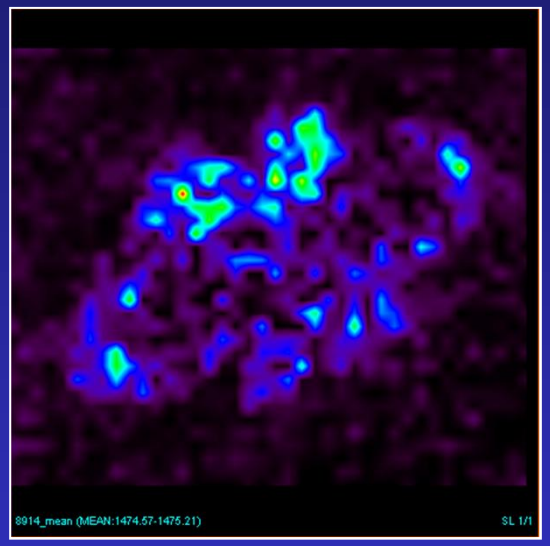
Imaging Mass Spectrometry (IMS): Searching for the invisible, a new tool for disease investigations



7th CEEPC Jena 16/10/13

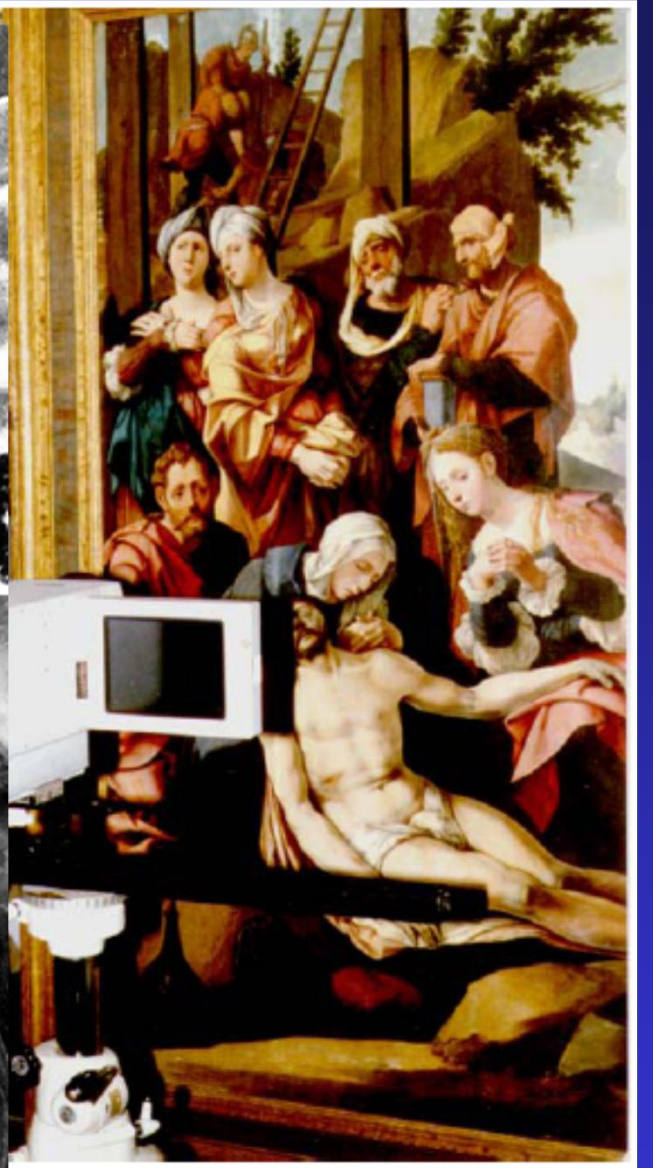
Meilahti Clinical Proteomics Unit (MCPU)
University of Helsinki, Faculty of Medicine
Biomedicum Helsinki and Haartman Institute
E-Mail: marc.baumann@helsinki.fi

(http://research.med.helsinki.fi/corefacilities/proteinchem)

Mass Spectrometry Imaging (tissue, cells, bacteria, viruses...)

Equals scanning with a mass spectrometer the invisible

IRR investigation of “the Lamentation of Christ” by Jan van Scorel



R investigation of “the
mentation of Christ”
by Jan van Scorel

Definition

MS is an analysis technique to determine by means of a mass spectrometer the molecule mass of free ions in high (semi high) vacuum

i.e. it sees everything with a mass!

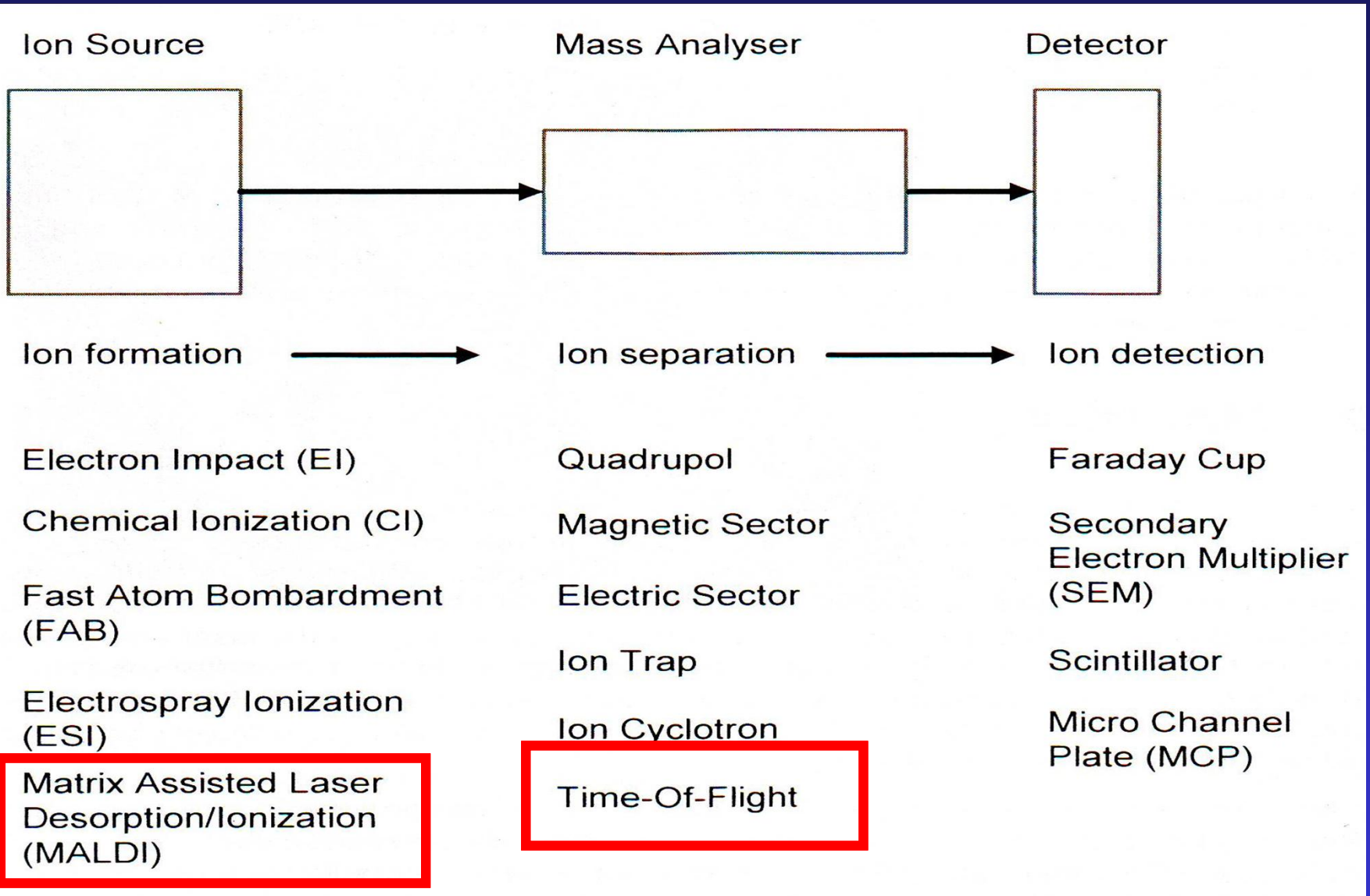
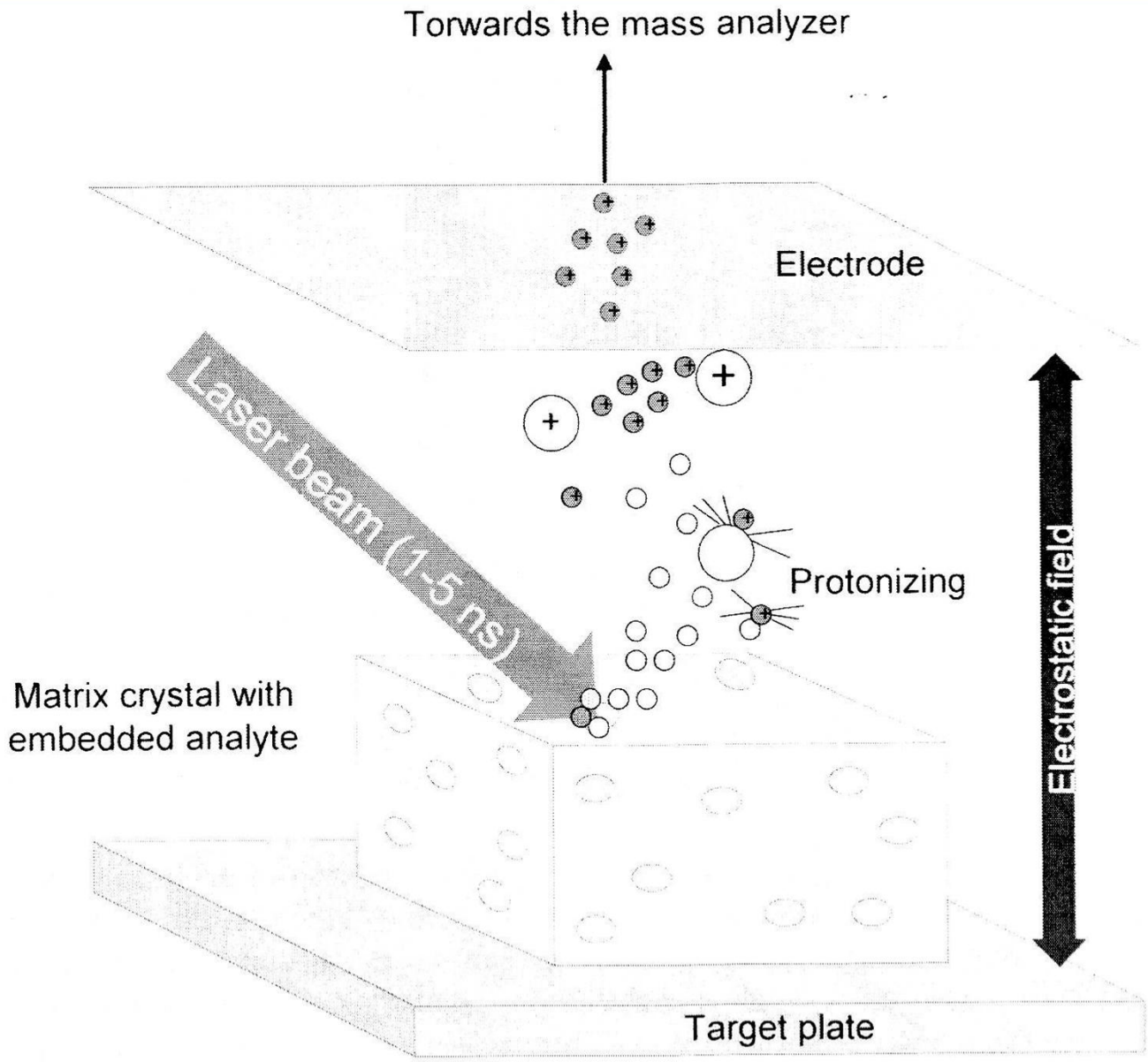


Figure 1 Components of a mass spectrometer

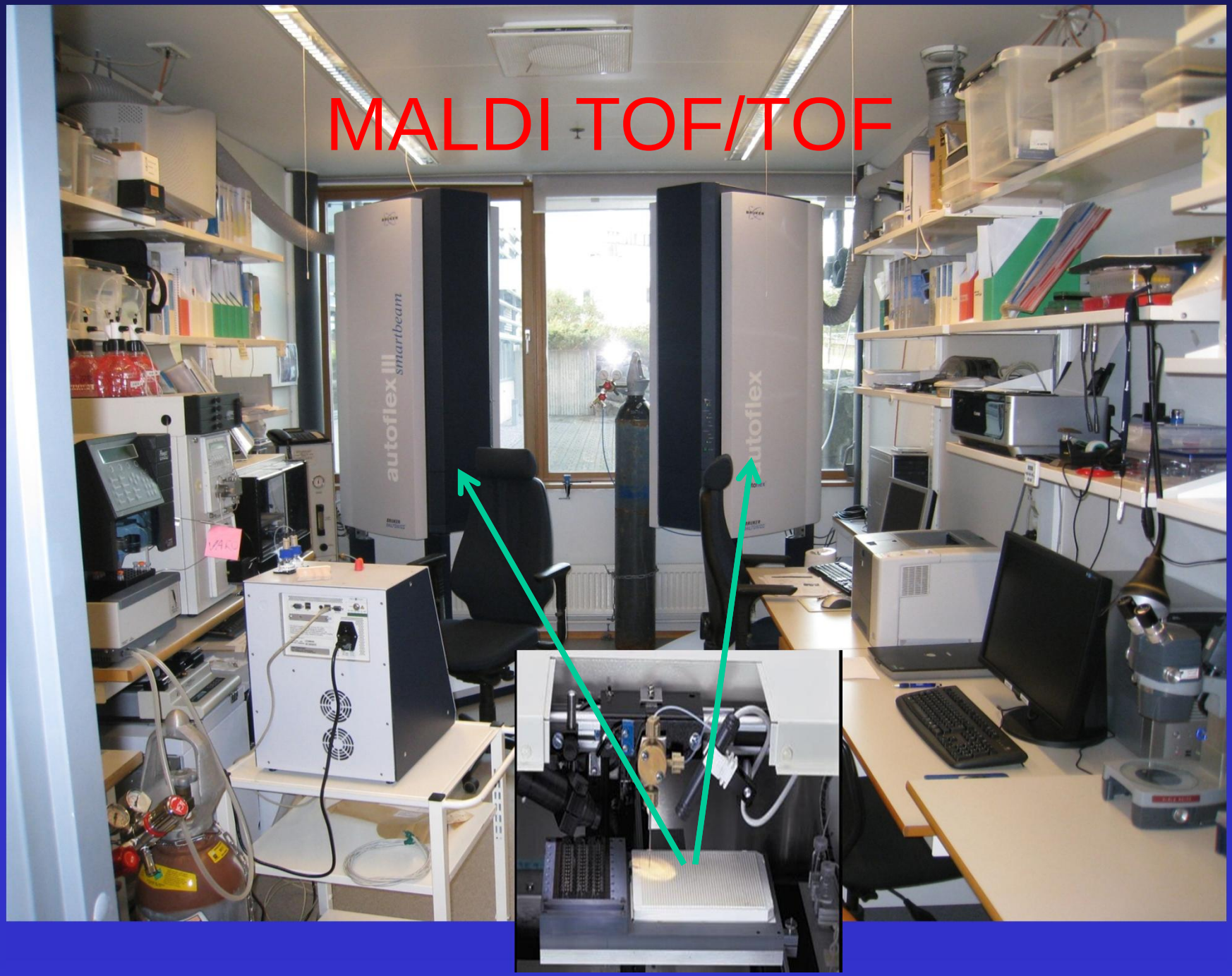
MALDI Principles

- Mix analytes with excess matrix compound to crystallize (1:1000 or 10 000)
- Matrix absorbs at the same laser wave length (commercially available N2-laser@337nm, YAG laser @355nm, infrared Er-YAG@2.94um)
- Short waved laser pulses



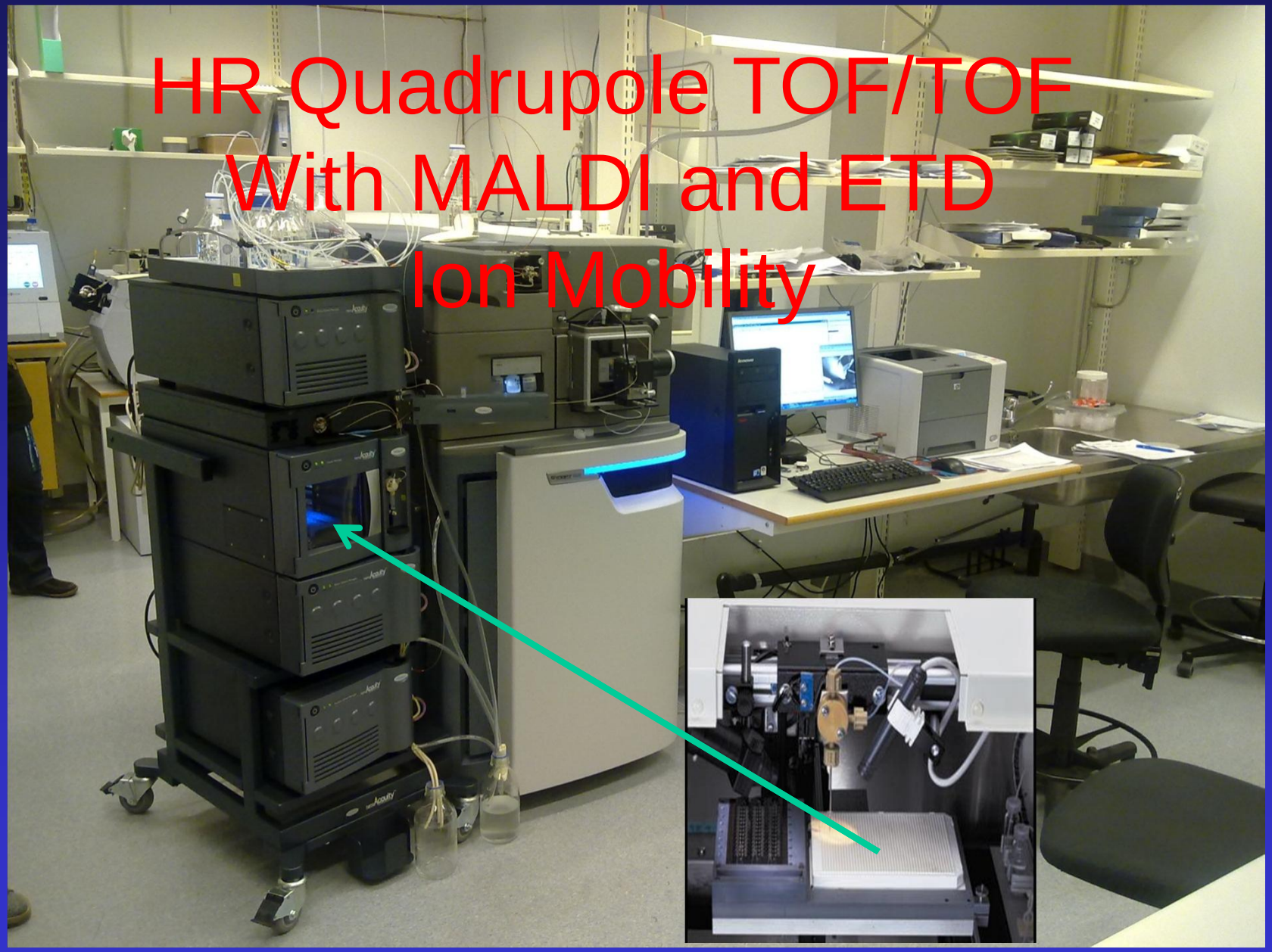
Principle of the MALDI process

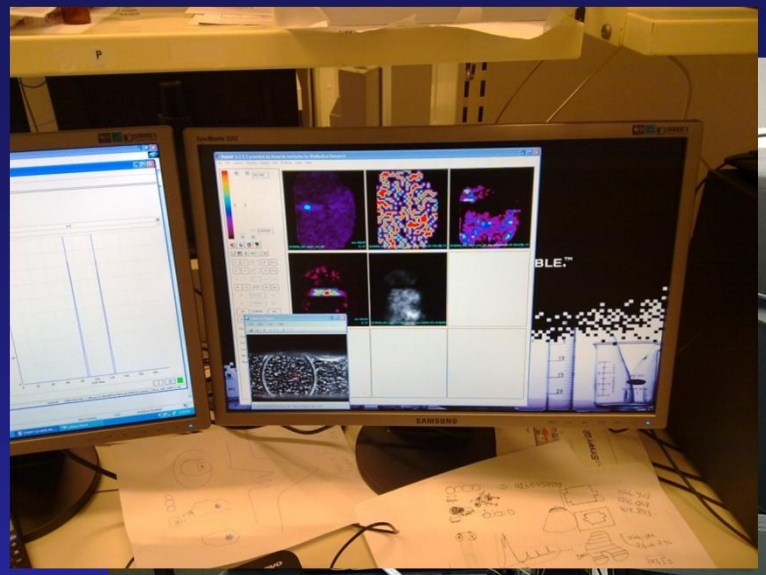
MALDI TOF/TOF



SLIDE ADAPTER

HR Quadrupole TOF/TOF With MALDI and ETD Ion Mobility




SAMSUNG

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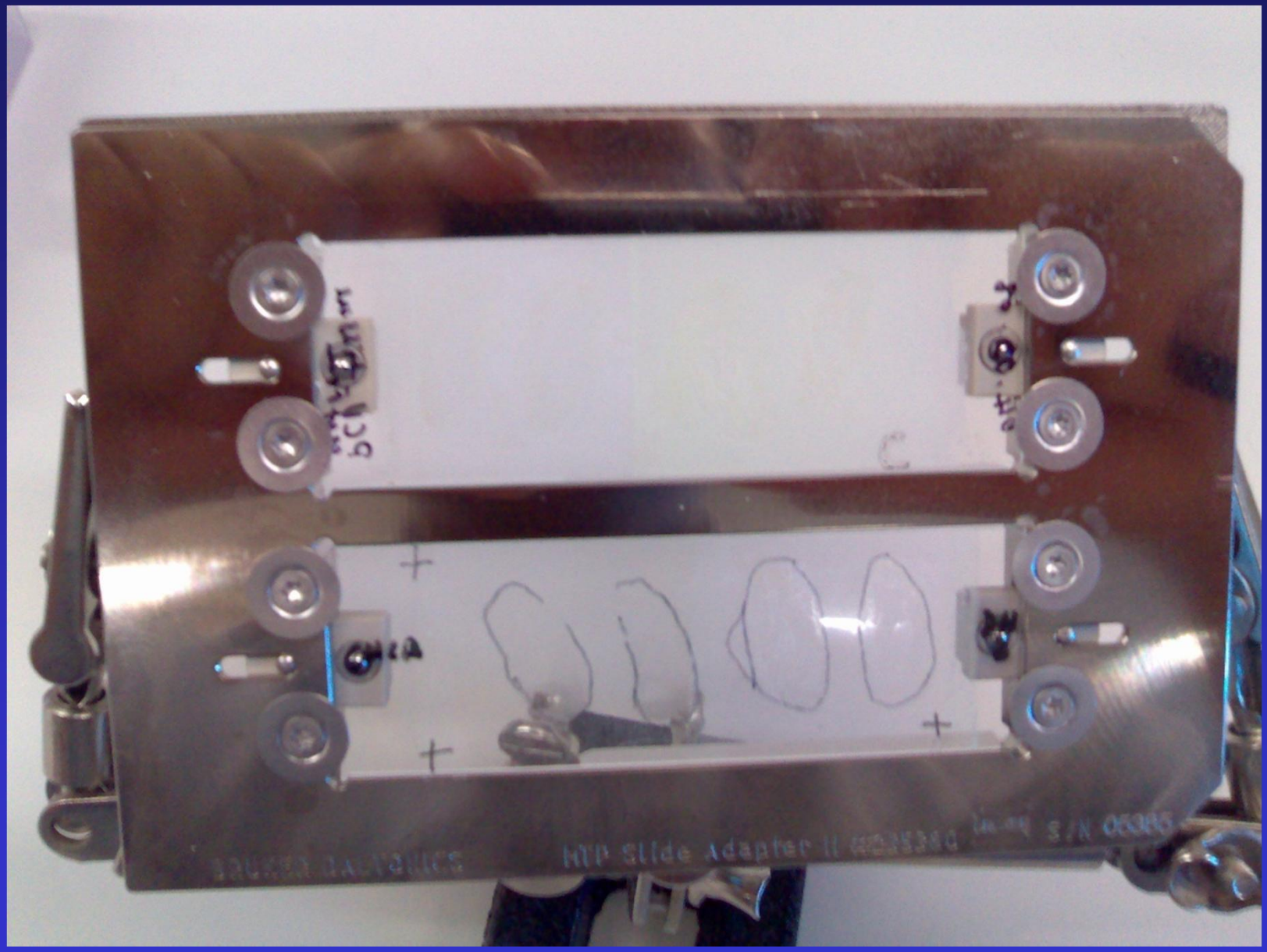
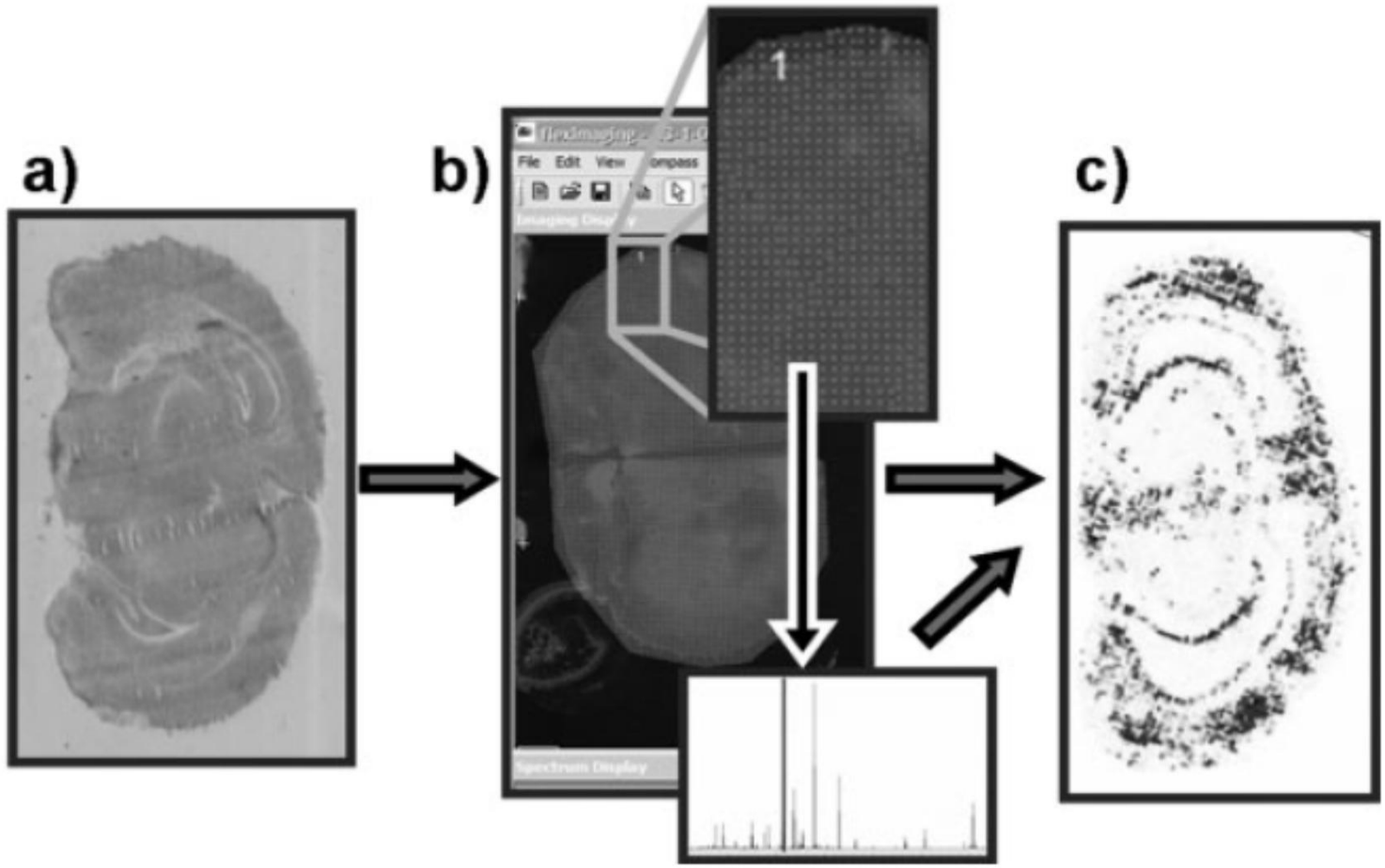
SYNAPT G2

Tissue slides for IMS-MS

Tissue slide for IMS-MS




a)
b)
c)
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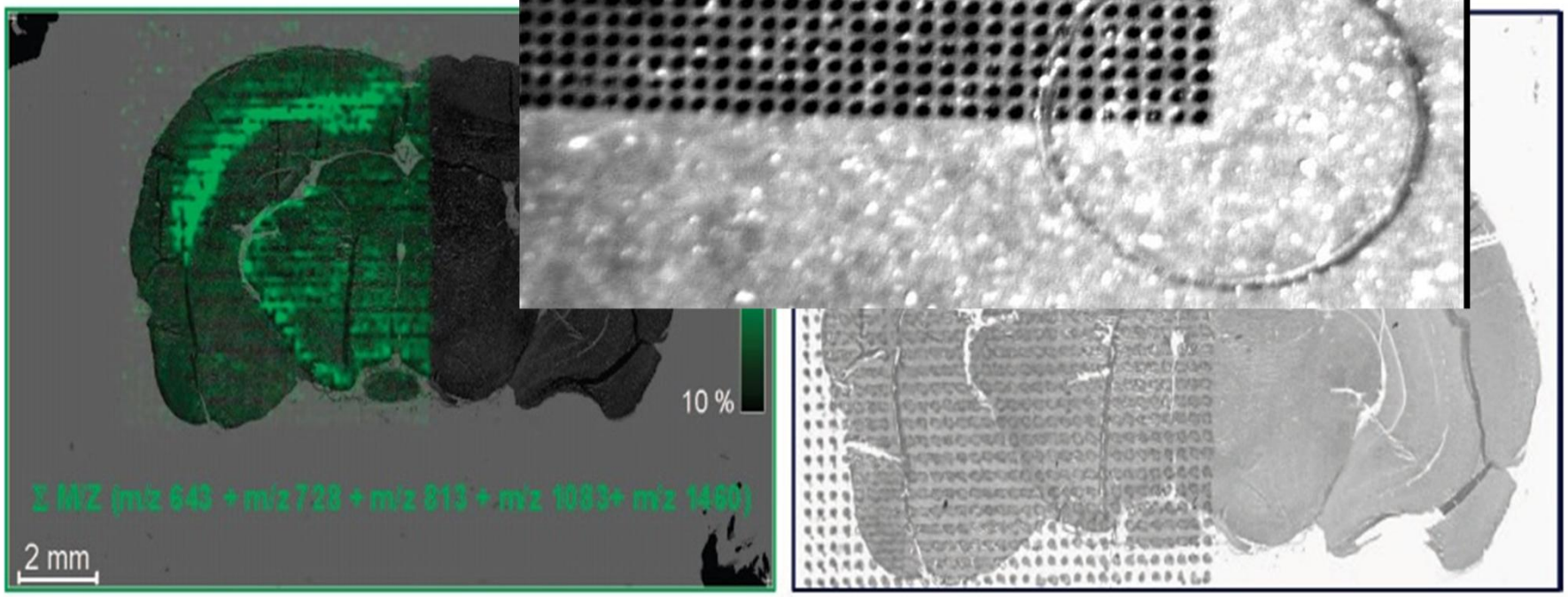
File Edit View

Proteomics 2008, *8*, 3785–3800 DOI 10.1002/pmic.200800320

REVIEW

Protein and pept…DI mass spectrome…

Richard J. A. Goodwin[1], *Ste…*

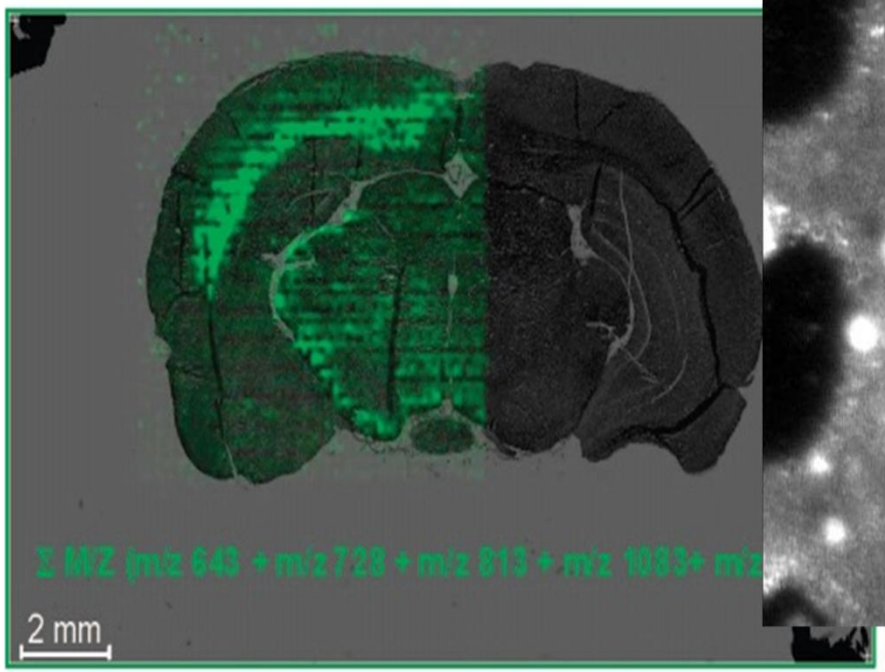


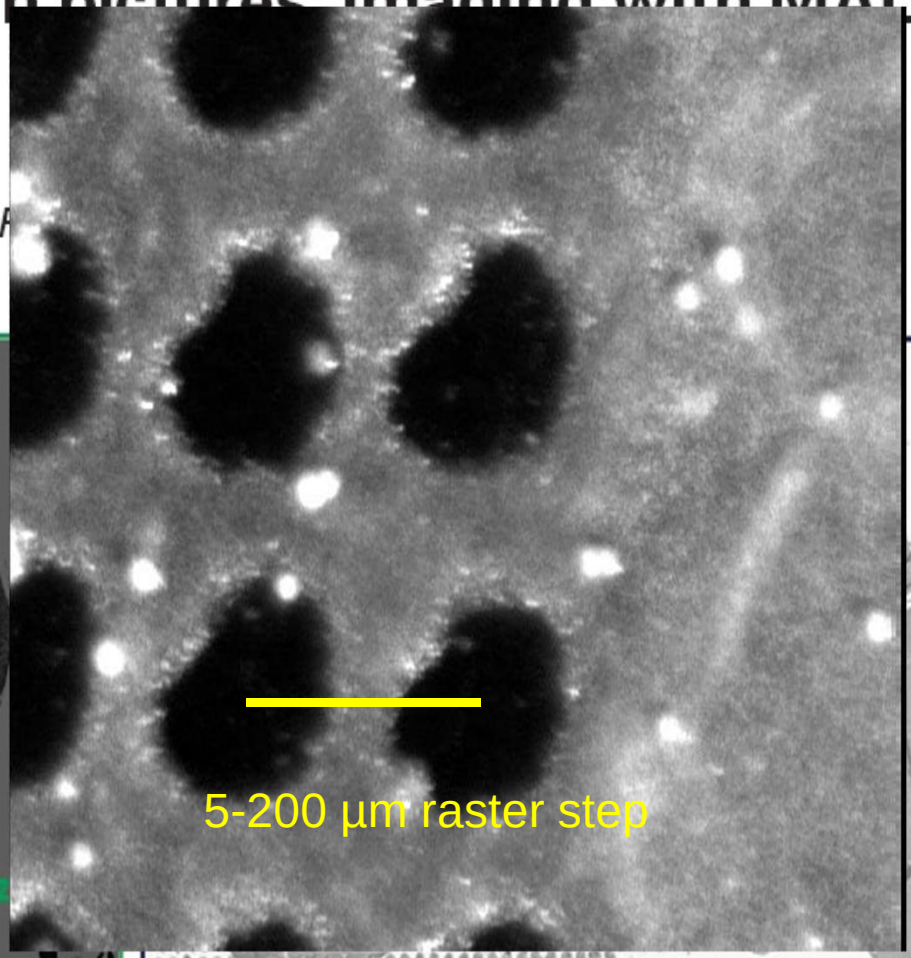
Proteomics 2008, *8*, 3785–3800 DOI 10.1002/pmic.200800320

REVIEW

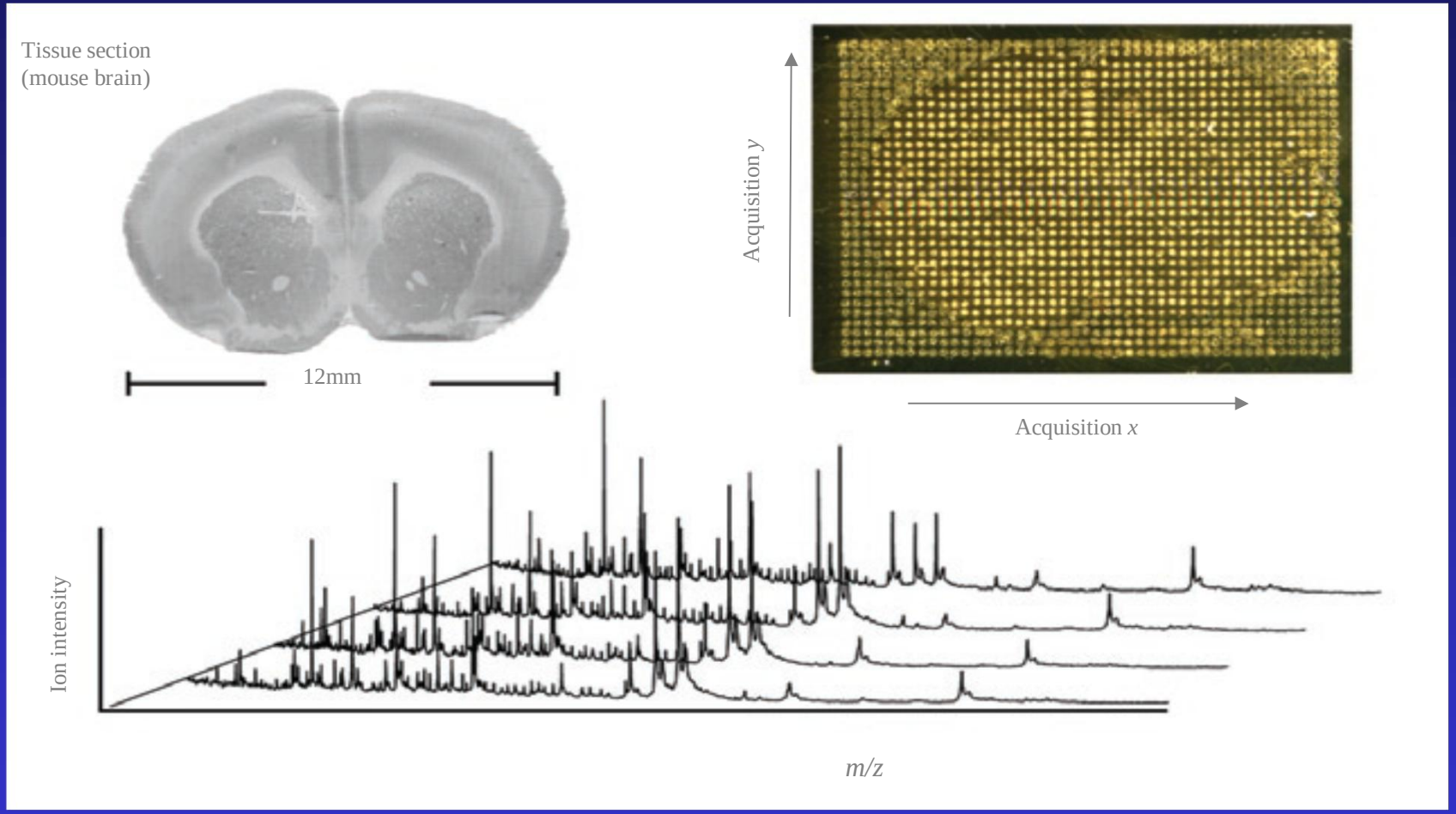
Protein and peptides in pictures: Imaging with MALDI mass spectrometry

Richard J. A. Goodwin[1], *Stephen R. P*





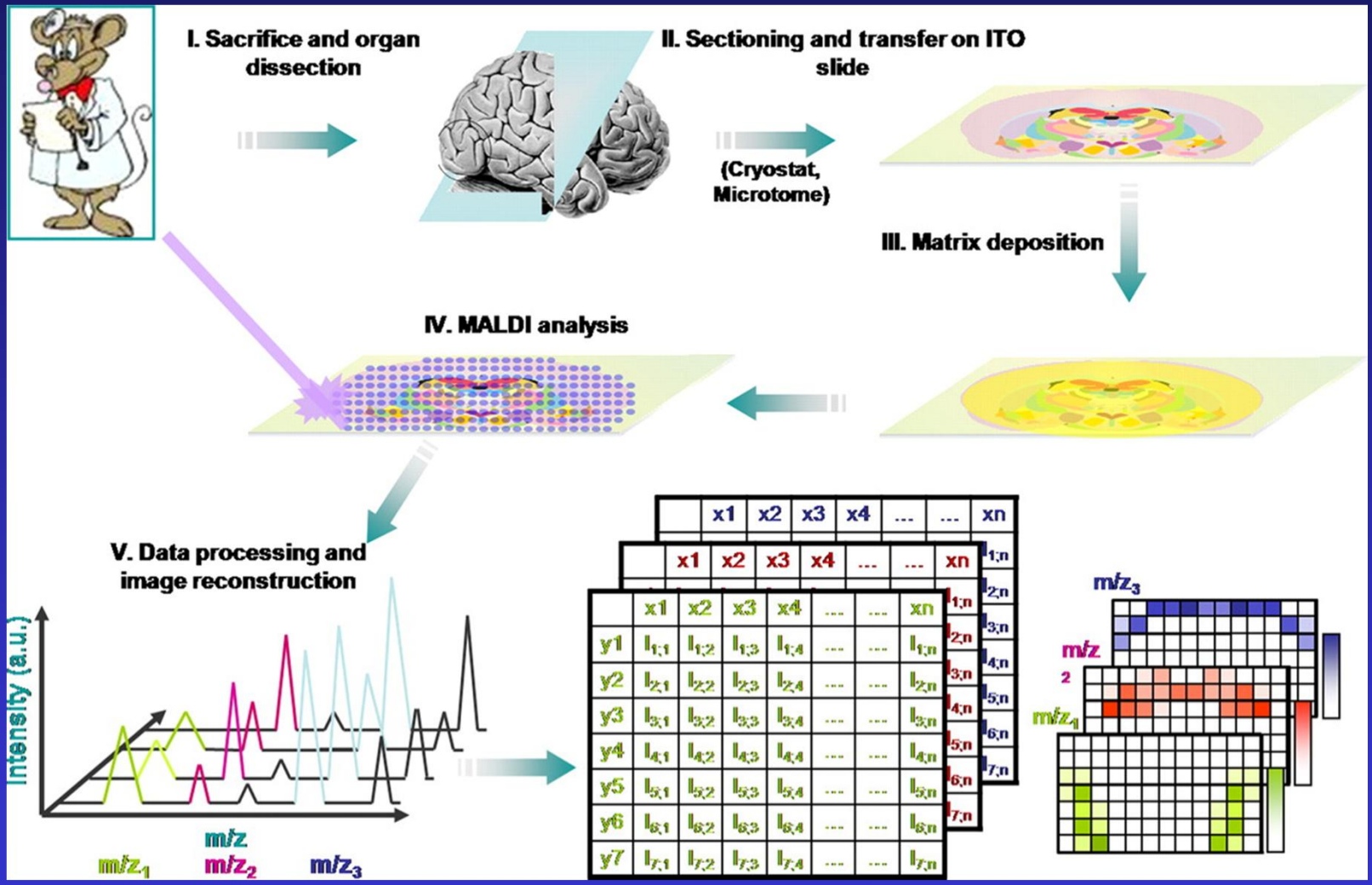
Principles



- A laser is rastered over a defined area while acquiring a complete mass spectrum from each position, resulting in molecular images for multiple analytes

  Cornett, et al., Nature Methods 2007

Schematic representation of the MALDI-MSI work flow



Franck J et al. Mol Cell Proteomics 2009;8:2023-2033

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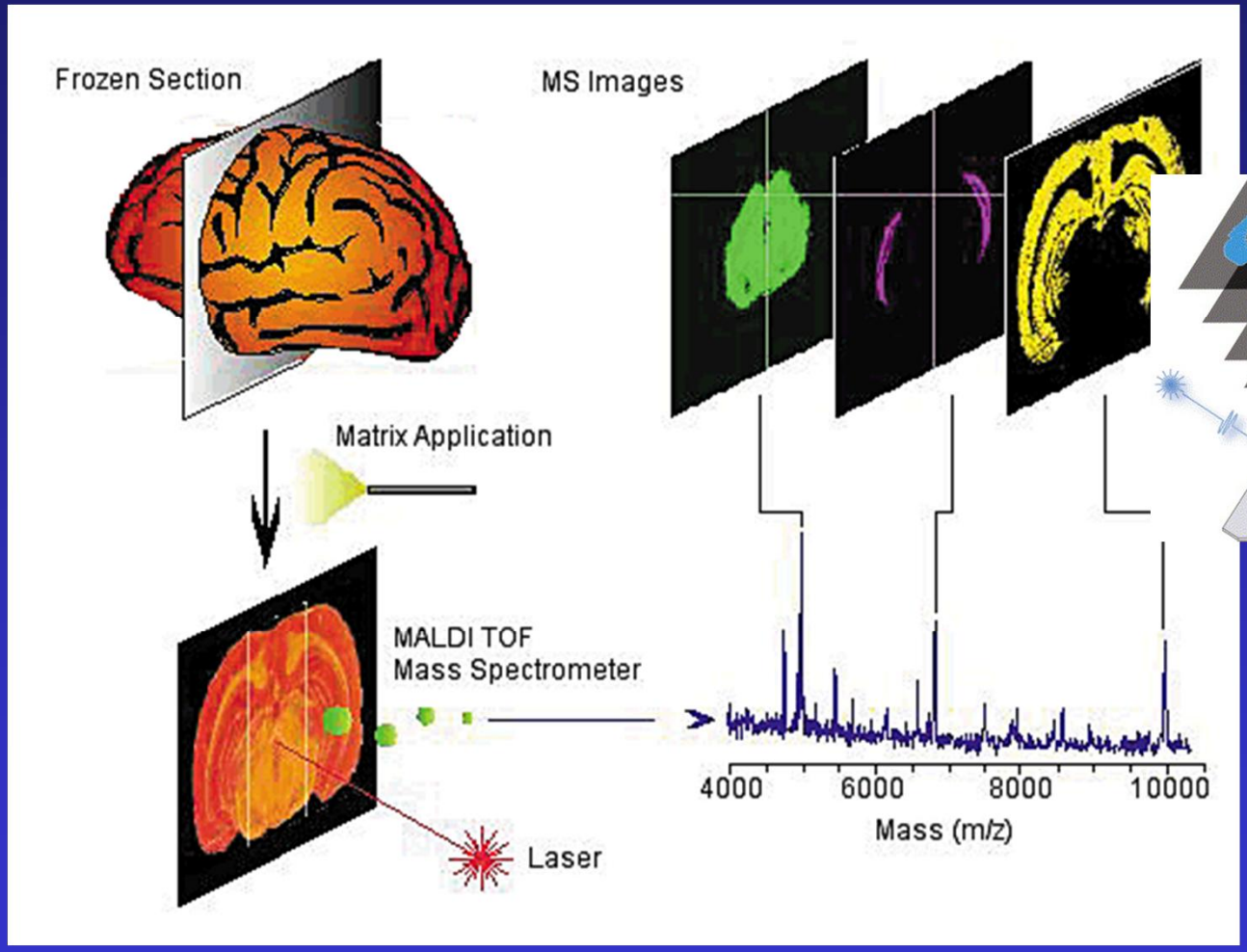
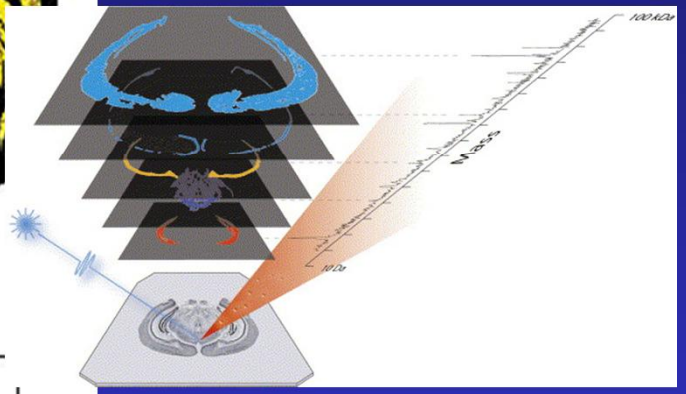
MSI workflow





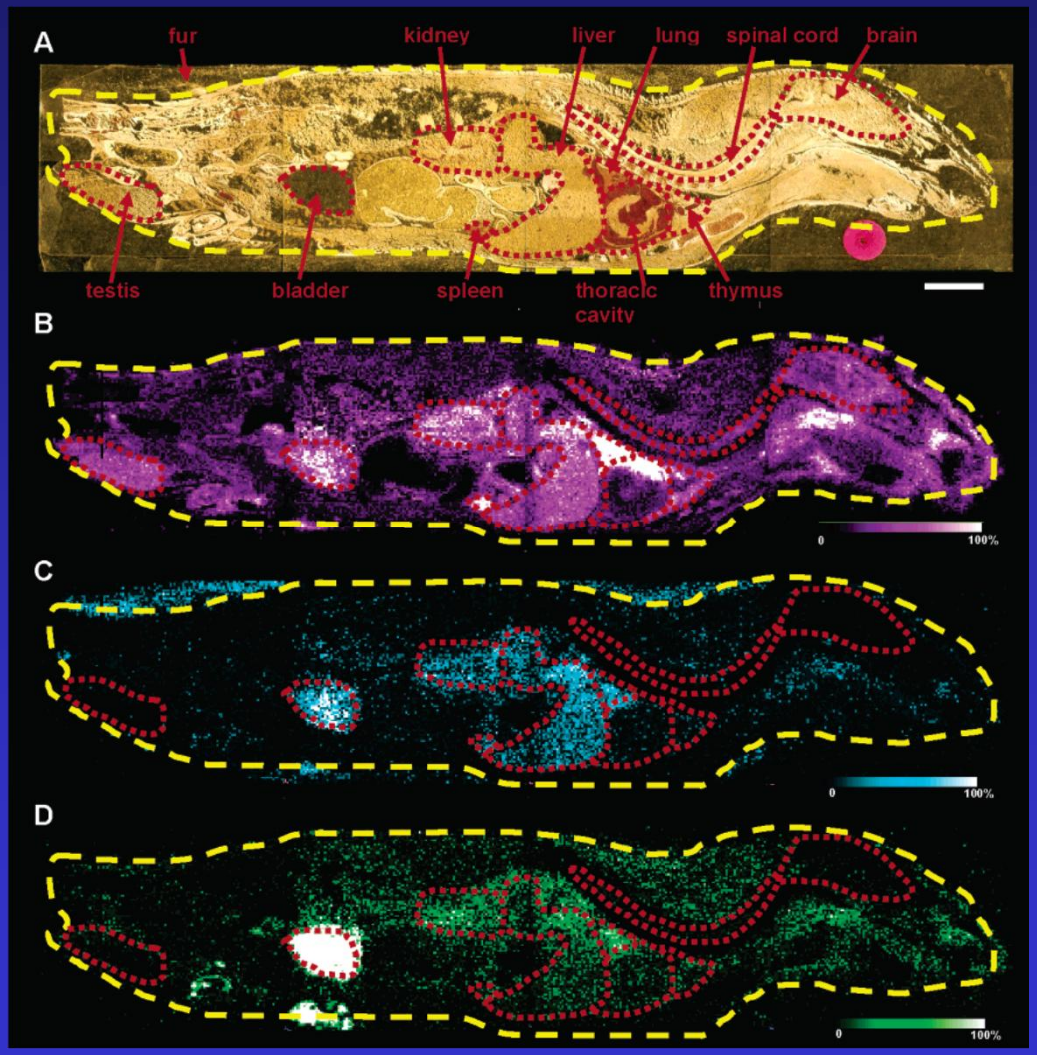
Stoeckli, M, et al., Analytical Biochemistry 2005

- Definitions:

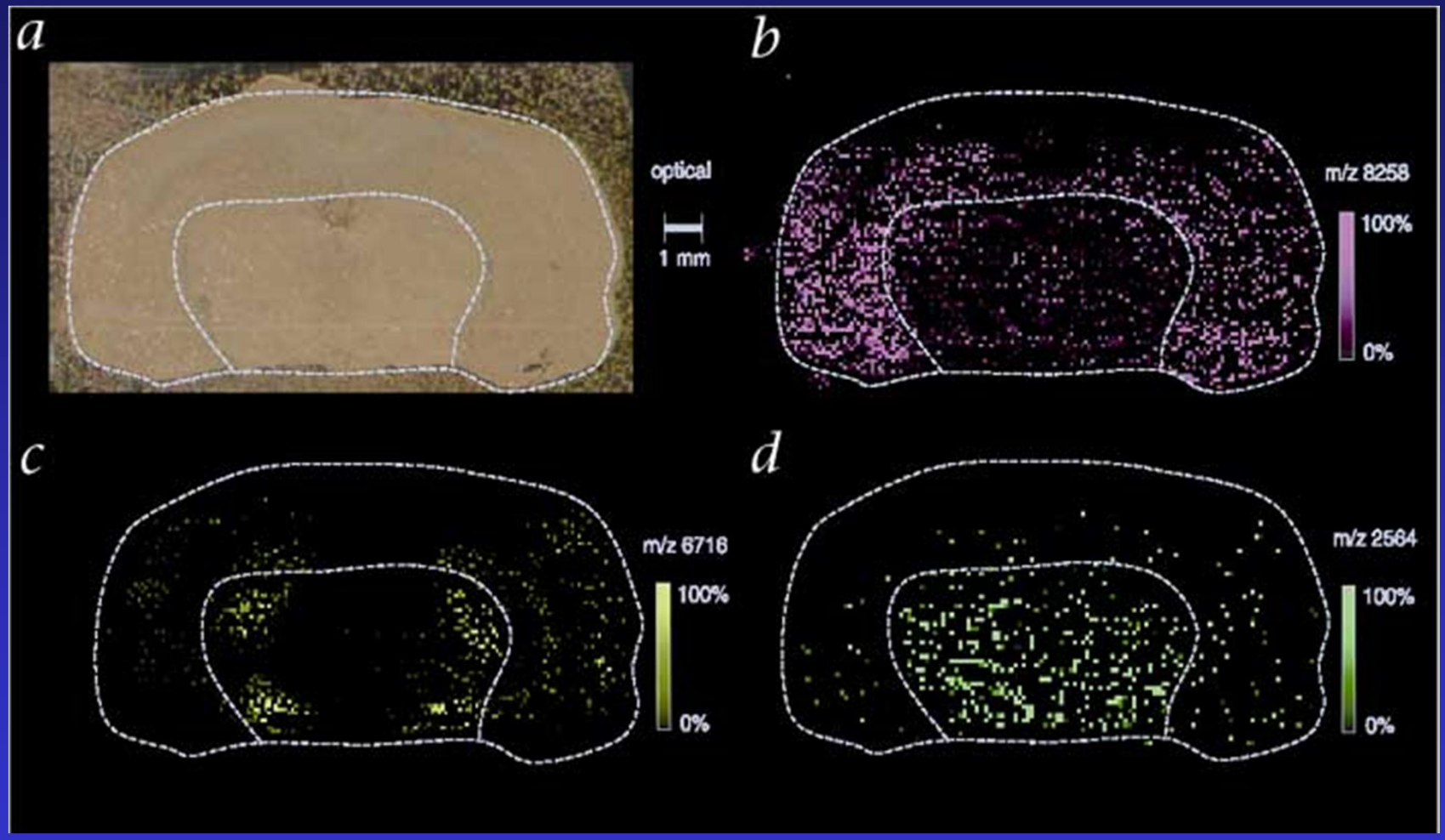
- MALDI Imaging (MALDI-IMS)(MS-IMS)
- A technique for analyzing the spatial arrangement of proteins, peptides, lipids, and small molecules in biological tissues
- A protein profiling technique which enables the direct sampling of histological section
- A technology that utilizes MALDI MS to map molecules of interest in thin tissue sections
- Potentially can deliver highly parallel, multiplexed data on the specific localization of molecular ions in tissue samples directly, and to measure and map the variations of these ions during development and disease progression or treatment

Benefits of MALDI-MSI

- Analysis of entire sample in one reading
- Previous knowledge of molecular composition is not necessary
- Allows for investigation of disease formation, progression, and treatment



www.maldi-msi.org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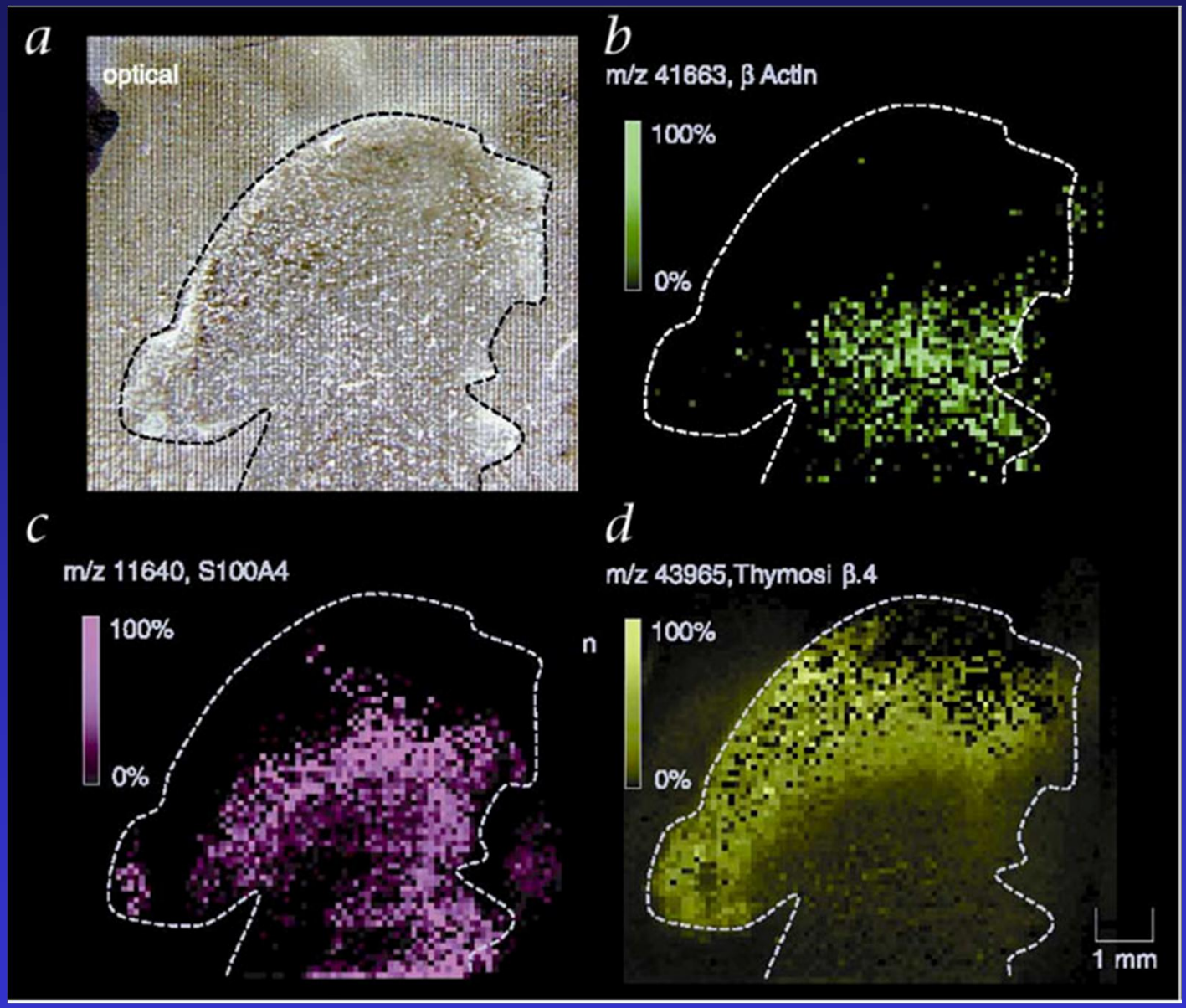
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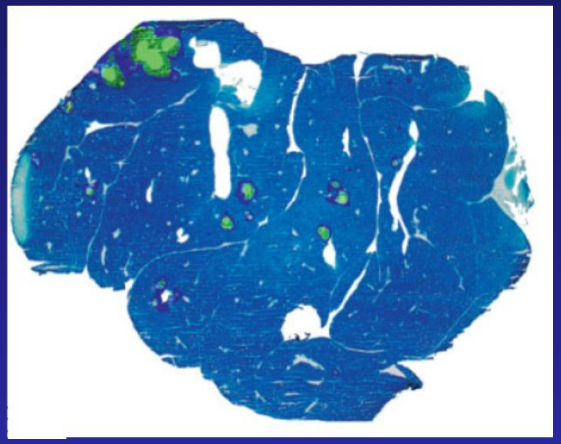
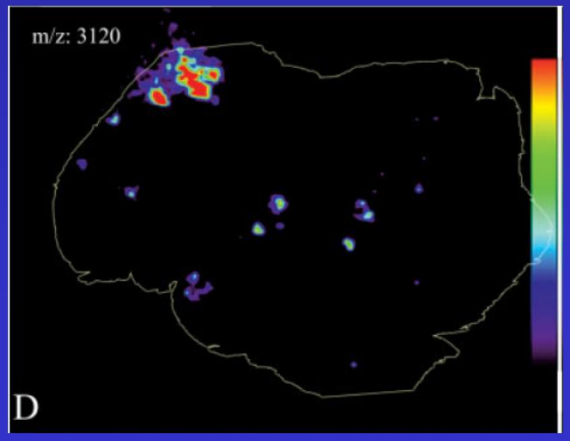
Targeted labeling



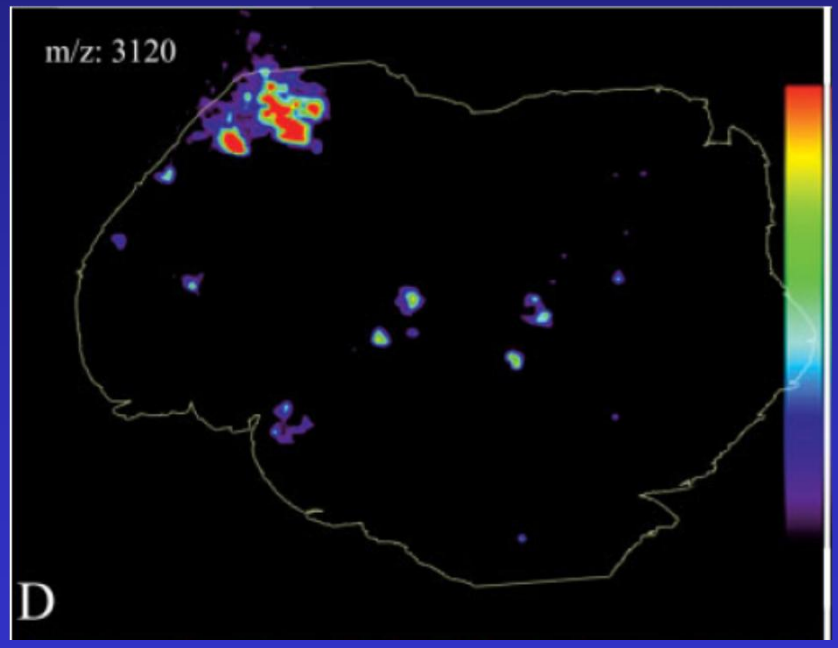
Label free imaging

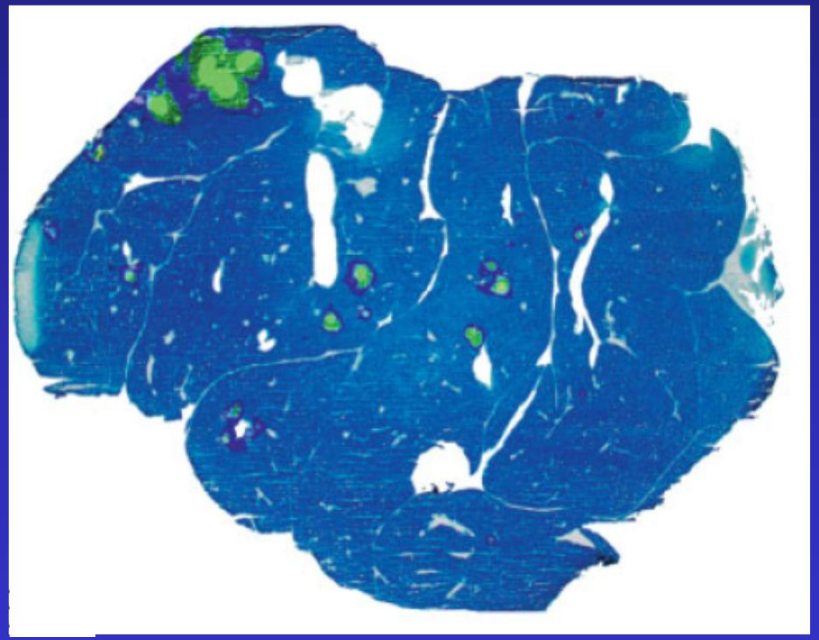


MS imaging advantages

- No labeling required Biomolecules are functionally unmodified
- Image biomolecular modifications PTM's, Metabolites
- Detailed information on molecular identity
- Large scope of different elements and molecules

MSI vs. histochemical stain





Imaging MS applications……

Imaging drugs and metabolites

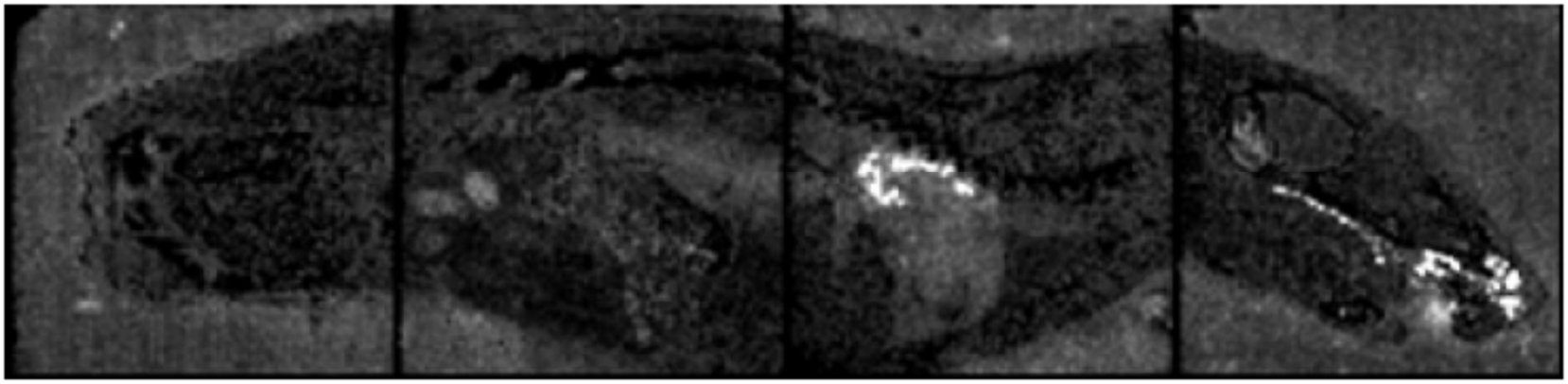
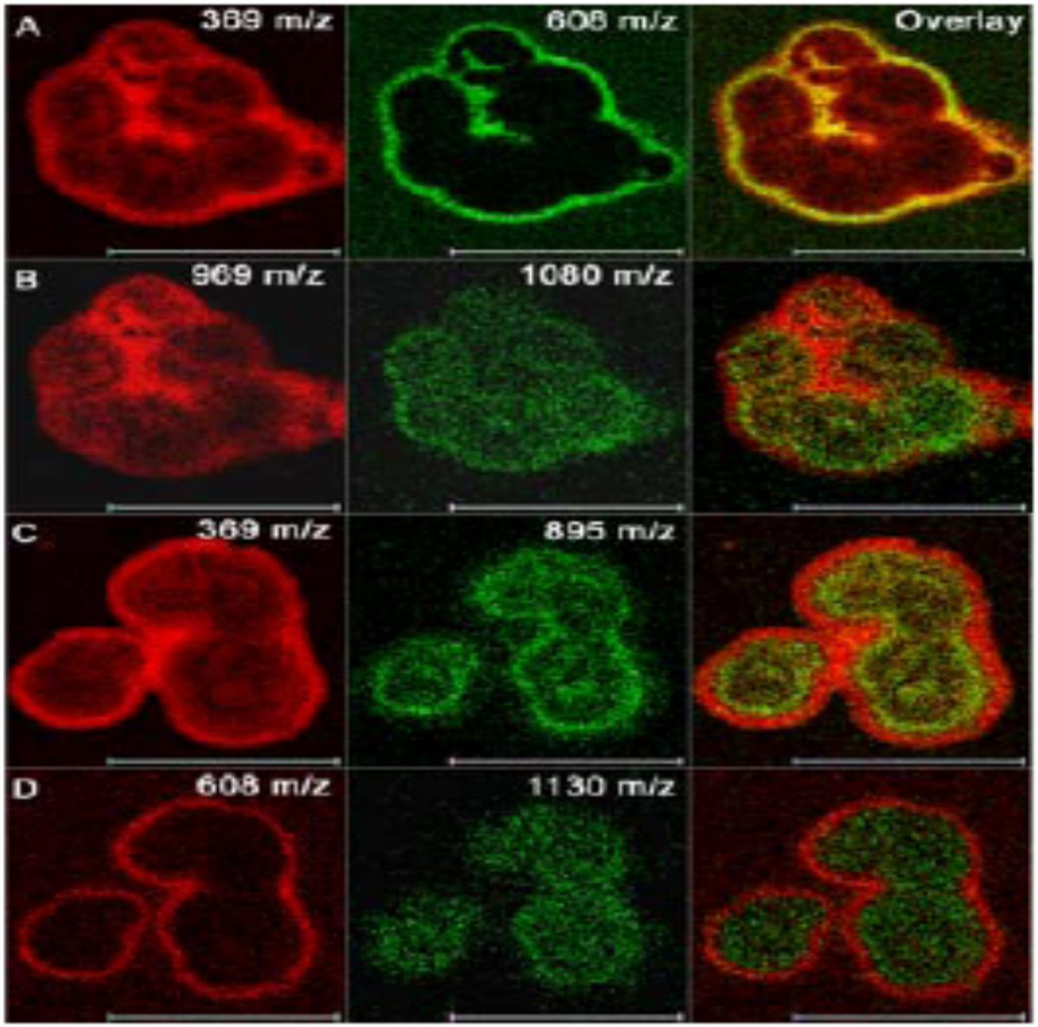


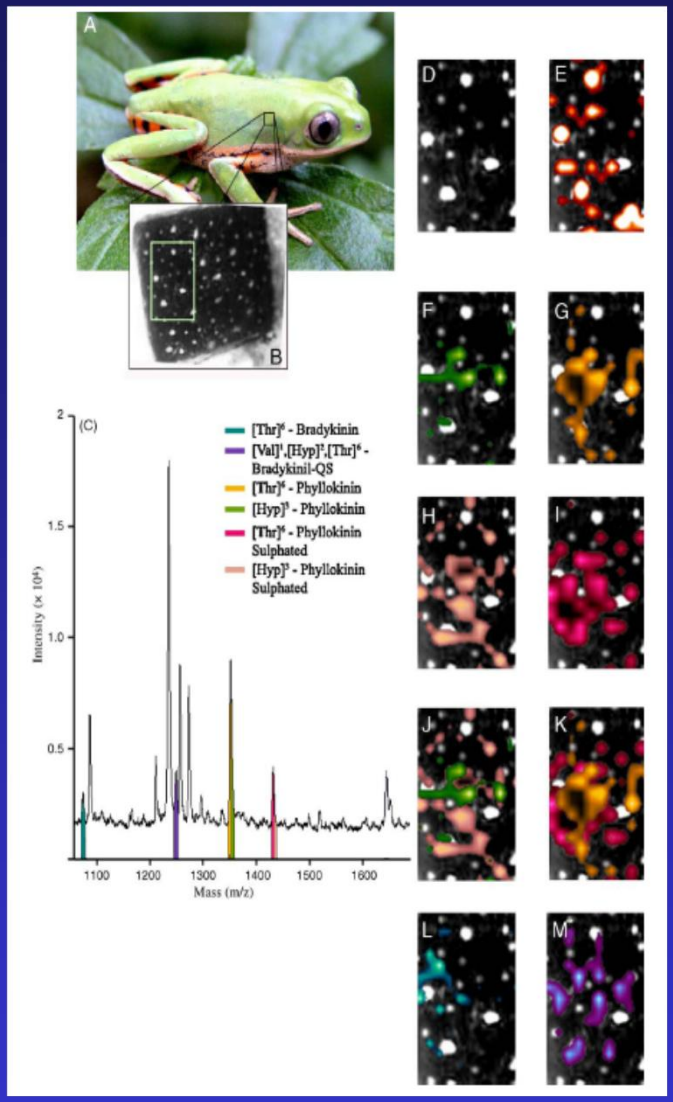
Image courtesy Markus Stoeckli

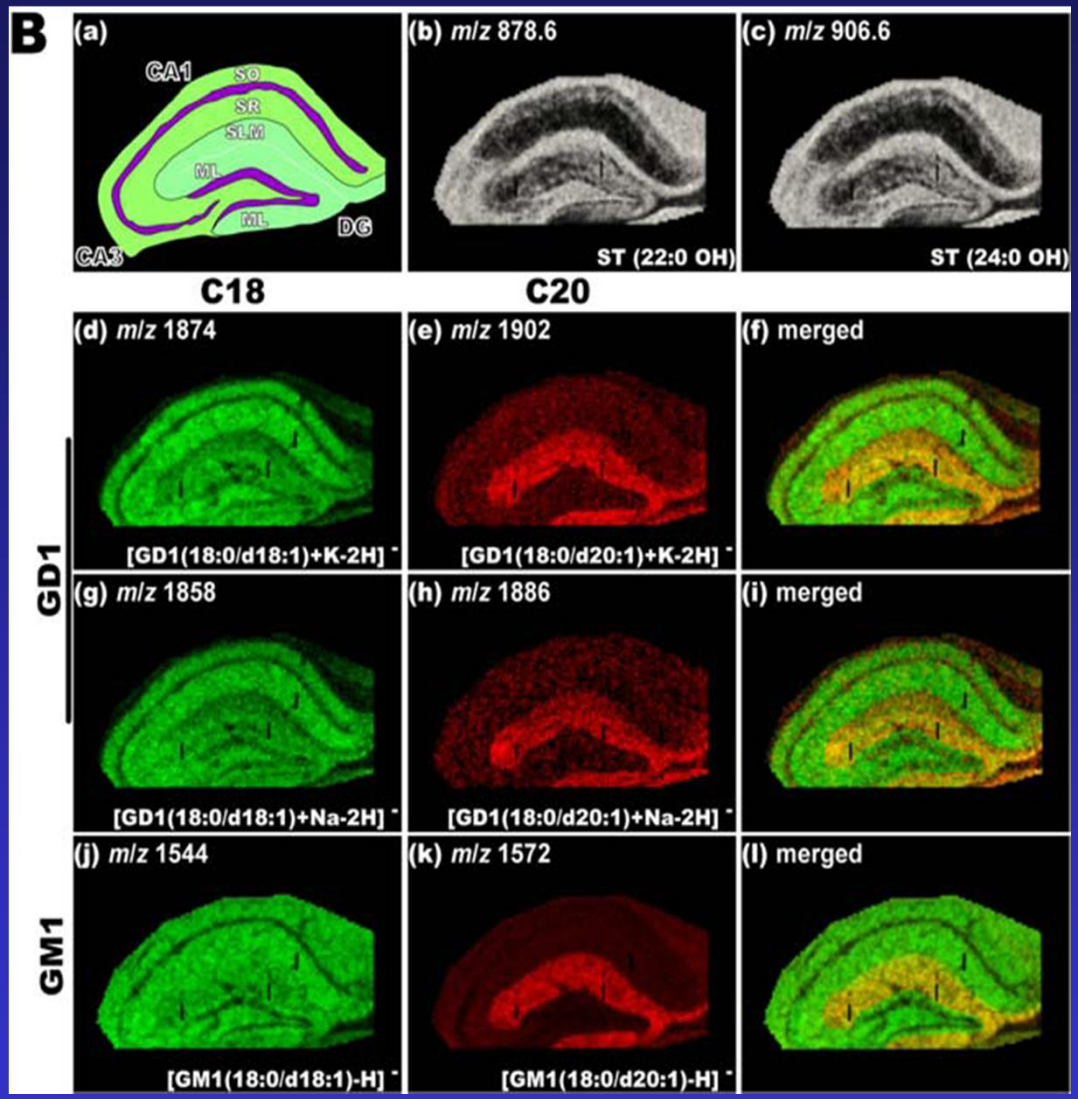
Drug administration and detection

This is probably where it all started?

Imaging Lipidomics







Imaging Endogenous peptides

Imaging Gangliosides

Localization of water-soluble carbohydrates in wheat stems using imaging matrix-assisted laser desorption ionization mass spectrometry

Sarah Robinson[1], Karen Warburton[3], Mark Seymour[2], Malcolm Clench[3] and Jane Thomas-Oates[1]

[1]Department of Chemistry, University of York, Heslington, York YO10 5DD, UK; [2]Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK; [3]Biomedical Research Centre, Sheffield Hallam University, Howard Street, Sheffield S1 1WB, UK

New Phytologist (2007) **173** : 438 –444

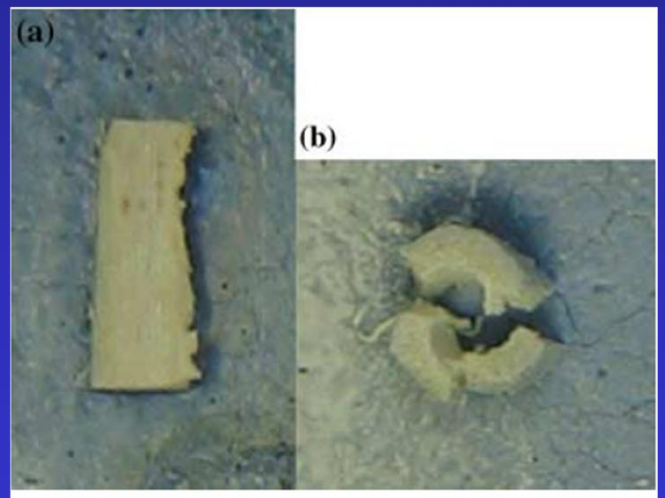
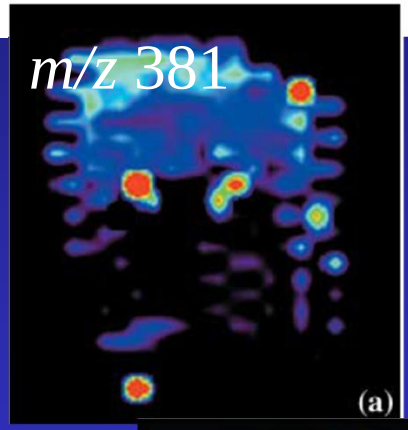
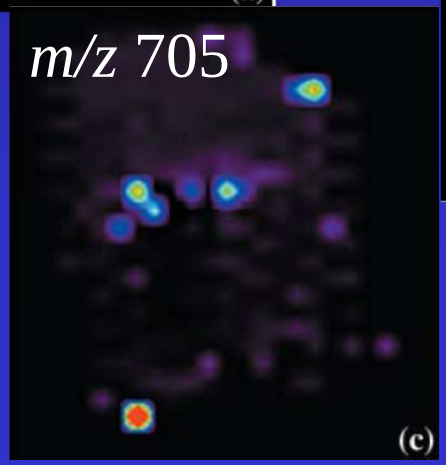
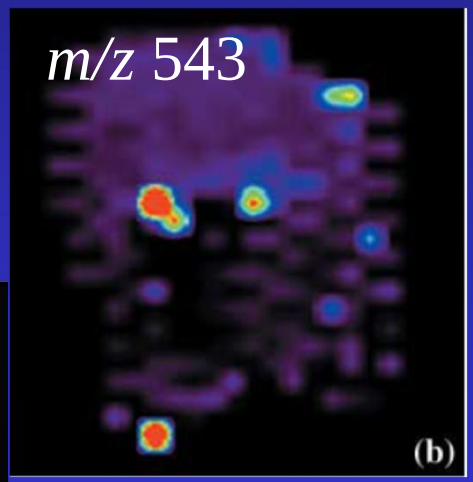
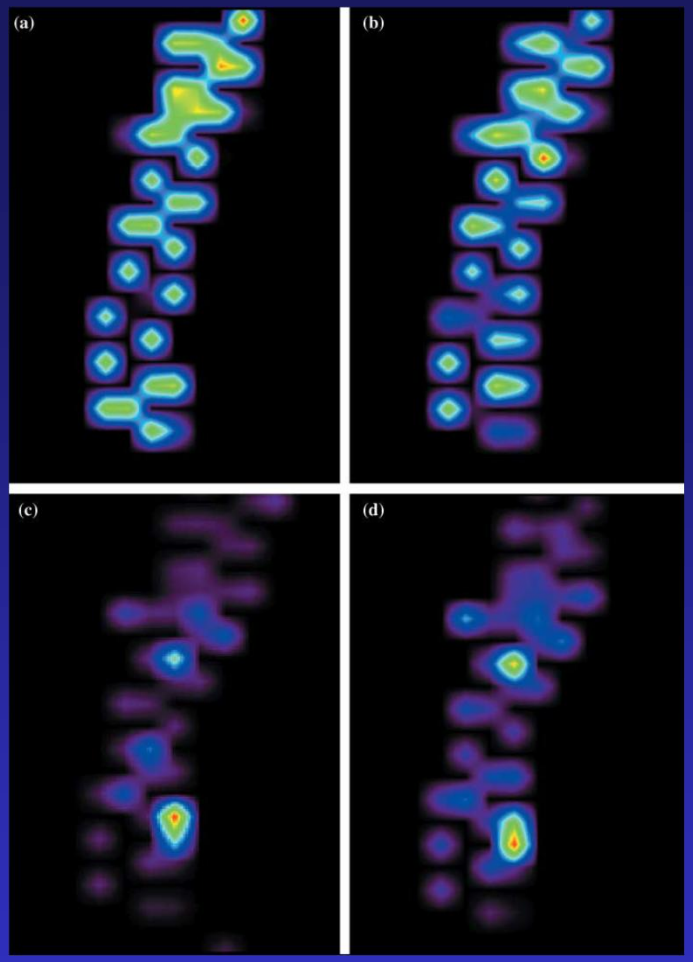


Fig. 2 Example photographs of (a) a longitudinal section and (b) a cross section through a piece of wheat (*Triticum aestivum*) stem before positioning in the matrix-assisted laser desorption ionization (MALDI) source of the mass spectrometer.

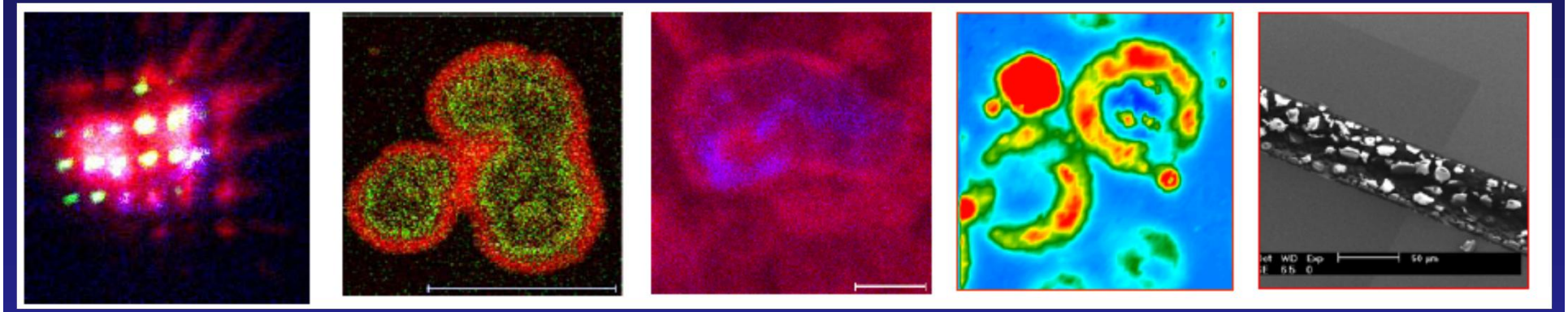








Reconstruction of the Carbohydrate moiety
Of WHEAT (*Triticum aestivum)*



Collection and storage of samples

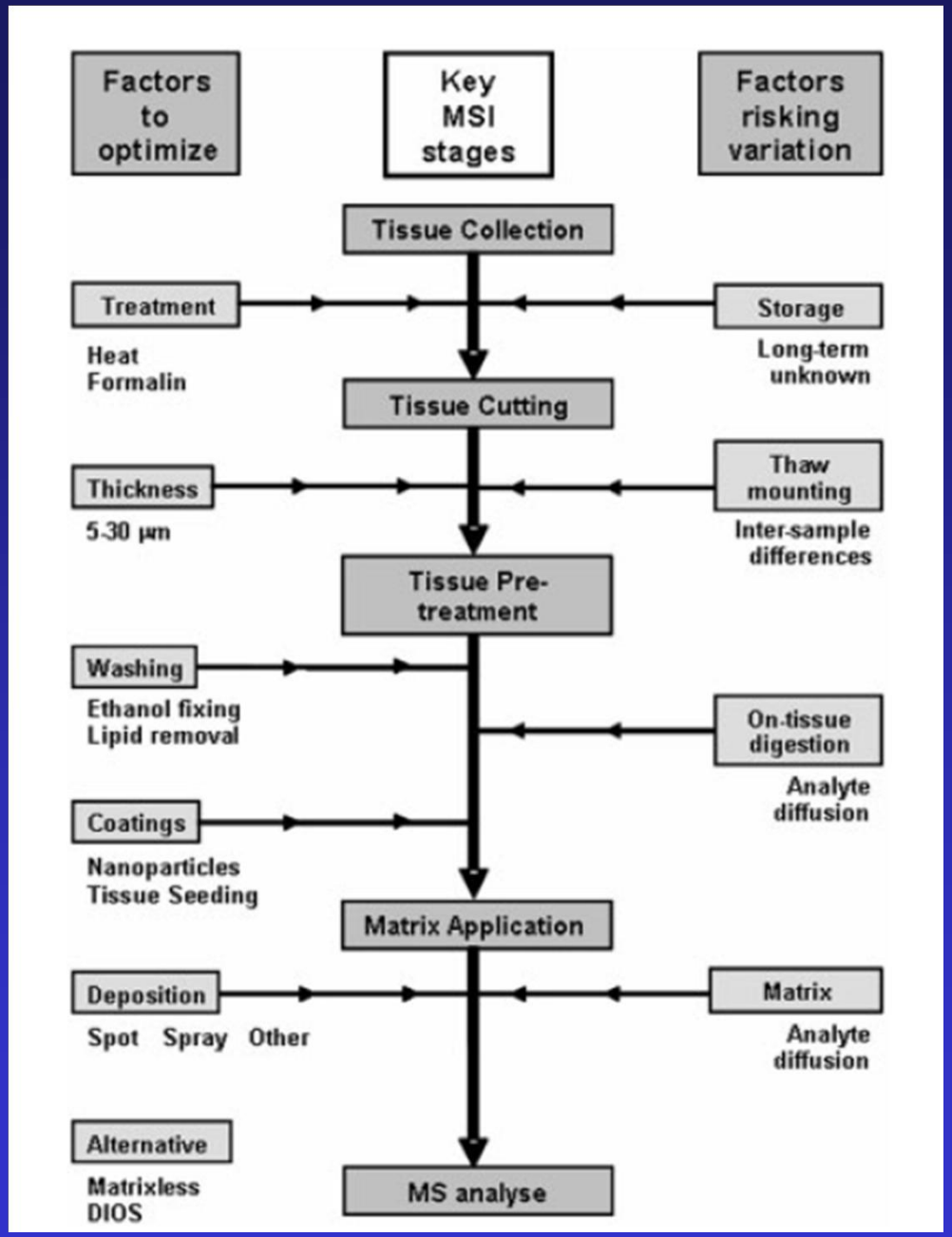
Embedding

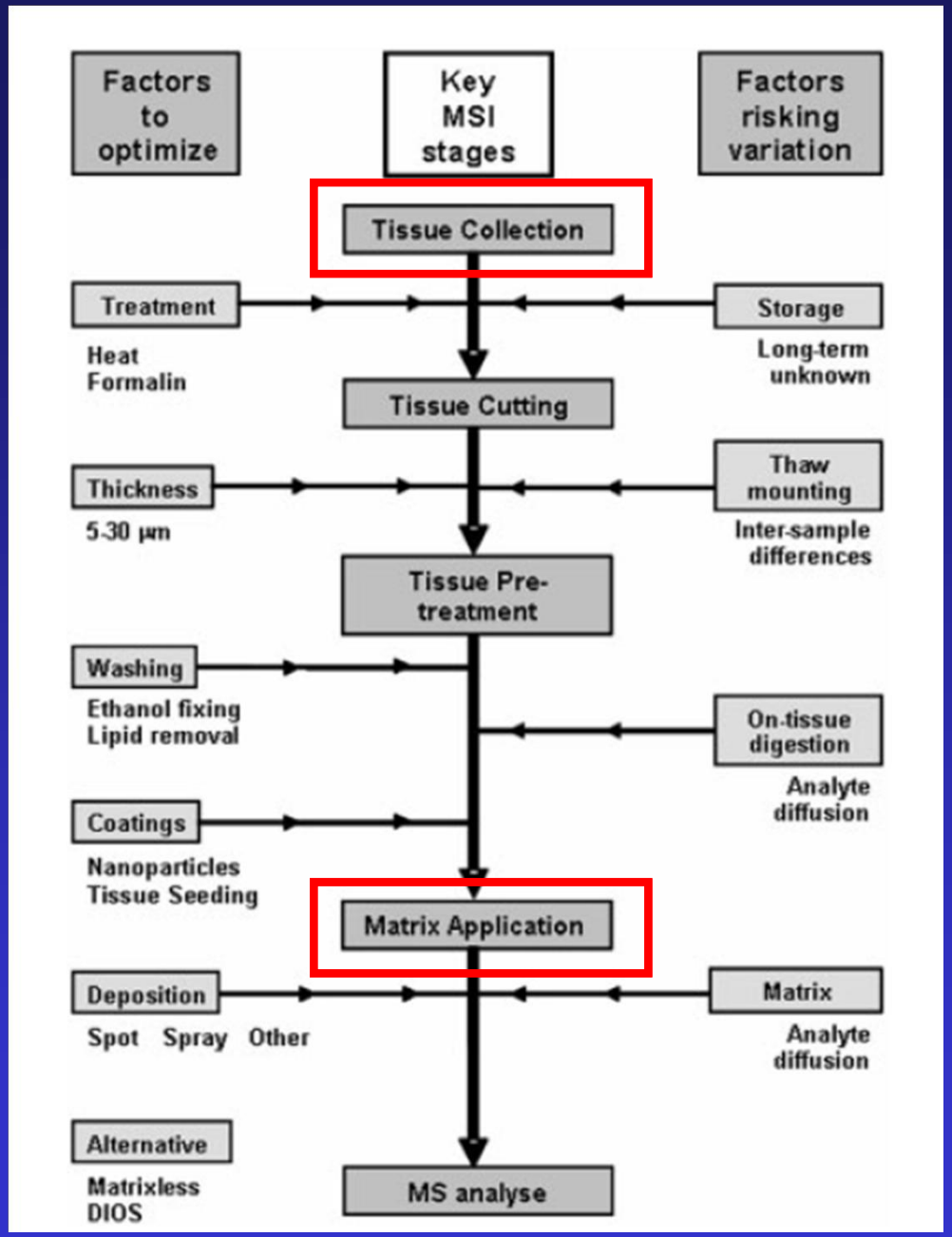
Tissue preparation and matrix application

Section mounting

Fixed *versus* fresh tissue

Heeren R, USA


Factors to optimize
Key MSI stages
Factors risking variation
Tissue Collection
Treatment
Heat
Formalin
Storage
Long-term unknown
Tissue Cutting
Thickness
5-30 µm
Thaw mounting
Inter-sample differences
Tissue Pre-treatment
Washing
Ethanol fixing
Lipid removal
On-tissue digestion
Analyte diffusion
Coatings
Nanoparticles
Tissue Seeding
Matrix Application
Deposition
Spot Spray Other
Matrix
Analyte diffusion
Alternative
Matrixless
DIOS
MS analyse


Factors to optimize
Key MSI stages
Factors risking variation
Tissue Collection
Treatment
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Tissue Cutting
Thickness
5-30 µm
Thaw mounting
Inter-sample differences
Tissue Pre-treatment
Washing
Ethanol fixing
Lipid removal
On-tissue digestion
Analyte diffusion
Coatings
Nanoparticles
Tissue Seeding
Matrix Application
Deposition
Spot Spray Other
Matrix
Analyte diffusion
Alternative
Matrixless
DIOS
MS analyse

Collecting the samples

1. Sample handling and preparation of sections for image analysis are critical to the spatial integrity of measured molecular distributions.

2. Any molecular degradation that occurs in the time between sample collection and analysis can adversely affect the results.

3. A typical study may involve samples collected over a lengthy period of time, and standardized procedures are therefore required to minimize experimental variability over the time course of the study.

4. Good communication among all personnel involved with collecting, storing and analyzing samples is critical.

5. Ideally, samples are frozen immediately after collection and stored at -80°C until sections for MALDI-IMS analysis are cut on a cryomicrotome just before analysis.

Cornett et al., *Nature Methods,* 2007

Preserving the tissues

1. Animals are usually killed by cervical dislocation, after which the tissue of interest has to be rapidly removed and immediately processed:

2. Flash frozen in liquid nitrogen (30-60 sec) and stored at -80°C

3. Flash frozen in liquid nitrogen cooled isopentane and stored at -80°C until sectioning in order to minimize proteolysis and conserve PTMs of peptides and proteins.

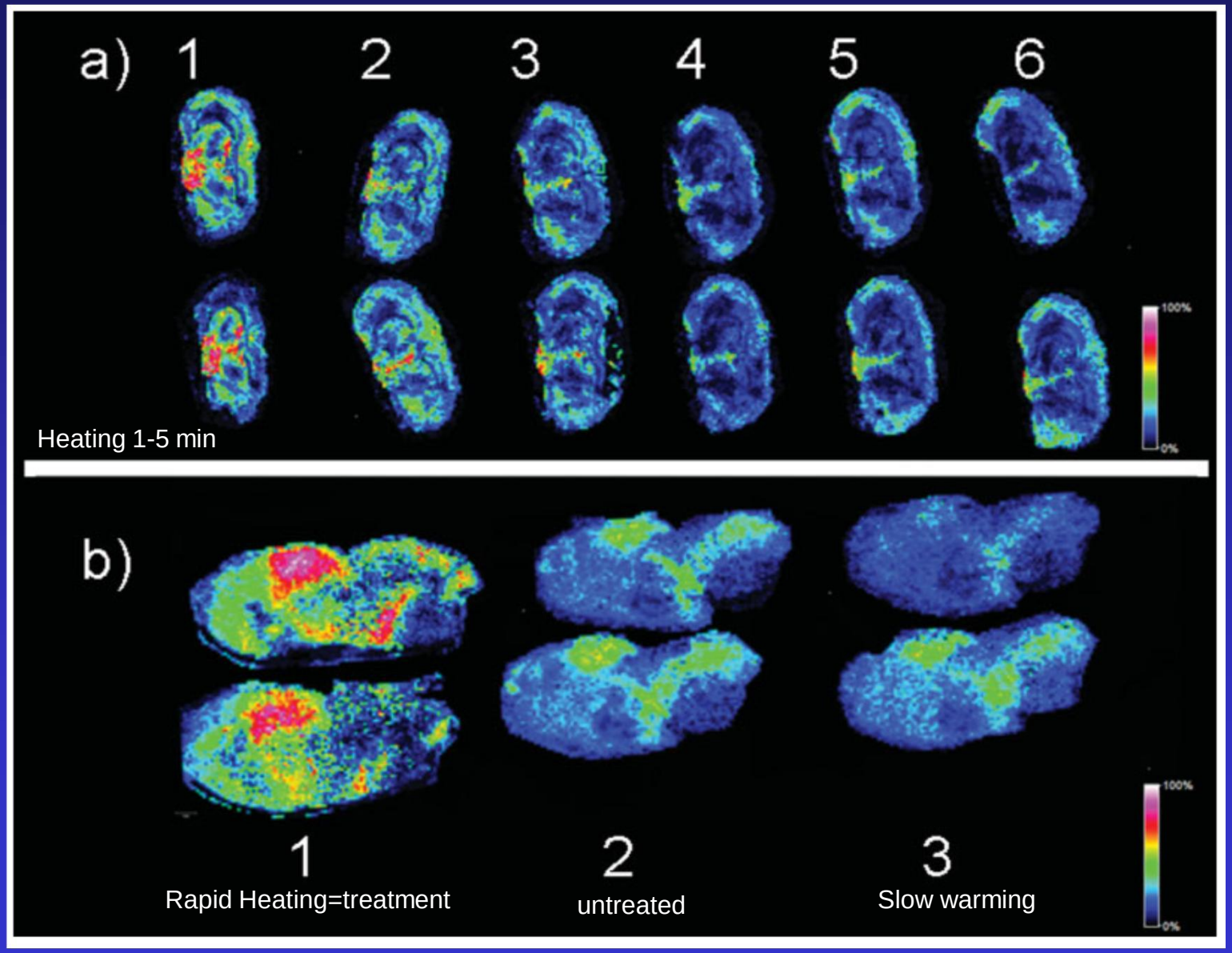
4. Small sections can also be frozen using dry ice and ethanol

5. Alternatively, the tissue may be frozen in a mixture of dry ice and hexane at -75°C, embedded in a 2% gel of sodium carboxymethylcellulose (CMC) and stored at -80°C until further use.

6. Embedding in gelatine has been used to facilitate handling of small or fragile samples (e.g., biopsies).

7. The freezing process can lead to sample cracking and fragmentation, as different parts of the tissue cool down at different rates and ice crystals may form. To avoid sample damage, the tissue may be loosely wrapped in aluminum foil and frozen in liquid nitrogen, ethanol, or isopropanol at temperatures below -70 °C by gently lowering the tissue into the liquid over a period of 30-60 s. This preserves the shape of the tissue and also protects the biological tissue components from degradation (Schwartz et al. *J Mass Spectr.*, 2003)

Effect of keeping the sample in RT



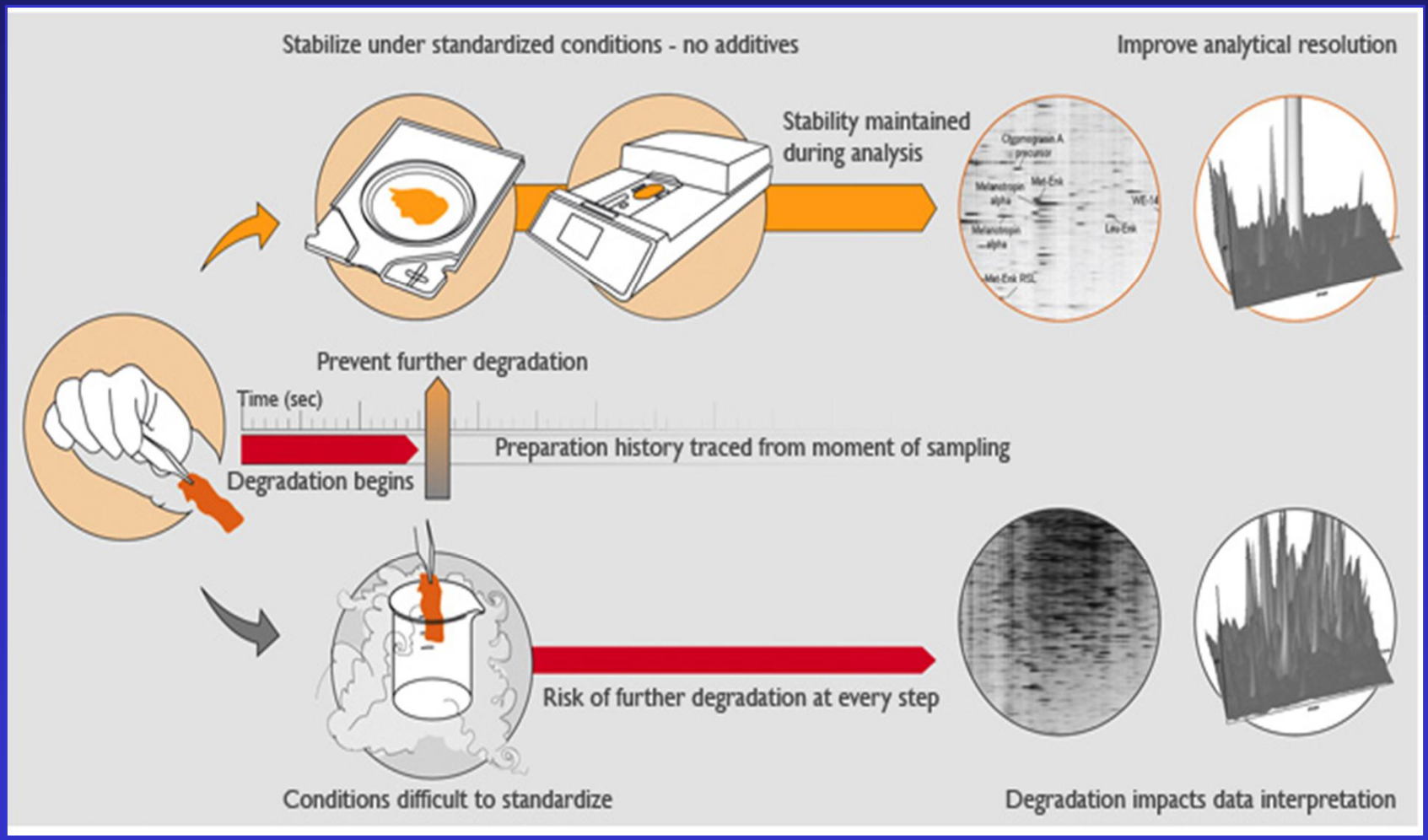
Heat stabilization

1. **Similarly, biopsy/autopsy human material can be stored at -80°C after being subjected to a conductive heat transfer. The methodology was developed to stabilize biological tissues and fluids at the moment of sampling (*Denator AB*, Gothenburg Sweden)**

2. **The tissue stabilization system utilizes a combination of heat and pressure under vacuum and its utility was demonstrated by monitoring the PTMs and stability of proteins and by checking the enzymatic activities in the mouse and human brain.**

www.denator.com

Heat stabilization



www.denator.com

Step 2: sectioning the tissues

1) Contamination with embedding media for cryosection, such as agar, a polysaccharide, Tissue-Tek® and OCT (optimal cutting temperature compound), a combination of polyvinyl alcohol and polyethylene glycol polymers, should be avoided as they suppress ion formation in MALDI MS.

2) To facilitate handling of small or fragile samples (i.e., biopsies), embedding in gelatine or agarose has also been used.

3) At present, the most widely used technique is to affix flash frozen tissue on a cold MALDI target plate or to a conductive surface, i.e. nickel or ITO-coated (indium-tin-oxide) glass slide with a minimal amount of OCT so that it is not in direct contact with the sectioned tissue or microtome blade during sectioning.

4) The microtome blades (preserved in mineral oil) should also be washed with acetone or methanol to prevent chemical contamination if no disposable blades are used.

OCT effect on IMS spectra

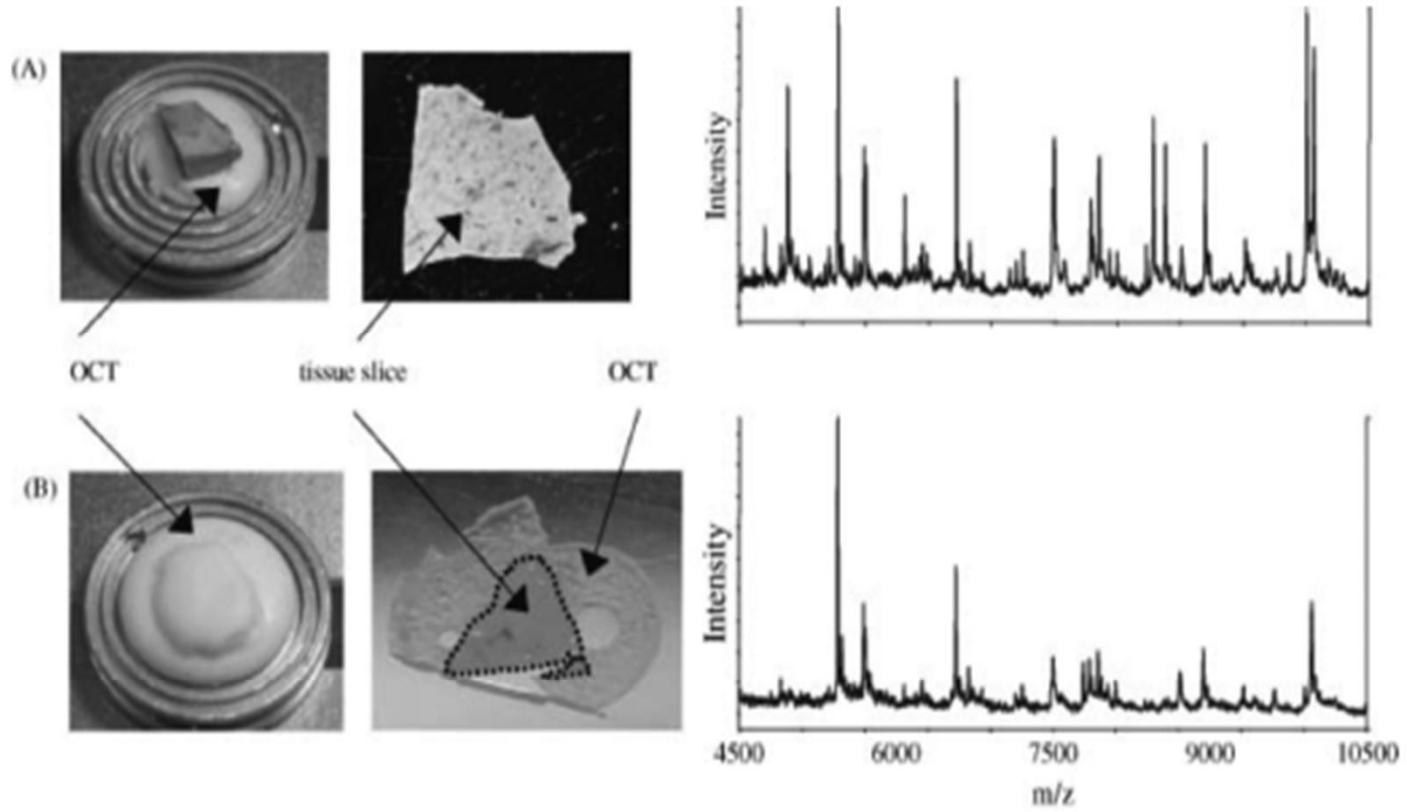
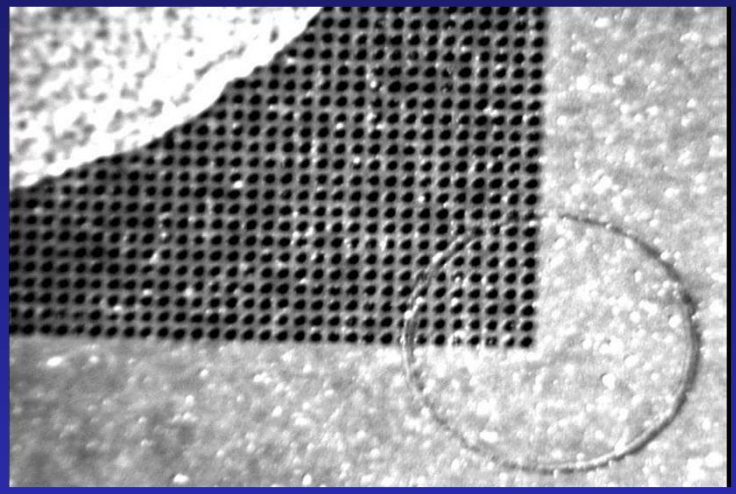


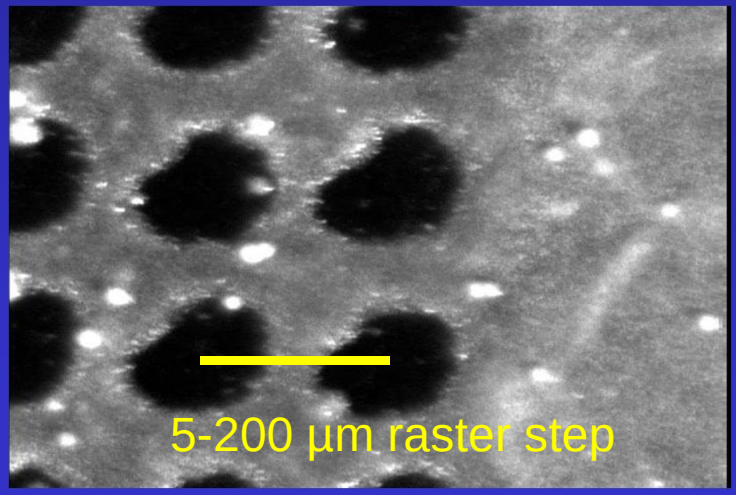
Figure 7. Analysis of the effect of OCT on MALDI signals from rat liver. (A) Procedure where OCT is used to adhere the tissue to the sample stage but does not come into contact with the sliced tissue. The resulting spectrum shows many intense signals between *m/z* 4500 and 10 500. (B) The tissue was embedded in OCT and attached to the sample stage. The resulting spectrum contains only about half of the signals as that in part A. Reprinted with permission from ref 95. Copyright 2003 Wiley Interscience.

(95) Schwartz, S. A.; Reyzer, M. L.; Caprioli, R. M. *J. Mass Spectrom.* **2003**, *38*, 699.

Matrix Application

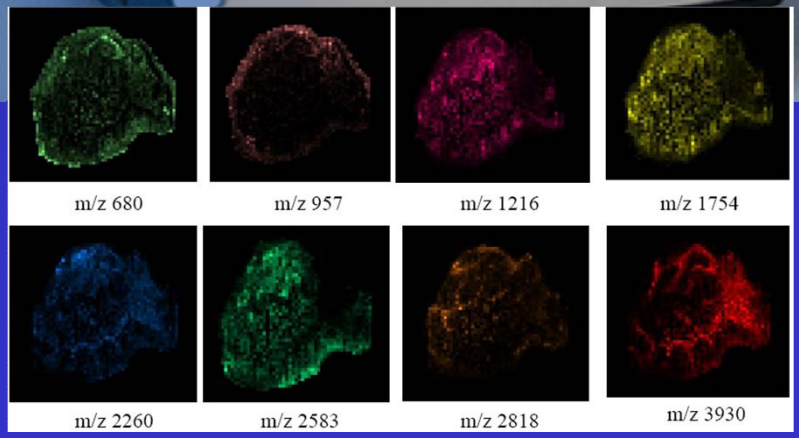
- Matrix application is vital for quality image resolution
- Must contact sample as fine, liquid mist
- Current procedure involves manual application with airbrush



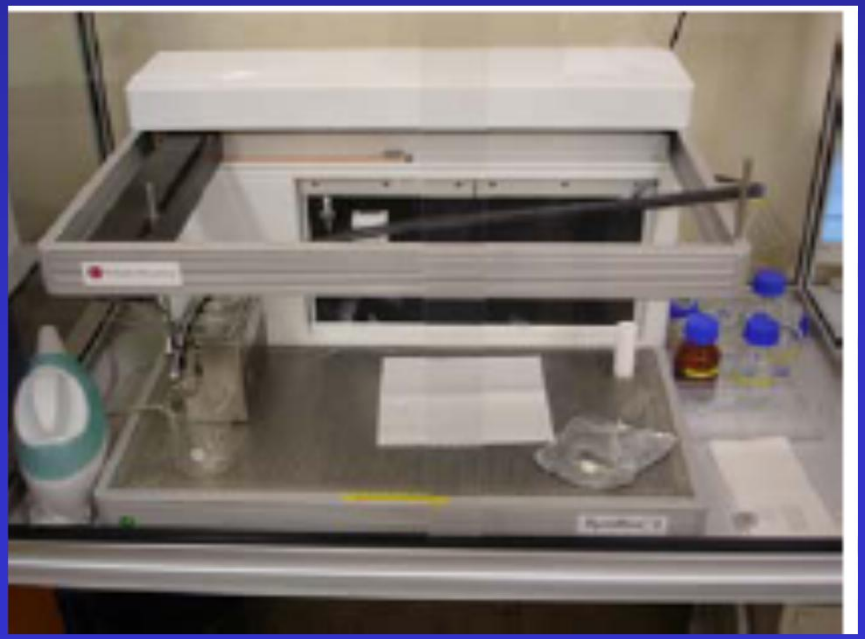


Barrett-Wilt, G., USA

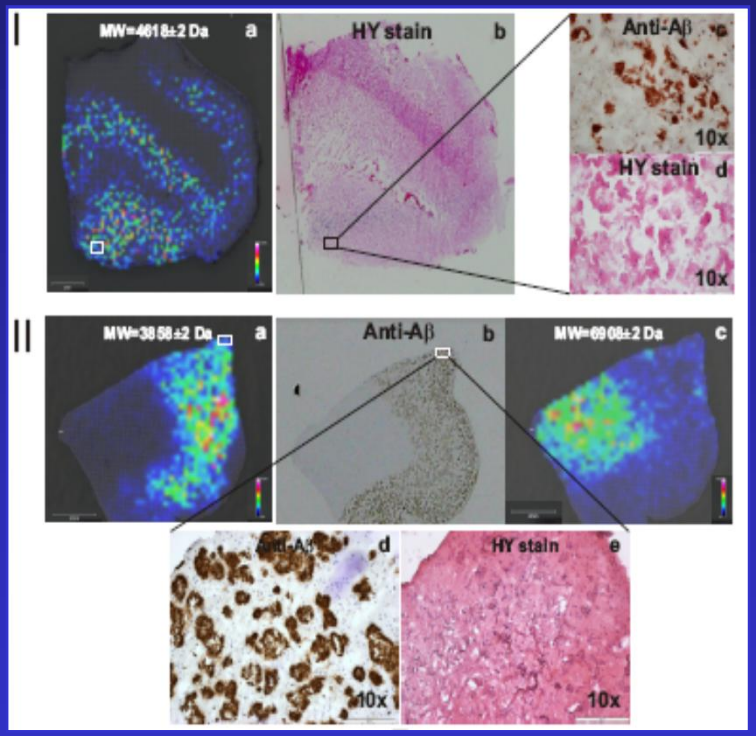
Bruker IMAGE-PREP With SPR



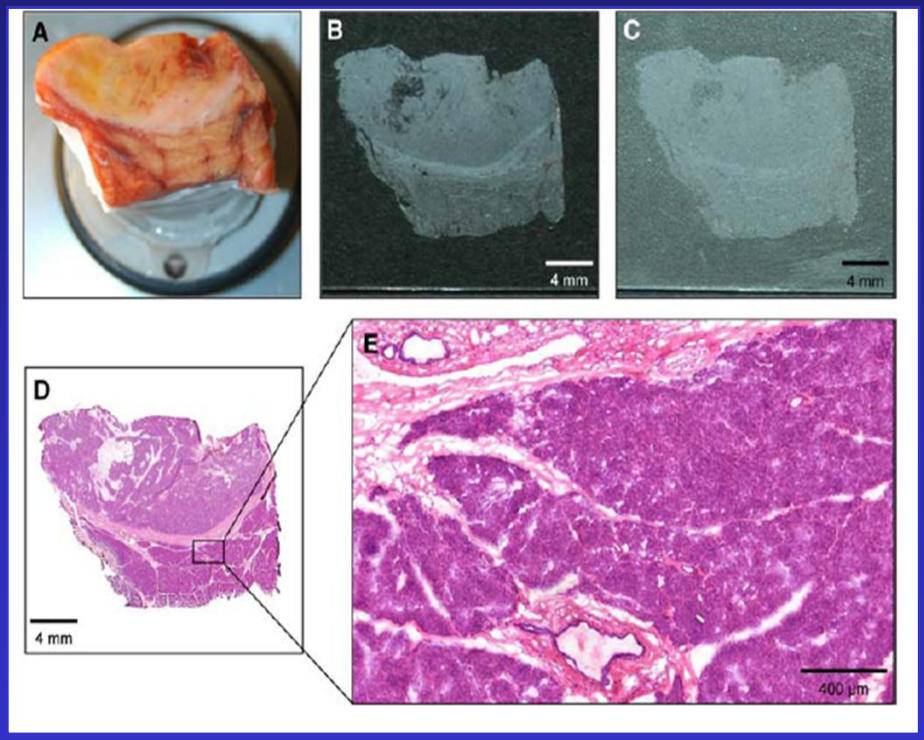
Nano spotter



(Immuno)-Histochemistry



Consecutive sections staining



Post analysis HE staining

Some clinical cases

Albuminuria and chronic kidney disease

>> Searching for non-immunogenic albumin peptide fragments

We know that:

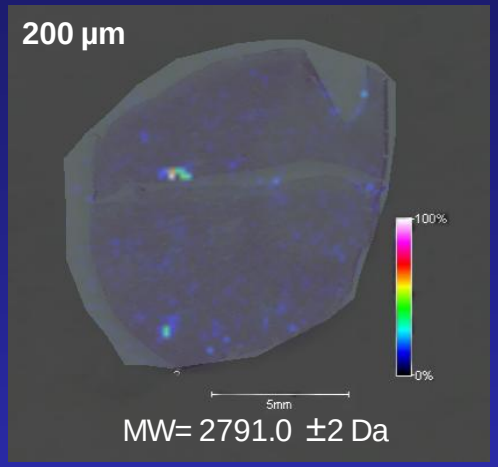
-> Albuminuria is linked to diabetes type 2

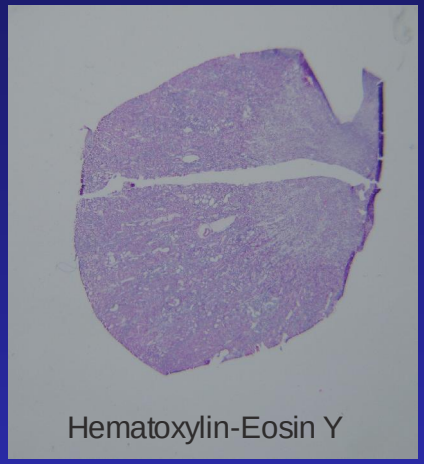
-> Albumin accumulation is linked to chronic nephropathy in these patients

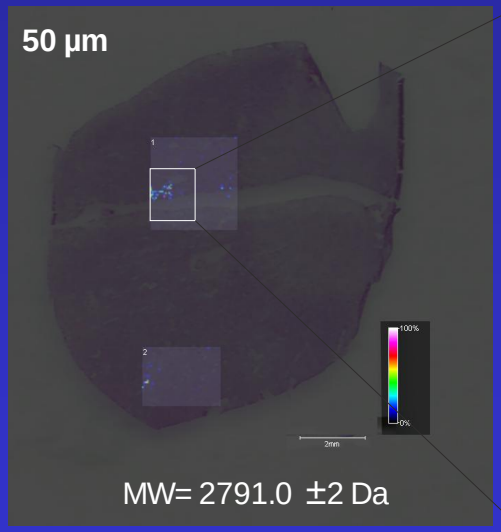
>> which part of albumin is “toxic”?

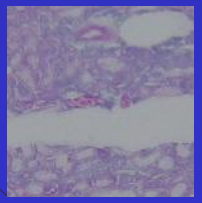
Preliminary data indicate a alb 1-24 peptide

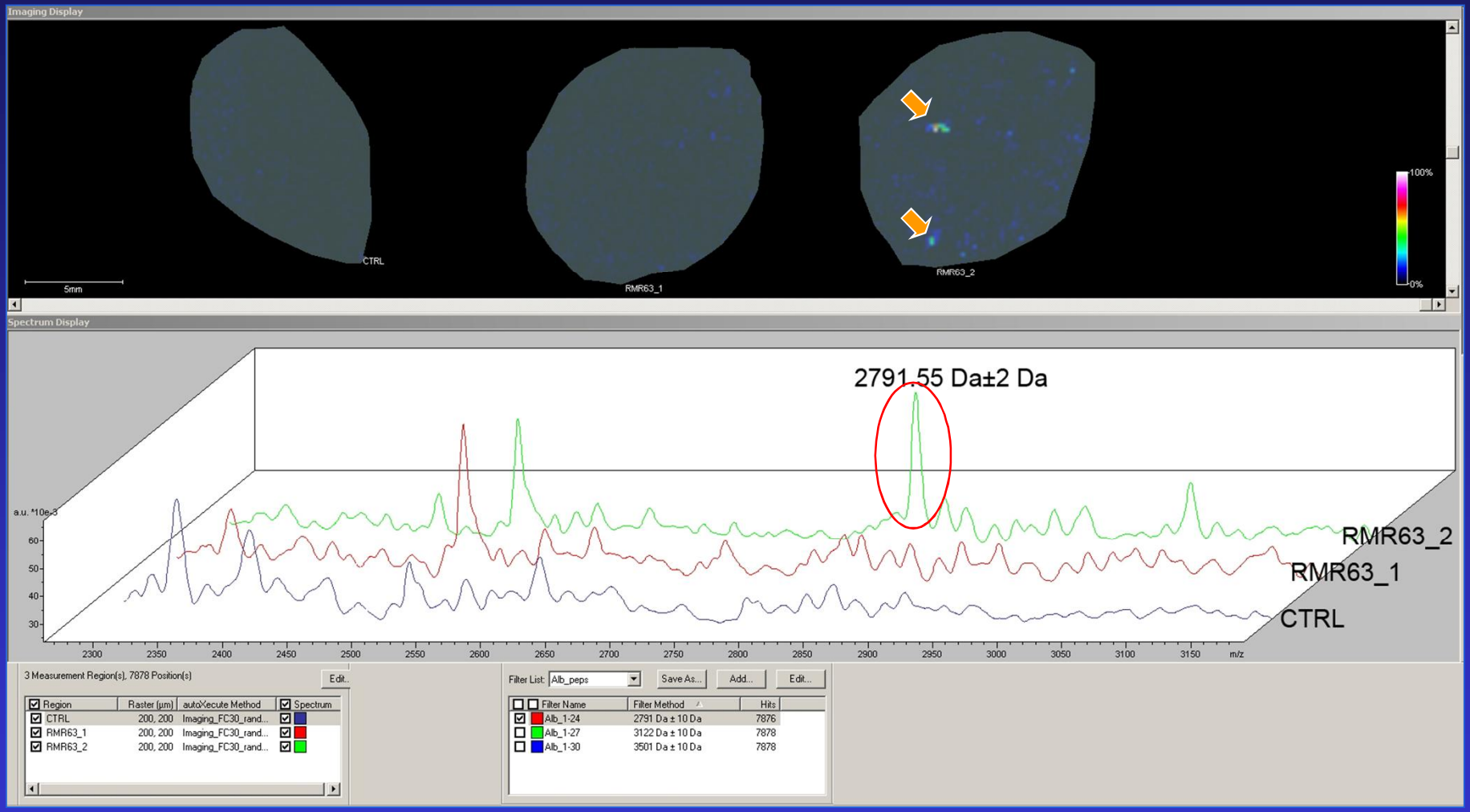
Analysis of endogenous peptides





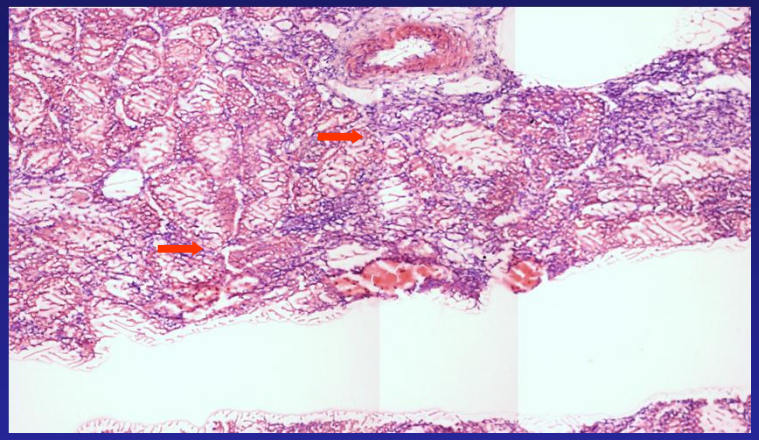




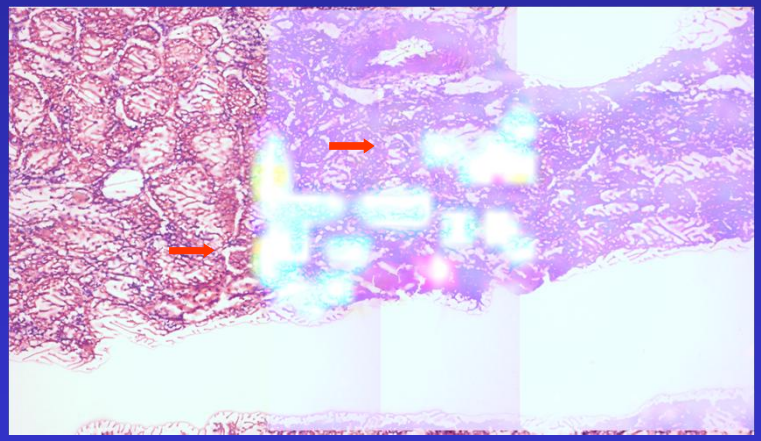


SA-coated slides were imaged within the same day by MALDI-TOF MS using an Autoflex III™ imager mass spectrometer, equipped with Smartbeam™ (Bruker Daltonics, Germany). The lateral resolution for the MALDI imaging was set to 200x200 µm for initial tissue screening and 50x50 µm for more detailed distribution analysis of MS peaks of interest.

Analysis of endogenous peptides

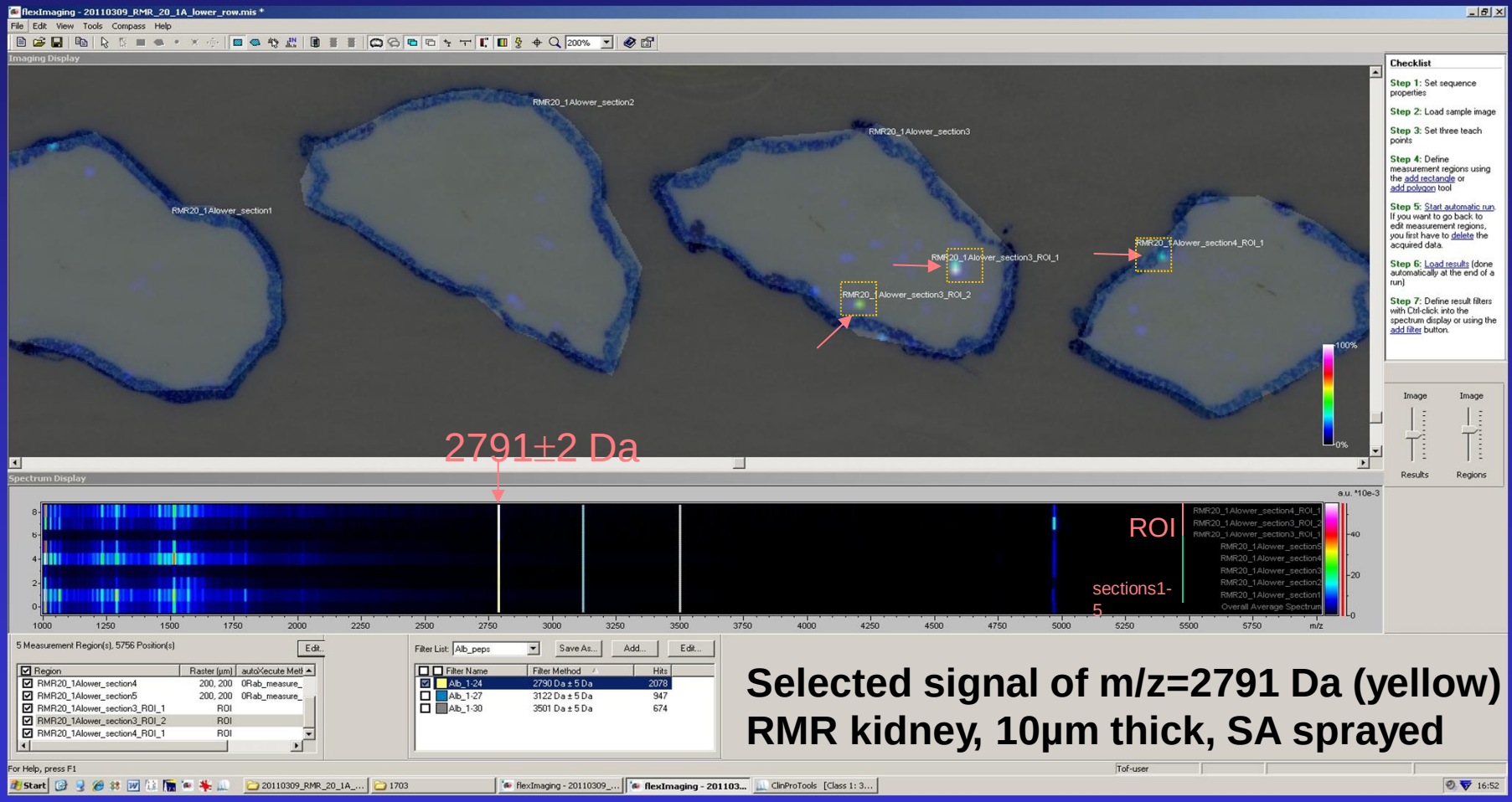


Post-analysis HE staining



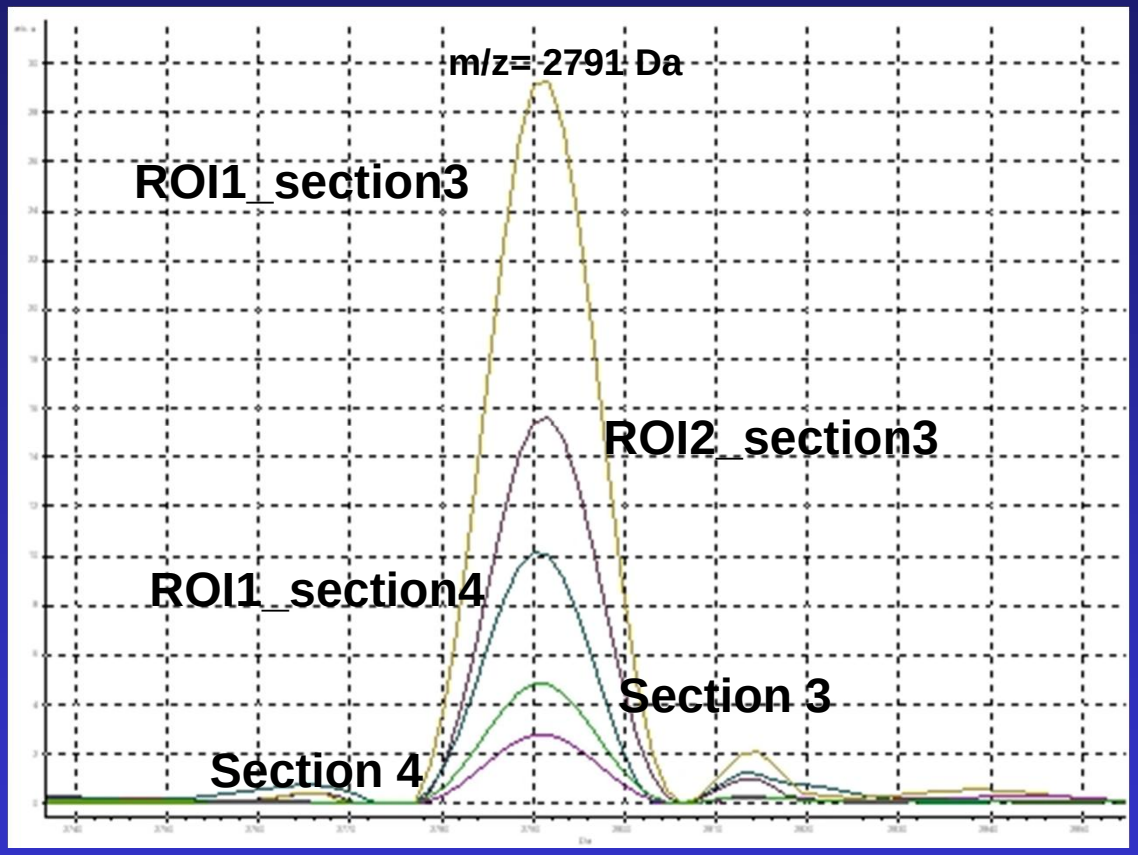
ALb_{1-24} peptide localizing to the cortex within, and in proximity of tubular cells

Analysis of selected signal on consecutive sections



Selected signal of m/z=2791 Da (yellow)
RMR kidney, 10µm thick, SA sprayed

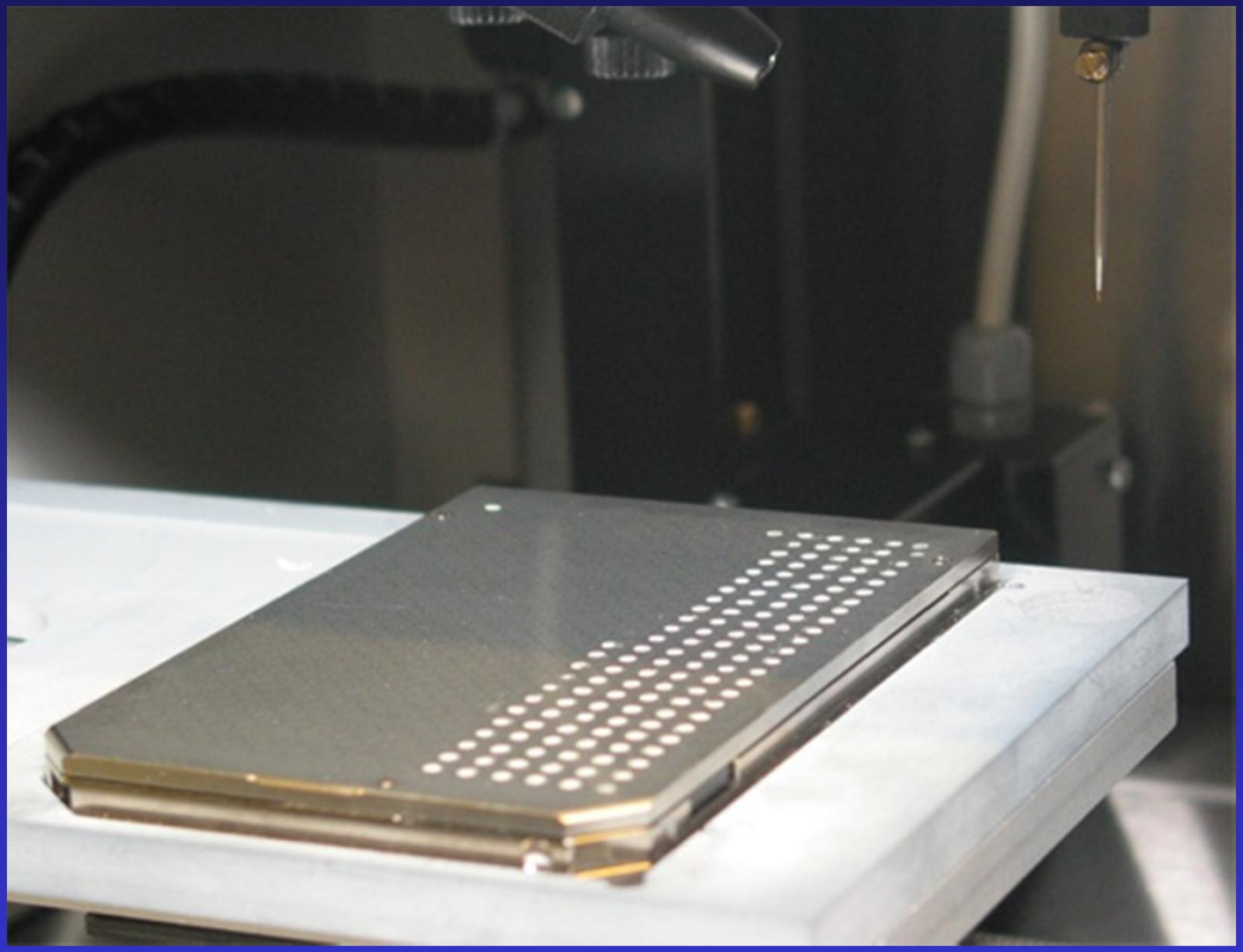
Comparison of average spectra from selected ROI



ROIX_sectionY= represents overall average spectrum from a given region
3 and 4=represents overall average spectrum from a scanned tissue section

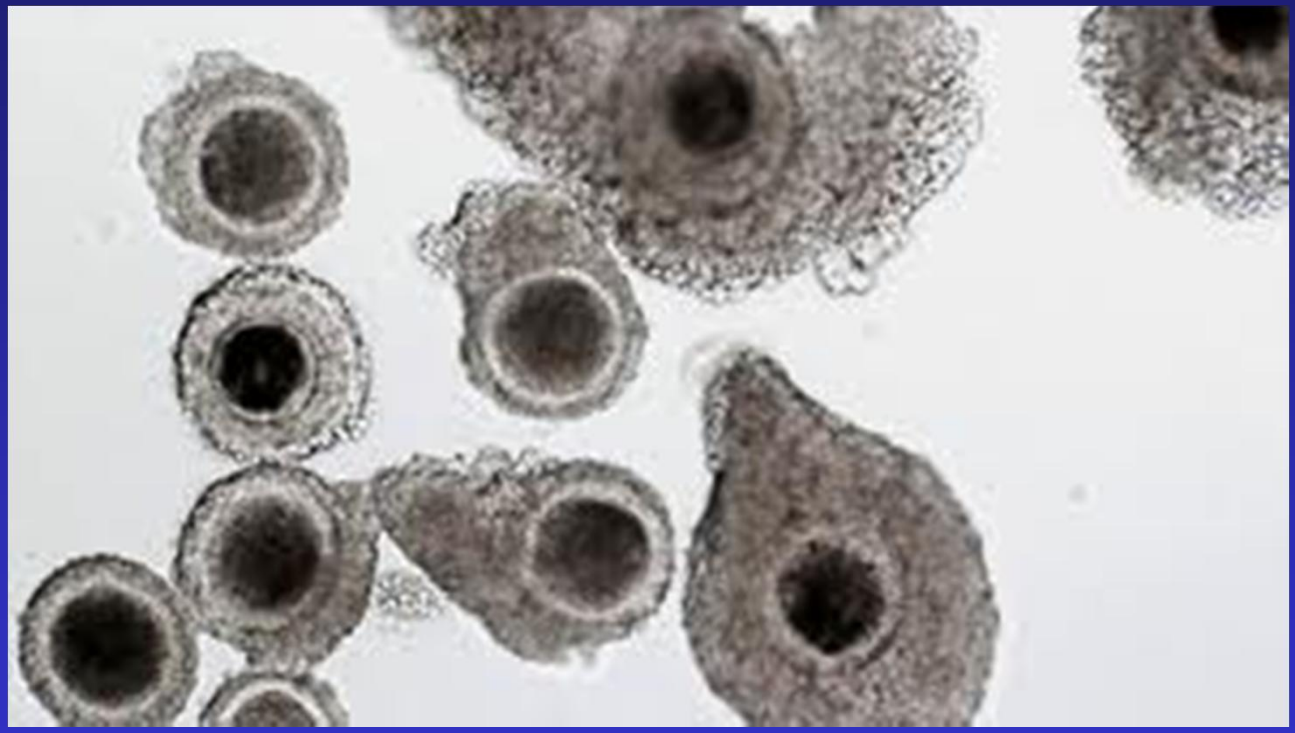
Determining the lipid content of early Embryonal stage of Oocytes

>> Searching for the possibility to biotype the Oocytes

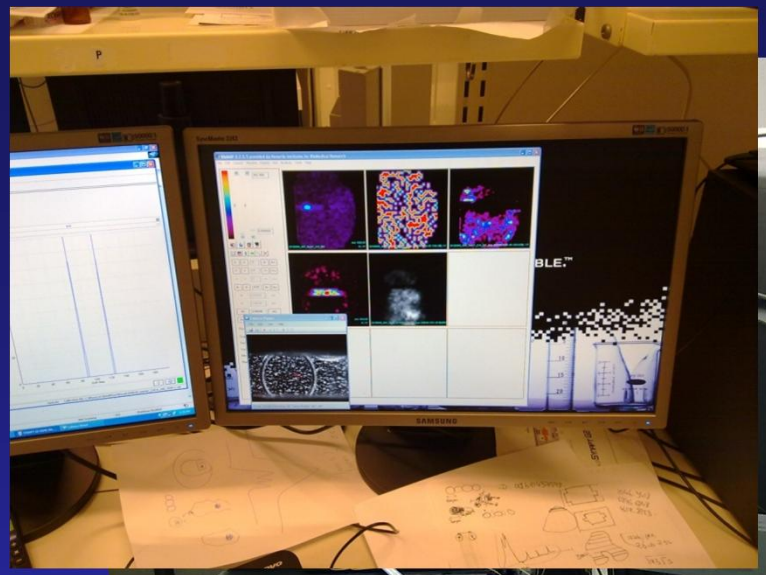


A 384 position MALDI-TOF sample target plate. To each position we place individual oocytes and cover them then with DHB matrix.

Bovine Oocytes



Bar: 150um


SAMSUNG

nanoAcquity
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nanoAcquity
SYNAPT G2

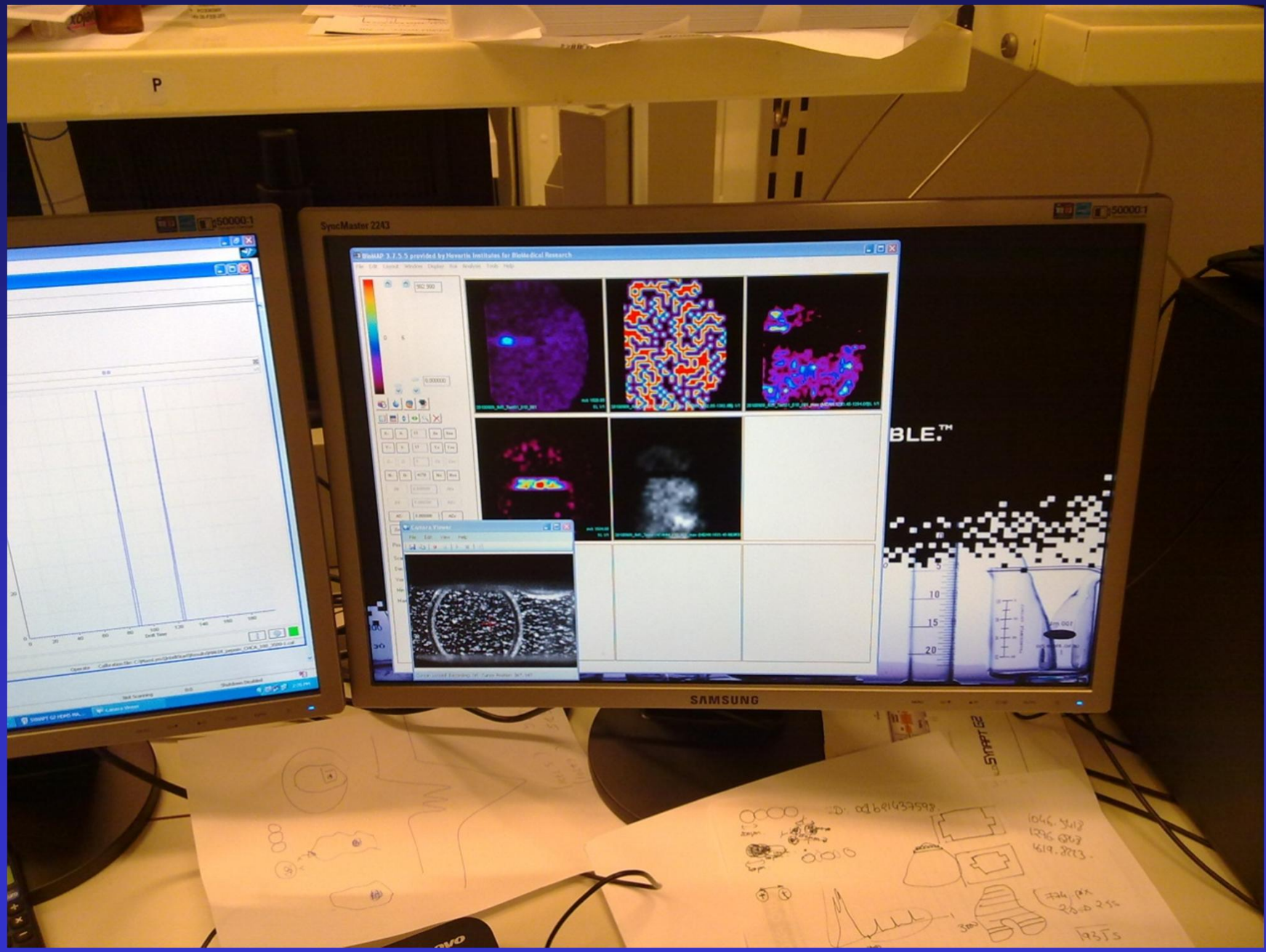
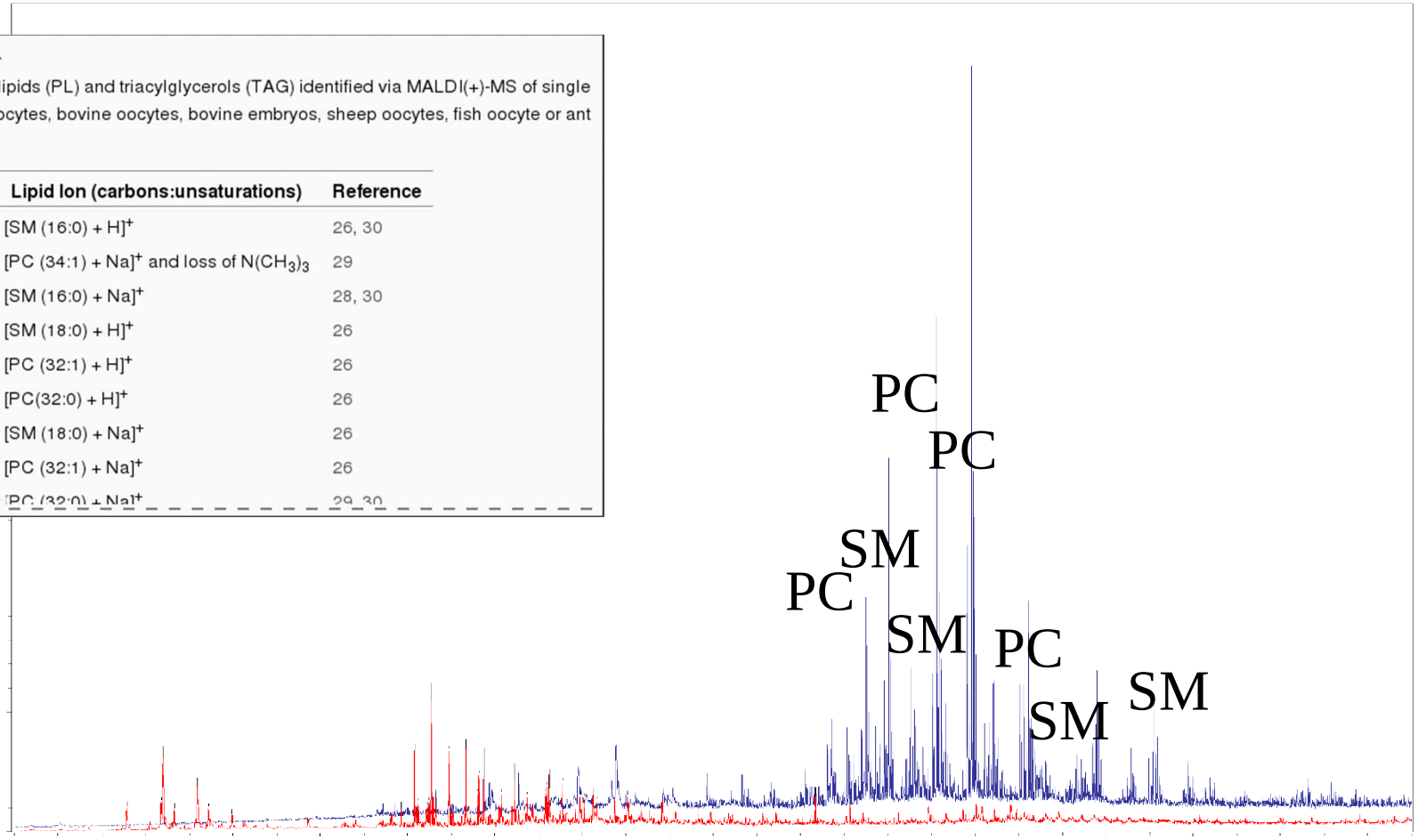
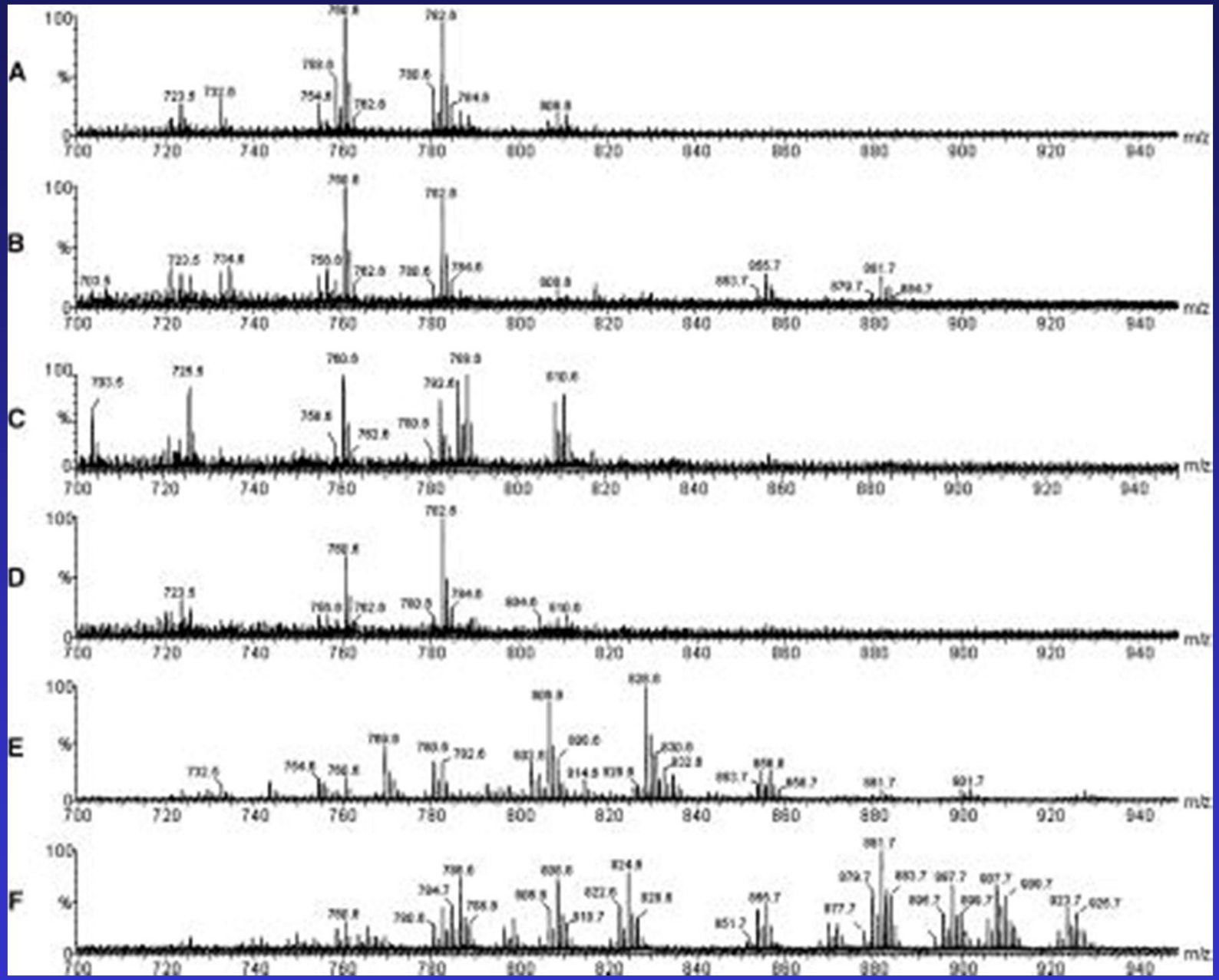

SyncMaster 2243
SAMSUNG

TABLE 1.

Phospholipids (PL) and triacylglycerols (TAG) identified via MALDI(+)-MS of single human oocytes, bovine oocytes, bovine embryos, sheep oocytes, fish oocyte or ant eggs

m/z	Lipid Ion (carbons:unsaturations)	Reference
703.5	$[SM\ (16:0) + H]^+$	26, 30
723.5	$[PC\ (34:1) + Na]^+$ and loss of $N(CH_3)_3$	29
725.5	$[SM\ (16:0) + Na]^+$	28, 30
731.5	$[SM\ (18:0) + H]^+$	26
732.5	$[PC\ (32:1) + H]^+$	26
734.6	$[PC(32:0) + H]^+$	26
753.6	$[SM\ (18:0) + Na]^+$	26
754.6	$[PC\ (32:1) + Na]^+$	26
	$[PC\ (32:0) + Na]^+$	29, 30





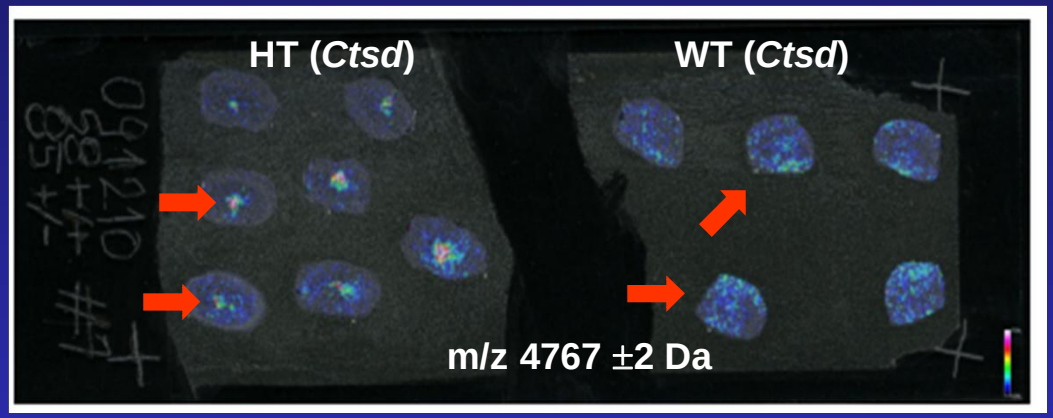
Ferreira CR et al, 2010

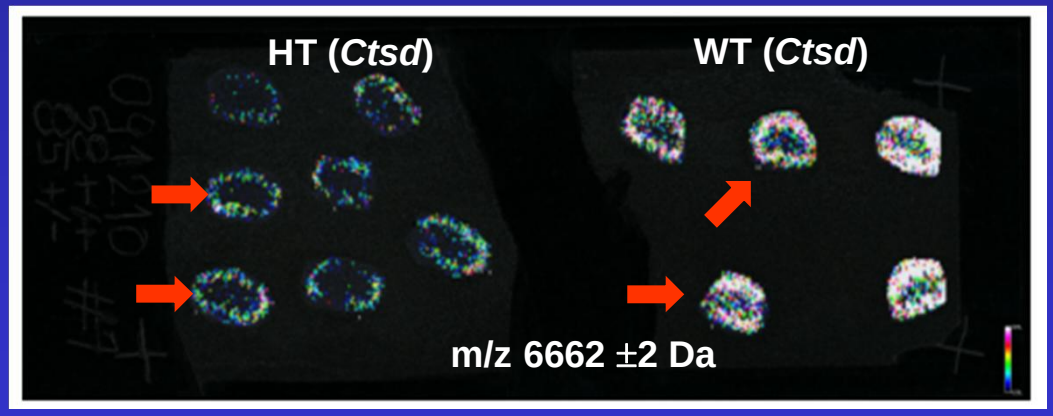
Neuronal Ceroild Lipofuscinosis (NCL)

>> Searching for changes in several accumulating peptide fragments

(misfolding of Saposin)

Visualization of molecular species allows differential comparison (*Ctsd* +/+ vs *Ctsd* +/-)

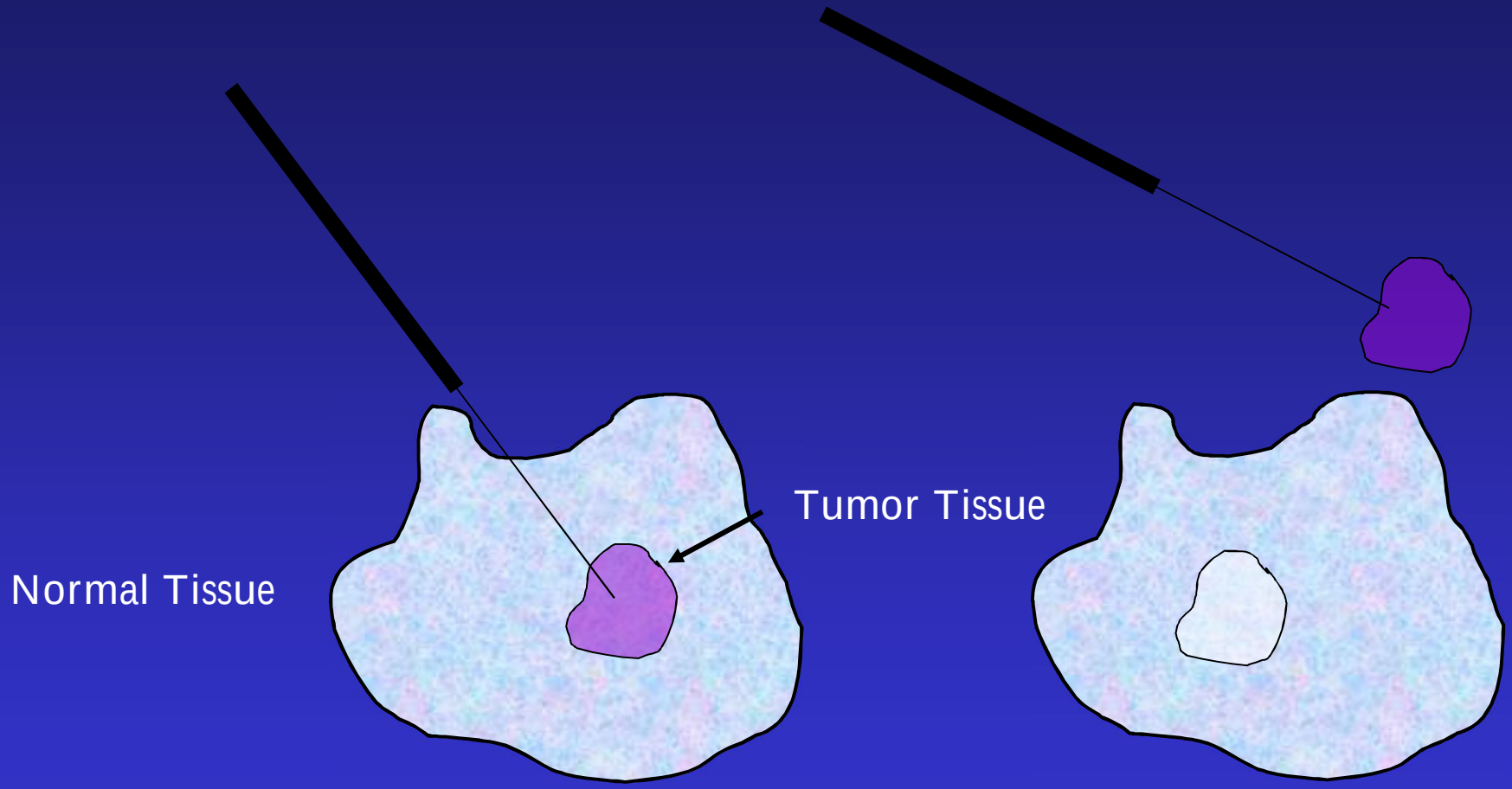




SA-coated kidney slides were imaged within the same day by MALDI-TOF MS using an Autoflex III™ imager mass spectrometer). The lateral resolution 150 x 150 µm for initial tissue screening (measurement range 2-30 kDa). WT-wild type *Ctsd* +/+, HT- heterozygous *Ctsd* +/-.

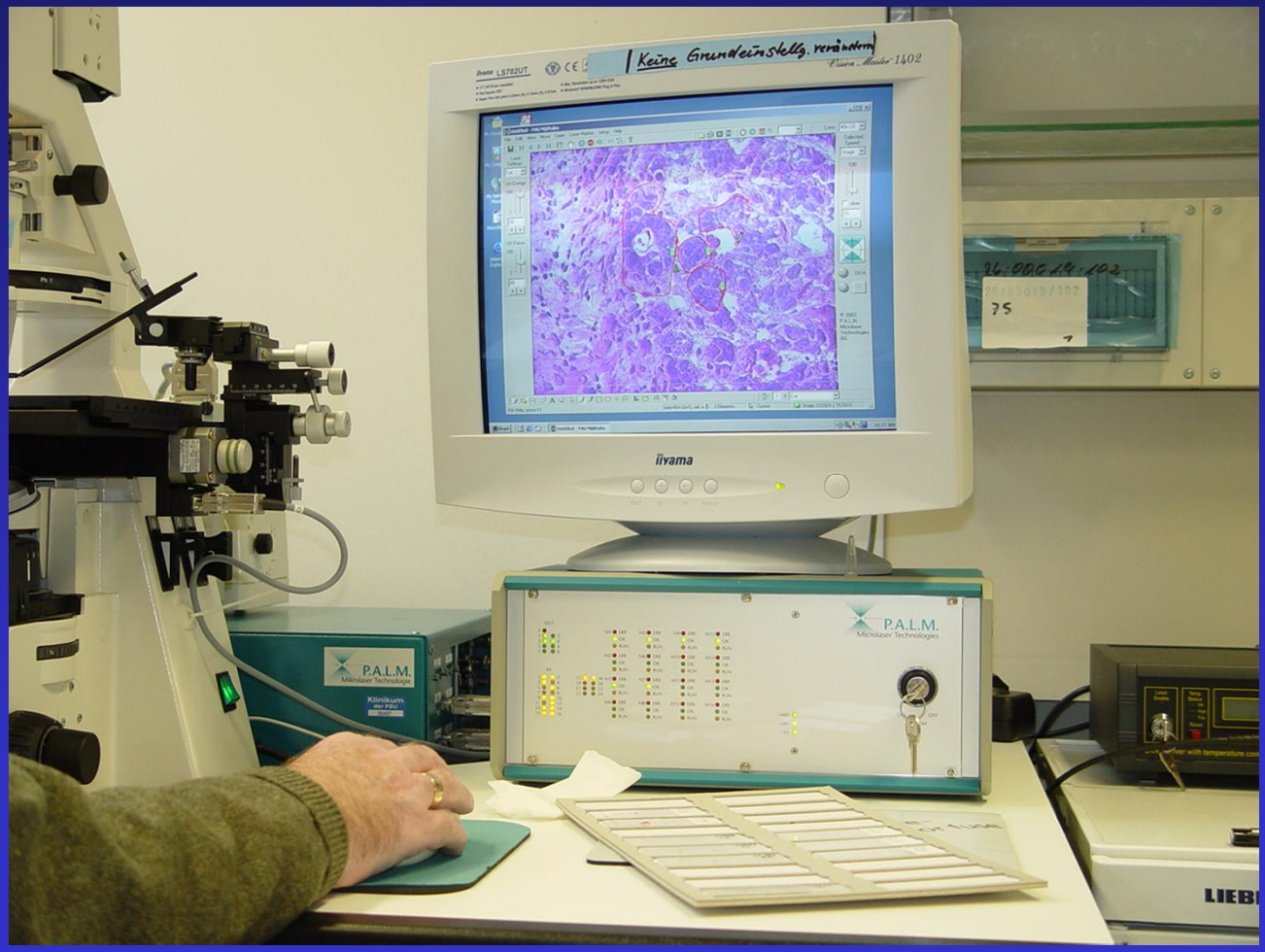
Tissue microdissection

Why Tissue Microdissection?



You would like to isolate only the targeted diseased tissue

Laser-based microdissection:

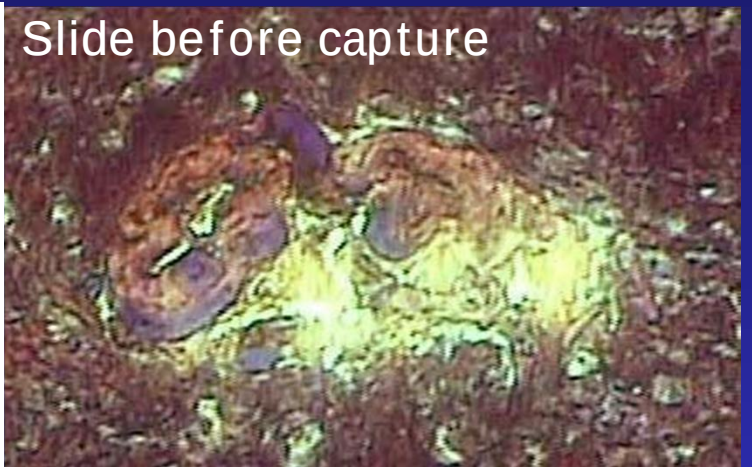


Laser Capture Microdissection (Arcturus Autopix)

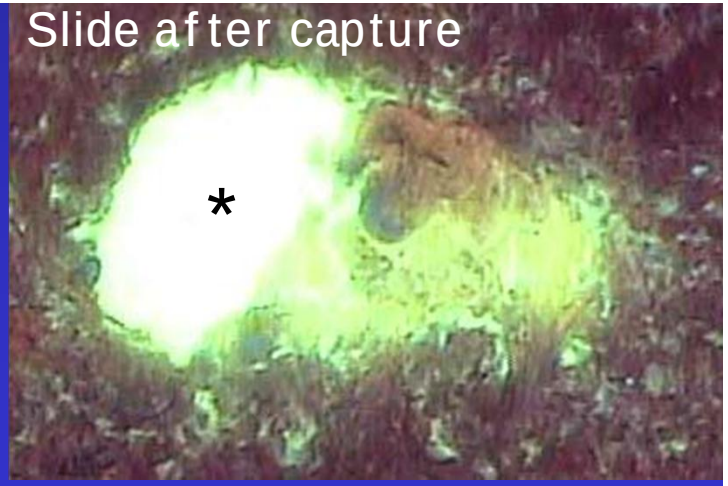
Capturing of the vessels in the control brain slide

Cap

Slide before capture



Slide after capture

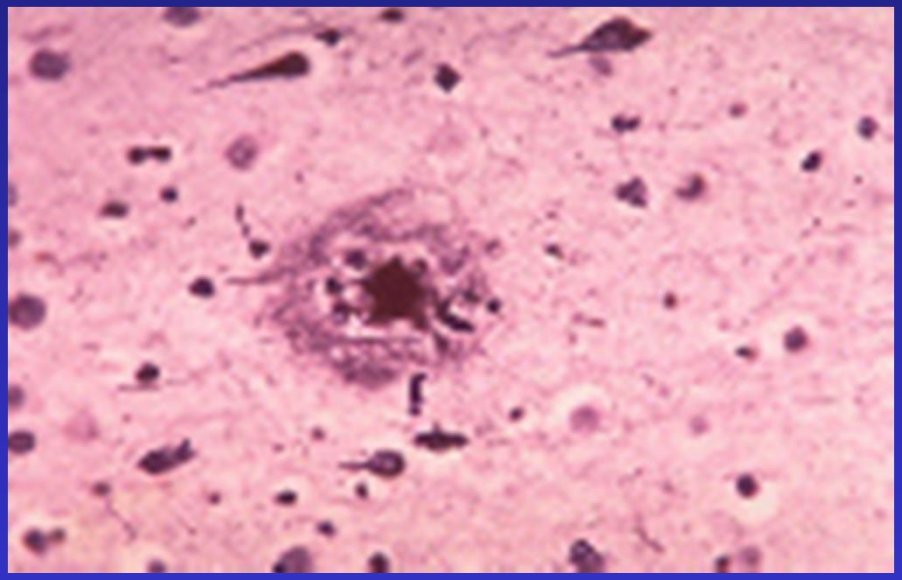


Cap with the selected tissue

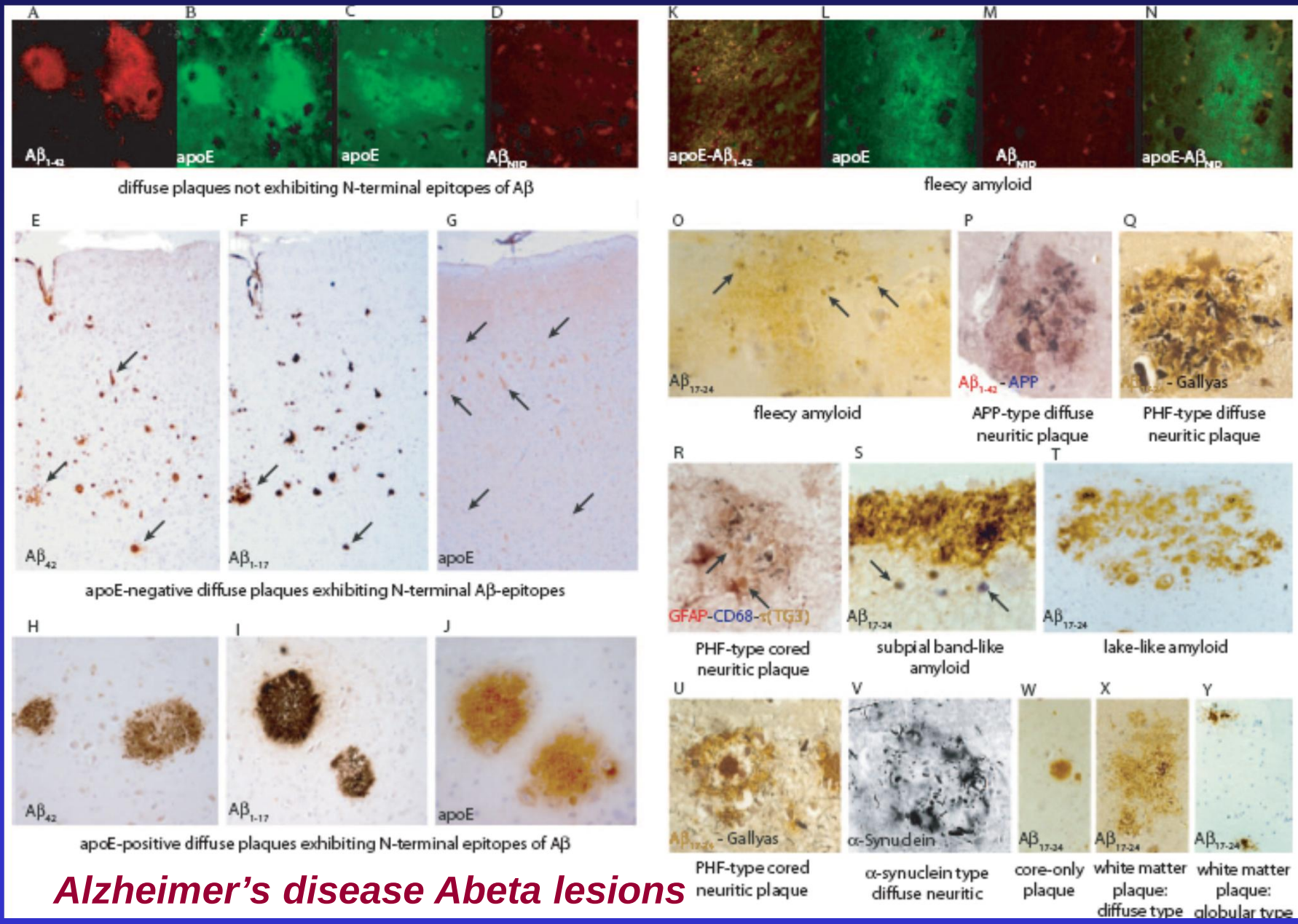
Determining the distribution of misfolded peptides in human brain (Alzheimer's diseases cases)

Alois Alzheimer

Alzheimer’s disease

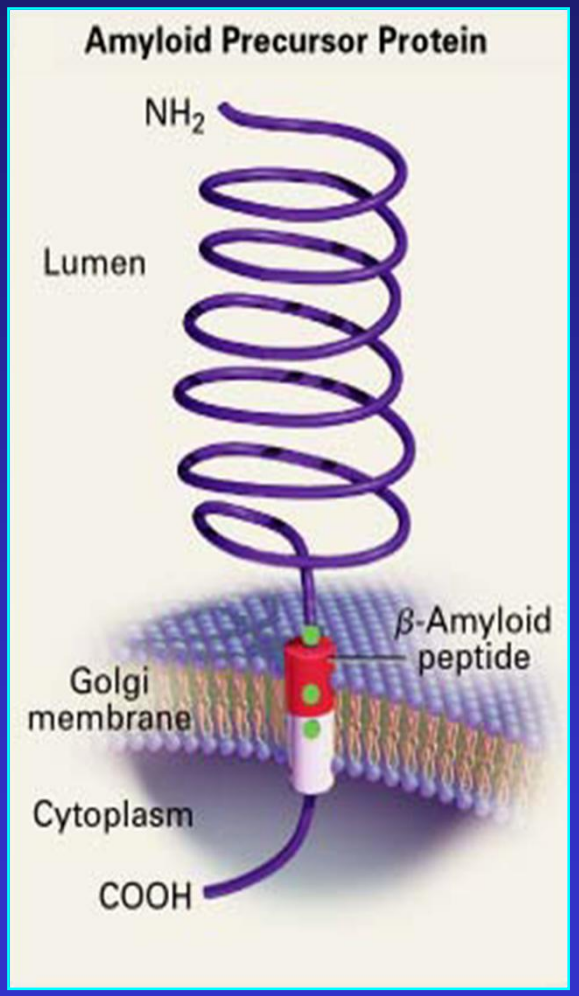


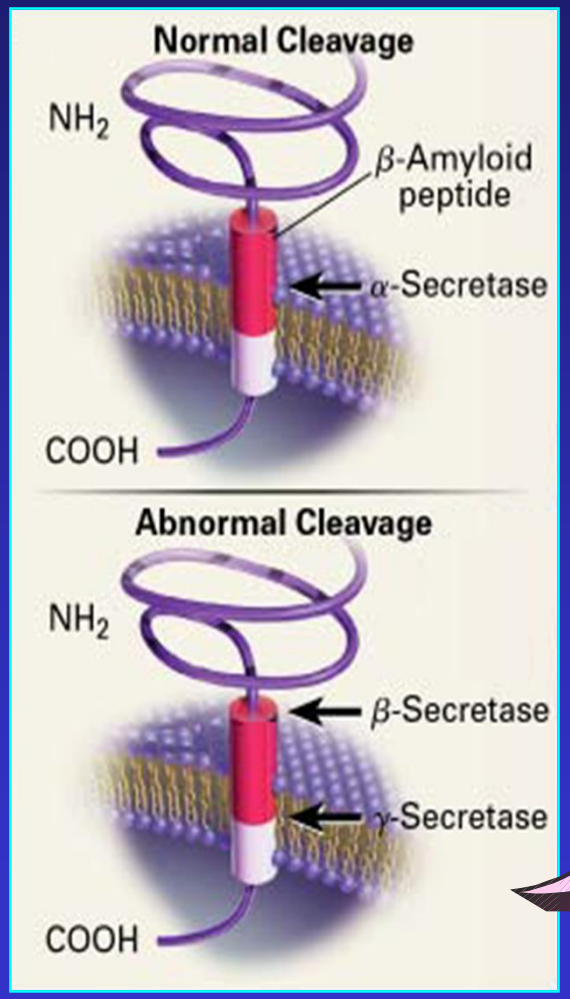
Amyloid protein plaques in the brain



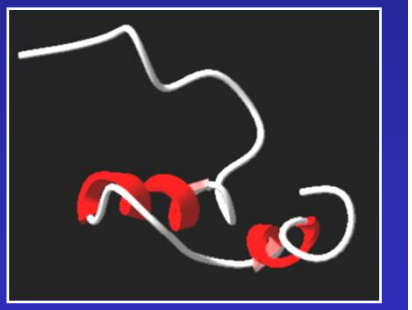
Alzheimer's disease Abeta lesions

Alzheimer's Amyloid Precursor Protein (APP) processing

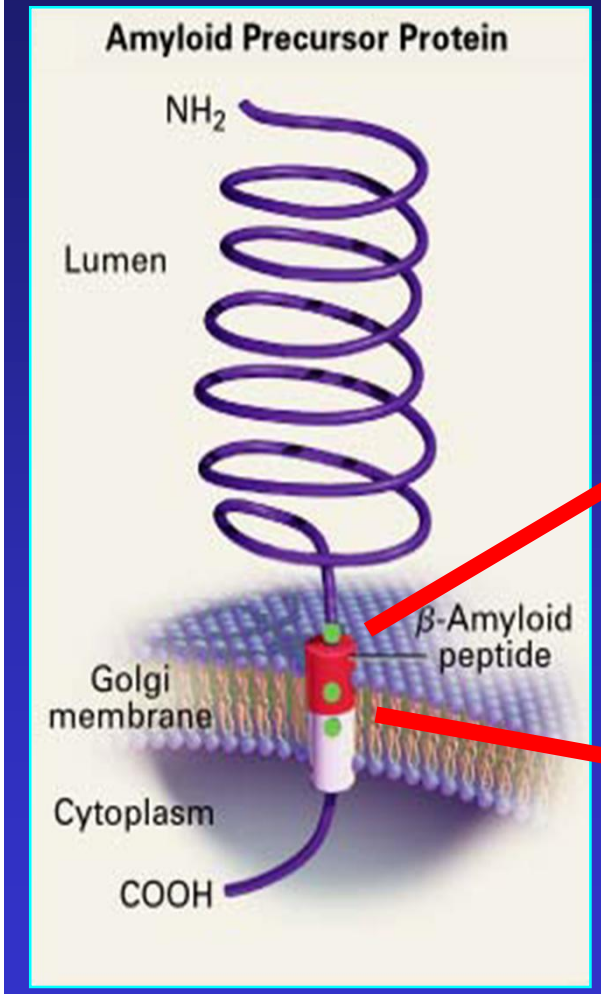


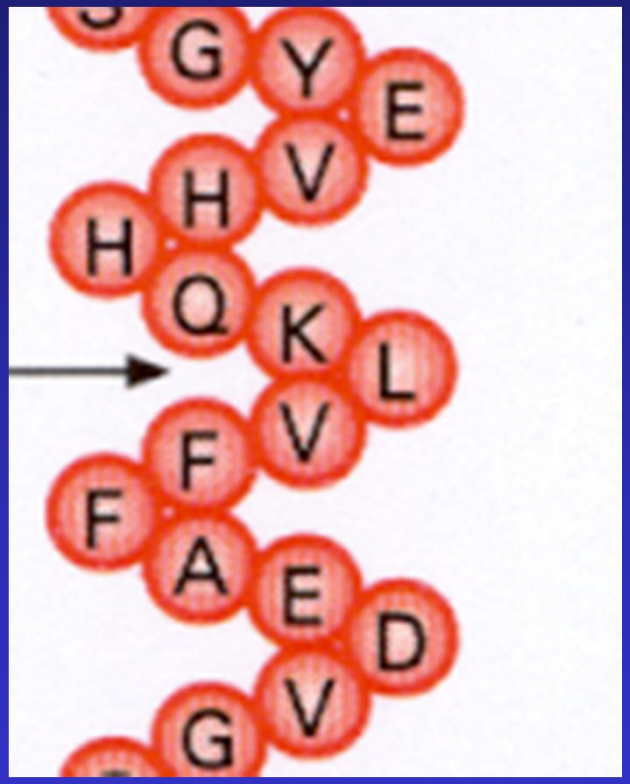


sAβ

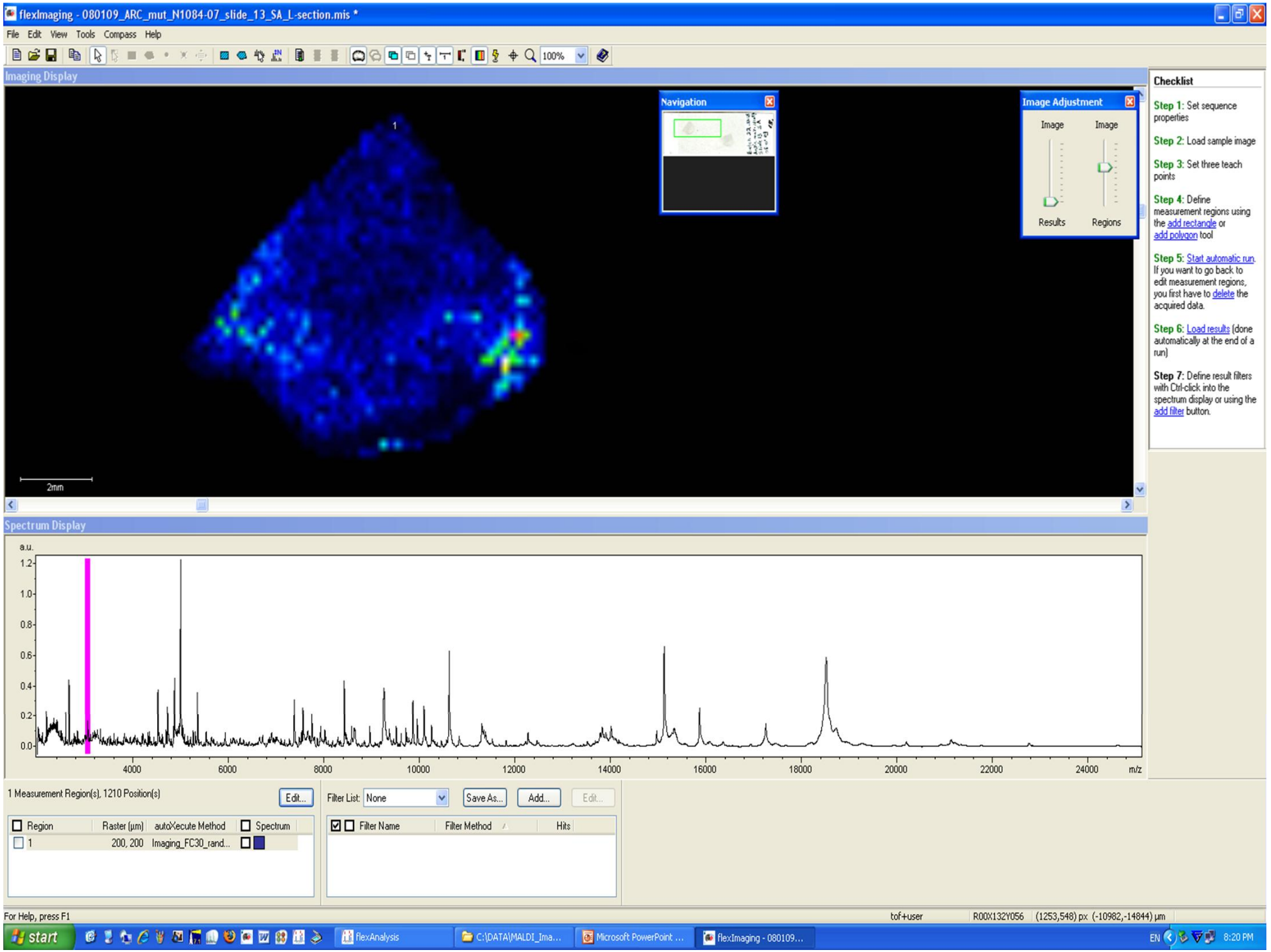


Various genetical mutations in amyloid protein lead to different forms of the amyloid protein





Observed by AB are the 1 – 40; 1 – 42
In the reality we have 1 minus something and 40 plus something!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Results
Regions
Checklist
Step 1: Set sequence properties
Step 2: Load sample image
Step 3: Set three teach points
Step 4: Define measurement regions using the add rectangle or add polygon tool
Step 5: Start automatic run. If you want to go back to edit measurement regions, you first have to delete the acquired data.
Step 6: Load results (done automatically at the end of a run)
Step 7: Define result filters with Ctrl-click into the spectrum display or using the add filter button.
Spectrum Display
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20000
22000
24000
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1 Measurement Region(s), 1210 Position(s)
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Filter List: None
Save As...
Add...
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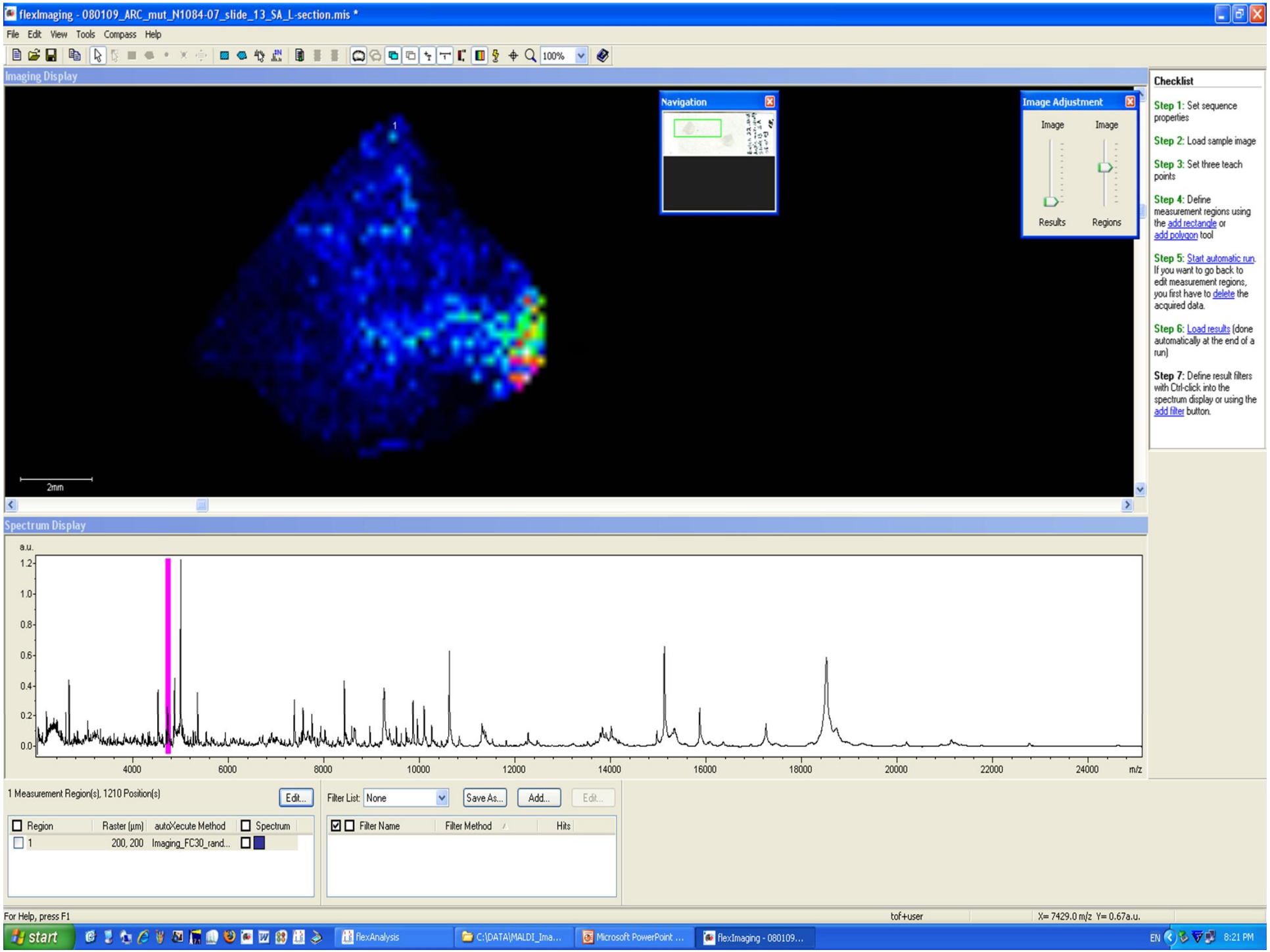
flexImaging - 080109_ARC_mut_N1084-07_slide_13_SA_L-section.mis *
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24000
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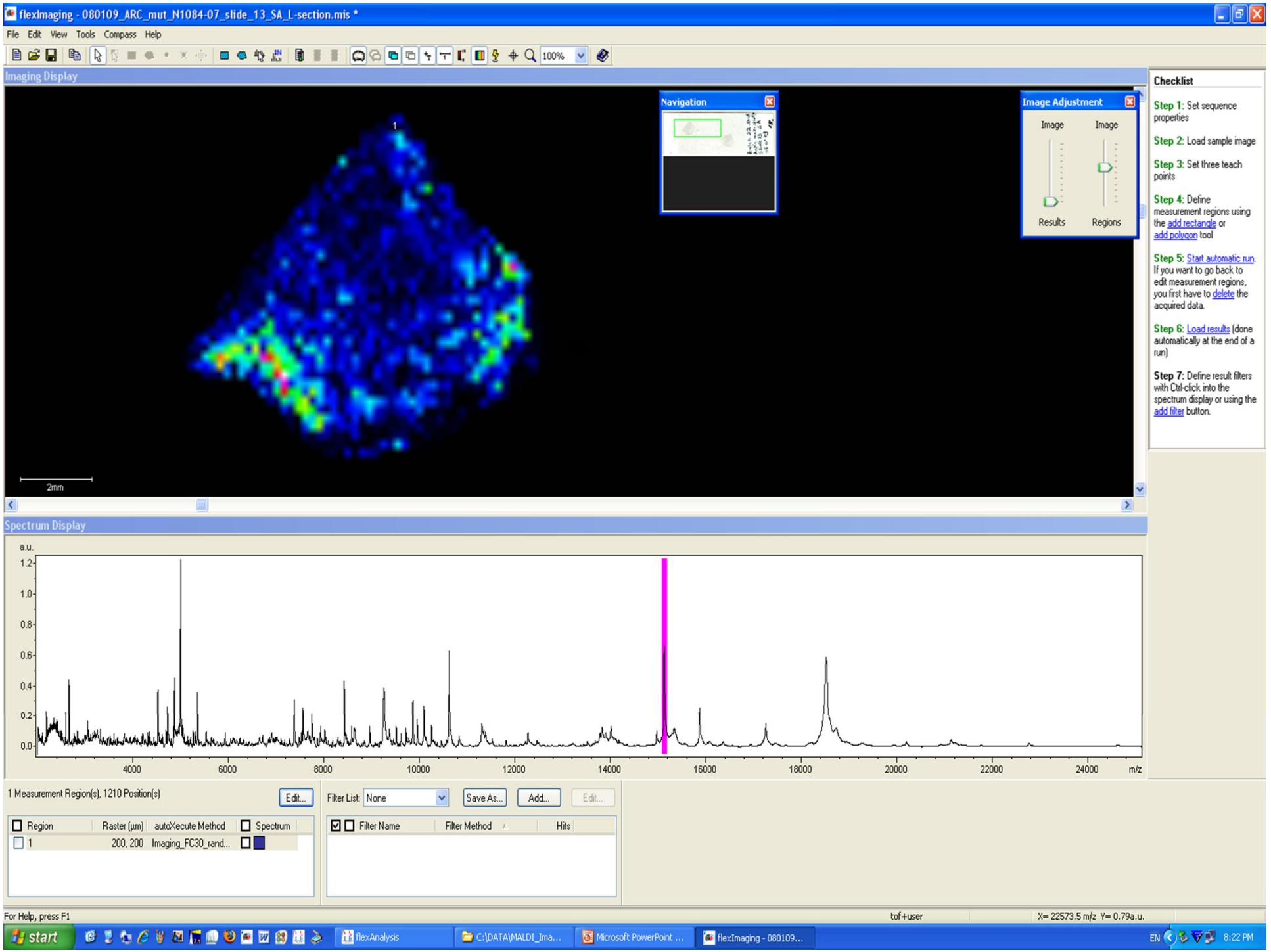
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20000
22000
24000
m/z
1 Measurement Region(s), 1210 Position(s)
Edit...
Filter List: None
Save As...
Add...
Edit...
Region
Raster (µm)
autoXecute Method
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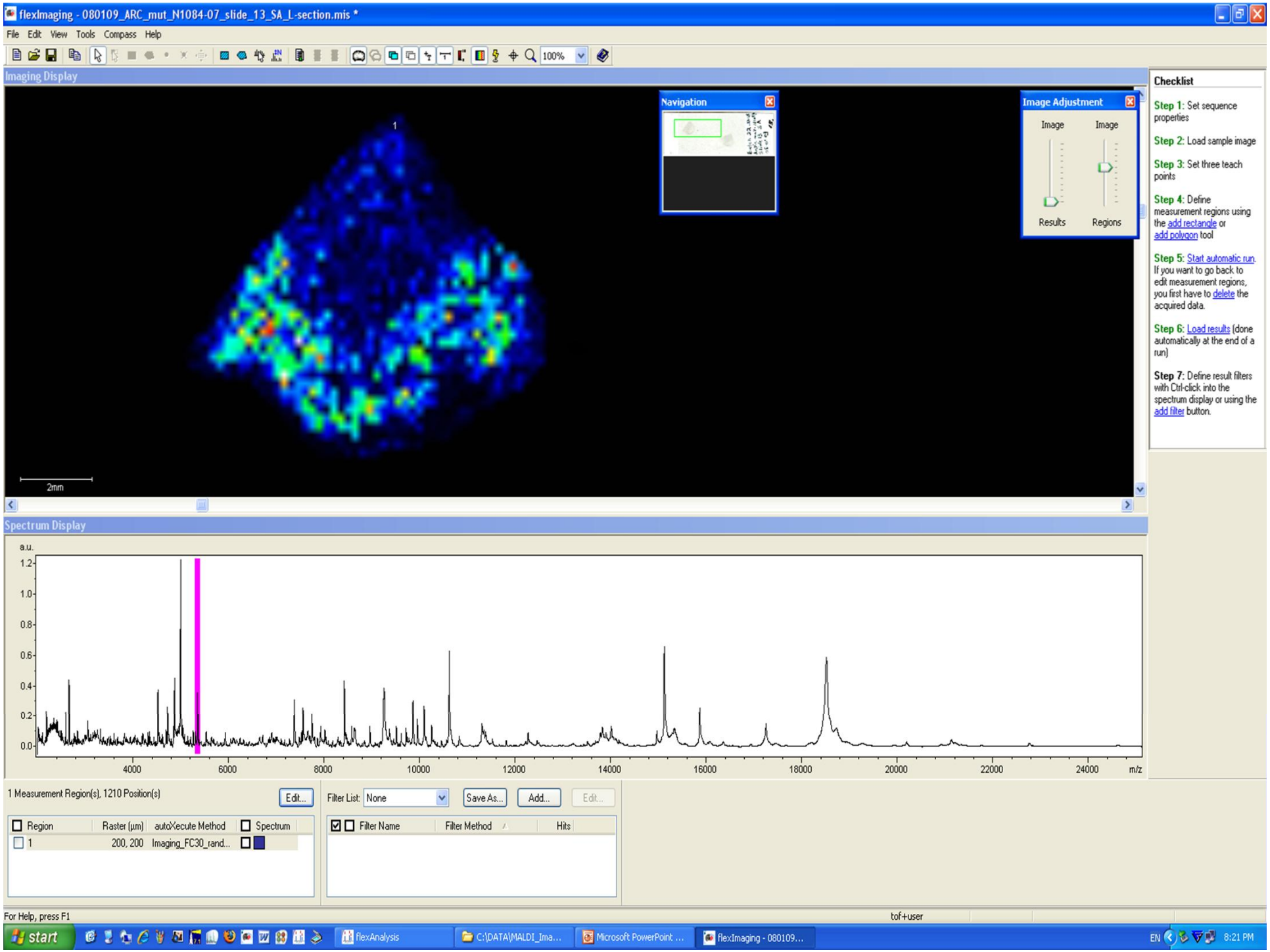
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Results
Regions
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Step 7: Define result filters with Ctrl-click into the spectrum display or using the add filter button.
2mm
Spectrum Display
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1.2
1.0
0.8
0.6
0.4
0.2
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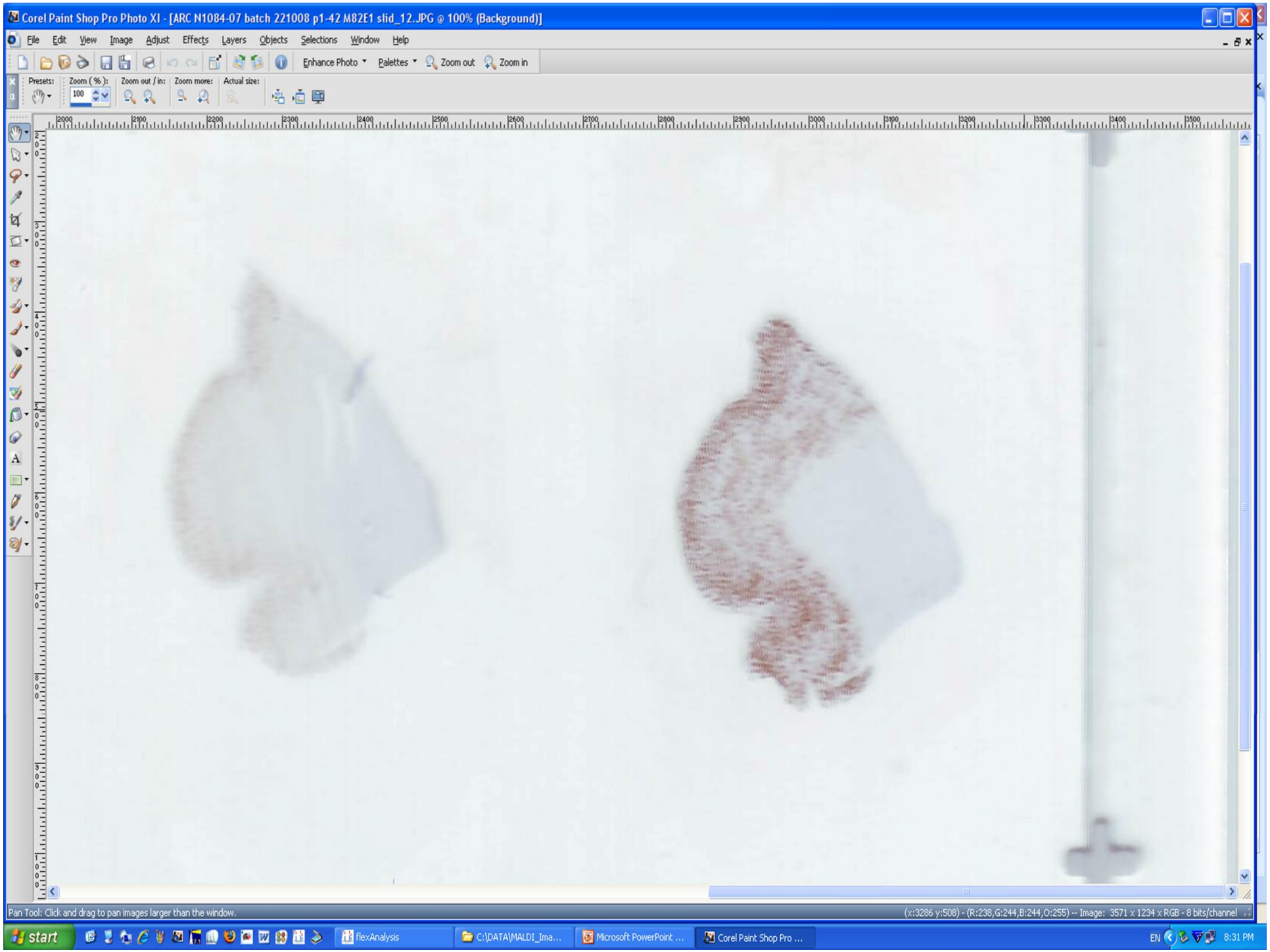
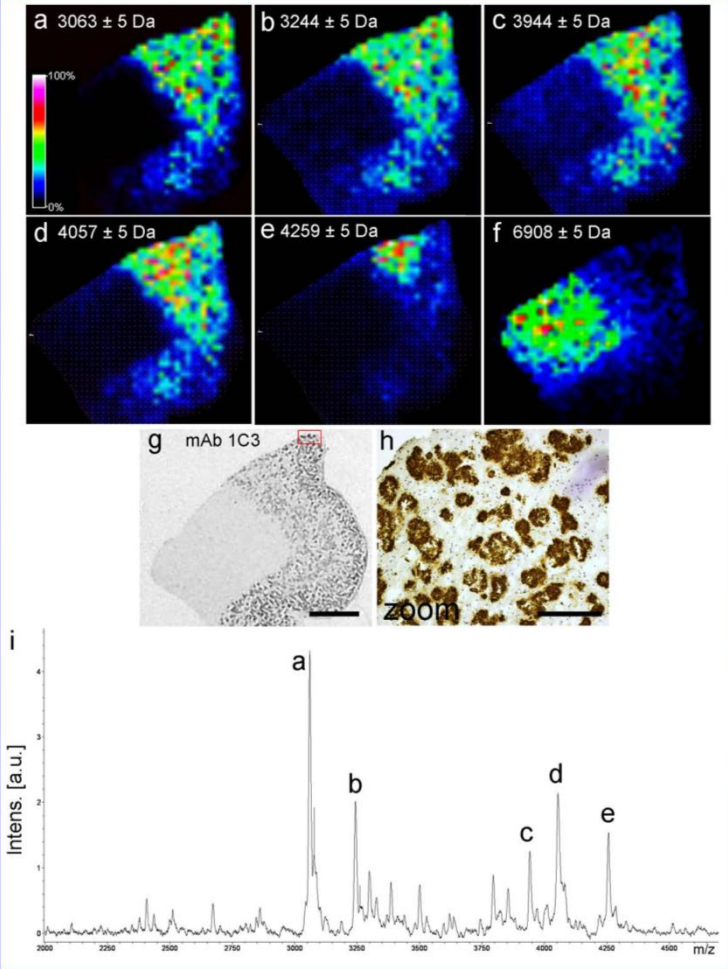
4000
6000
8000
10000
12000
14000
16000
18000
20000
22000
24000
m/z
1 Measurement Region(s), 1210 Position(s)
Edit...
Filter List: None
Save As...
Add...
Edit...
Region
Raster (µm)
autoXecute Method
Spectrum
1
200, 200
Imaging_FC30_rand...
Filter Name
Filter Method
Hits
For Help, press F1
tof+user
start
flexAnalysis
C:\DATA\MALDI_Ima...
Microsoft PowerPoint ...
flexImaging - 080109...
EN
8:21 PM


Corel Paint Shop Pro Photo XI - [ARC N1084-07 batch 221008 p1-42 M82E1 slid_12.JPG @ 100% (Background)]
File Edit View Image Adjust Effects Layers Objects Selections Window Help
Enhance Photo
Palettes
Zoom out
Zoom in
Presets:
Zoom (%):
100
Zoom out / in:
Zoom more:
Actual size:
Pan Tool: Click and drag to pan images larger than the window.
(x:3286 y:508) - (R:238,G:244,B:244,O:255) -- Image: 3571 x 1234 x RGB - 8 bits/channel
start
flexAnalysis
C:\DATA\MALDI_Ima...
Microsoft PowerPoint ...
Corel Paint Shop Pro ...
EN
8:31 PM

Validation of results

MALDI-IMS analyses on the Arctic AD brain



Aβ peptides detected with MALDI-TOF and MALDI-IMS analyses

Aβ peptide sequence	Aβ m/z predicted (Da)*a*	MALDI-TOF m/z observed (Da)*b*	MALDI-IMS m/z observed (Da)*c*	MALDI-IMS area
8-32 wt	2675.01	2676.26	2674.99	plaque area, cortex
3-25 wt	2675.91	2676.26	2674.99	plaque area, cortex
7-31 wt	2676.94	2676.26	2674.99	plaque area, cortex
1-25 wt	2862.08	2861.39	2863.28	plaque area, cortex
12-41 Arc	3064.66	3060.5	3063.23	plaque area, cortex
11-40 Arc	3080.63	3083.56	3081.67	plaque area, cortex
10-40 Arc	3243.79	3244.13	3244.73	plaque area, cortex
11-42 (p3E) Arc	3246.84	3244.13	3244.73	plaque area, cortex
9-40 Arc	3300.84	3298.25	3302.97	plaque area, cortex
8-40 Arc	3387.92	3384.96	3387.41	plaque area, cortex
10-42 wt	3500.09	3502.12	3503.61	plaque area, cortex
8-41 Arc	3501.08	3502.12	3503.61	plaque area, cortex
7-40 Arc	3503.01	3502.12	3503.61	plaque area, cortex
7-41 Arc	3616.17	3618.12	na	plaque area, cortex
5-40 Arc	3796.34	3795.56	3797.33	plaque area, cortex
7-43 wt	3860.41	3855.31	3858.02	plaque area, cortex
4-40 Arc	3943.52	3943.23	3945.40	plaque area, cortex
5-42 wt	4052.64	4054.19	4057.67	plaque area, cortex
3-40 (p3E) Arc	4054.62	4054.19	4057.67	plaque area, cortex
4-41 Arc	4056.68	4054.19	4057.67	plaque area, cortex
1-38 Arc	4060.53	4054.19	4057.67	plaque area, cortex
2-41 Arc	4256.87	4257.67	4259.48	plaque area, cortex
3-42 Arc	4256.87	4257.67	4259.48	plaque area, cortex
3-41 wt	4257.85	4257.67	4259.48	plaque area, cortex
1-40 Arc	4258.80	4257.67	4259.48	plaque area, cortex
1-43 Arc +Na	4564.63	4544.14	4566.16	plaque area, cortex

In grey: peptides within range of +/5 Da in positive linear mode of MS analysis of predicted m/z
a Aβ m/z predicted: predicted m/z of the monoisotopic protonated molecules [M+ H]+.
b MALDI-TOF m/z observed: observed m/z in the extraction experiments
c MALDI-IMS m/z observed: observed m/z average [+H$^+$] of the Aβ in MALDI-IMS analyses

Resolution is 200 x 200 µm, positive linear mode. Scale bar in (**a**-**g**) represents 2 mm.

Diagnostic value?

Imaging Mass Spectrometry (IMS) of a Specific Fragment of Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Kinase Kinase 2 Discriminates Cancer from Uninvolved Prostate Tissue

Lisa H. Cazares,1,2,3 Dean Troyer,1,3 Savvas Mendrinos,3 Raymond A. Lance,3,5, Julius O. Nyalwidhe,1,2,3 Hind A. Beydoun,4 Mary Ann Clements,1,2,3, Richard R. Drake,1,2,3 and O. John Semmes1,2,3

Clin Cancer Res 2009;15(17) September 1, 2009

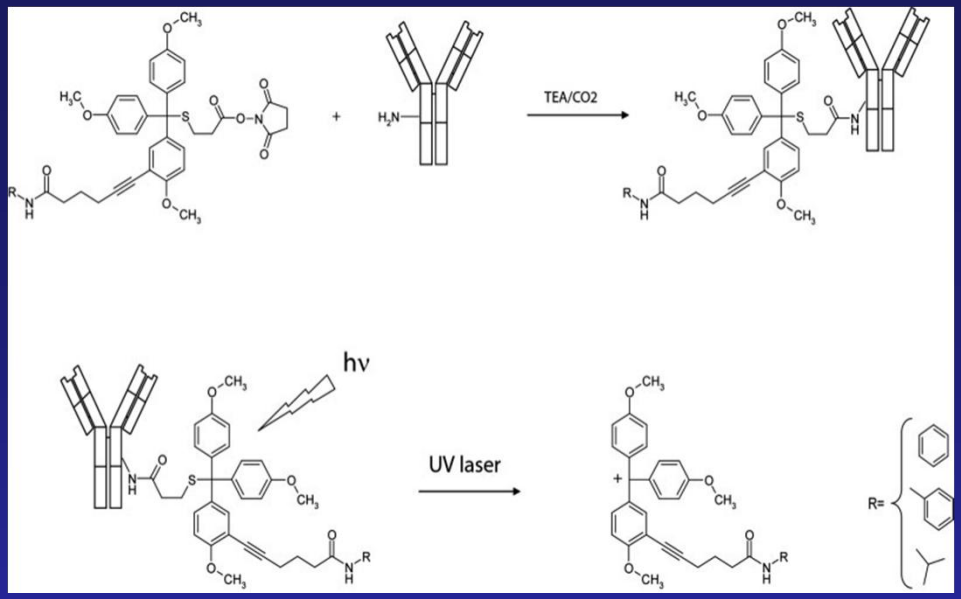
Special Applications for IMS

Multiplex Targeted Secondary Detection (MTSD)

- Targeting molecule of interest with specific antibodies which carry a photocleavable mass tag sensor
- The mass tag sensor is a small molecule of known mass easily detectable by MALDI MS (indirect detection)
- The tag is released just before detection step due to irradiation with MALDI laser (photolabile linker)

Immuno-IMS

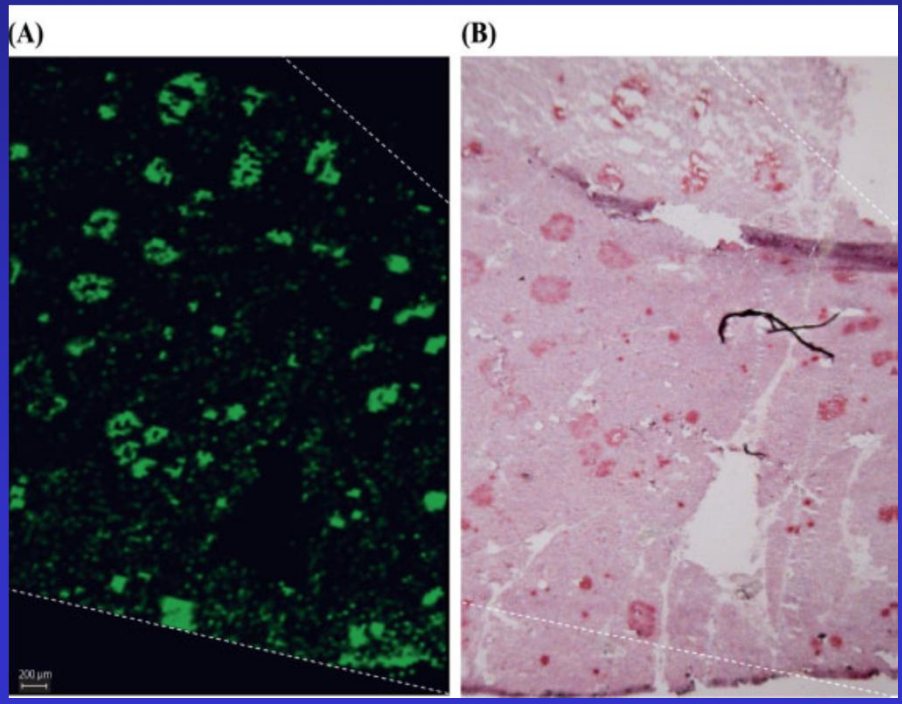
TAMSIM (TArgeted Multiplexed MS Imaging)



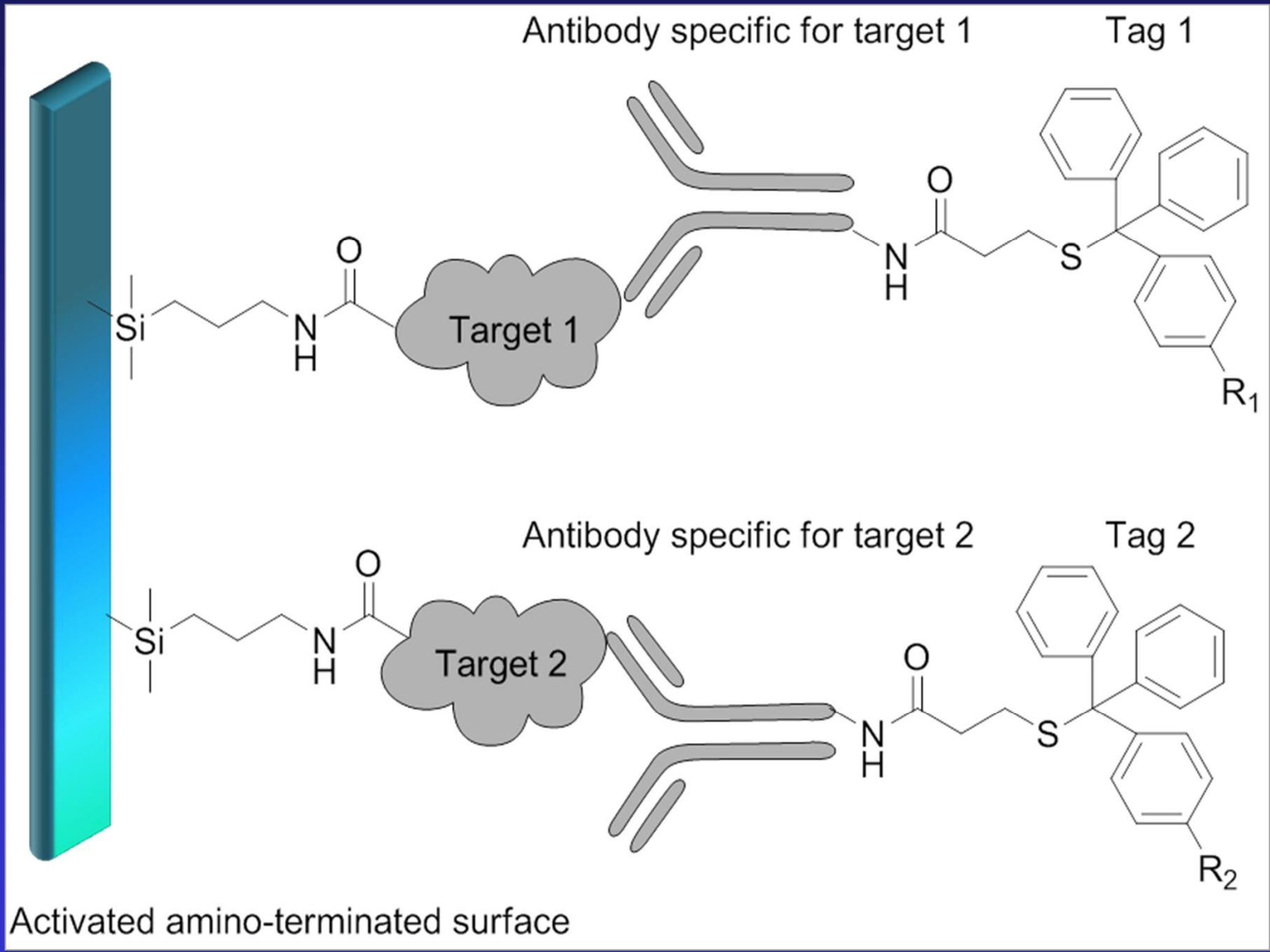
Imaging of regions immunoreactive with anti-synaptophysin Ab in healthy human pancreas.

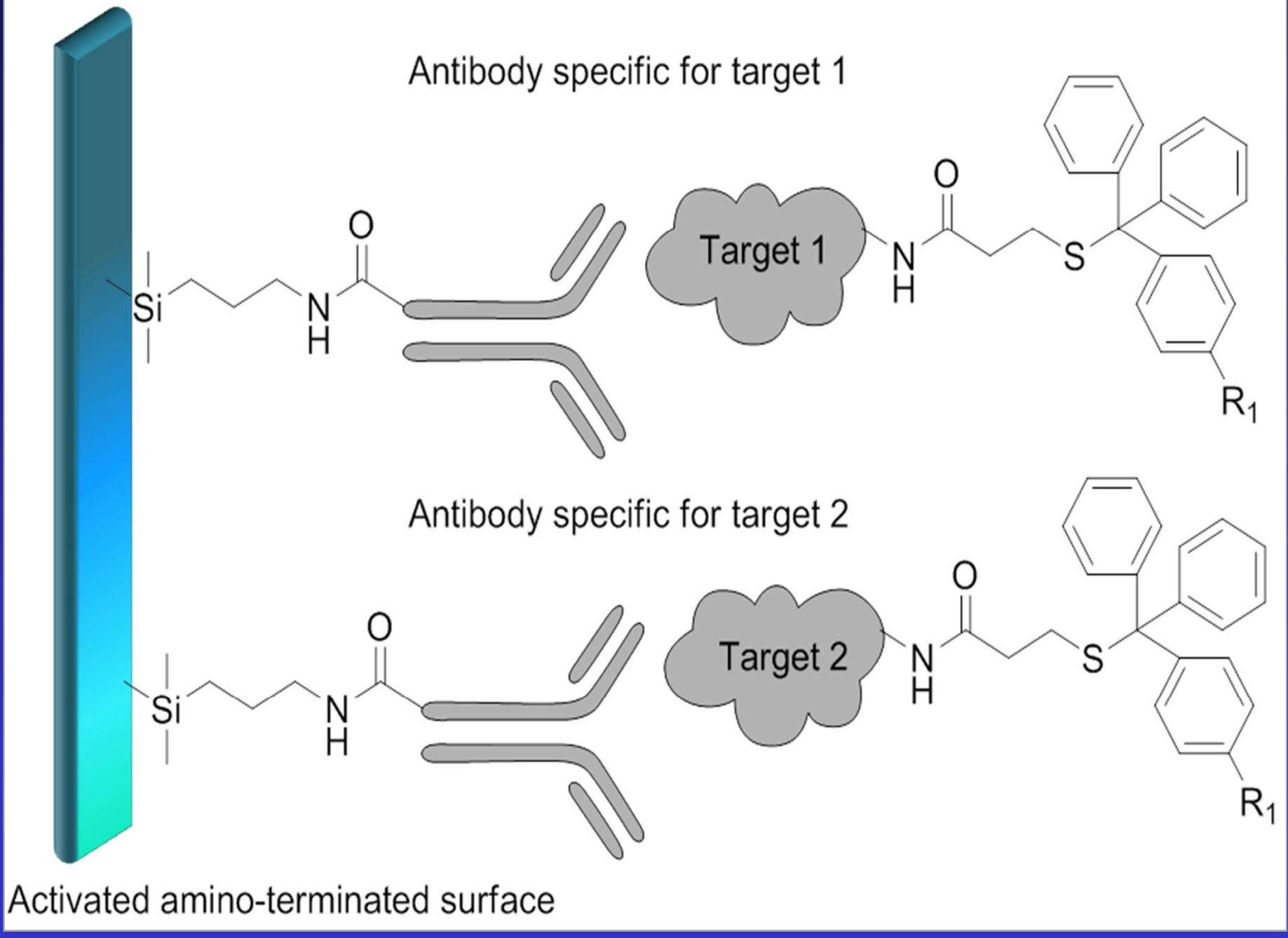
(A) Localization of synaptophysin positive cells by TAMSIM. The monoclonal rabbit anti-synaptophysin is conjugated with the tag EI 307 (498 *m/z*). The false color green points in the section show the presence of the tag EI 307 and thus synaptophysin positive cells.

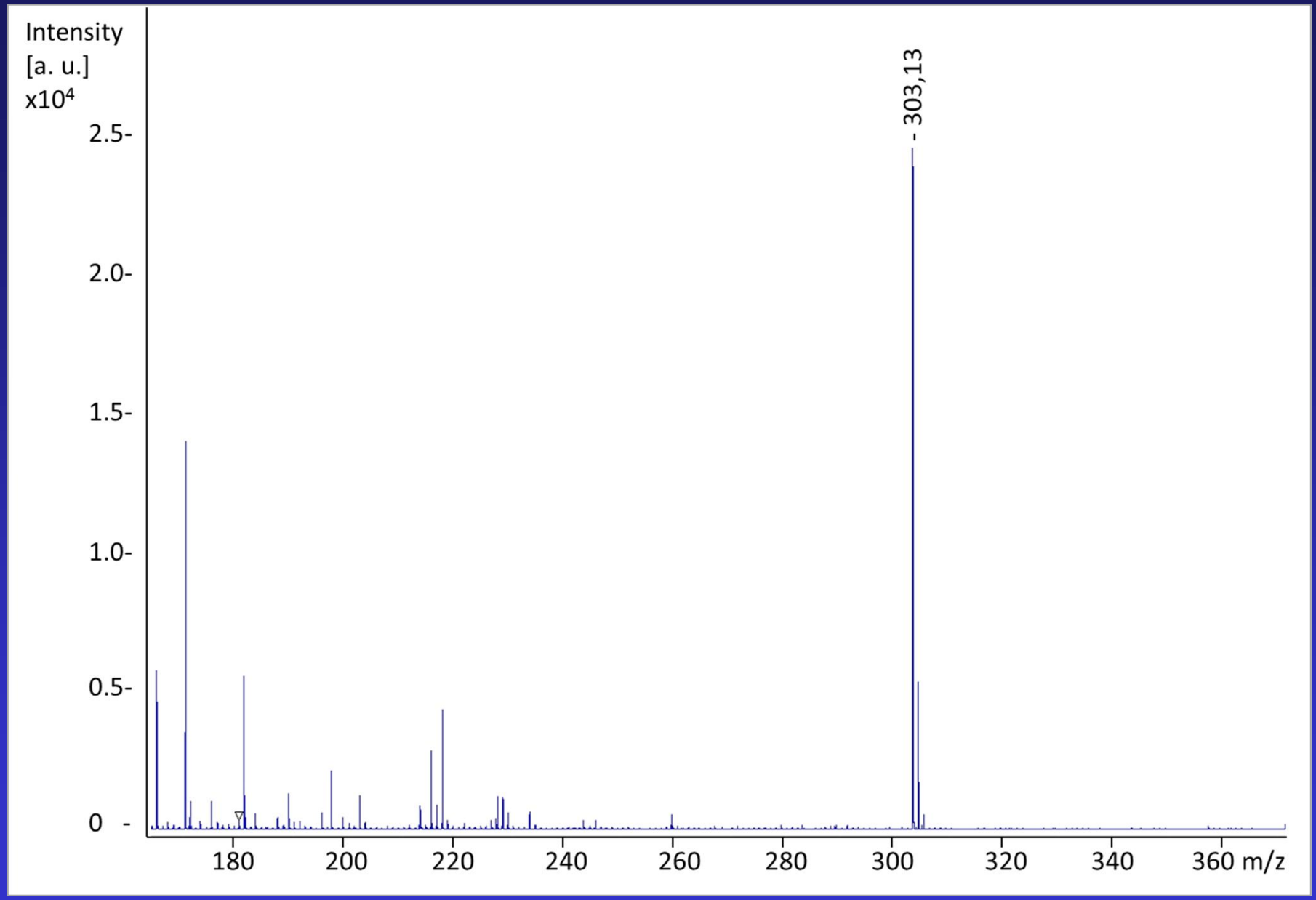
(B) Classical IHC image with the anti-insulin Ab. The dark pink spots correspond to Langerhans islets and so the synaptophysin-positive cells. The distribution of synaptophysin positive cells in (A) is very similar to that in (B).



Thiery, G., et al., Proteomics 2008

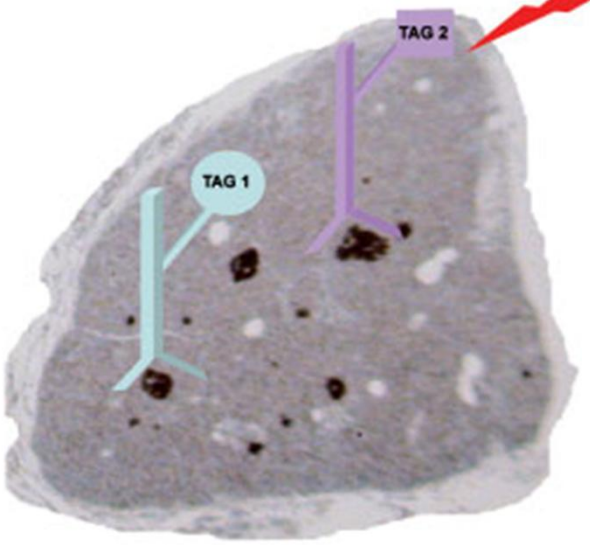

Antibody specific for target 1
Tag 1
Si
N
H
O
Target 1
O
N
H
S
R1
Antibody specific for target 2
Tag 2
Si
N
H
O
Target 2
O
N
H
S
R2
Activated amino-terminated surface

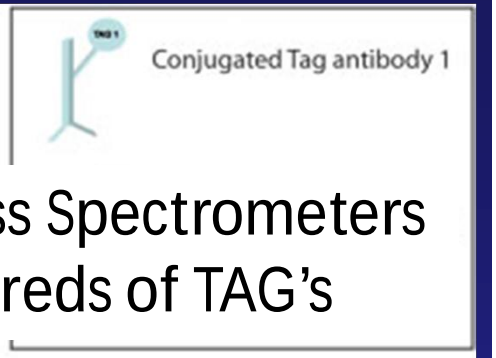

Antibody specific for target 1
Target 1
Antibody specific for target 2
Target 2
Activated amino-terminated surface


Intensity
[a. u.]
x10^4
2.5-
2.0-
1.5-
1.0-
0.5-
0 -
- 303,13
180
200
220
240
260
280
300
320
340
360 m/z

1 DIRECT IMMUNOHISTOCHEMISTRY

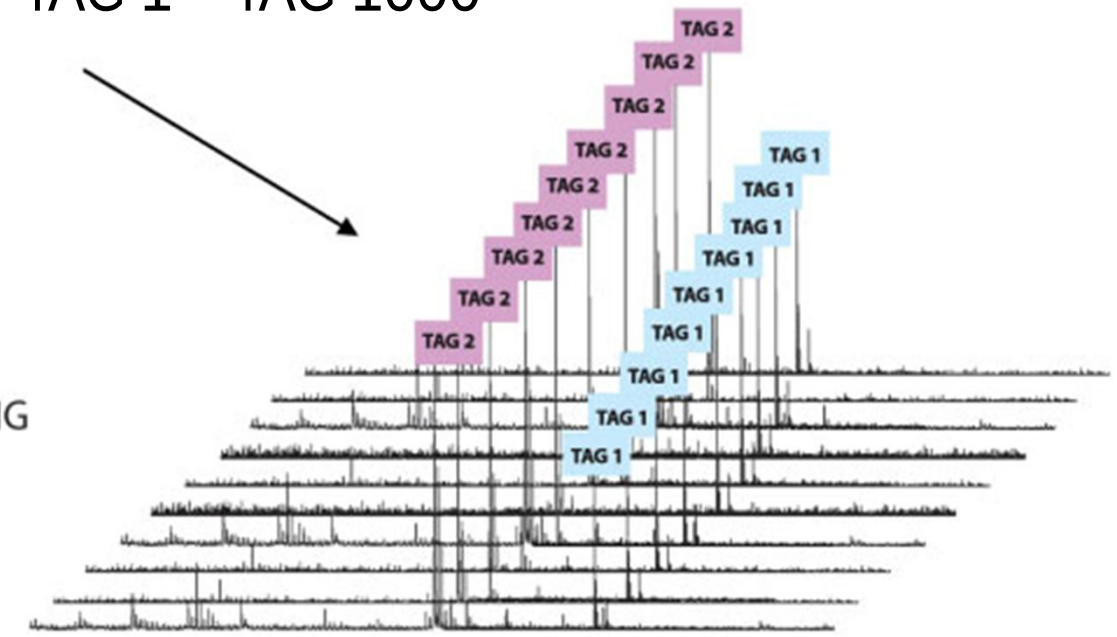
MALDI UV LASER



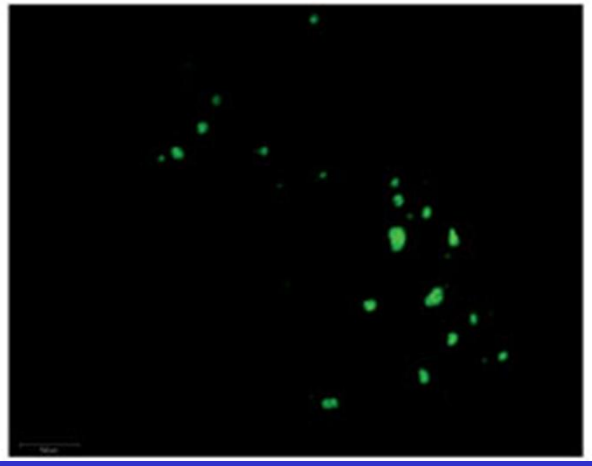


With High Resolution Mass Spectrometers we can have several hundreds of TAG's

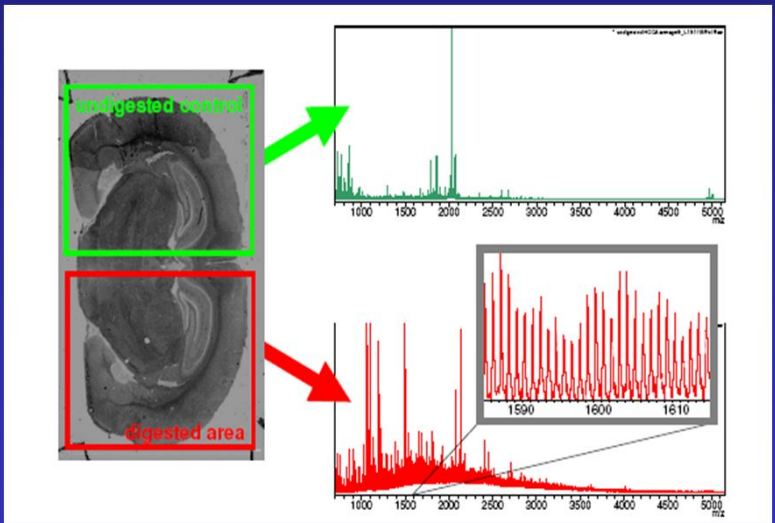
TAG 1 – TAG 1000



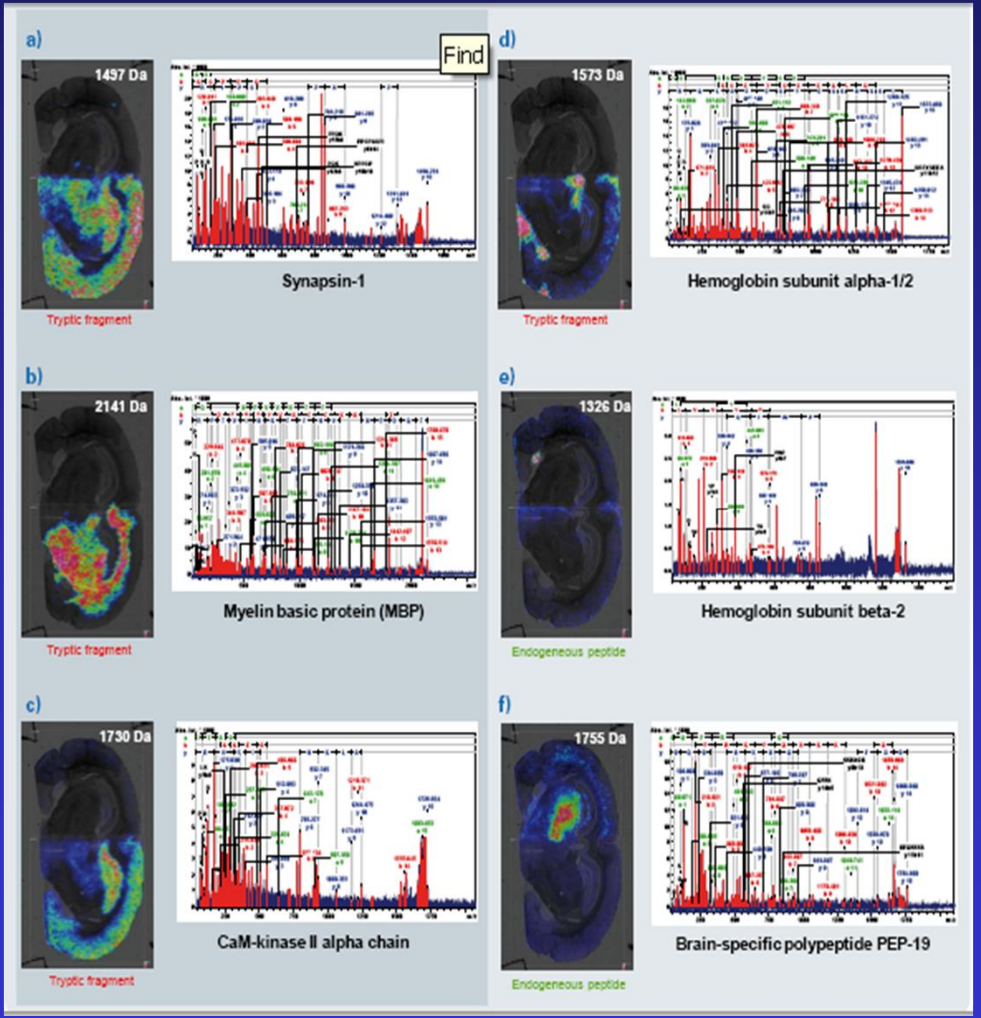
3 SPECIFIC MASS SPECTROMETRY IMAGING



MS/MS directly on tissues



Comparison of average sum spectra acquired from the digested vs. the undigested rat brain tissue area.



Spatial distribution and MS/MS spectra of selected peptides

www.bdal.com

FFPE archaised tissues

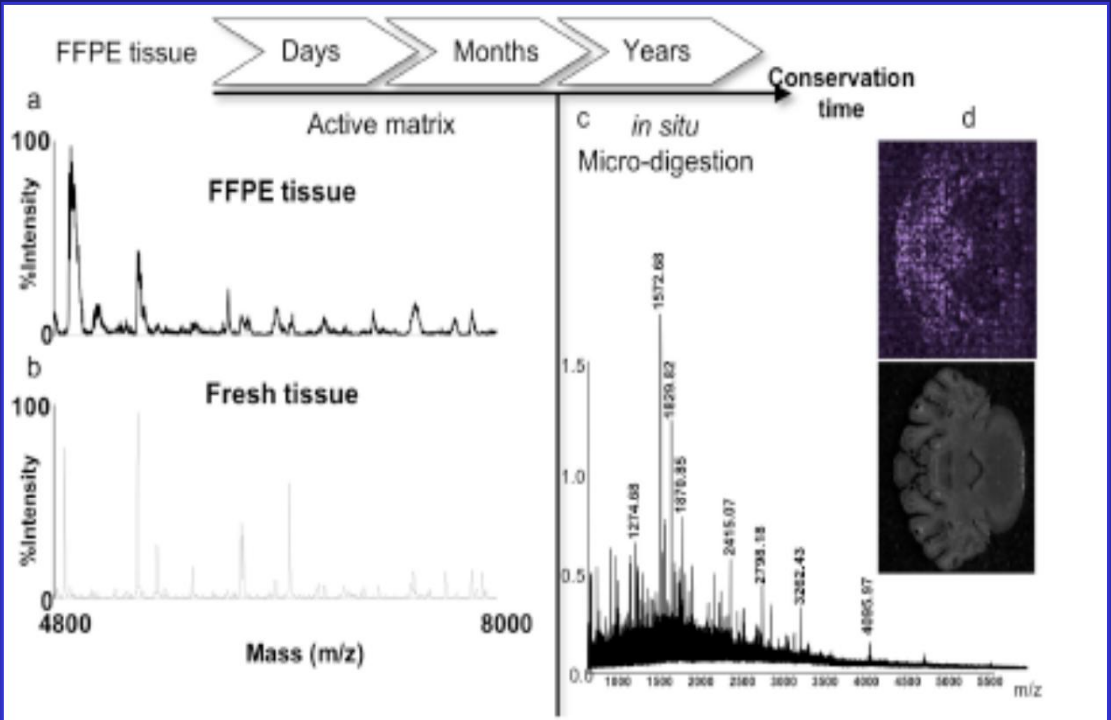


Fig. (4). Strategies used for FFPE tissue (a) stored less than 6 months, (b) stored more than 6 months.

a: Compared MALDI mass spectra in the linear positive mode of the direct analysis of a <1year old FFPE and fresh frozen rat brain tissues recorded in the same region With sinapinic acid as matrix.

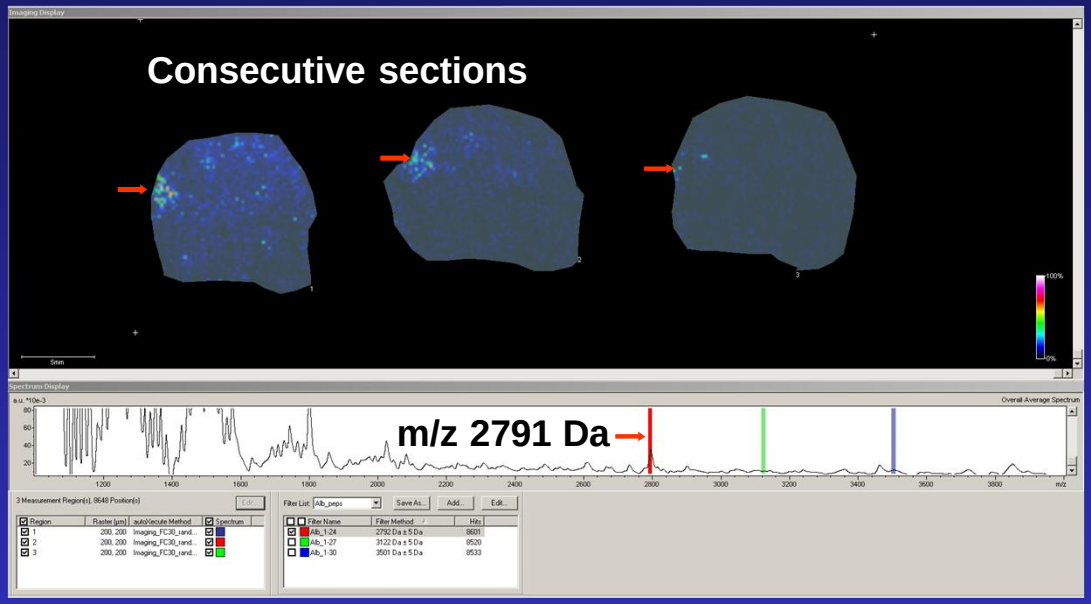
b: MALDI mass spectrum in the linear positive mode of the direct analysis of a <1year old FFPE tissue using 2,4-DNPH as matrix. Zooming compared this spectrum to the one recorded in the same conditions and in the same region of the rat brain of a fresh frozen tissue.

c: MALDI mass spectrum in the linear positive mode of the direct analysis of a 2 years old FFPE tissue section after *in situ* trypsin digestion of the whole tissue section (15 min).

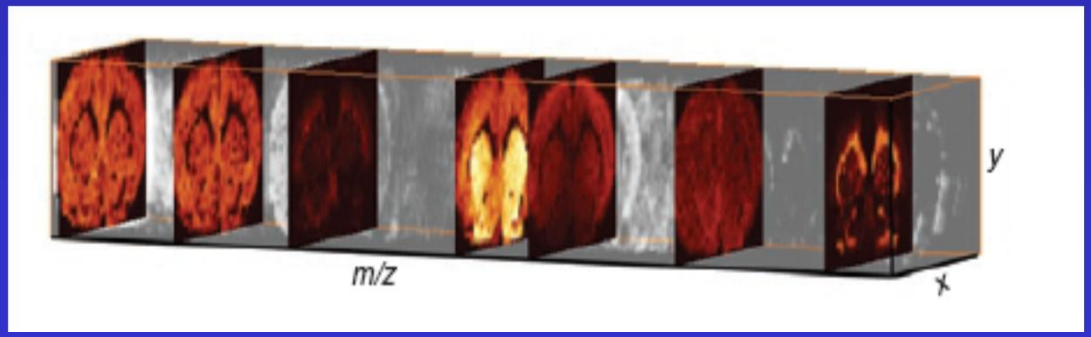
d: MALDI molecular images reconstructed from the data recorded on the 2 years old FFPE rat brain tissue section after micro-spotted *in situ* trypsin digestion followed by extraction and performed on MALDI-TOF-TOF/TOF using HCCA as matrix and compared to rat brain picture and morphology.

MALDI tissue profiling was combined with *in situ* tissue enzymatic digestion,
which appears to be mandatory for FFPE tissue analysis.
Wisztorski et al., 2007.

Towards 3D reconstruction

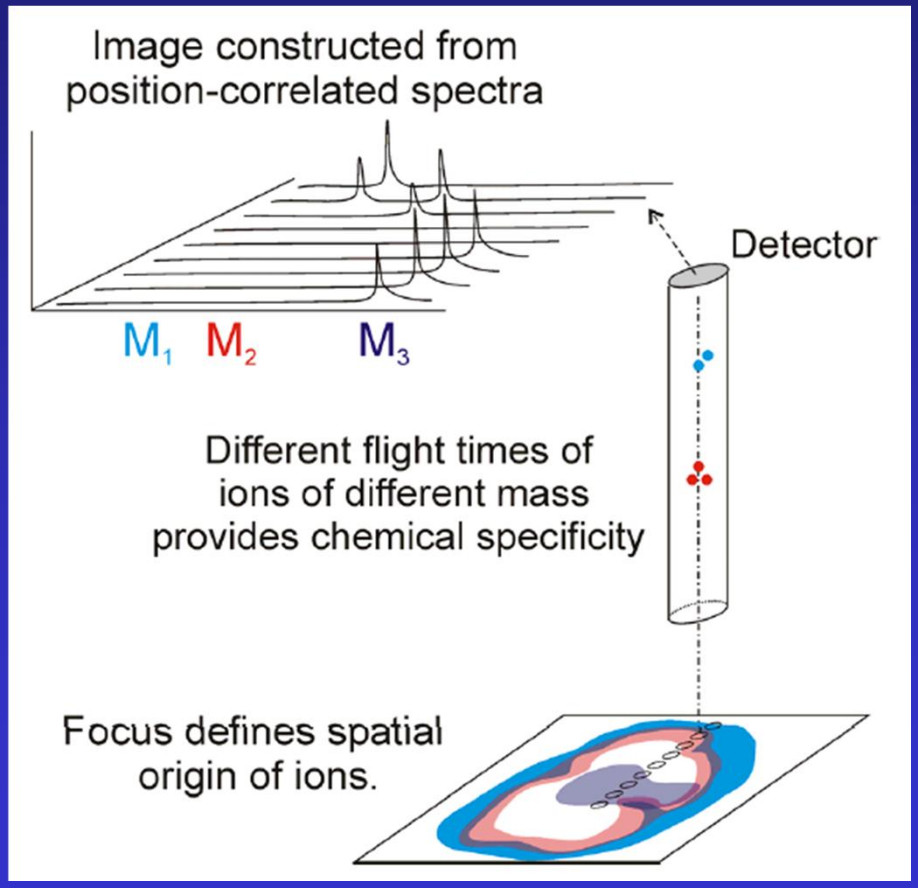


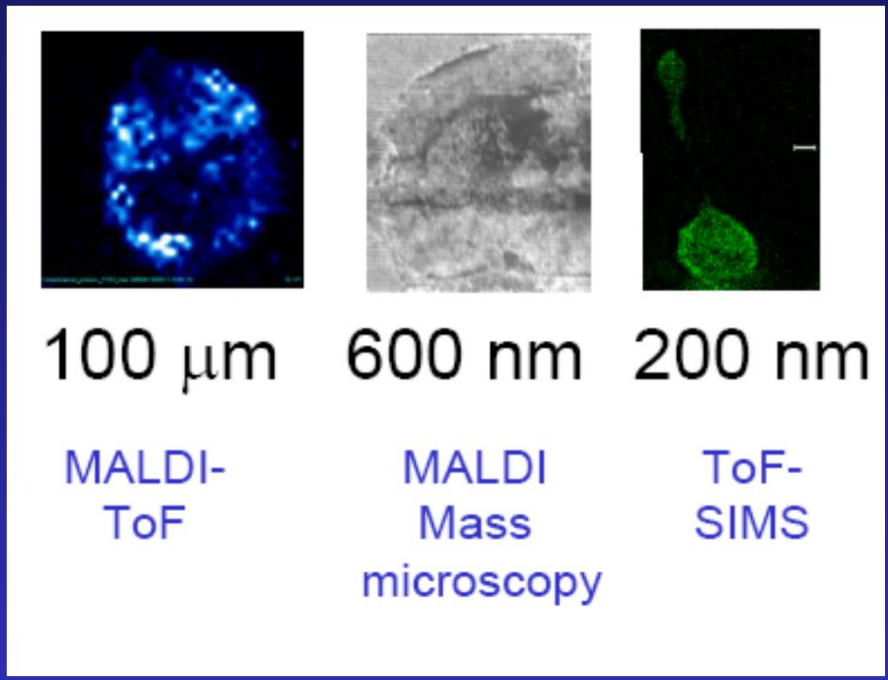
SA-coated slides were imaged within the same day by MALDI-TOF MS using an Autoflex III™ imager mass spectrometer. The signal can be seen in focal regions in several consecutive sections cut at 10µm.

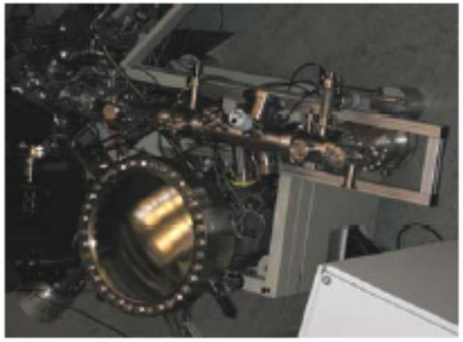


Three-dimensional volumetric plot of complete dataset with selected *m/z* 'slices' or ion images. Principal axes are *x*, *y* and *m/z*. Color of each voxel is determined by ion intensity.

Resolving power of an MS

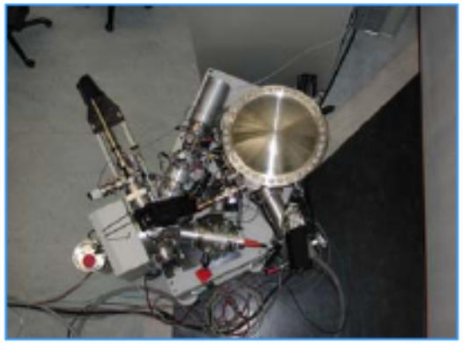




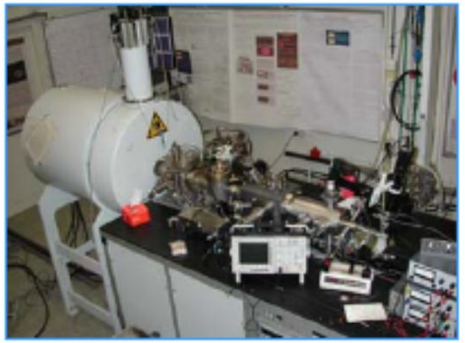


BioTRIFT
(MALDI + ESI-Beam)

ULTRAFLEX III
(MALDI ToF/ToF)

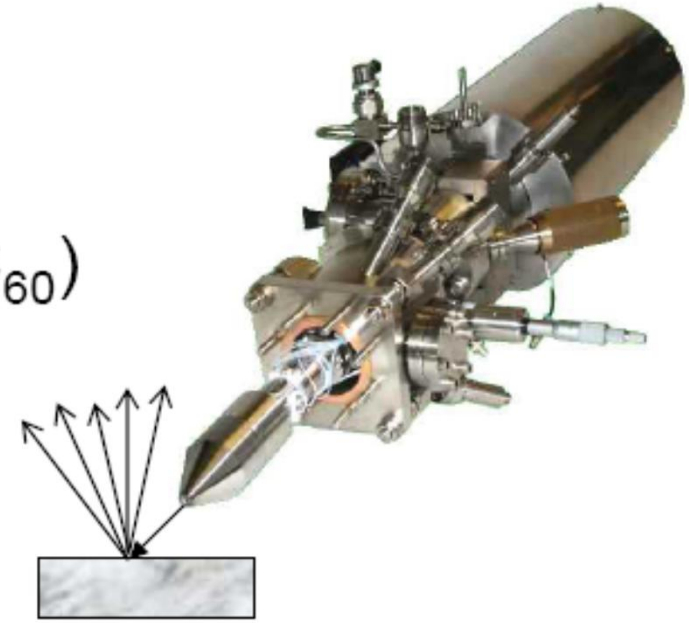


TRIFT-II



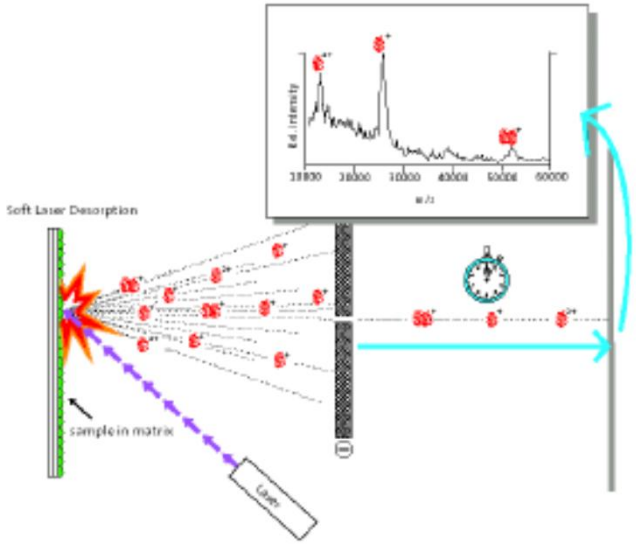
7T-FTMS
(DESI,
MALDI + MS/MS)

- Secondary Ion Mass Spectrometry (SIMS)
- Cluster SIMS (Au_n, Bi_n, C_{60})
- ME-SIMS / Meta-SIMS
- DESI



Charged Particles

- Laser desorption /ionization (LDI)
- Matrix Assisted LDI
  - UV-MALDI
  - IR-MALDI

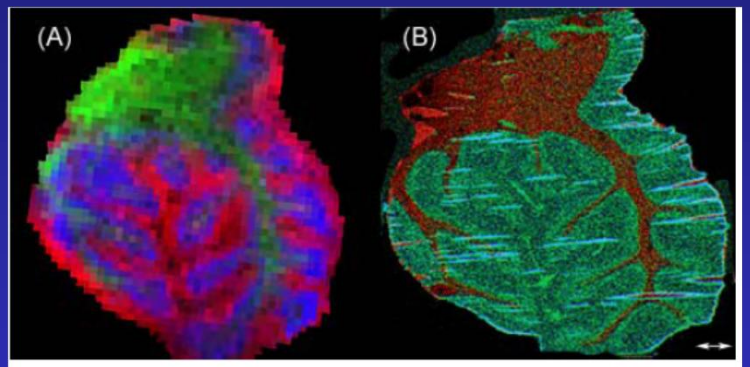


Photons

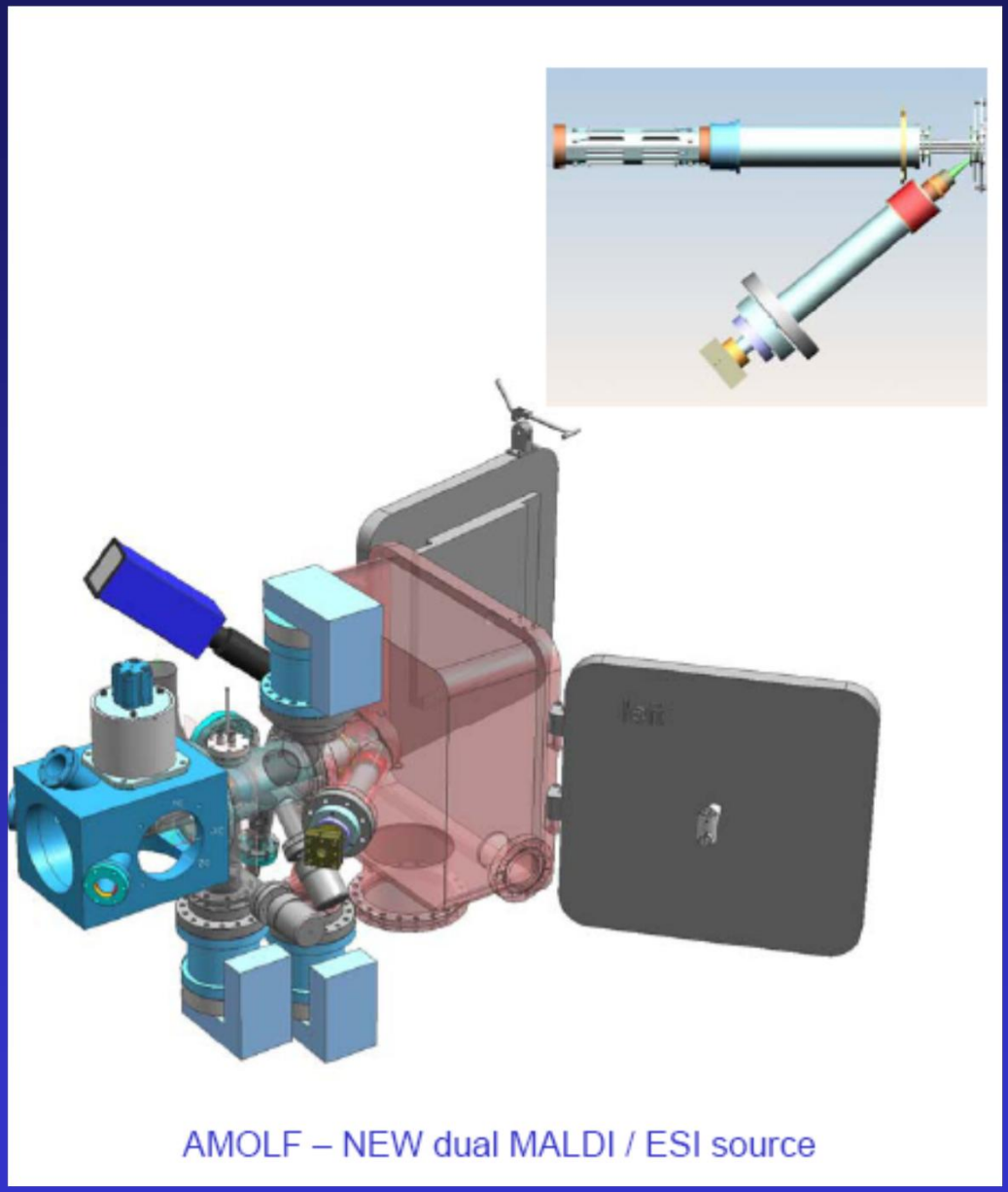
IMS FT-ICR-MS

(Fourier Transformation Ion Cyclotron Resonance MS)

Sensitivity in the zmol range

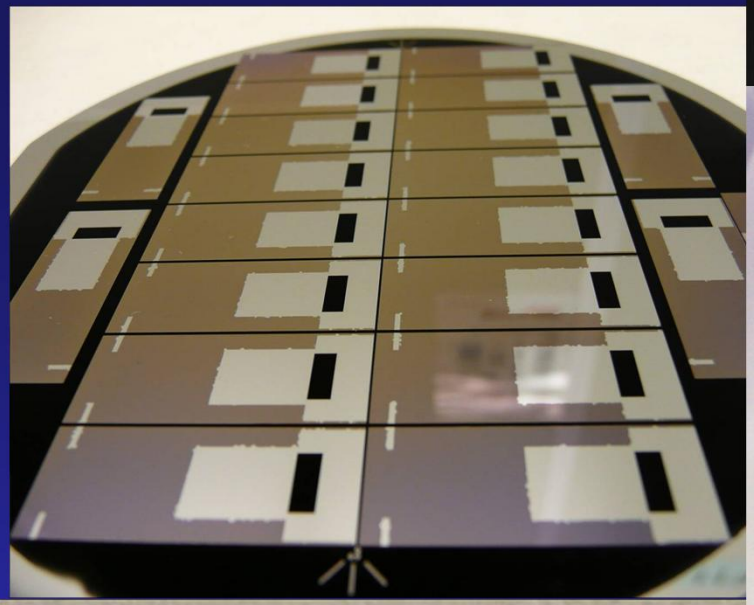


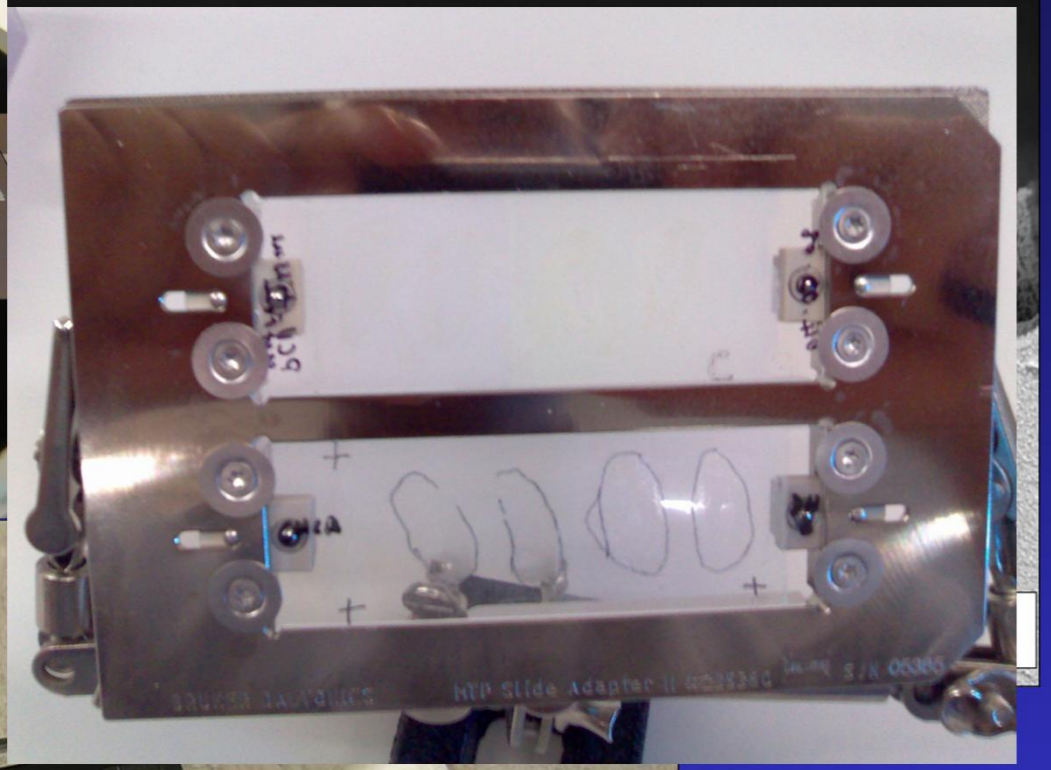
MALDI-ToFMS SIMS -ToFMS

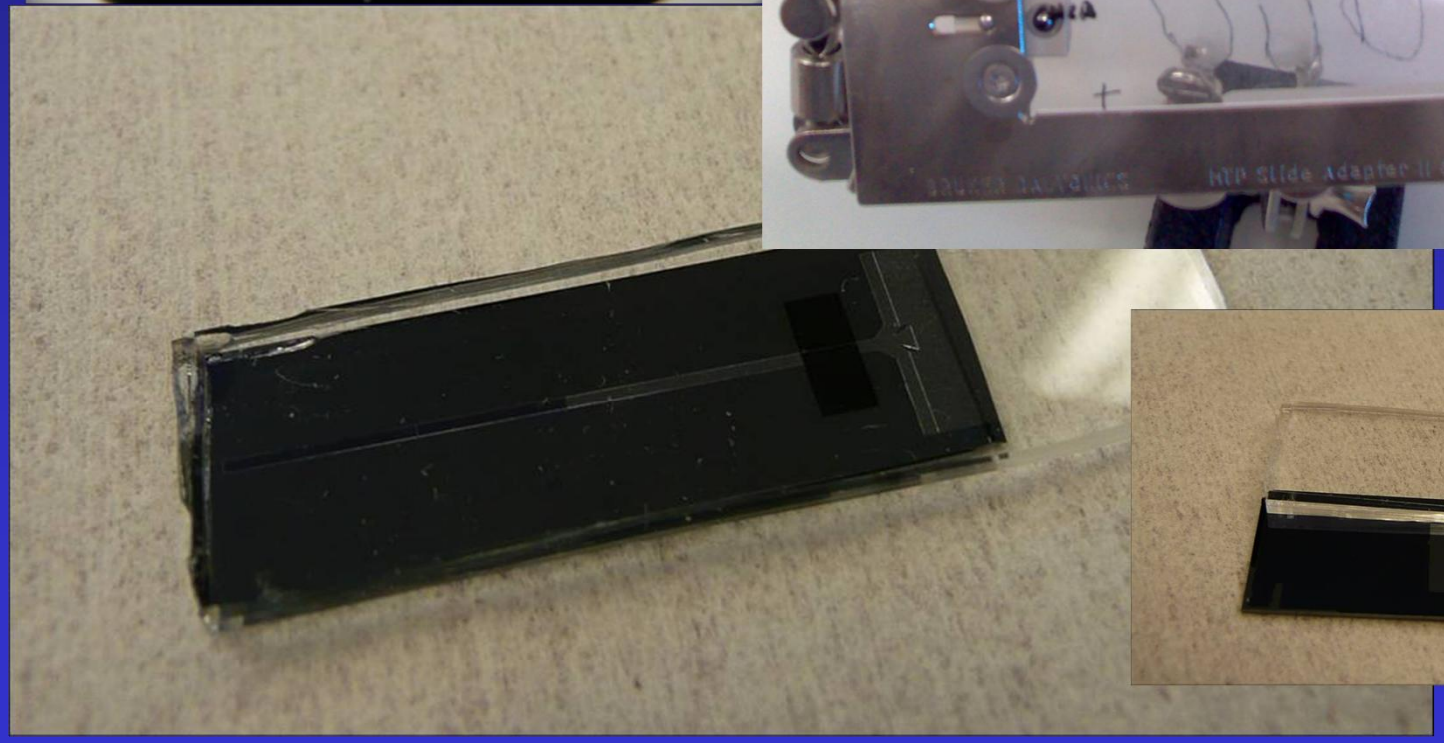


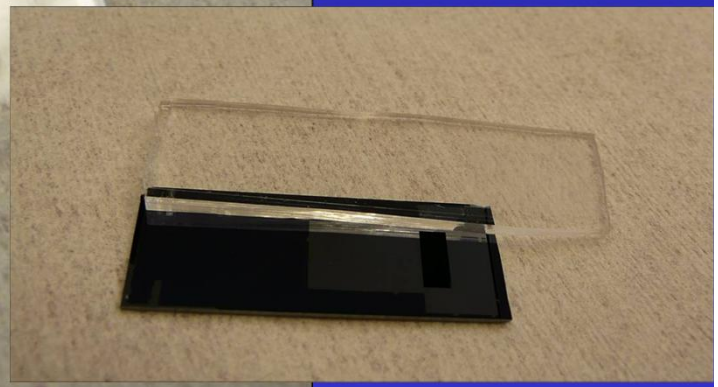
AMOLF – NEW dual MALDI / ESI source

Microchips on IMS




HTP Slide Adapter II

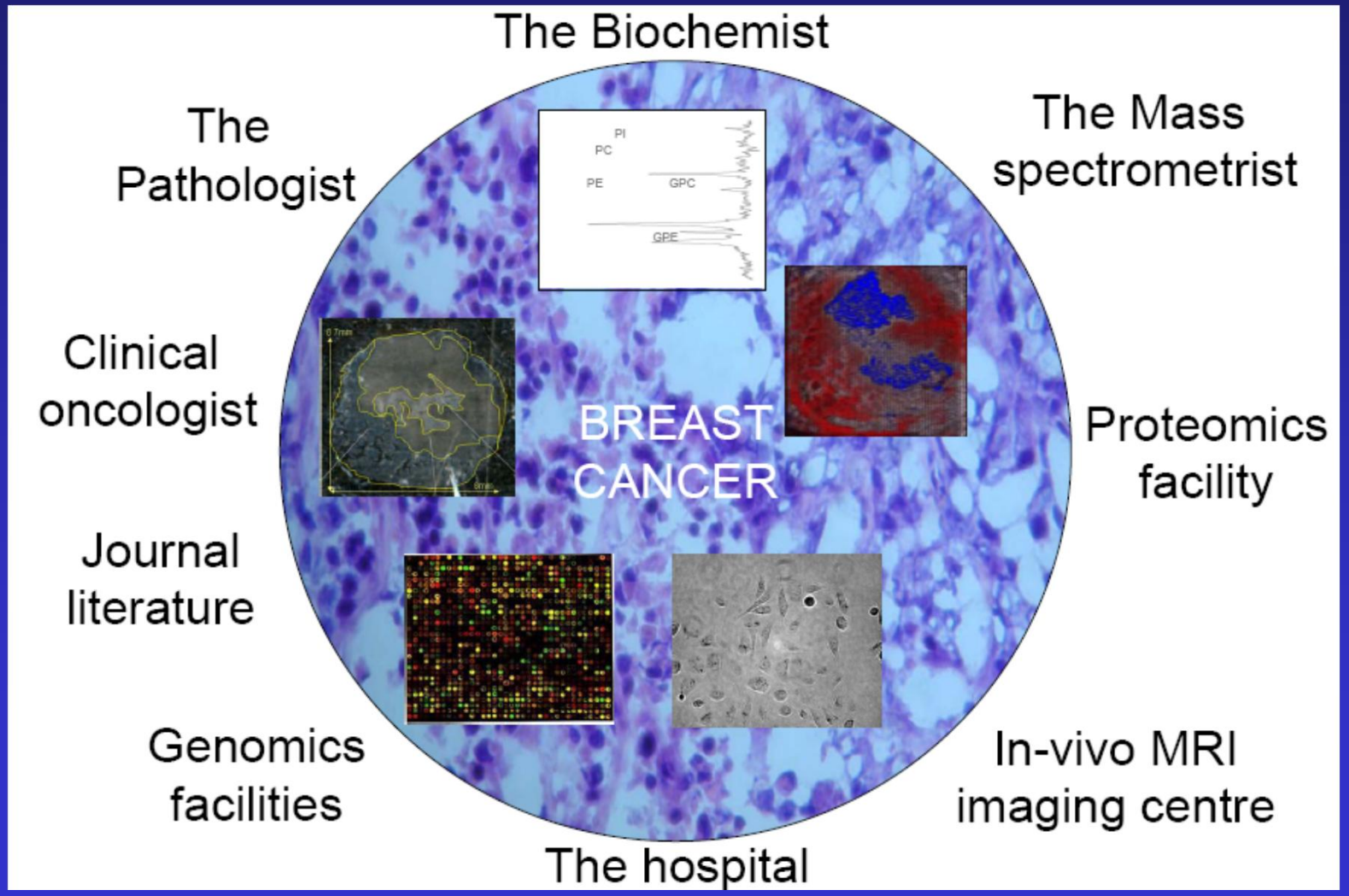




Limitations, so far …

- IMS Scans are best performing for <30 kDa m/z range
- Sample preparations challenges:
  - Tissue cryosections (mostly)
  - Autolysis possible
  - Tissue fixation
  - Suppression effect
  - Reproducibility is a concern
- Interpretation of complex spectra is not straight forward
  - Protein identifications challenges
  - Possible displacement or loss of small molecules after trypsinization for MS/MS
- Minor proteins enrichment is a handicap
- Requires other staining methods for comparison

IMS-MS combined with other technologies



main funding:

BIOMEDICUM HELSINKI

ACADEMY OF FINLAND

Juselius Foundation

EU FP7 DEMCHILD

COST actions:

EuroKUP

IMS Action

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Evanthia Monogioudi

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University of Uppsala, Sweden
Ola Philipson
Lars Lannfelt
Lars Nielsen

Mario Negri Institute for Pharmacological Research, Bergamo, Italy
Daniela Macconi
Ariela Benigni

University of Helsinki

GYÓGYÁSZATI SEGÉDESZKÖZÖK
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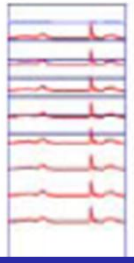
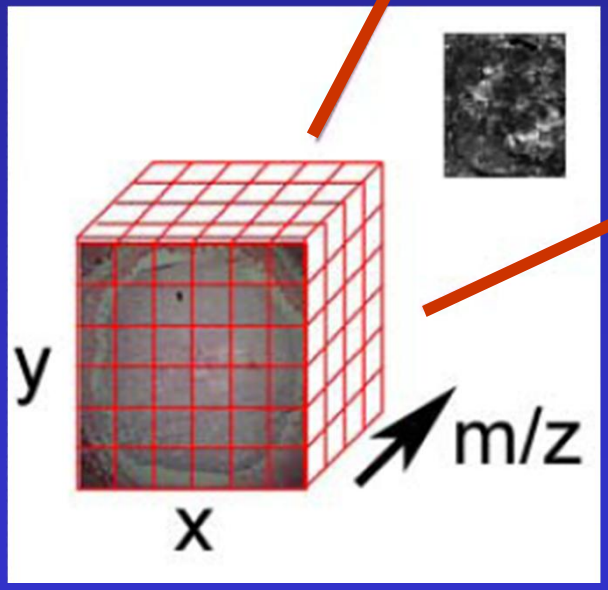
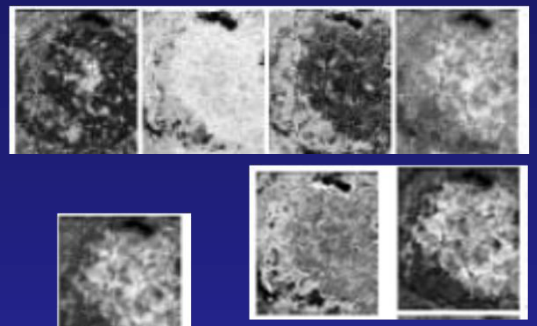
3D IMS-MS

a spectrum per pixel