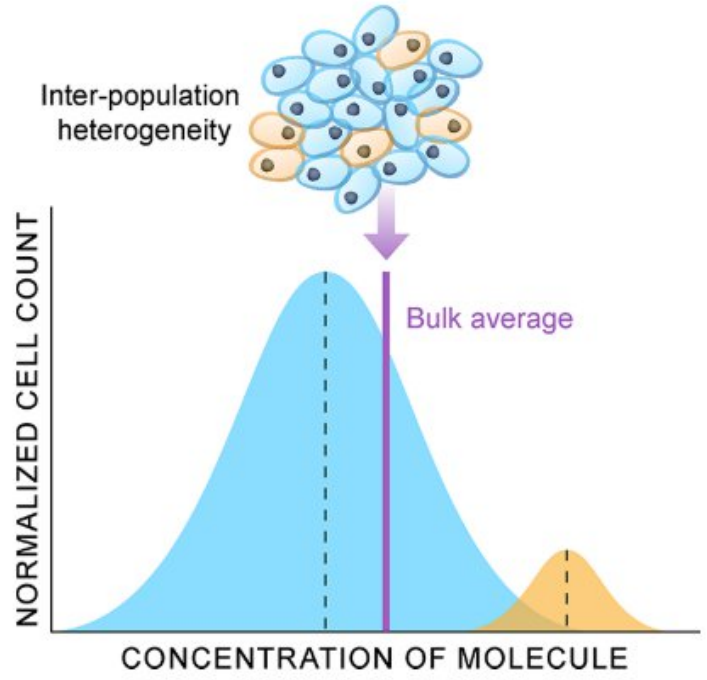
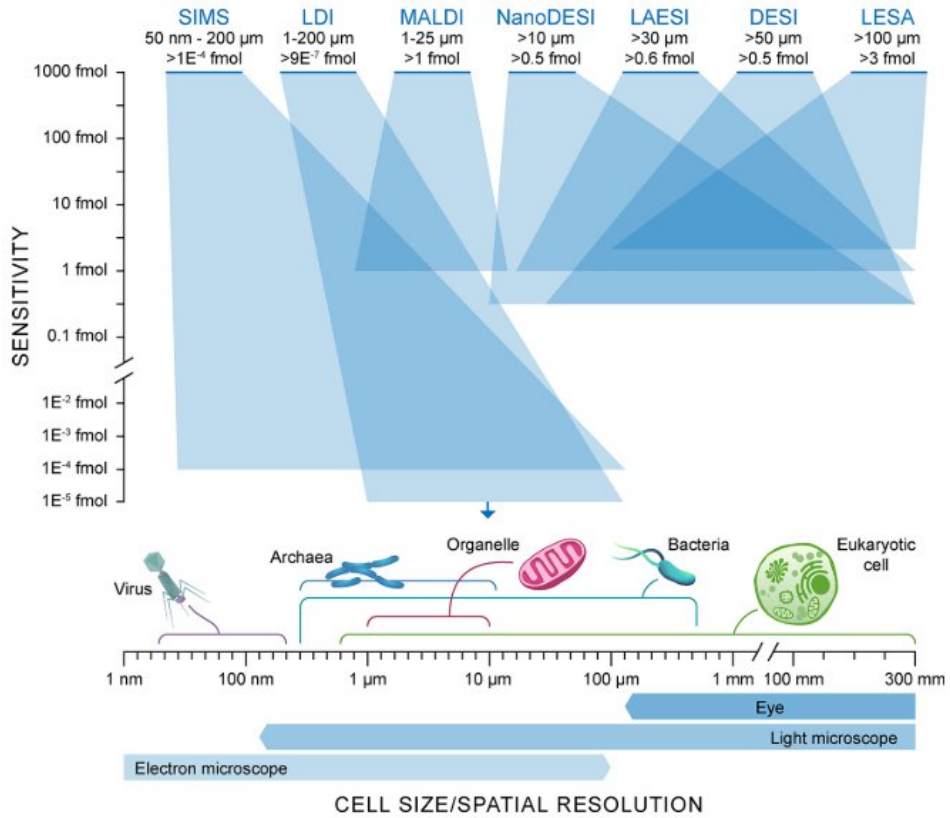
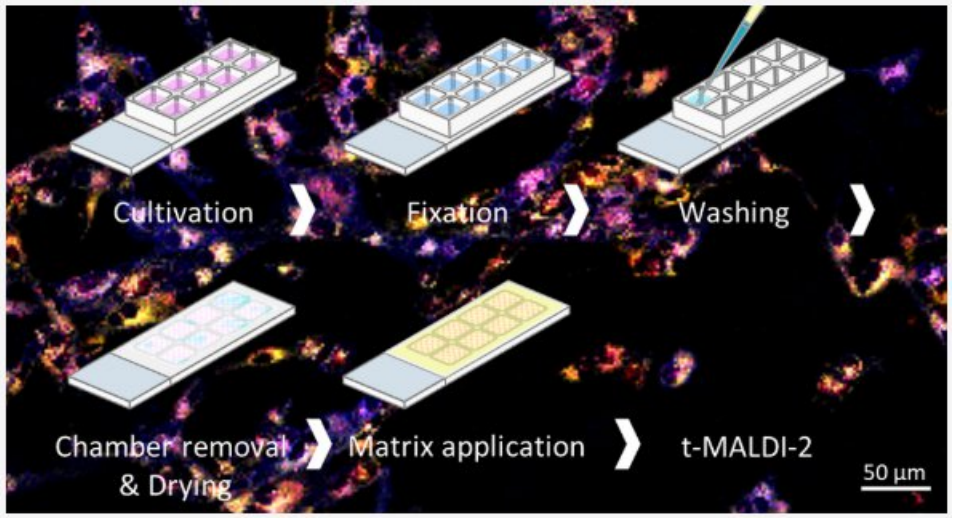


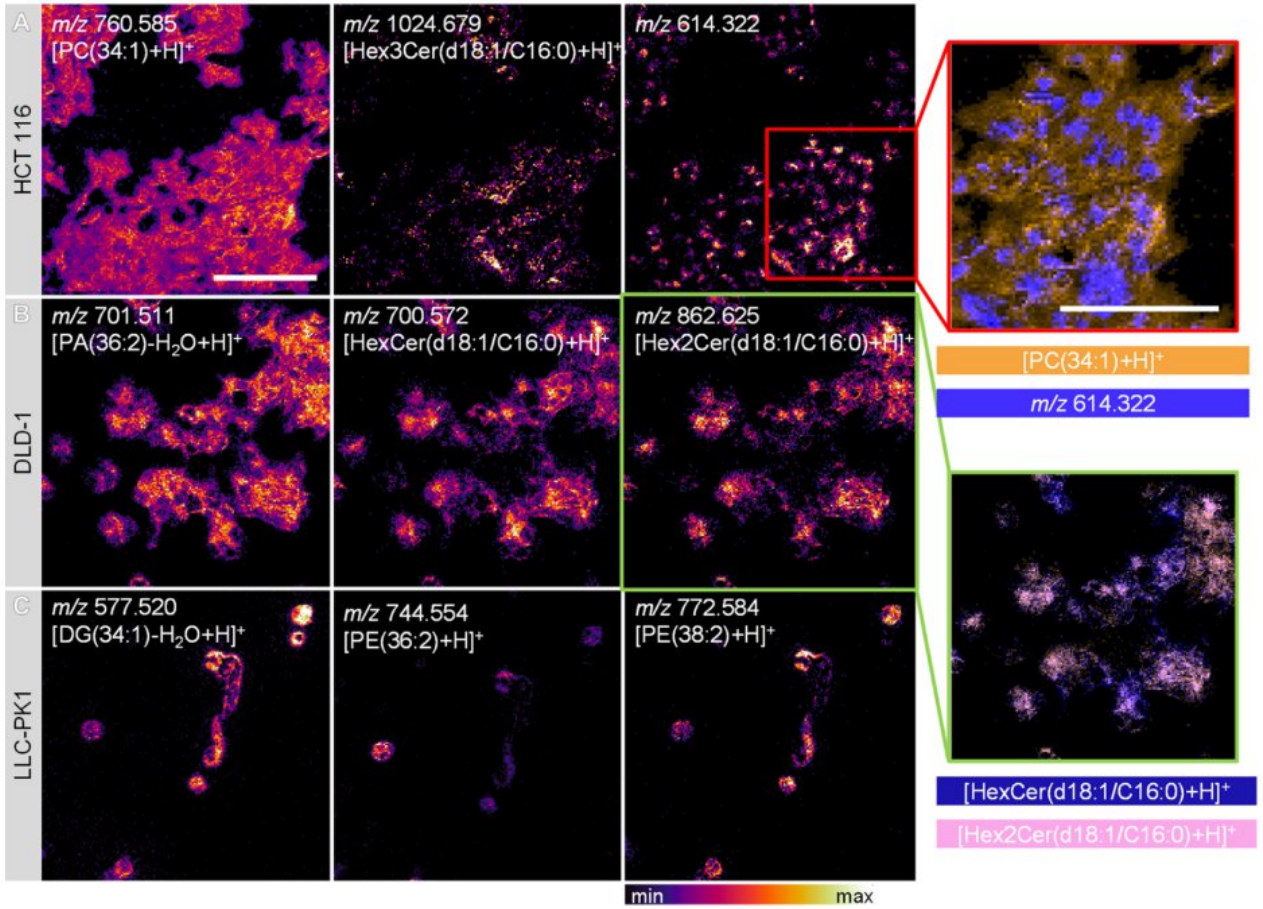
Illustration of how physiological state heterogeneity occurring across a cell population within a sample may not be resolved by bulk omics measurements (purple line). In this example, two discrete subpopulations of cells can be resolved by measuring each cell, which will resolve the predominate cell population (blue) from a minor cell population (yellow)



10.1021/jasms.0c00439

Transmission-Mode MALDI Mass Spectrometry Imaging of Single Cells





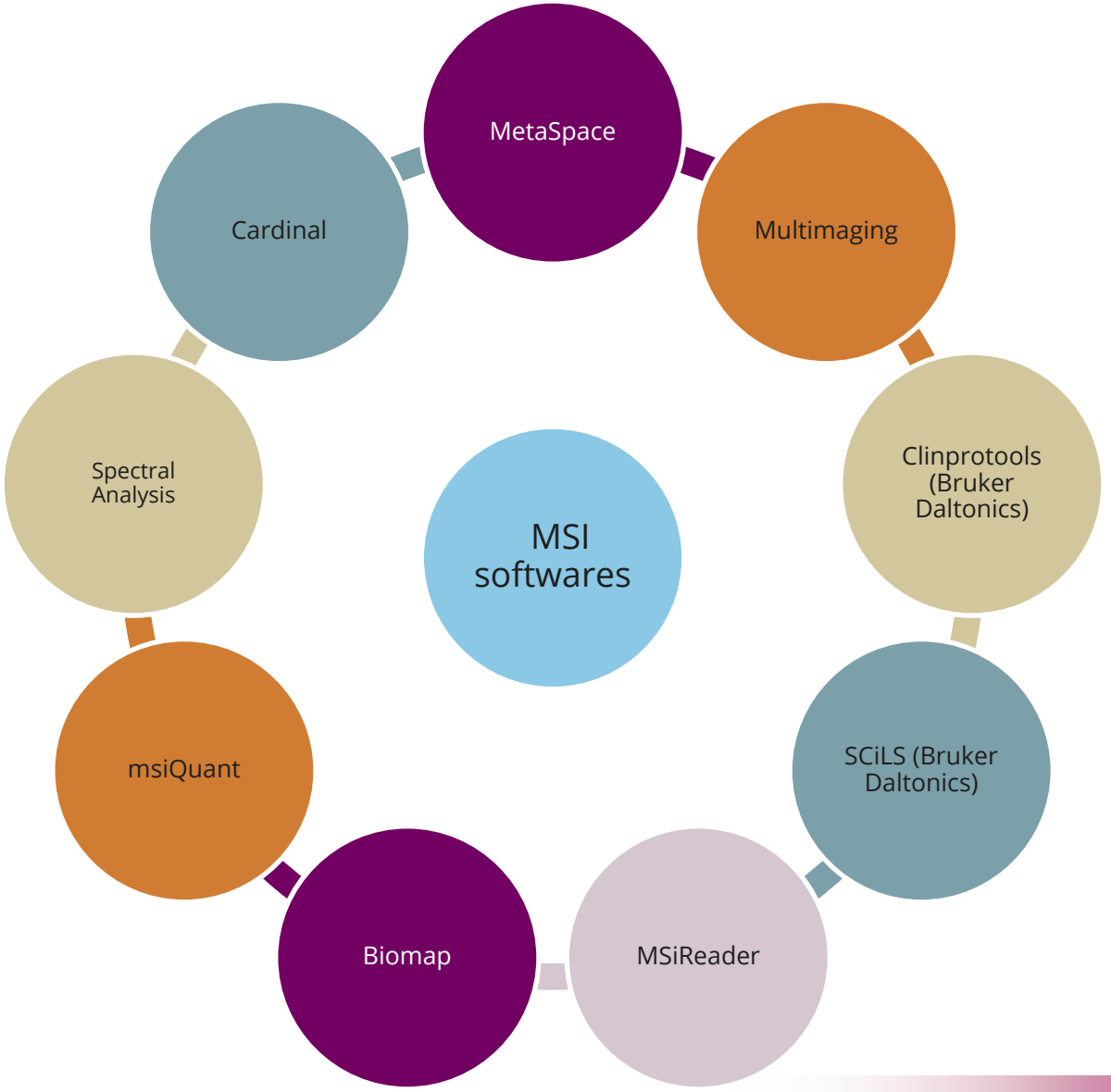
Ion distribution images of selected glycosphingo- and phospholipids and an unknown compound at m/z 614.322 analyzed by t-MALDI- 2-MSI from :

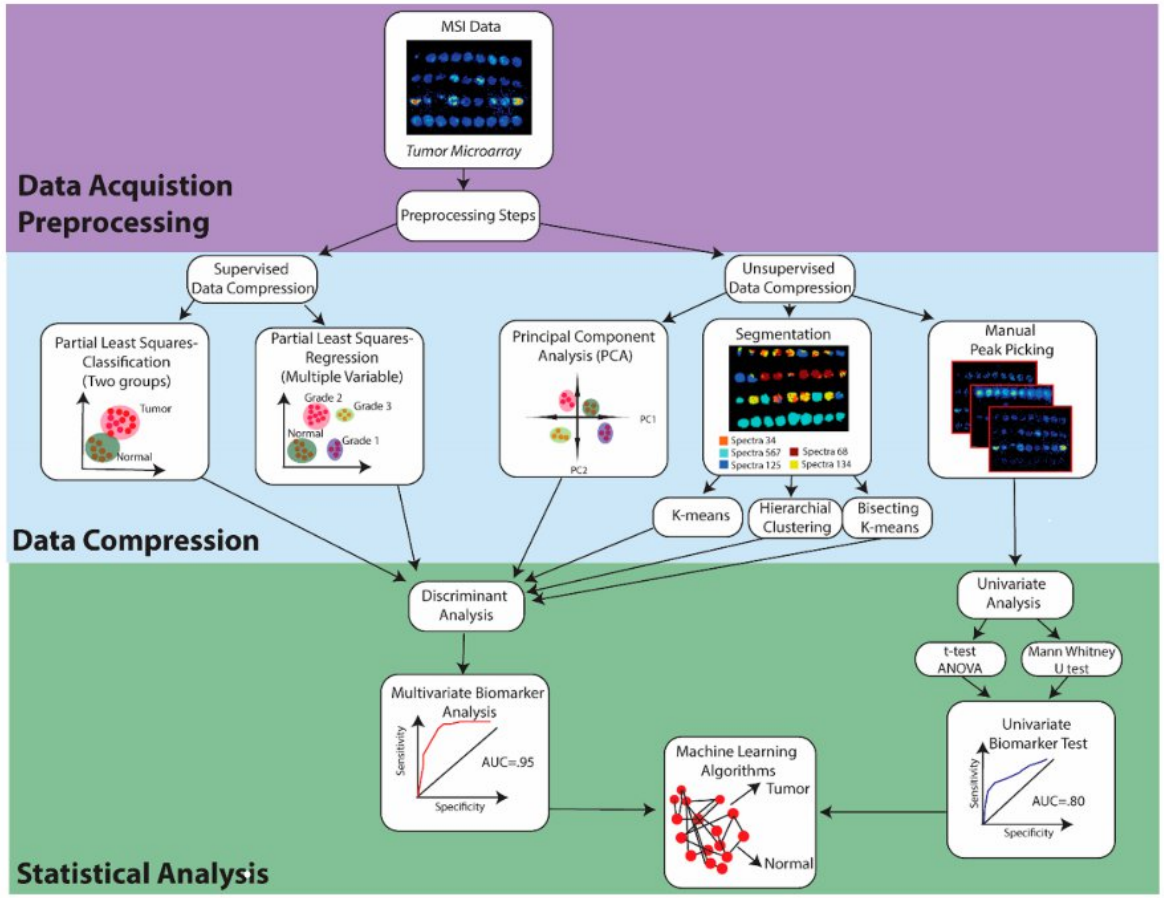
(A) HCT 116
(B) DLD-1
(C) LLC-PK1 cells

10.1021/acs.analchem.0c04905

Data Processing and visualization

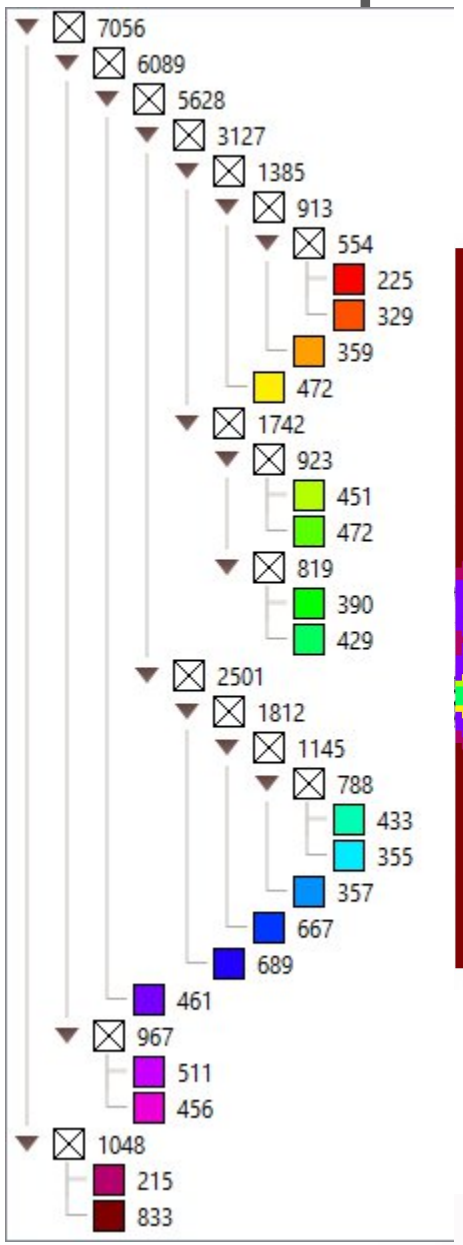
MSI software packages

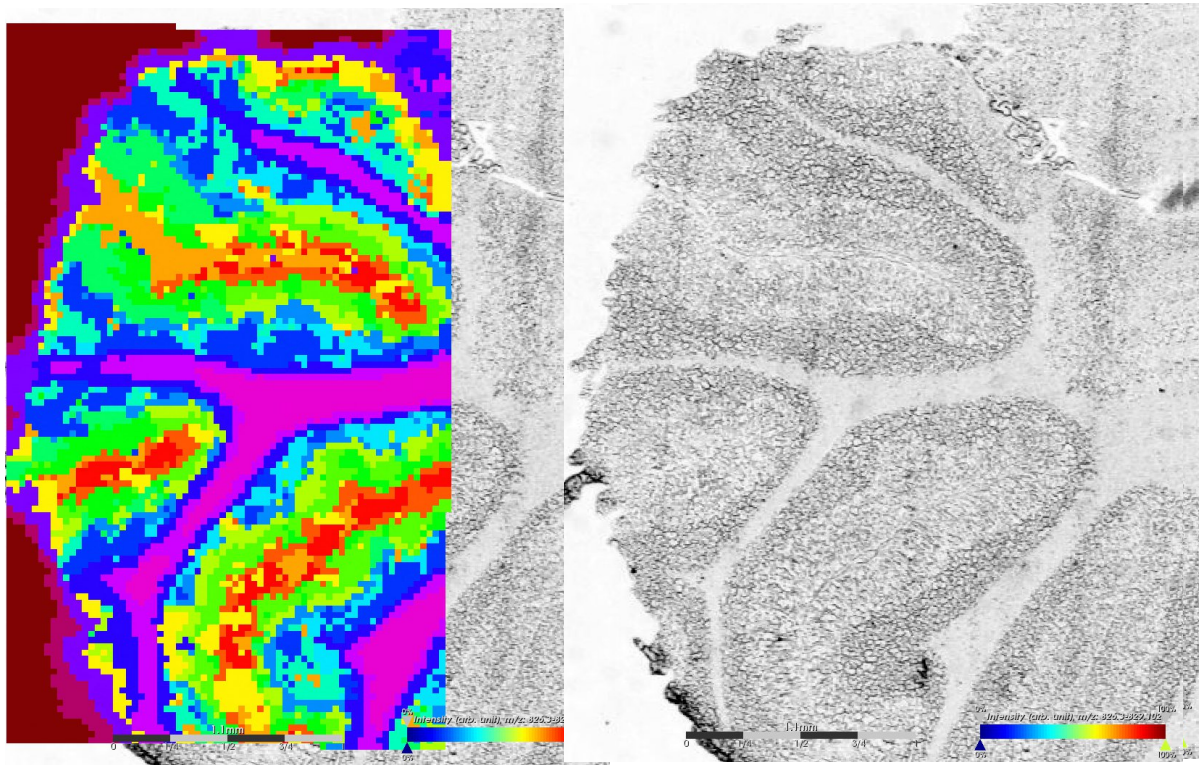




10.1021/acs.analchem.7b04733

Spatial segmentation





18 different classes

Deninger et al., J. Proteom. Res, 2008, 7, 5230-5236

SFSM

THANKS FOR YOUR ATTENTION