Institute for Atomic and Molecular Physics

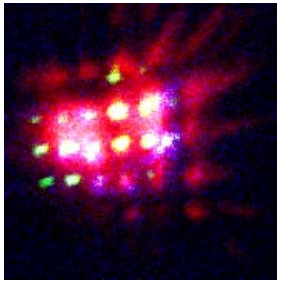
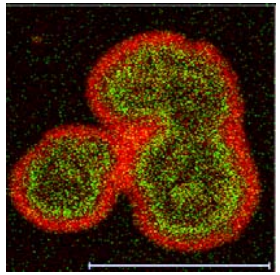
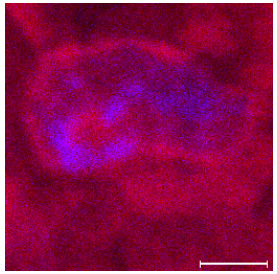
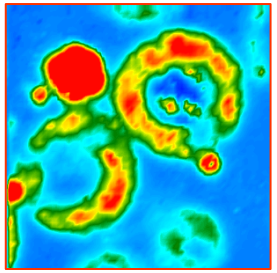
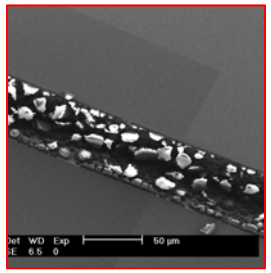
"New Horizons in High Resolution Imaging Mass Spectrometry: The need for speed.."

Or

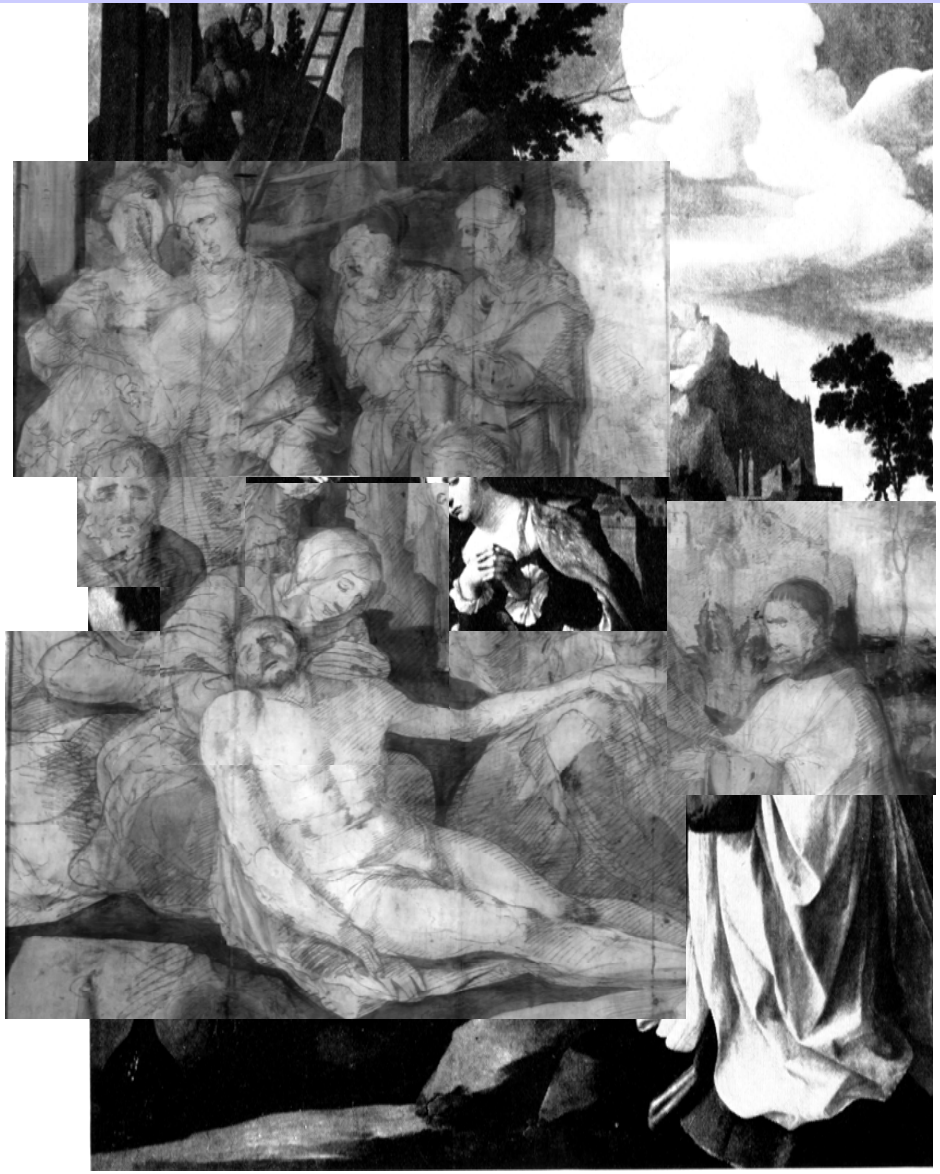
Novel FTICR-MS instrumentation

Ron M.A. Heeren

NHMFL, January 16th, 2008,
Tallahassee, FL, USA

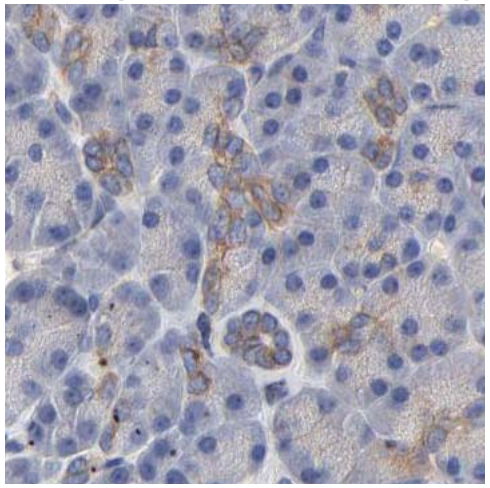
Imagery



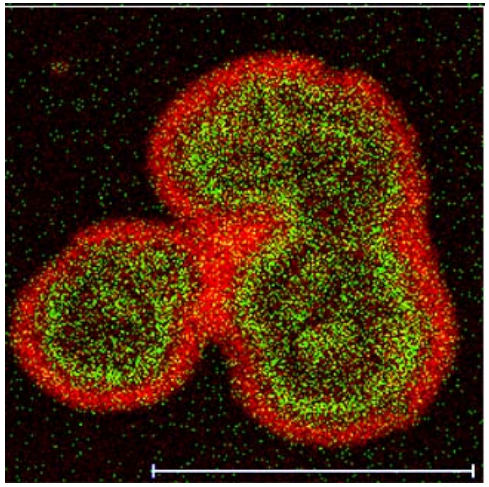
IRR investigation of “the Lamentation of Christ” by Jan van Scorel

Molecular imaging strategies……

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

Imaging MS applications……

Imaging drugs and metabolites

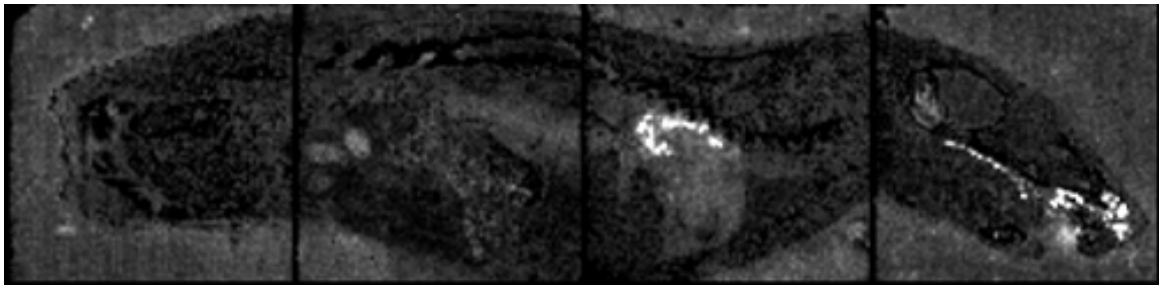
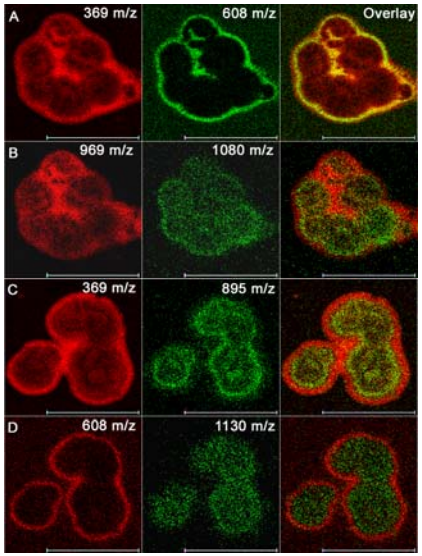
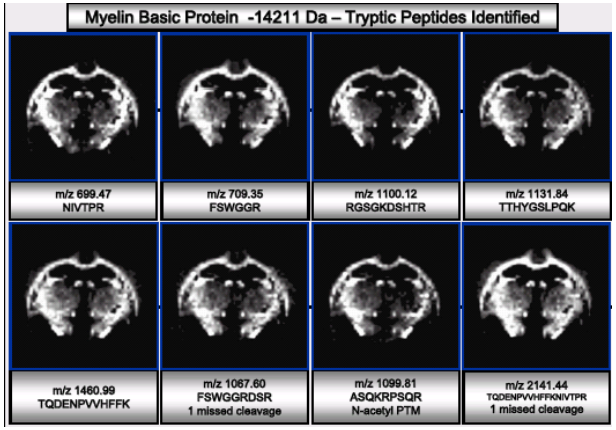


Image courtesy Markus Stoeckli

Imaging Lipidomics



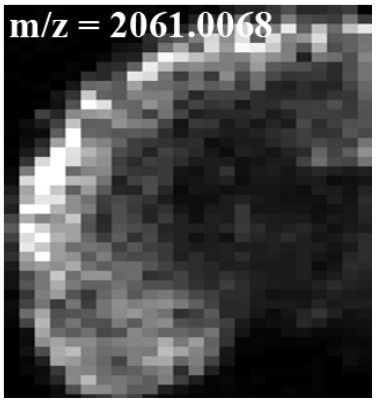
Imaging Proteomics

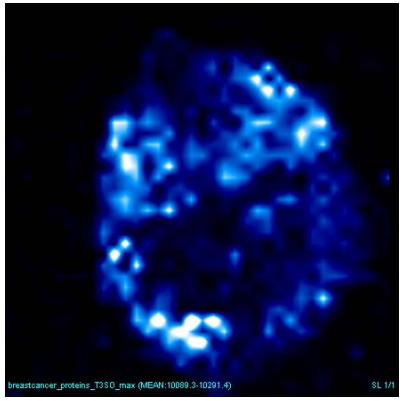


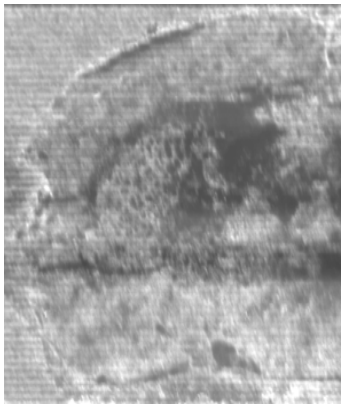
M. Reid Groseclose, Malin Andersson, Richard M. Caprioli ASMS 2006, poster presentation

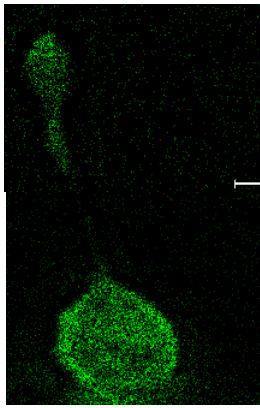
Resolving power in imaging Mass Spectrometry

Increasing Spatial detail







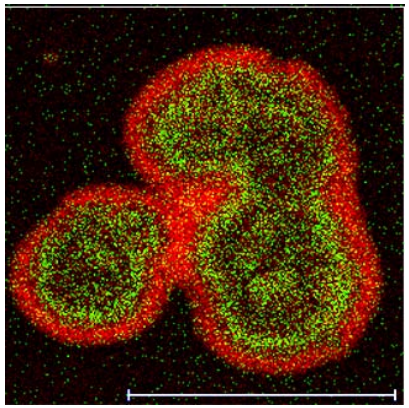


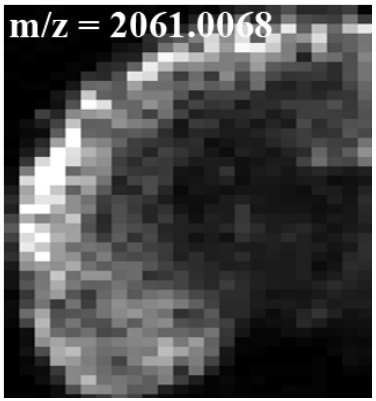
1 mm	200 μm	100 μm	600 nm	200 nm
DESI-FTMS	MALDI-FTMS	MALDI-ToF	MALDI Mass microscopy	ToF-SIMS

Increasing spectral detail

New research possibilities when…

Combined high quality spatial and spectral detail



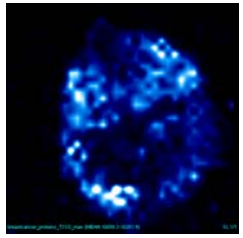


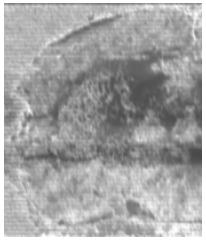
300 nm | <100 μm

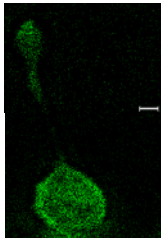
SIMS-FTMS | MALDI-FTMS

Highest spectral detail

Highest spatial detail







100 μm | 600 nm | 200 nm

MALDI-ToF | MALDI Mass microscopy | ToF-SIMS

Image mode I : Microprobe MS Imaging

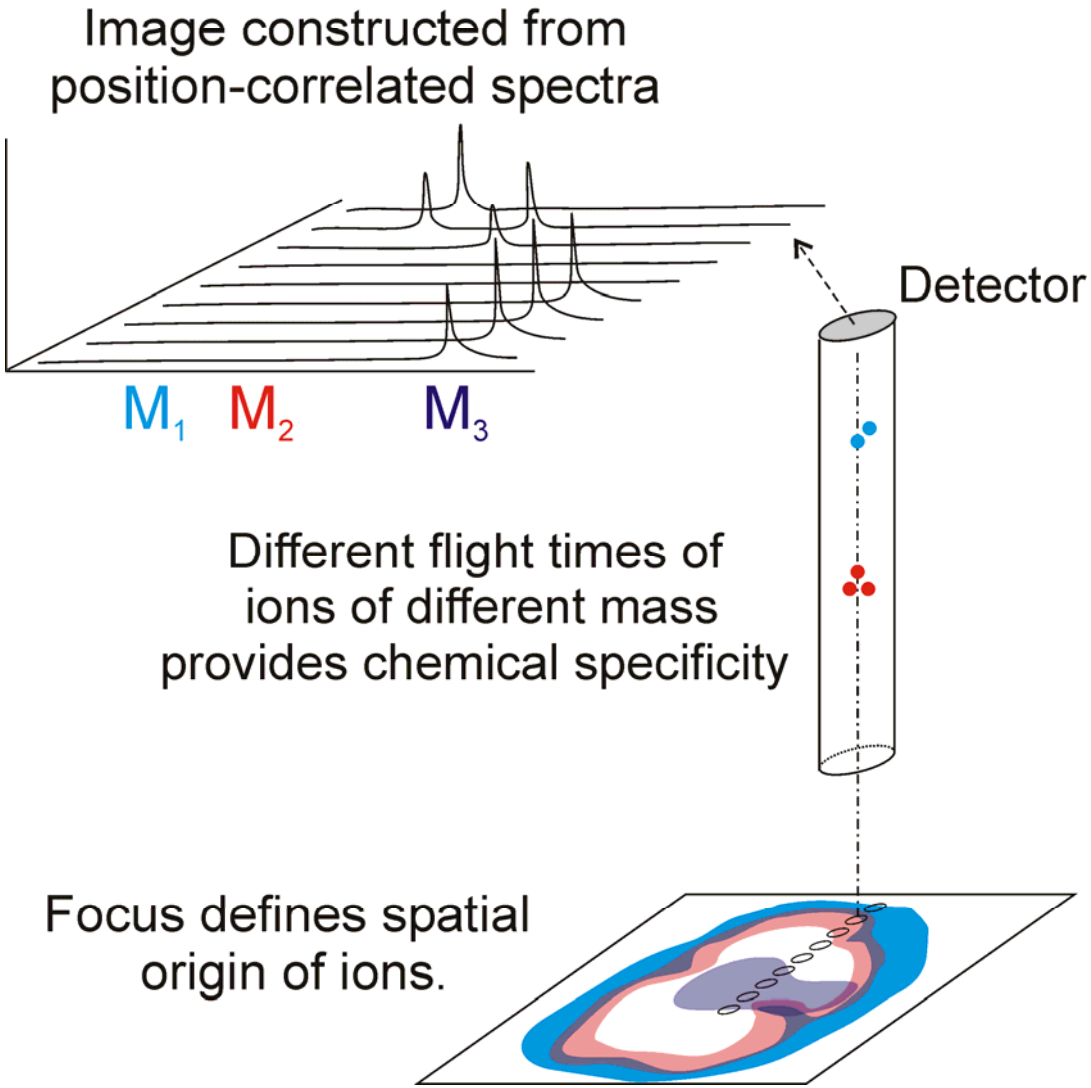
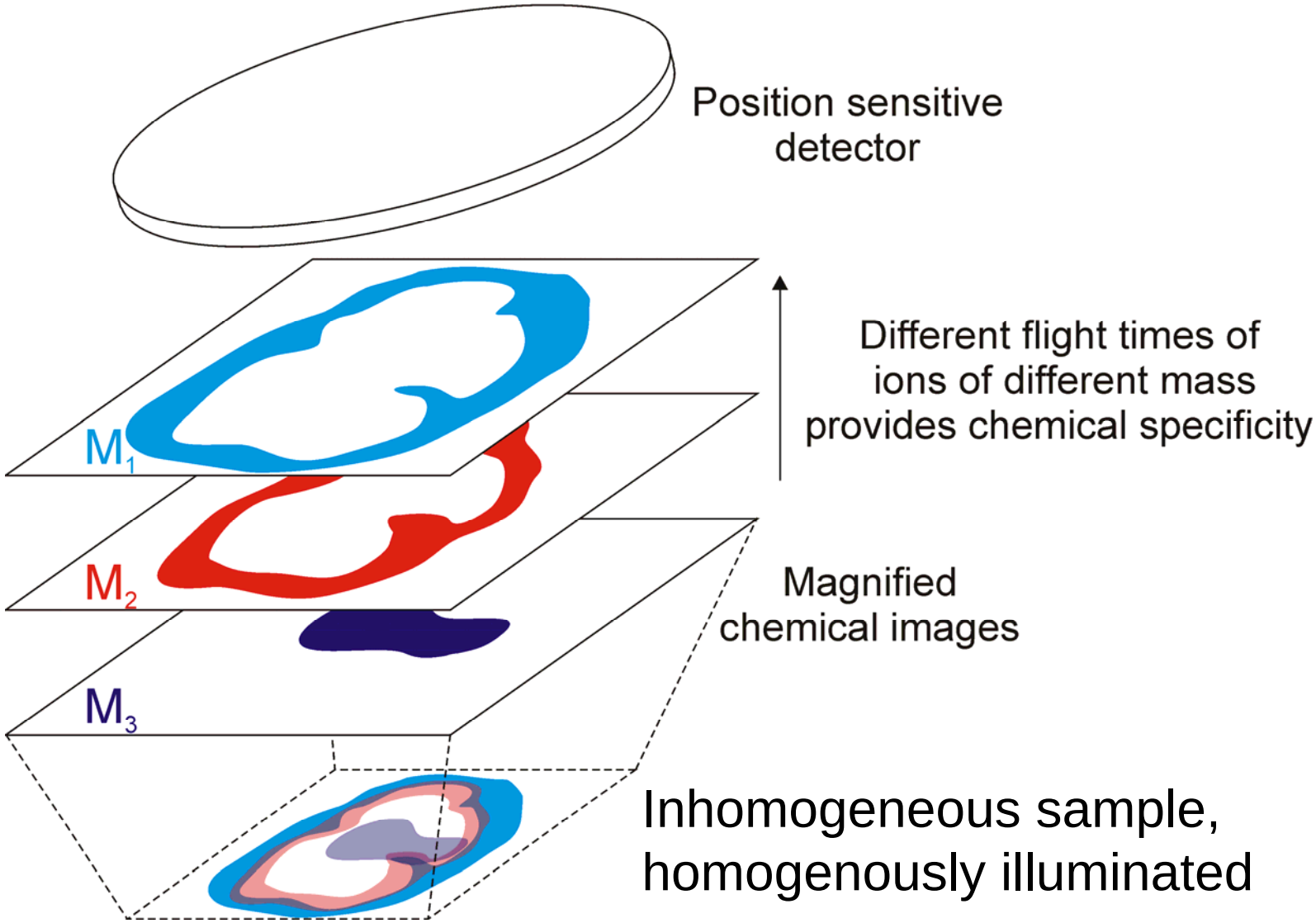


Image mode II: Microscope (Stigmatic) MS Imaging



Inhomogeneous sample, homogenously illuminated

Microprobe vs. microscope MS

Microprobe analysis

- Resolution governed by probe size
- selected spot is analyzed
- Only focussed analytical beams
- Image reconstructed after completion of scans, raster or sample positioning
- “Slow”

Microscope analysis

- Resolution governed by optics and detector
- Entire surface is irradiated (global analysis)
- Focussed and Unfocussed analytical beams
- Direct image acquisition
- “Fast”

The Need for Speed….

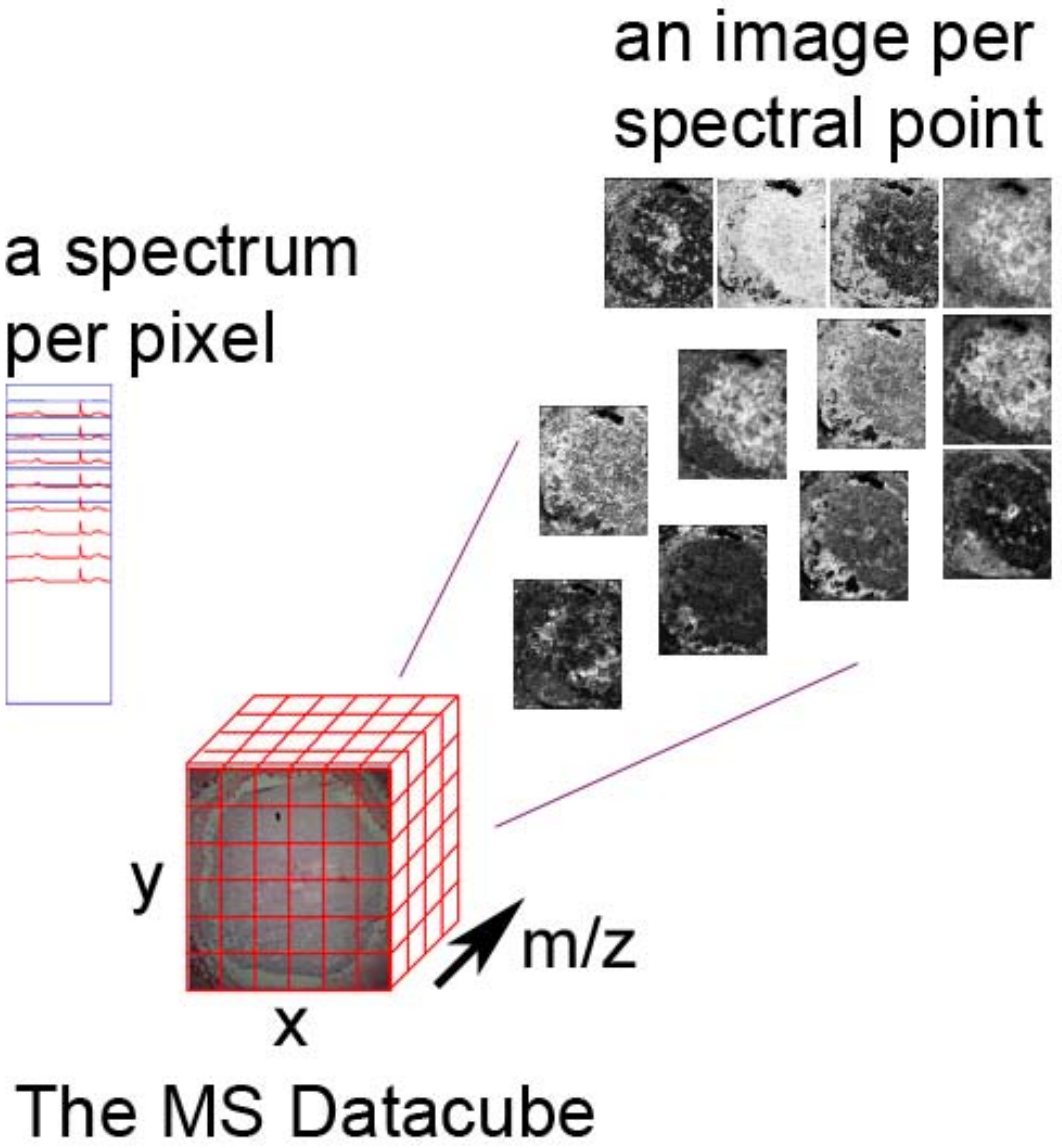
- Increase speed reduces total experiment time (Larger FOV)
- Increased speed can improve spatial resolution
- Mass resolution interchangeable for spatial resolution in FTICR-MS

To image a 1x1 mm area

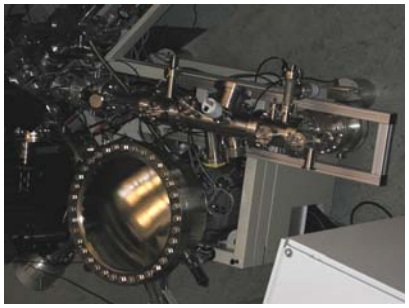
Microprobe mode imaging			
Pixel size	No. of pixels	Experimental Resolution	Time @ 1 Hz
100 μm	100	> 100 μm	< 2 min
25 μm	1600	> 25 μm	< 27 min
5 μm	40000	> 5 μm	< 11.5 hrs
2 μm	250000	4 μm	< 3 days

10 X speed increase from 9.4-14.5 T
~100X from 9.4-21 T ?

MS hyperspectral image datacube……



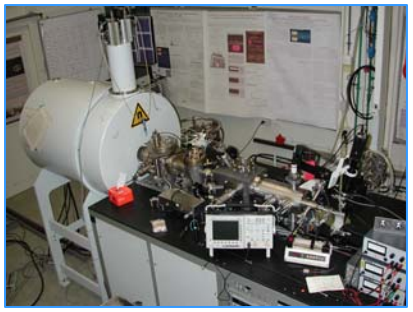
Imaging Mass Spectrometry at FOM-AMOLF



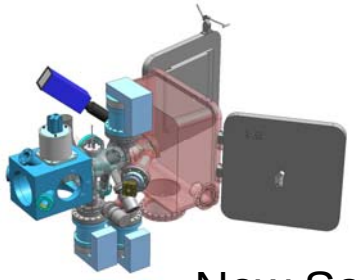
BioTRIFT
(MALDI + ESI-Beam)

ULTRAFLEX III
(MALDI ToF/ToF)

TRIFT-II
(In beam + DLD
+ Au Upgrade soon)



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



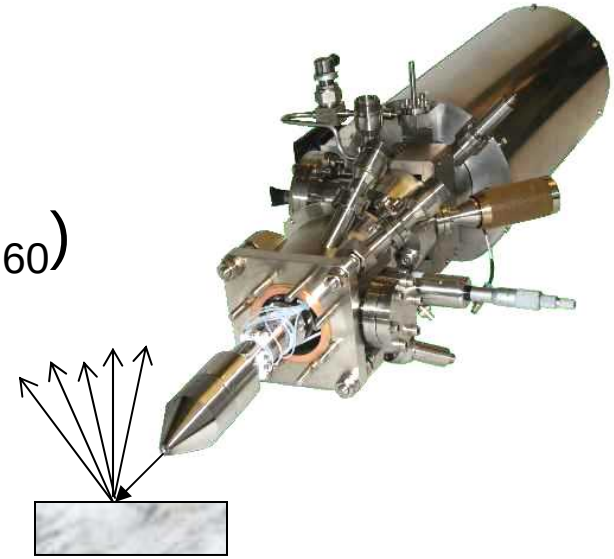
New Source
under
construction

Imaging MS considerations….

- Ionisation technique
  - ✓ Elements or molecules
  - ✓ Type of molecules
- Spatial Resolution and FOV
  - ✓ Organism level
  - ✓ Tissue level
  - ✓ Cellular level
- Type of mass analyzer
- Speed
- Sensitivity
- Sample preparation / modification
- In vacuo or Atmospheric

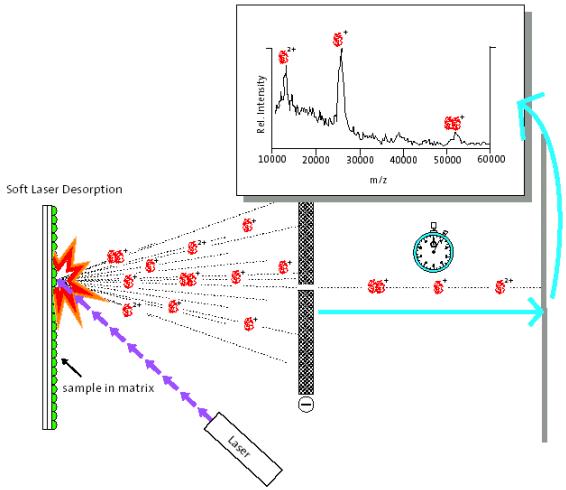
(Macro)molecular ionization at surfaces

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



Charged Particles

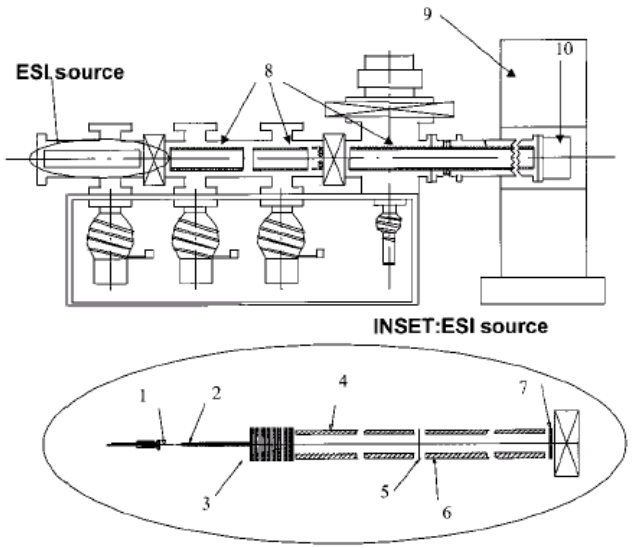
- Laser desorption /ionization (LDI)
- Matrix Assisted LDI
  - UV-MALDI
  - IR-MALDI



Photons

Imaging FTICR-MS Challenges: Sensitivity

Belov et al. *Anal. Chem.* **72**, 2271 (2000)

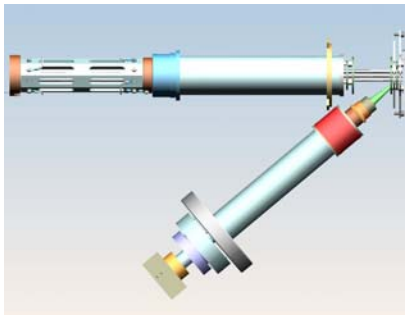


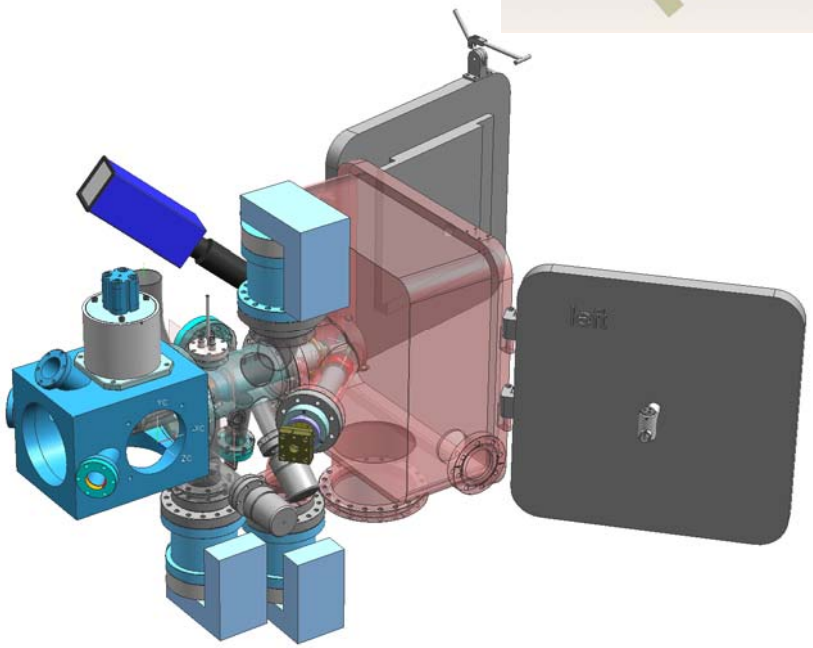
overall transmission efficiency of 7-10%

detection ≈ 30 zmol

amol sensitivity regularly achieved

zmol regularly achieved in leading labs

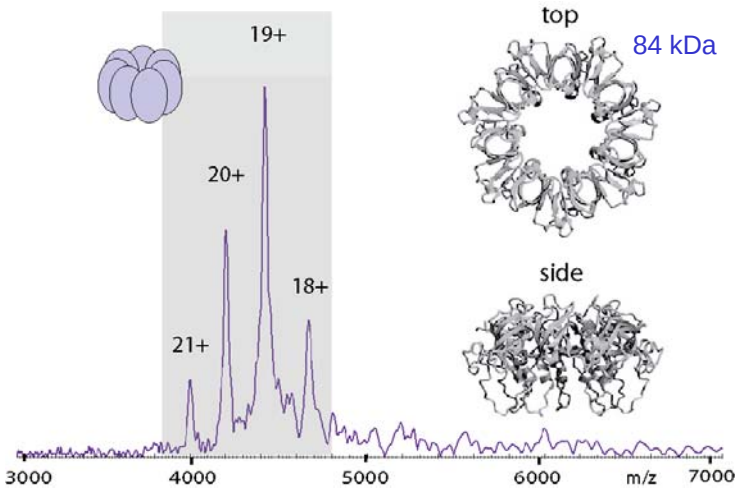




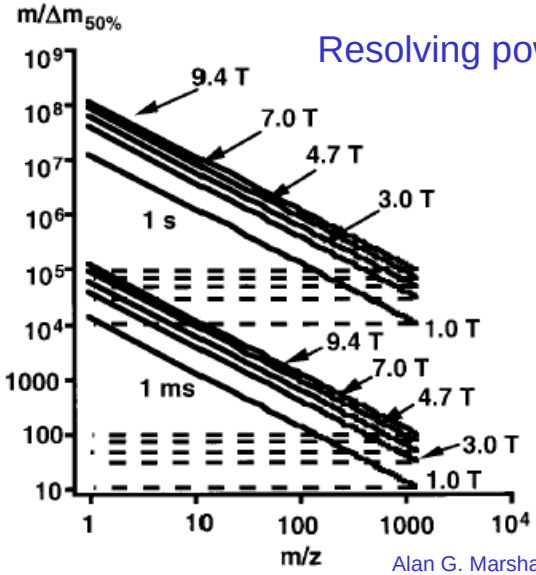
AMOLF – NEW dual MALDI / ESI source

Imaging FTICR-MS Challenges: Mass range

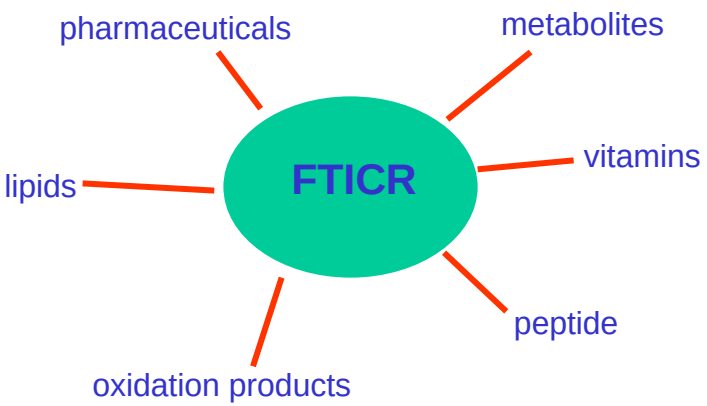
GP31 heptamer



Geels *et al*, *Anal. Chem.* **78**, 7191 (2006)



Alan G. Marshall, *MSR* **17**, 1 (1998)

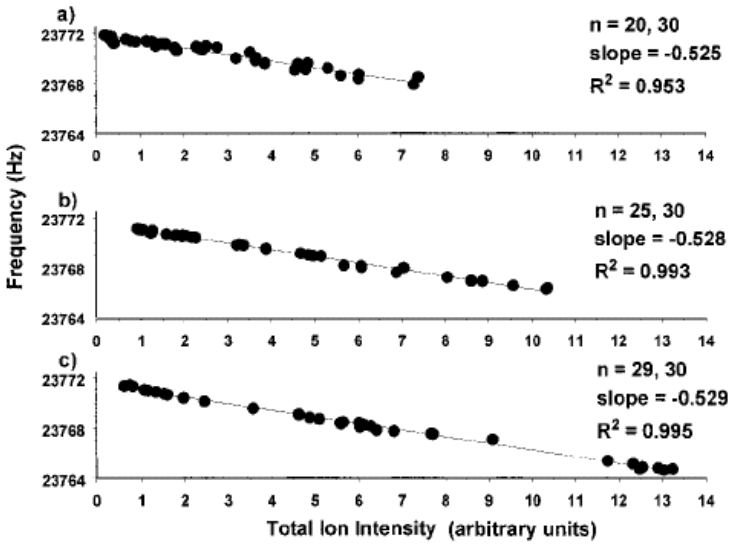


High magnetic fields

increase upper mass limit

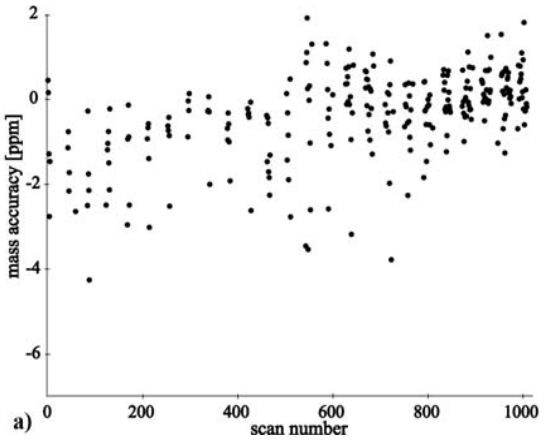
FTICRMS Challenges: Calibration + Accuracy

frequency dependent on space charge



Easterling *et al*, *Anal. Chem.* **71**, 624 (1999)

Correct calibration dependent on space charge (number of ions in ICR cell)



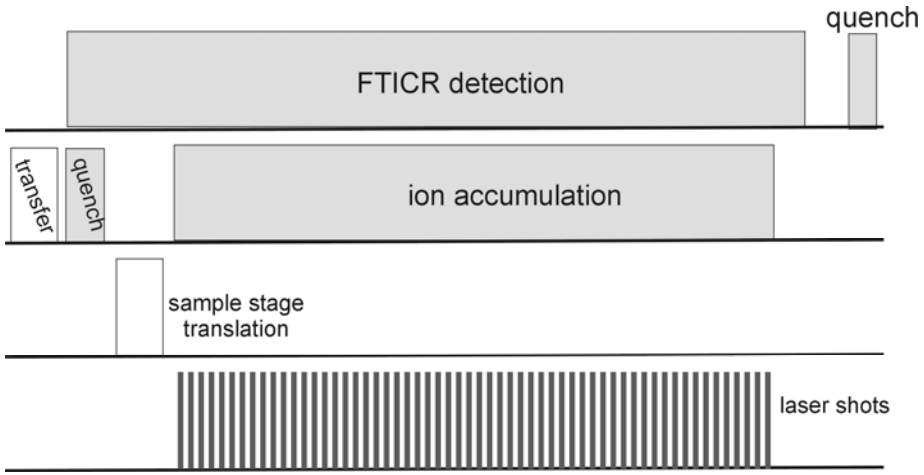
Taban *et al*, *JASMS.* **18**, 145 (2007)

High magnetic fields

larger ICR cells and new ICR cell designs reduce space charge effects

Review: Zhang *et al*. Mass Spectrom. Rev. **24**, 286 (2005)

Imaging FTICR-MS Challenges: Speed



FTICR-MS : 100 000 resolution @ *m/z* 400, scan rate 1 Hz @ 7T

100 000 resolution @ *m/z* 400, scan rate 3 Hz @ 21T

33 000 resolution @ *m/z* 400, scan rate 9 Hz @ 21T

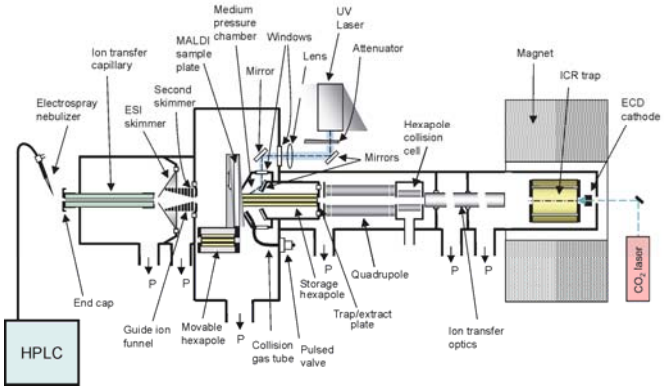
Increased magnetic field allows higher data acquisition rates

Trade-offs for imaging:

- Acquisition rate compromise with for mass resolution
- Practical spatial resolution determined by acquisition rate

MALDI FTICR-MS imaging

Identification of biomarkers in MS imaging requires accurate mass analysis and tandem mass spectrometry



Imaging:

- 200 x 300 micrometer spot
- Left hemisphere 42x24 positions
- Microprobe !
- Bruker 9.4T with combi-source
- Right hemisphere with mass microscope

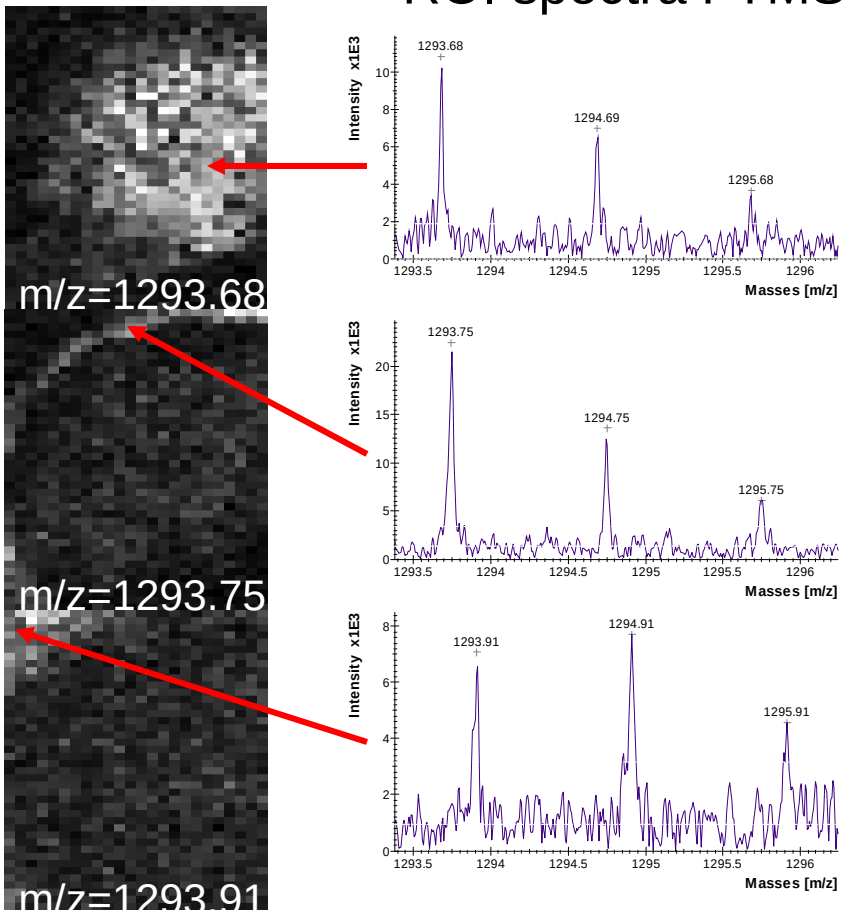
Tandem MS profiling:

- External CAD (In hex-trap)
- In –cell IRMPD

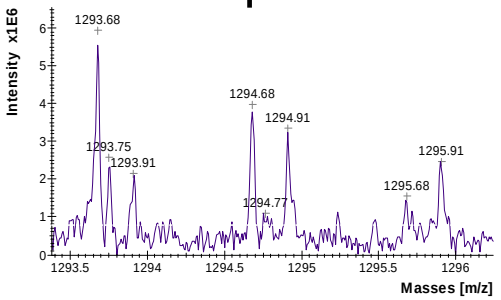
High resolution MALDI reveals different localization

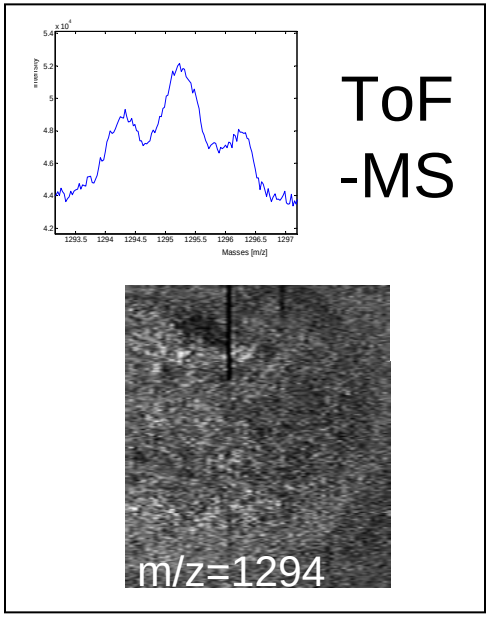
At 7 T 12 hour experiment!

ROI spectra FTMS

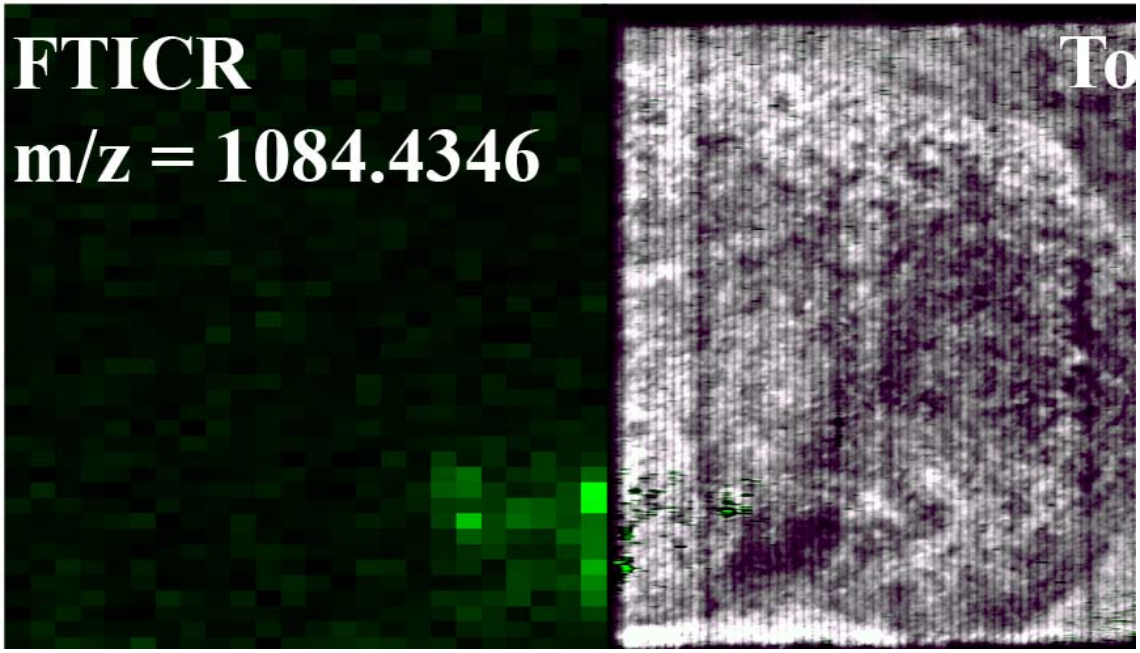


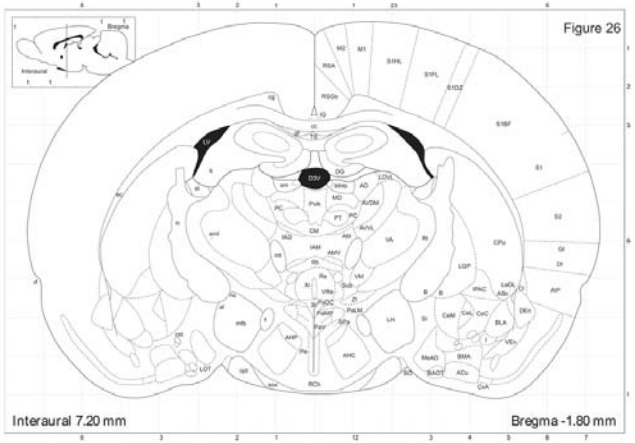
Summed spectra FTMS



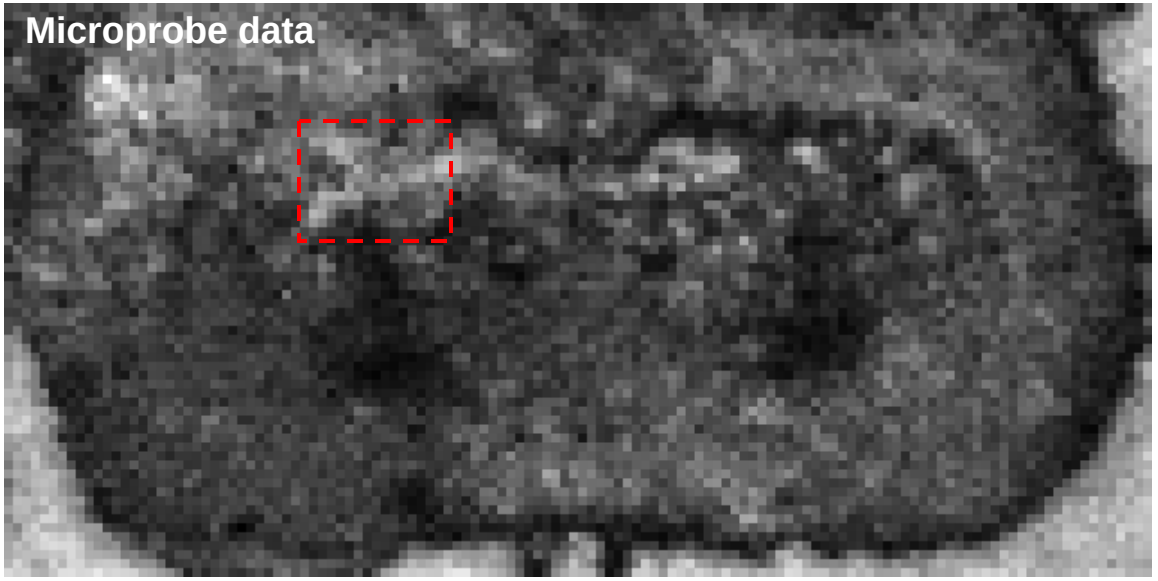


FTMS/TOF microscope image comparison

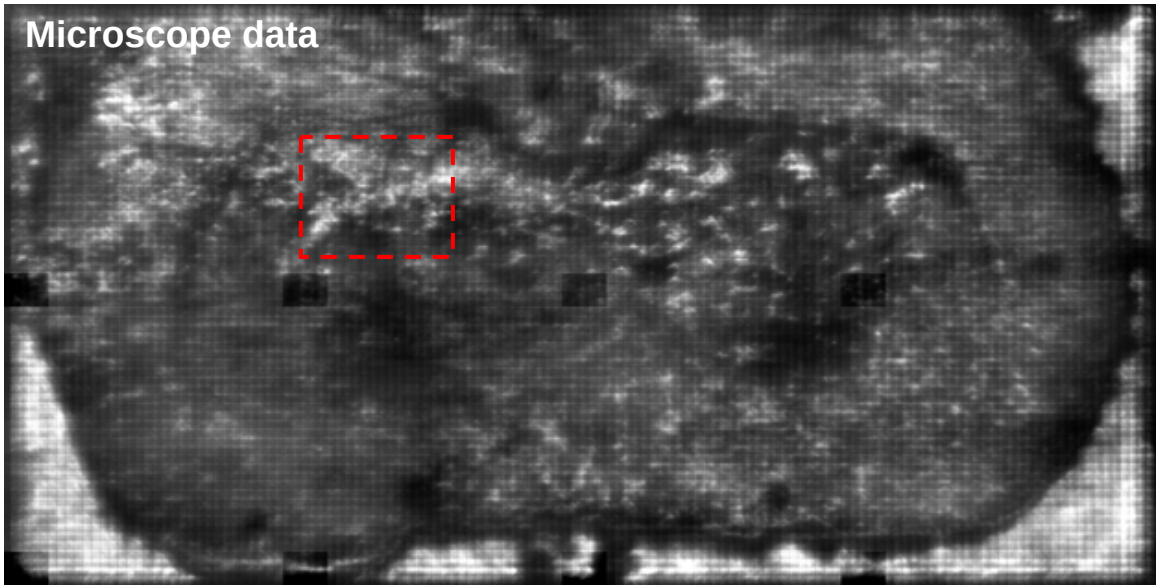




Spatial detail in stigmatic imaging

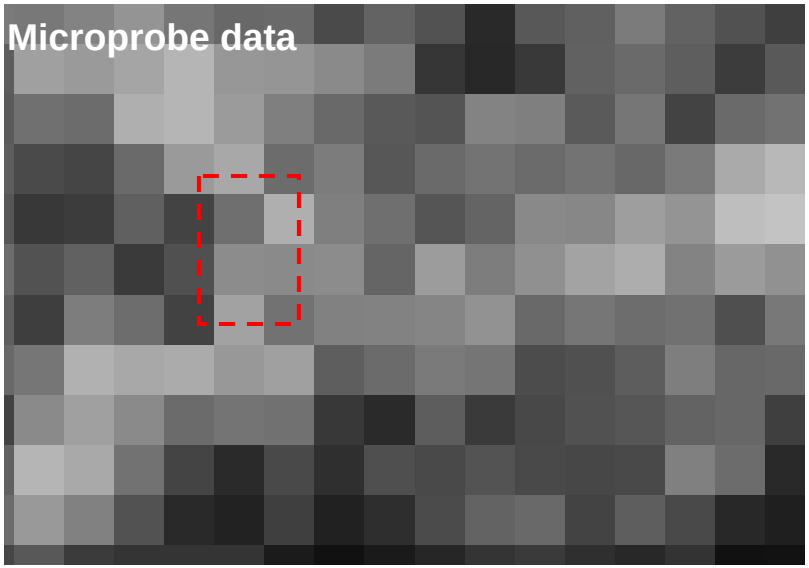


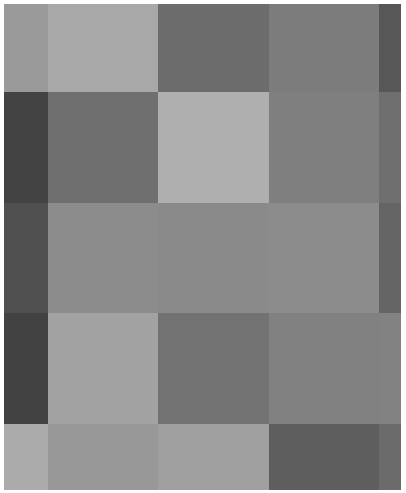
80 um resolution

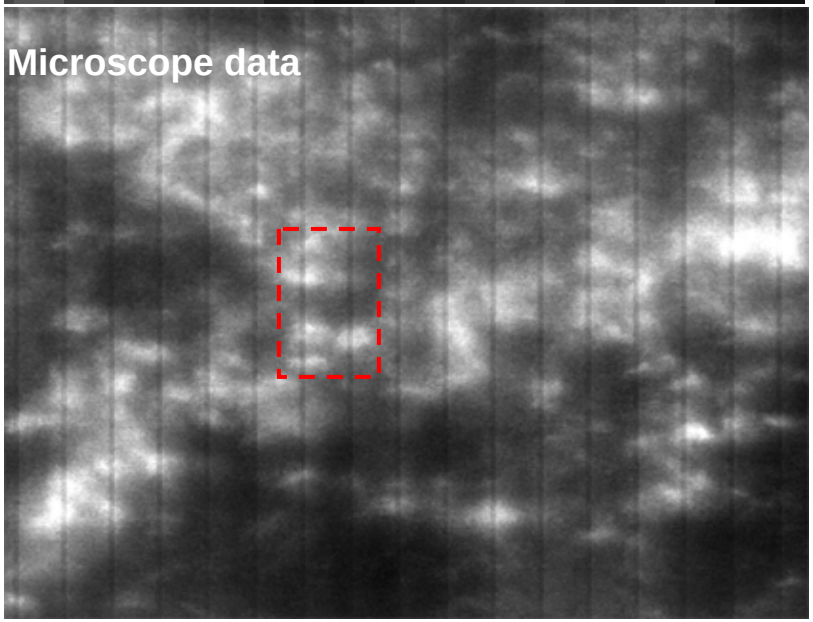


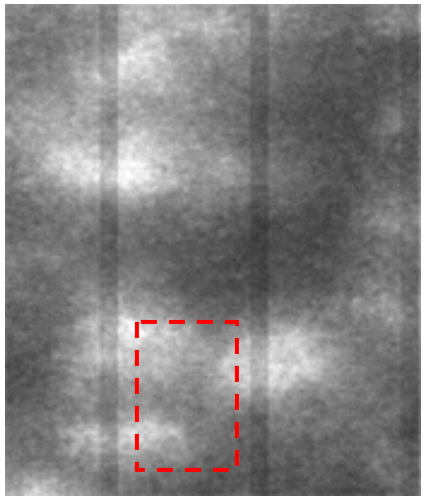
600 nm resolution

Spatial detail in stigmatic imaging

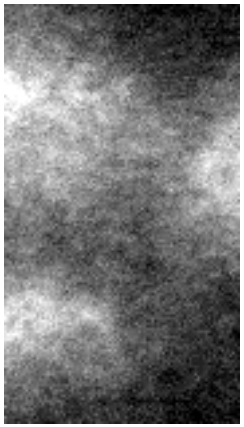








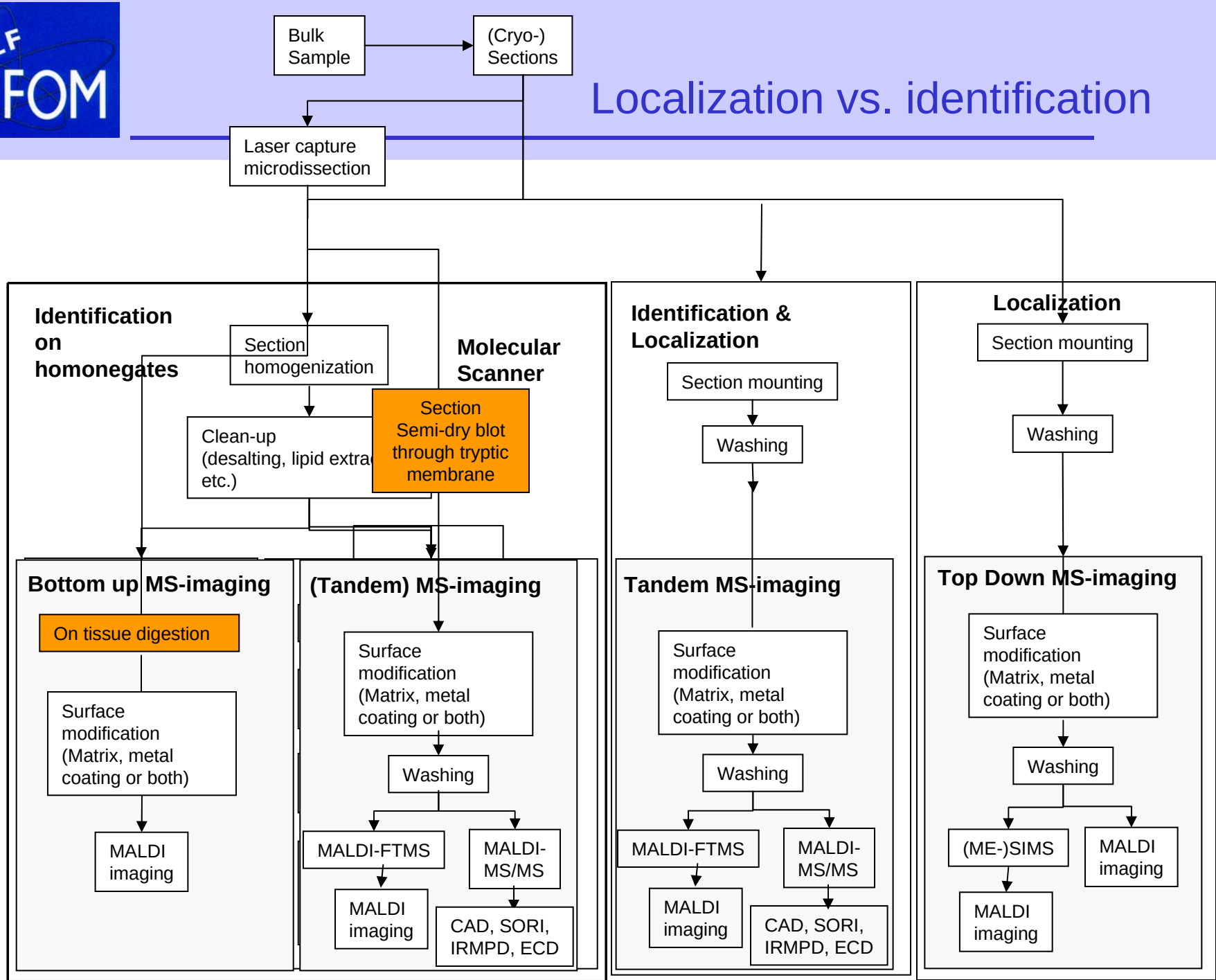
100%



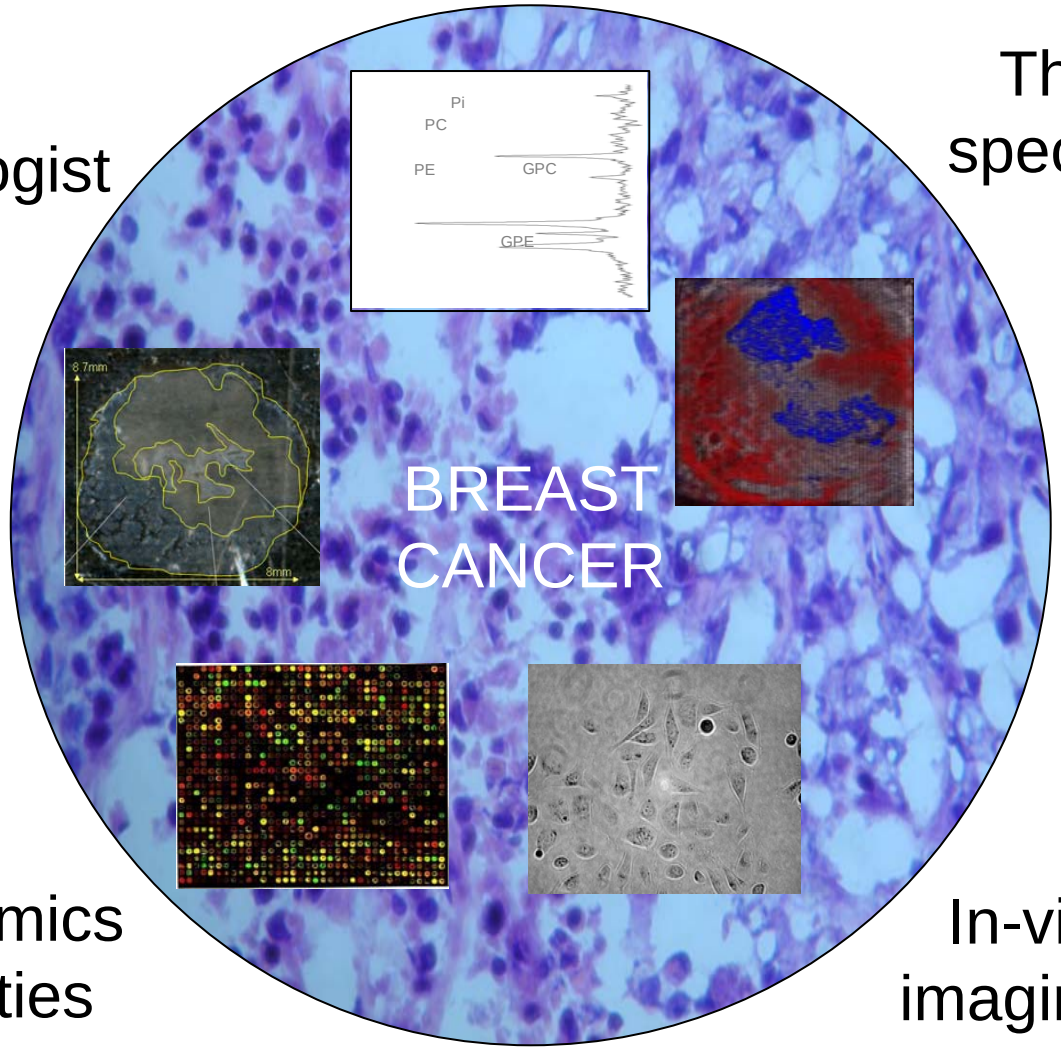
New imaging FTMS possibilities at high fields

- *Reveal more spatial details*
- *C60-SIMS (lipidomics) (Fast DC beam) (combined with MALDI)*
- *Deploy improved proteomics capabilities*
- *high speed or high resolution imaging MALDI*
- *Combination with Ion Mobility (both MALDI and ESI)*
- *Higher ion density in cell improves identification capabilities*
- *Fast Dissociation IRMPD and ECD*

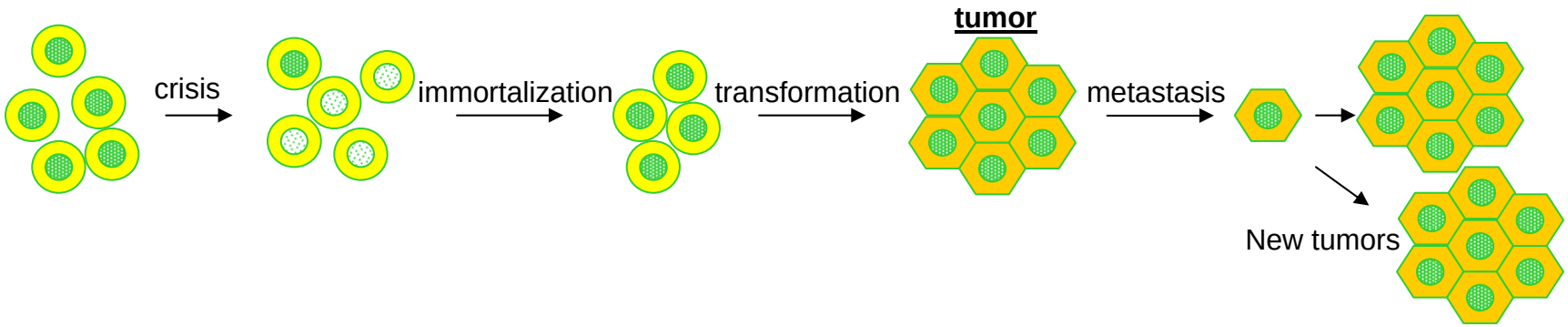
Localization vs. identification



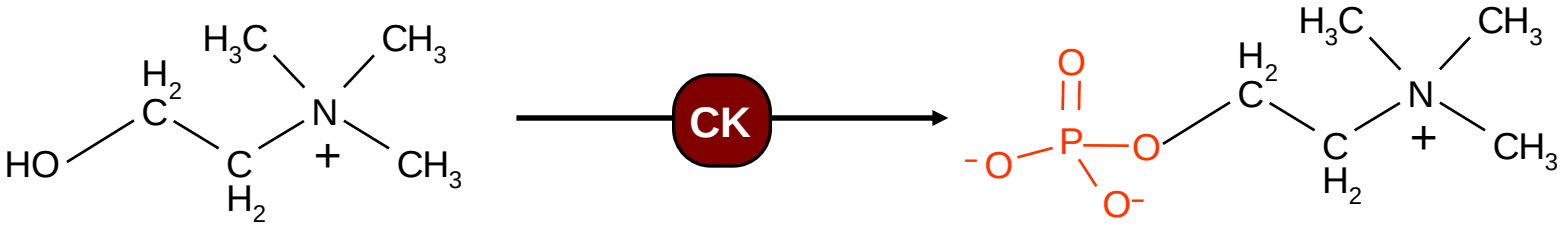
Intermezzo: Protein imaging alone is not enough !



MS imaging in breastcancer



“Choline kinase is overexpressed in breast cancer cells and activated by oncogenes and mitogenic signals, making it a potential target for cancer therapy”



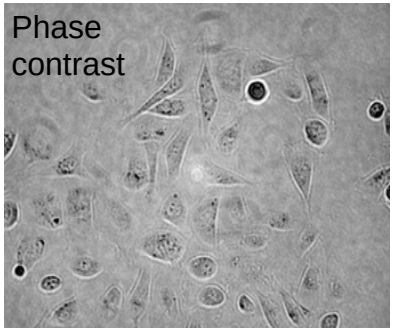
Choline (Cho)

Phosphocholine (PC)

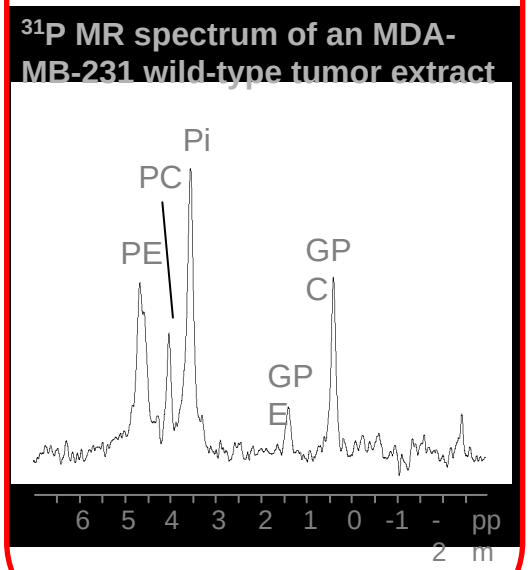
Glunde *et al*, *Cancer Res* **65**, 11034-11043, 2005

In-vivo study of tumor xenografts

From studies on choline kinase in cancer cells



To *in vivo* studies on choline kinase in solid tumors

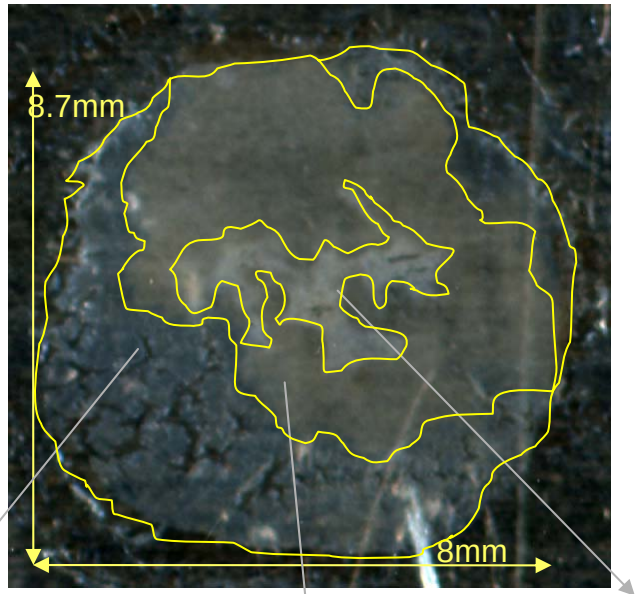


The Johns Hopkins University School of Medicine

Xenotransplantation is the transplantation of cells, tissues or organs from one specie to another. Such cells, tissues or organs are called xenografts.

Optical images

MALDI prepared Optical image

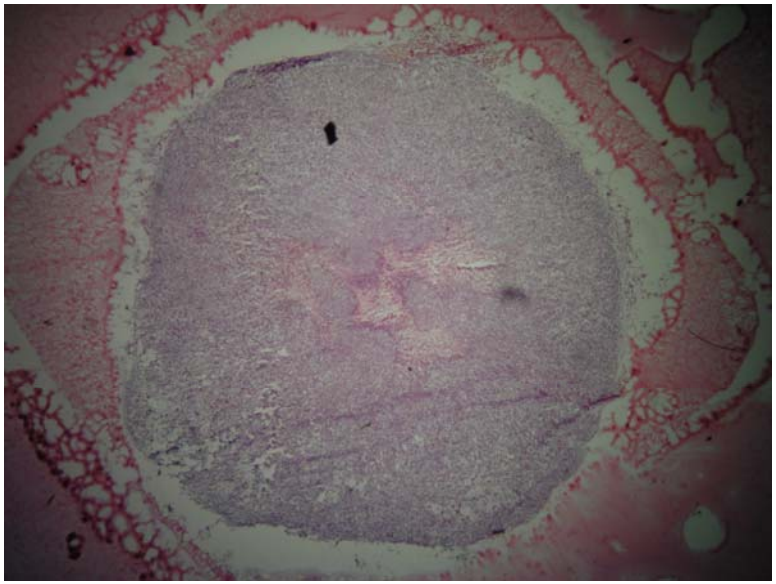


Angiogenesis, vascularized region,

Viable tumor tissue, more hypoxic towards the center

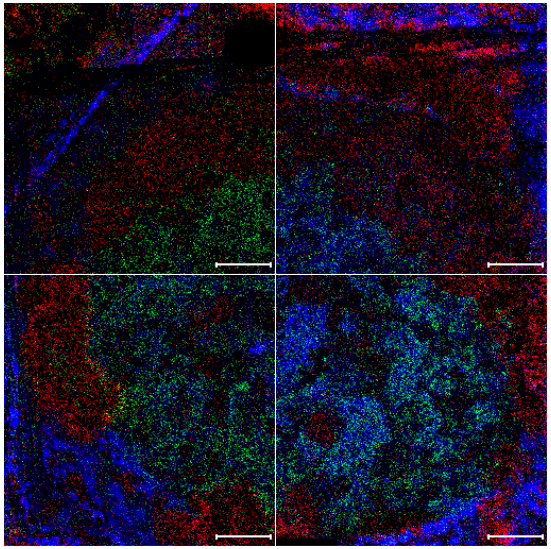
Necrotic or very hypoxic region

H&E stained Optical image



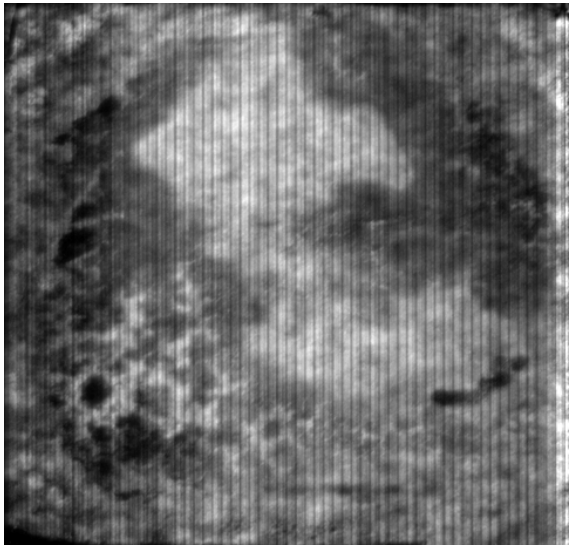
Complementary MS images breast cancer xenografts

R=578=DAG
G=184=PC
B=23=Na

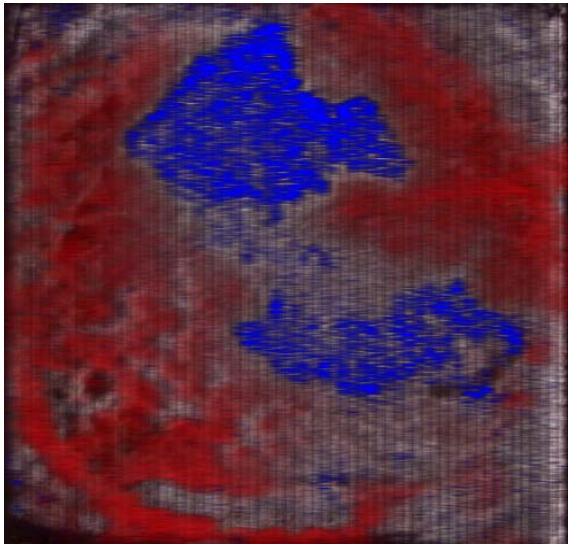


ME-SIMS MICROPROBE

TIC image

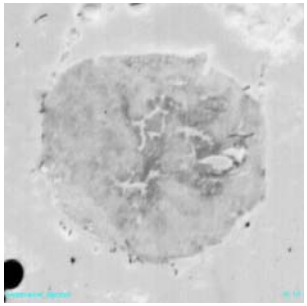


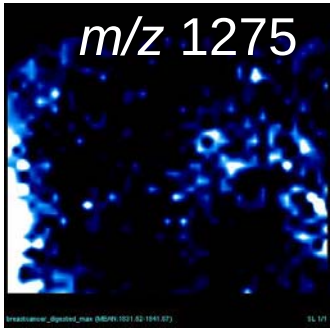
TIC,
PC and PL overlay



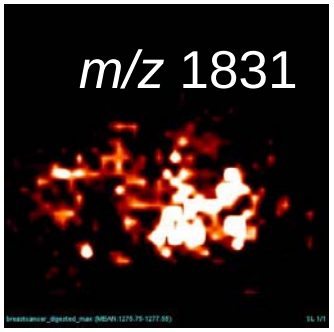
MALDI MICROSCOPE

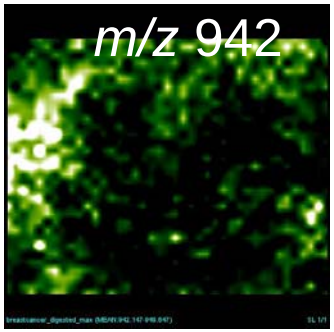
MALDI-ToF microprobe imaging of BC xenografts



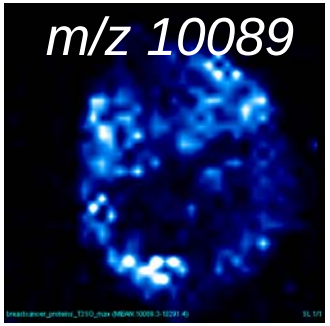


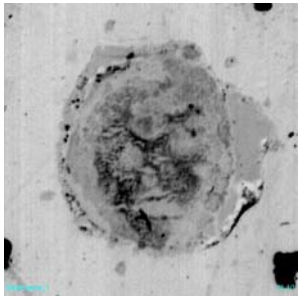
- Tissue section
- Washing
- DTT sprayed
- Incubation
- Trypsin sprayed
- Incubation
- Matrix nebulized
- IMS experiment
- Data analysis



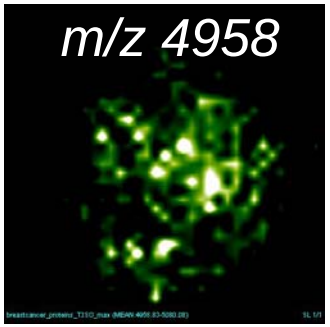


On-tissue digestion





- Tissue section
- Washing
- Matrix nebulized
- IMS experiment
- Data analysis

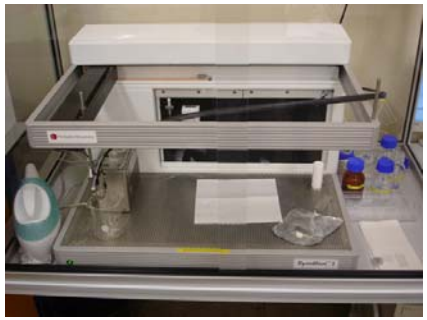


Direct Tissue analysis

Sample prep and matrix coating are crucial

Requirements

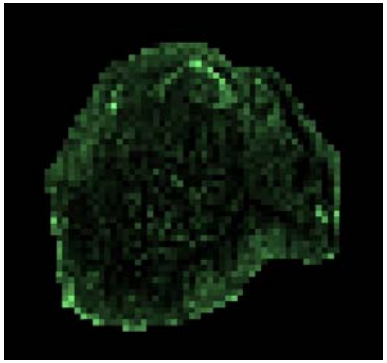
- Good wetting
- Limited diffusion
- Homogeneous
- Different X-tal sizes
- Controlled environment
- Reproducible



Methods

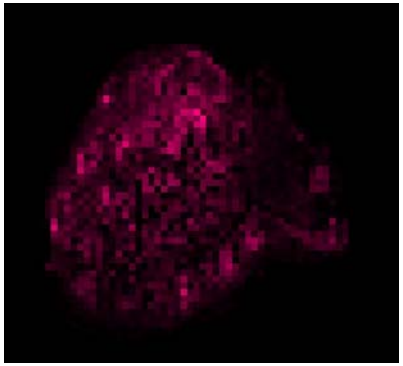
- Dried Droplet
- Automatic droplet spotter (CHIP….)
- Manual TLC sprayer
- Sonic Spray
- Electrospray deposition
- Automated Pneumatic nebulisation (SprayStation)
- ImagePrep

MALDI-ToF undigested results

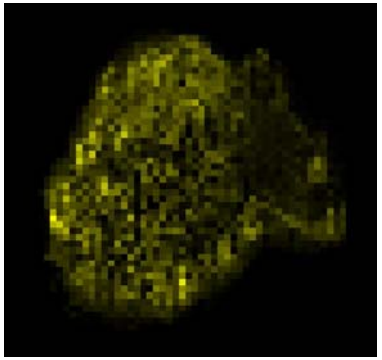


m/z 680

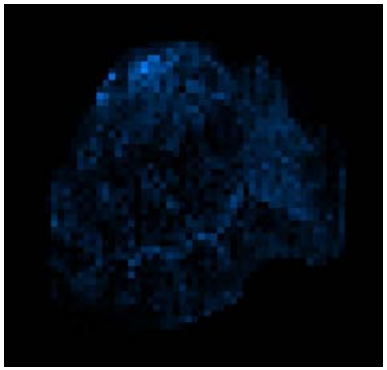
m/z 957



m/z 1216



m/z 1754



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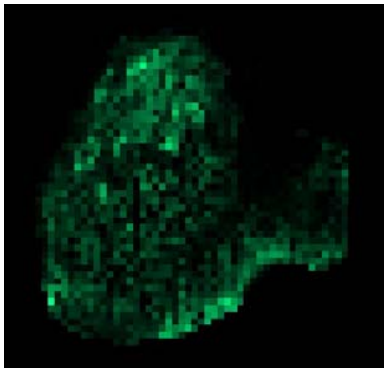
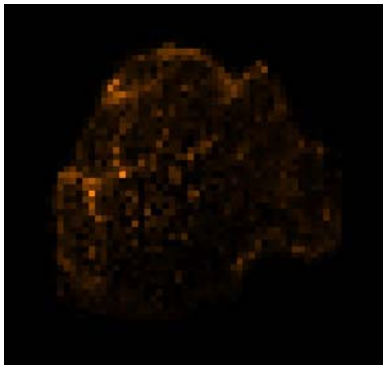
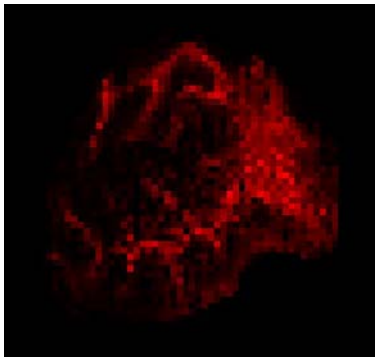


m/z 2583



m/z 2818



m/z 3930

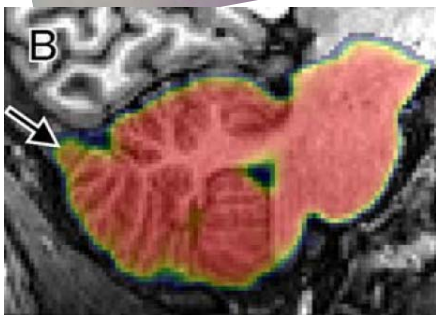
Matrix:

7 mg/ml CHCA, 50% ACN, 0.2% TFA

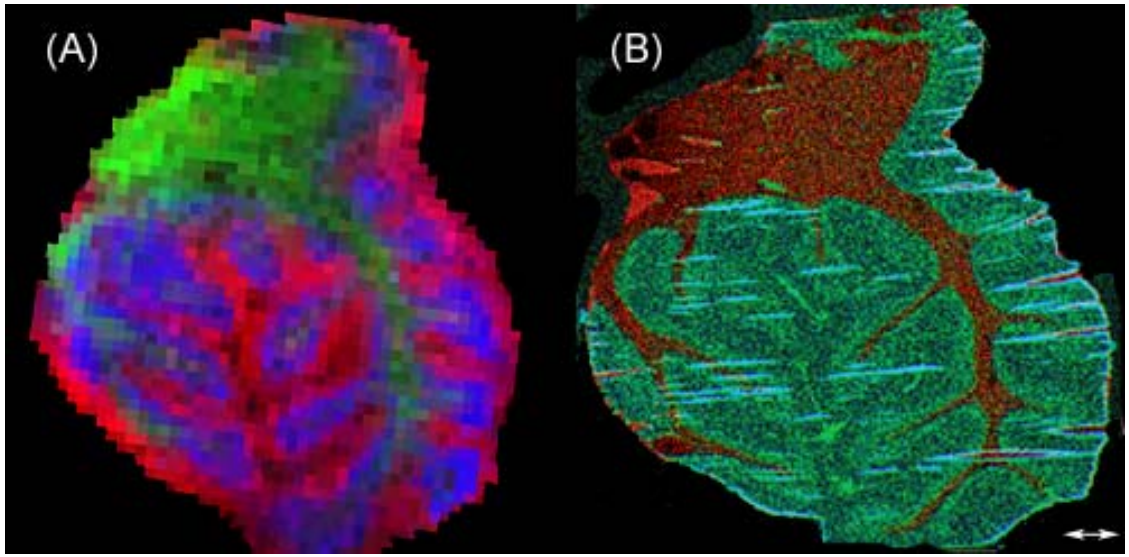
- Application method: ImagePrep
- Pre-treatment : washed with 2x (1 min) ice cold EtOH

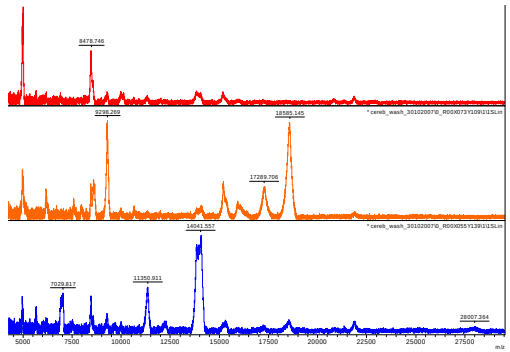
Intact proteins in human cerebellum

HE



MRI





5000 *30000*

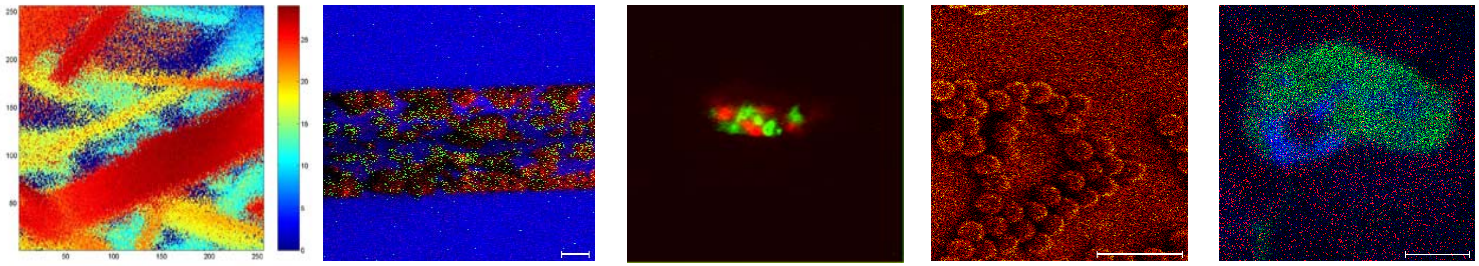
MALDI-ToFMS
Matrix,
heavy washing!

SIMS -ToFMS
No matrix,
no washing!

- Washing steps remove lipids and salts and reduces ion surpression

Summary

- Complementary imaging techniques yield completer pictures
- FTICR-MS imaging needs high magnetic fields to be come practically applicable.
- Heroism becomes routine at high fields!



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