

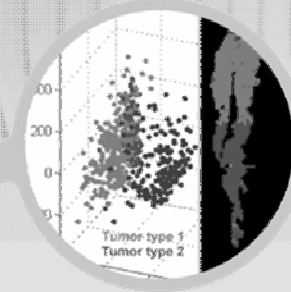
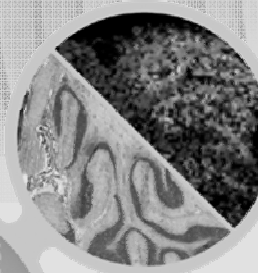
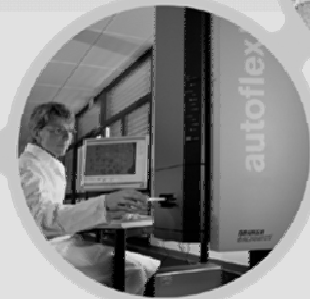
1000 Hz



## MALDI-Tissue Imaging at High Resolution and Speed: Essential Steps Towards its Applications in Histology

Sören-Oliver Deininger, Detlev Suckau,  
Michael Becker, Martin Schürenberg,

Bruker Daltonik, Bremen, Germany



# Principle of MALDI Imaging

34

*M. Stoeckli et al. / Analytical Biochemistry 311 (2002) 33–39*

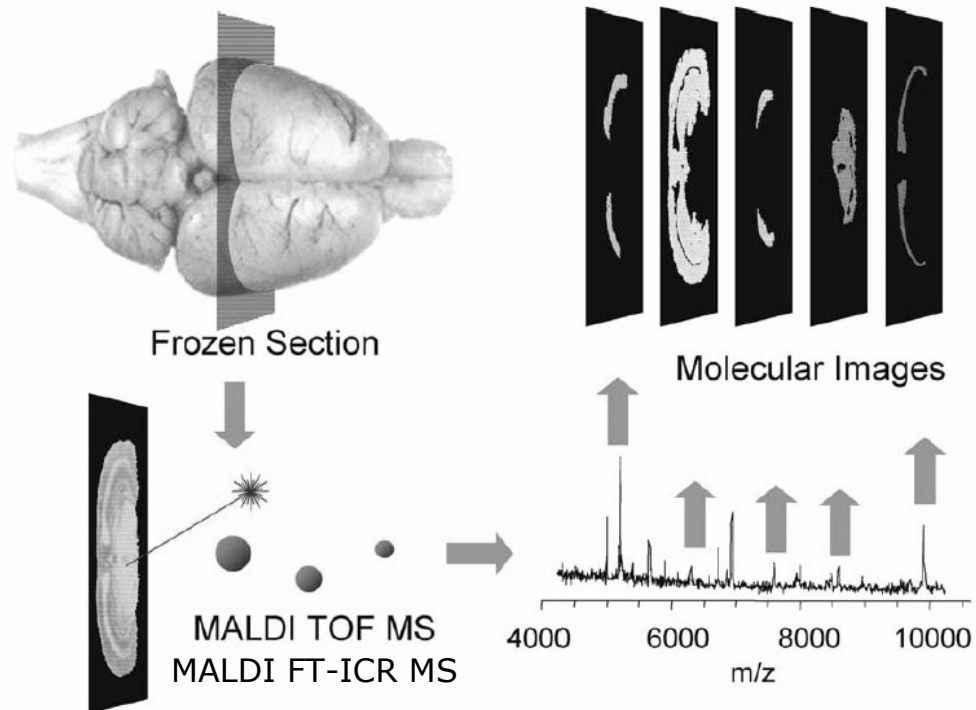
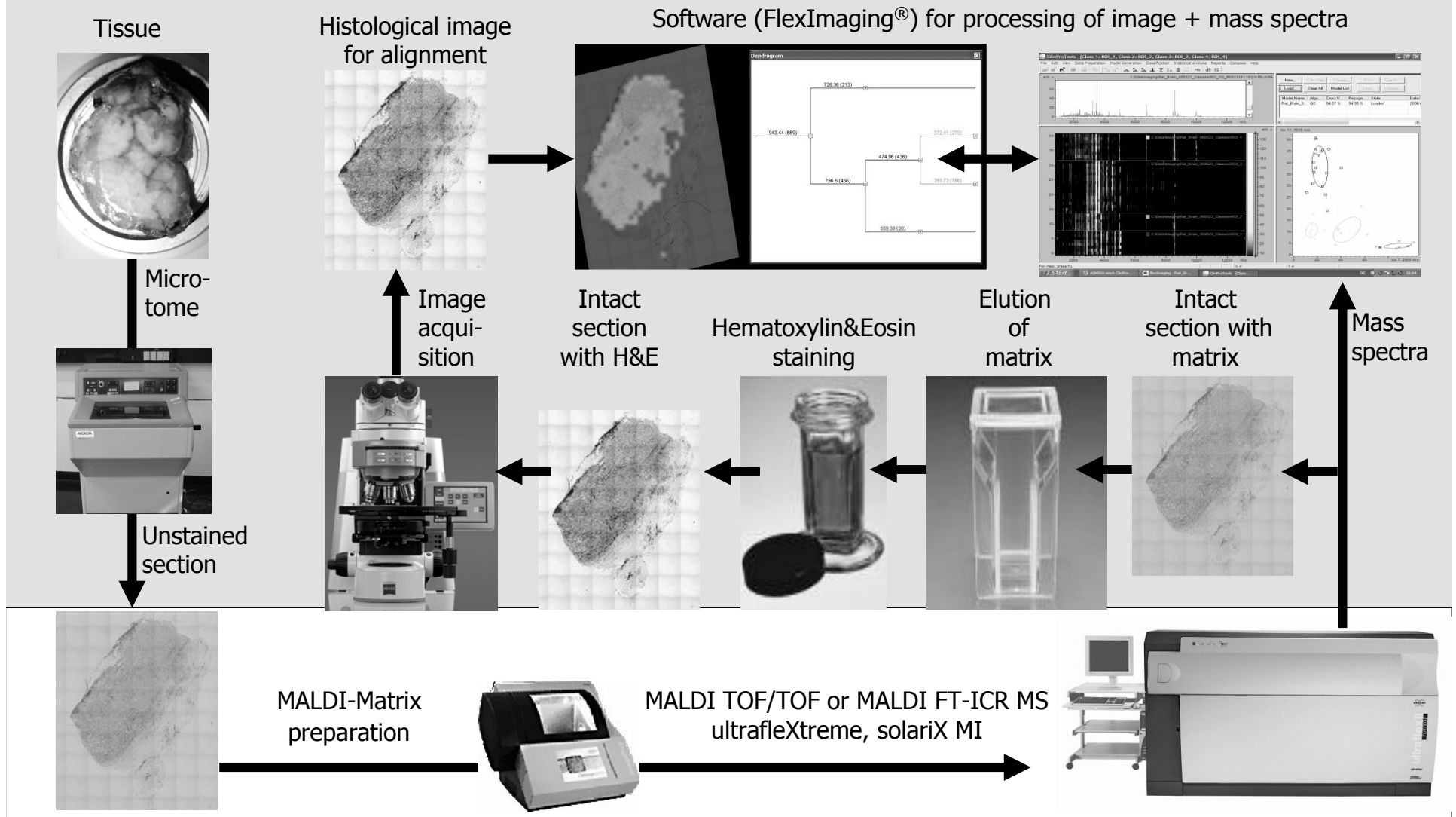


Fig. 1. MALDI MS imaging process: Cryostatic sections are fixed and coated with matrix, before they are analyzed in a TOF MS. Specific software is used for image acquisition and calculation of image data.

## Monitoring of Biomarker Tissue Distribution

# Molecular Histology Workflow "Bimodal Imaging"

From Dr. Walch, GSF Munich



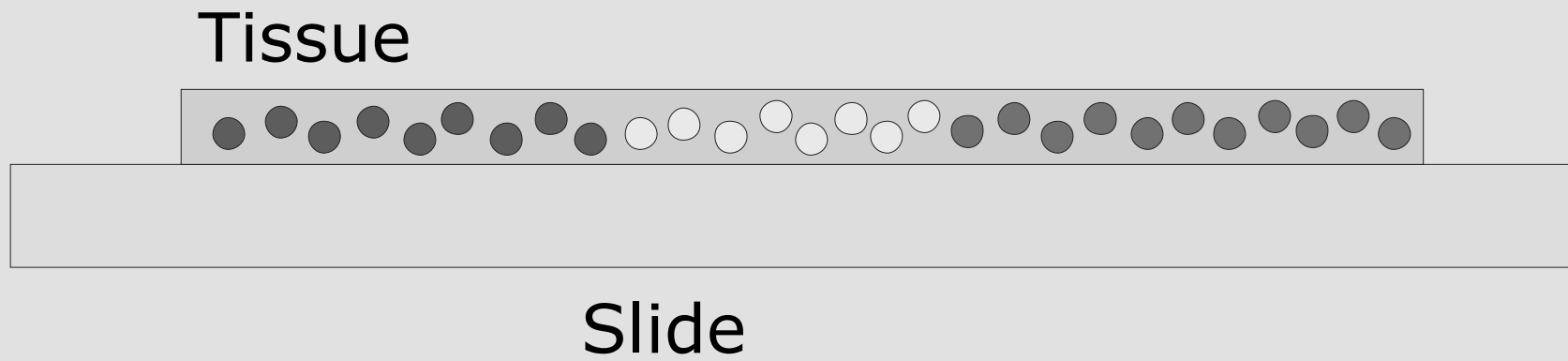
# Conditions: the three crucial steps

- 1. Tissue Preparation**
- 2. MS Analysis**
- 3. Data Interpretation and Information Retrieval**



# MALDI Imaging

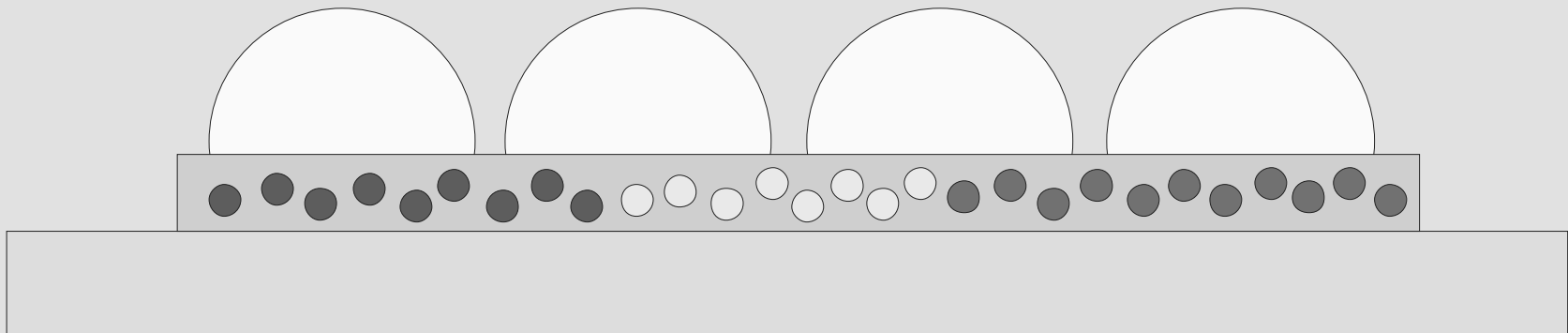
the challenge of sample preparation



# MALDI Imaging

the challenge of sample preparation

Matrix solvent needs to extract proteins from tissue

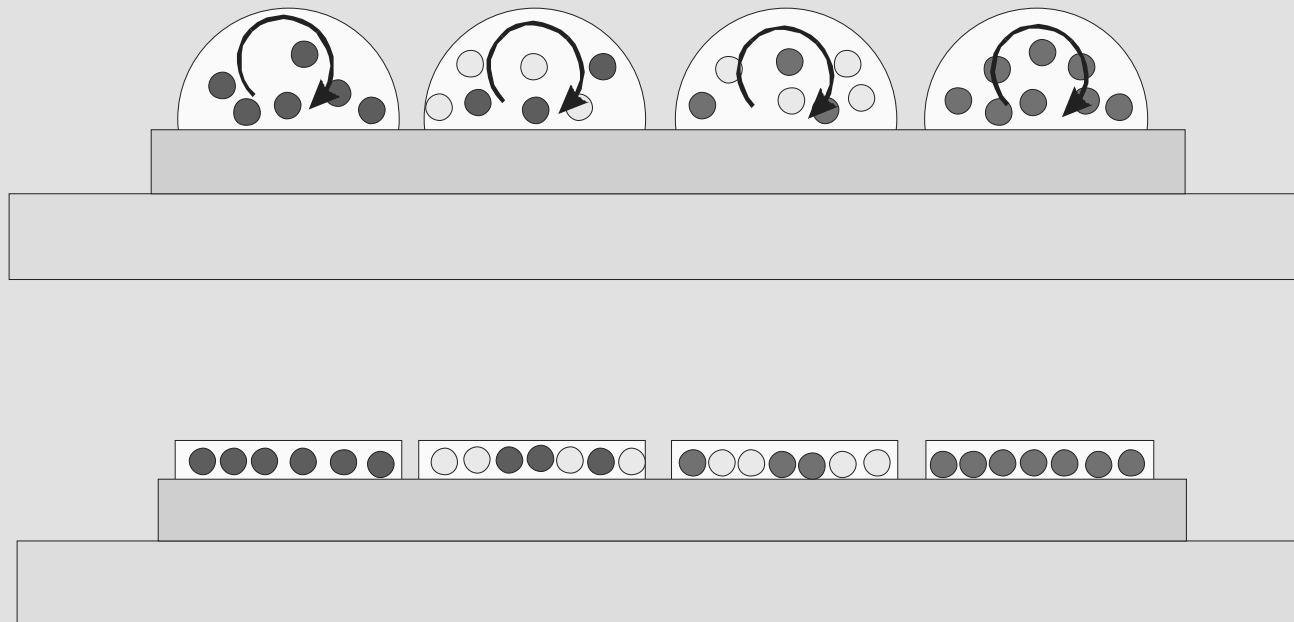


The longer the matrix incubates on the slide, the more efficient the protein extraction

# MALDI Imaging

the challenge of sample preparation

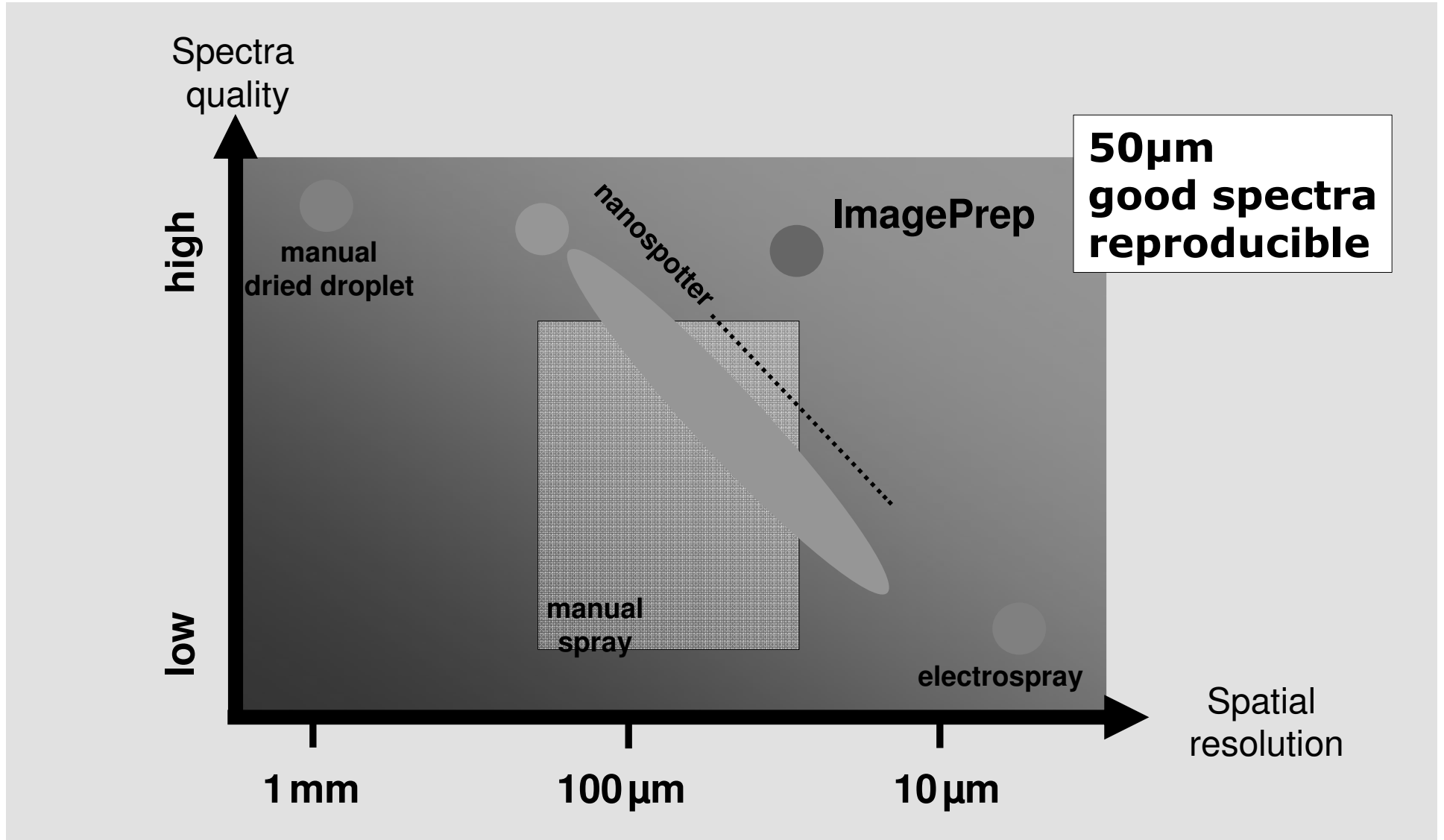
Inside droplets: delocalization of proteins



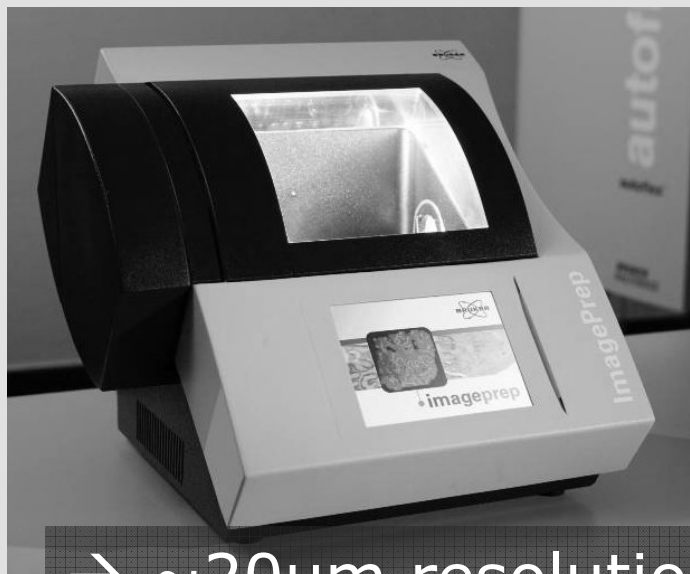
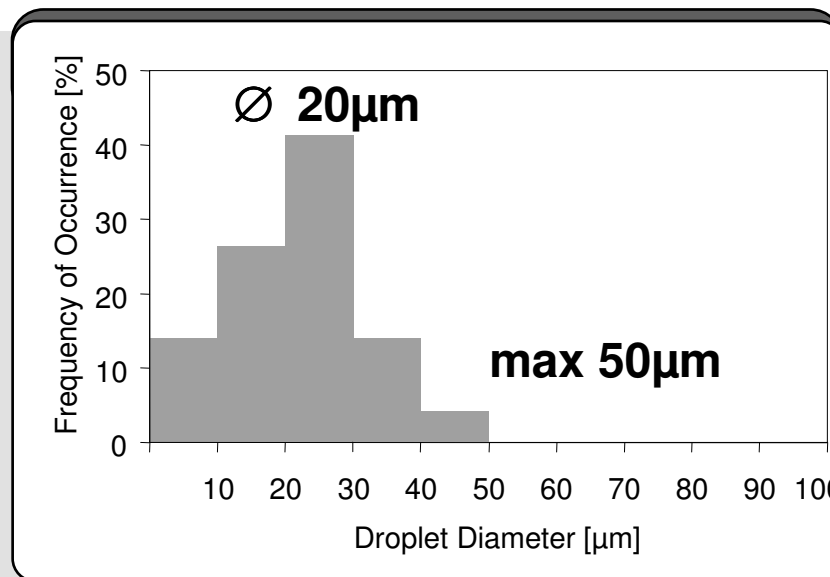
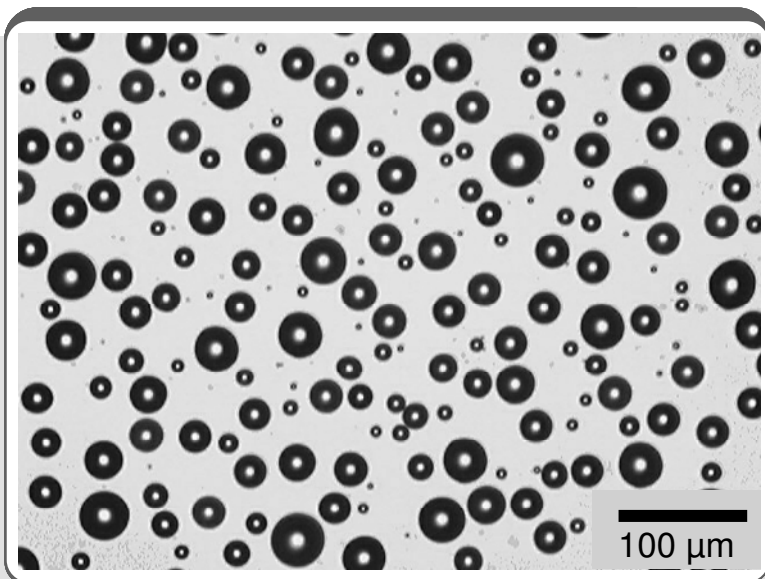
Large droplets: Good spectra, low lateral resolution

Smaller droplets: better lateral resolution, worse spectra

# Spatial Resolution vs Spectra Quality



# Droplet and Crystal Size $\sim 20 \mu\text{m}$

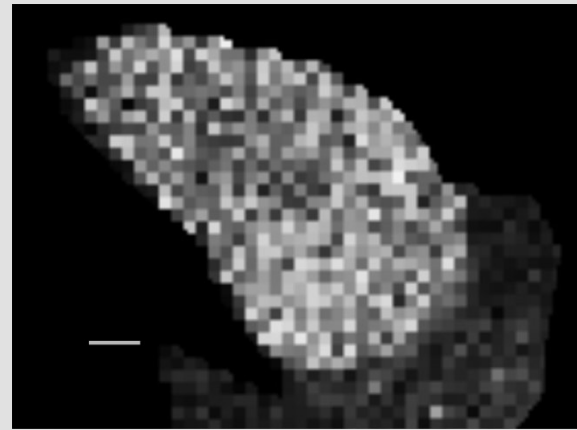
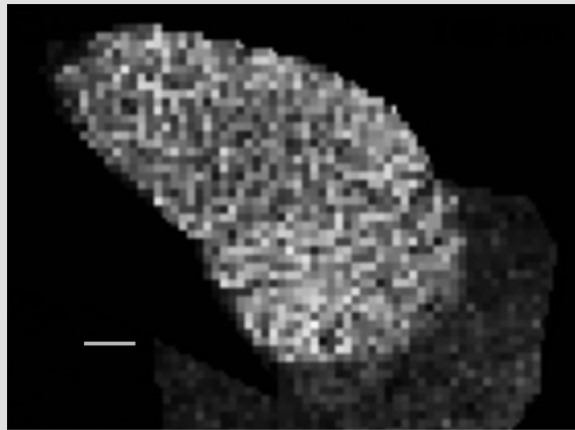
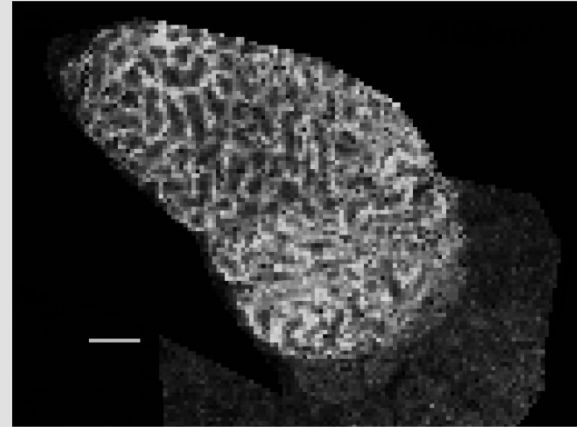
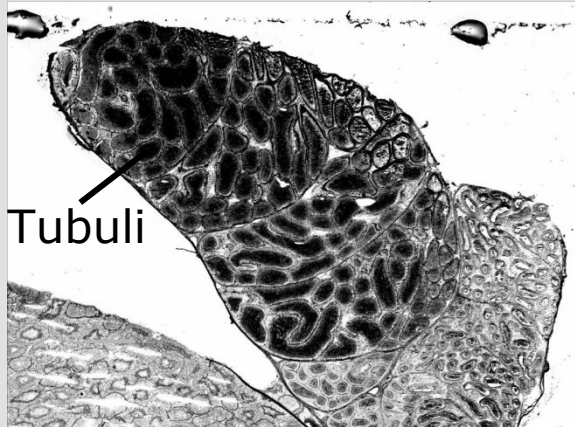


$\rightarrow \sim 20\mu\text{m}$  resolution



Sinapinic Acid (20g/l in 50% ACN)

# Lateral Resolution is Important to Understand Tissue Morphology



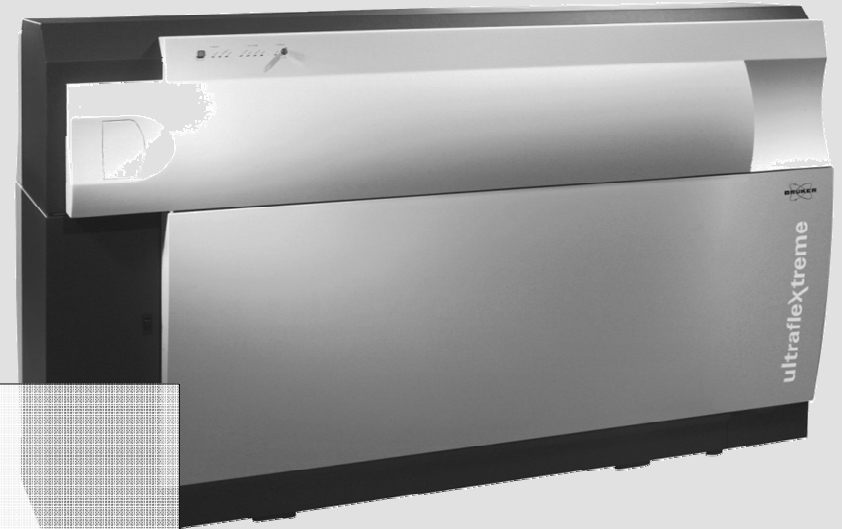
Scale bar: 1mm

Rat Testis

# Conditions: the three crucial steps

- 1. Tissue Preparation**
- 2. MS Analysis**
- 3. Data Interpretation and Information Retrieval**

# ultrafleXtreme 1 kHz MALDI-TOF/TOF used for MALDI-Tissue Imaging

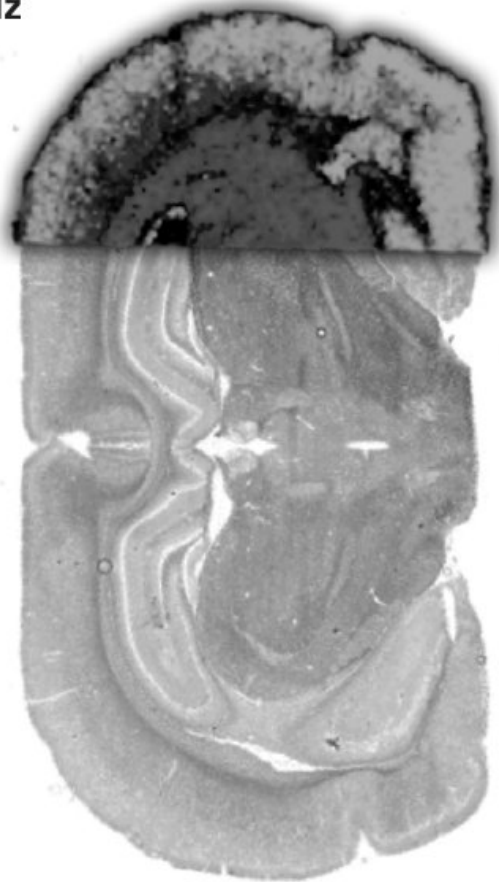


- smartbeam-II laser: 1 kHz, 20  $\mu\text{m}$  res. in protein images
- 1 kHz electronics/ data handling for operation in MS and MS/MS mode
- 2 pixel/sec image acquisition
- low down-time:  
fast self-cleaning ion source
- Virtual microscopy

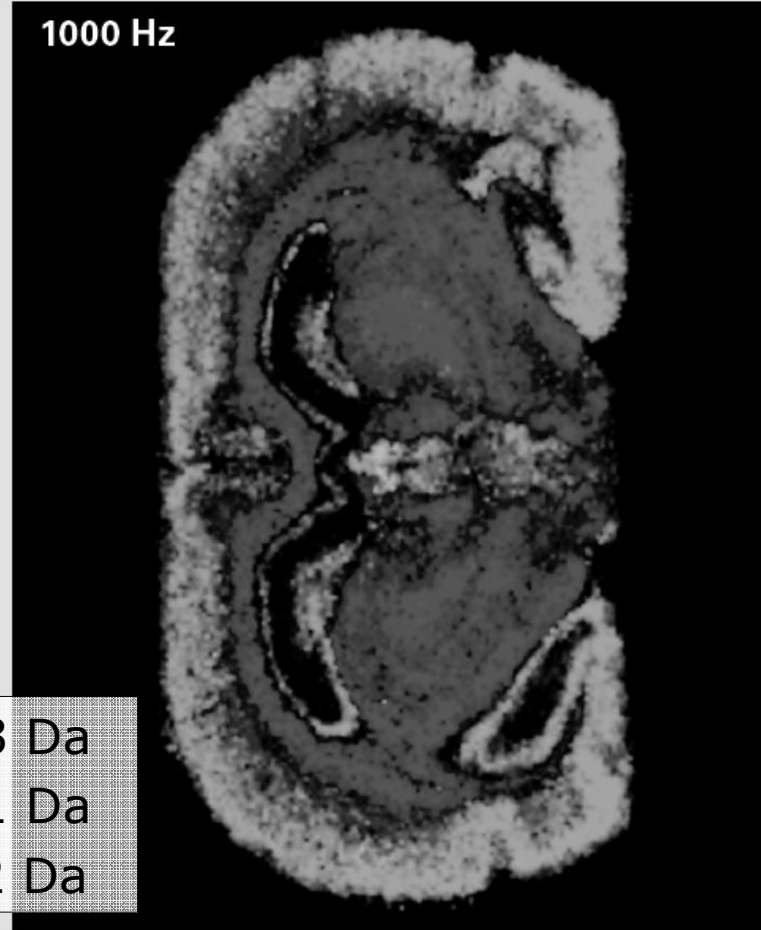


# 1 kHz Protein Imaging: 2pixel/sec

200 Hz

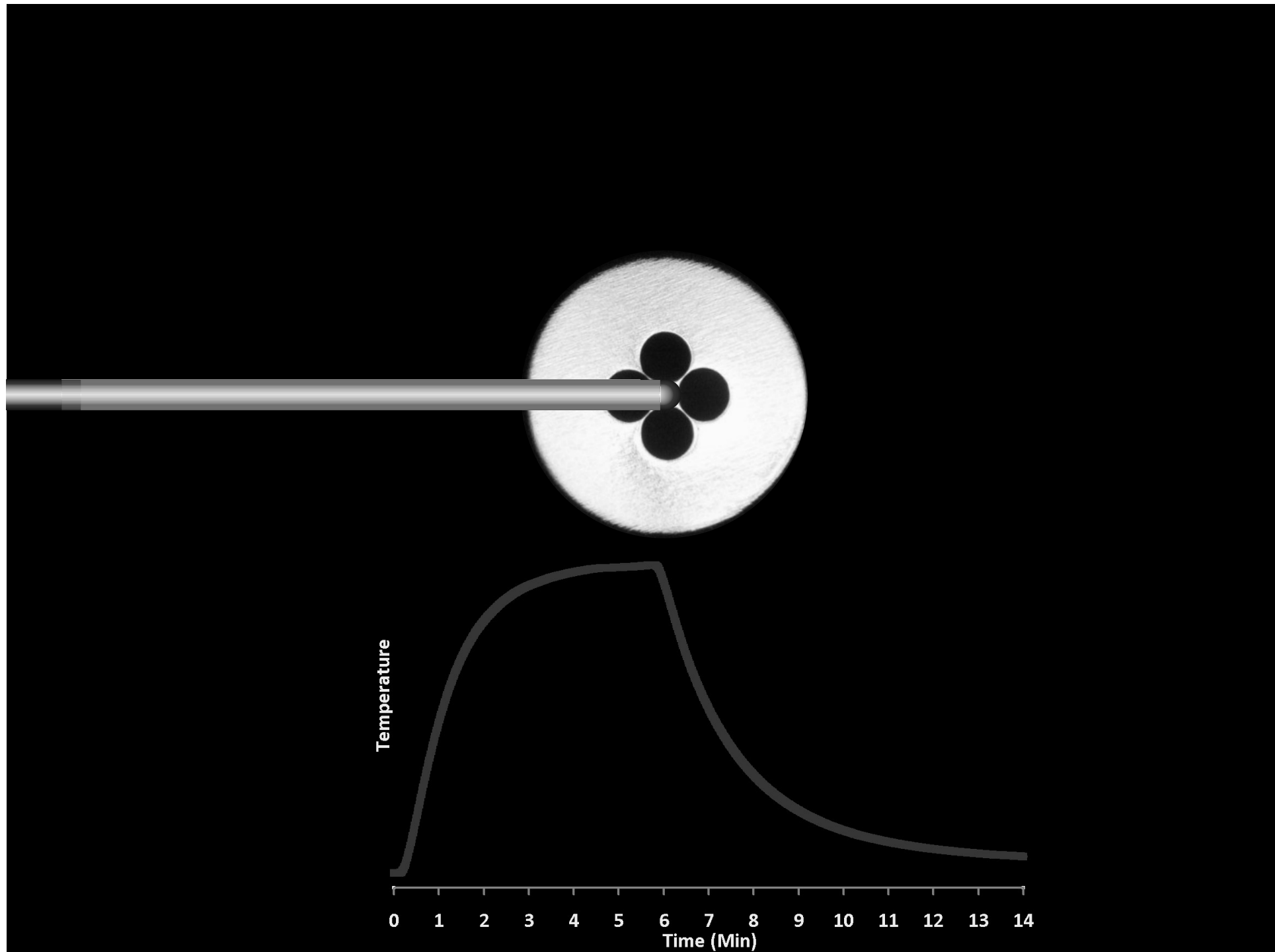


1000 Hz

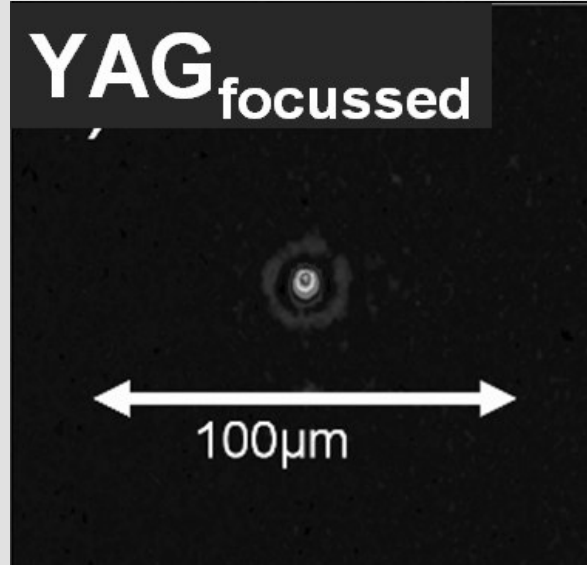
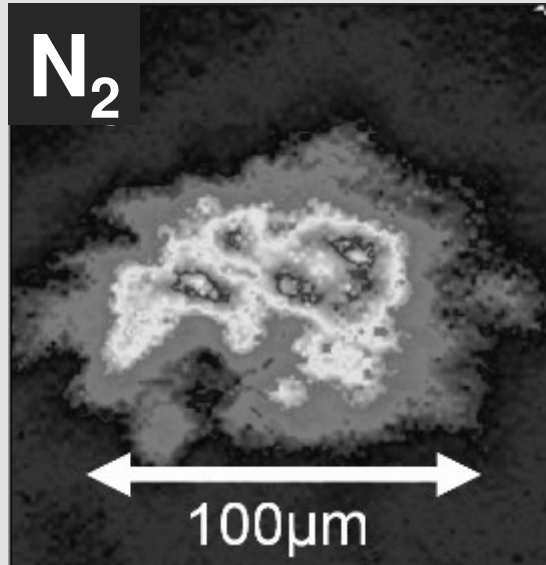


6713 Da  
6551 Da  
14092 Da

25,000 pixel in 3.5 h



# Laser Beam Profiles of MALDI Lasers

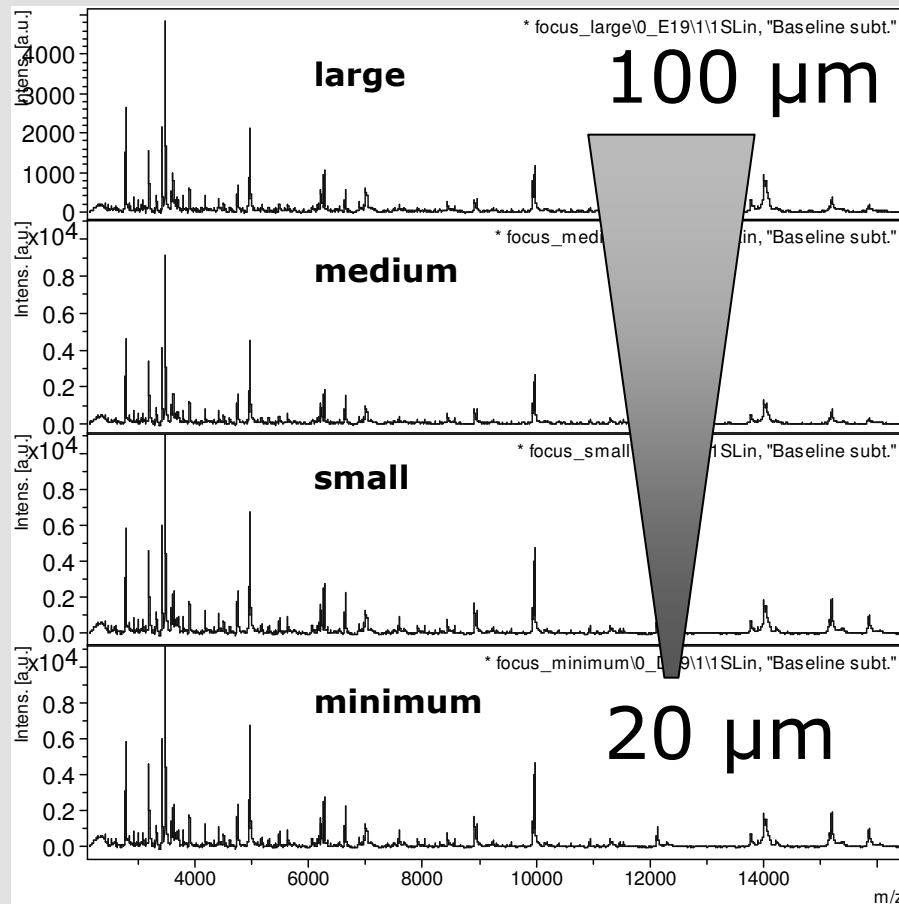


- < 100 Hz rep rate
- ~100 M shot lifetime
- ~50  $\mu$ m focus
- variable beam profile

- 1000+ Hz
- >1 G shot lifetime
- <10-100  $\mu$ m spot size
- Gaussian beam profile

- 1000+ Hz
- >1 G shot lifetime
- <10-100  $\mu$ m spot size
- variable beam profile

# Protein Imaging Spectra Quality as Function of smartbeam-II Laser Focus Size



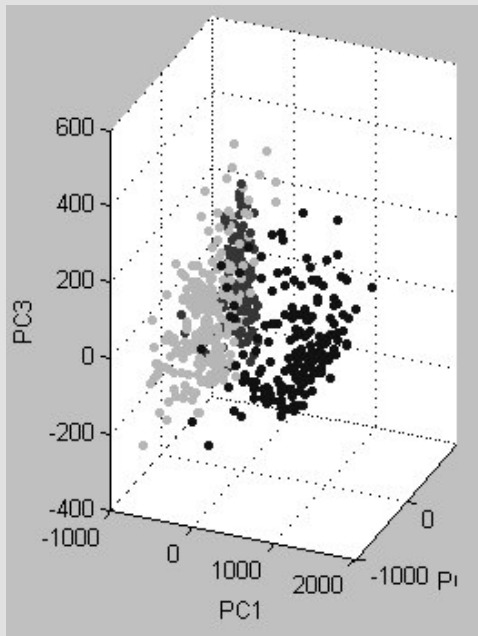
2000 Shots on tissue sample, different laser focus

Analyzed area identical for all focus settings

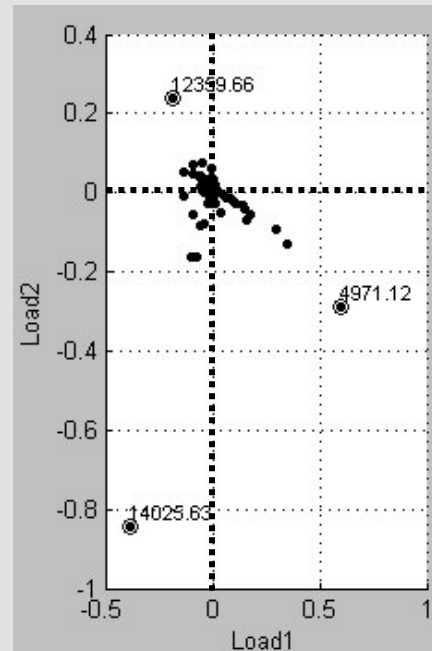
# Conditions: the three crucial steps

- 1. Tissue Preparation**
- 2. MS Analysis**
- 3. Data Interpretation and Information Retrieval**

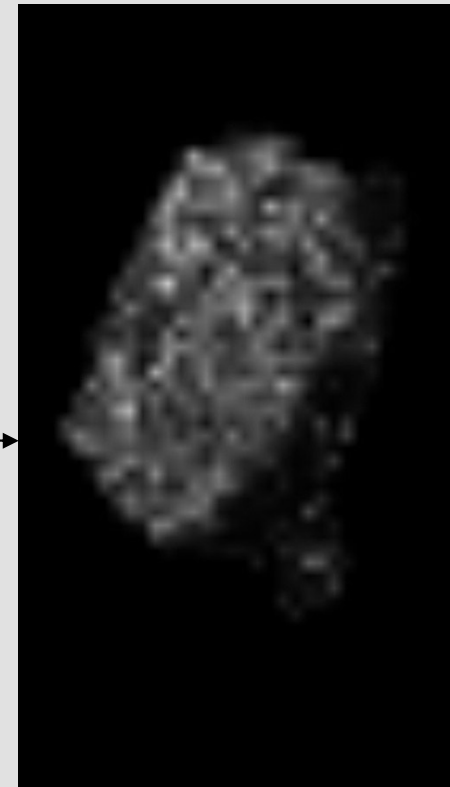
# Unsupervised Analysis of Image Data with PCA – Principal Component Analysis



Each point represents one spectrum in the PCA-vector space (colors as guide to the eye only)

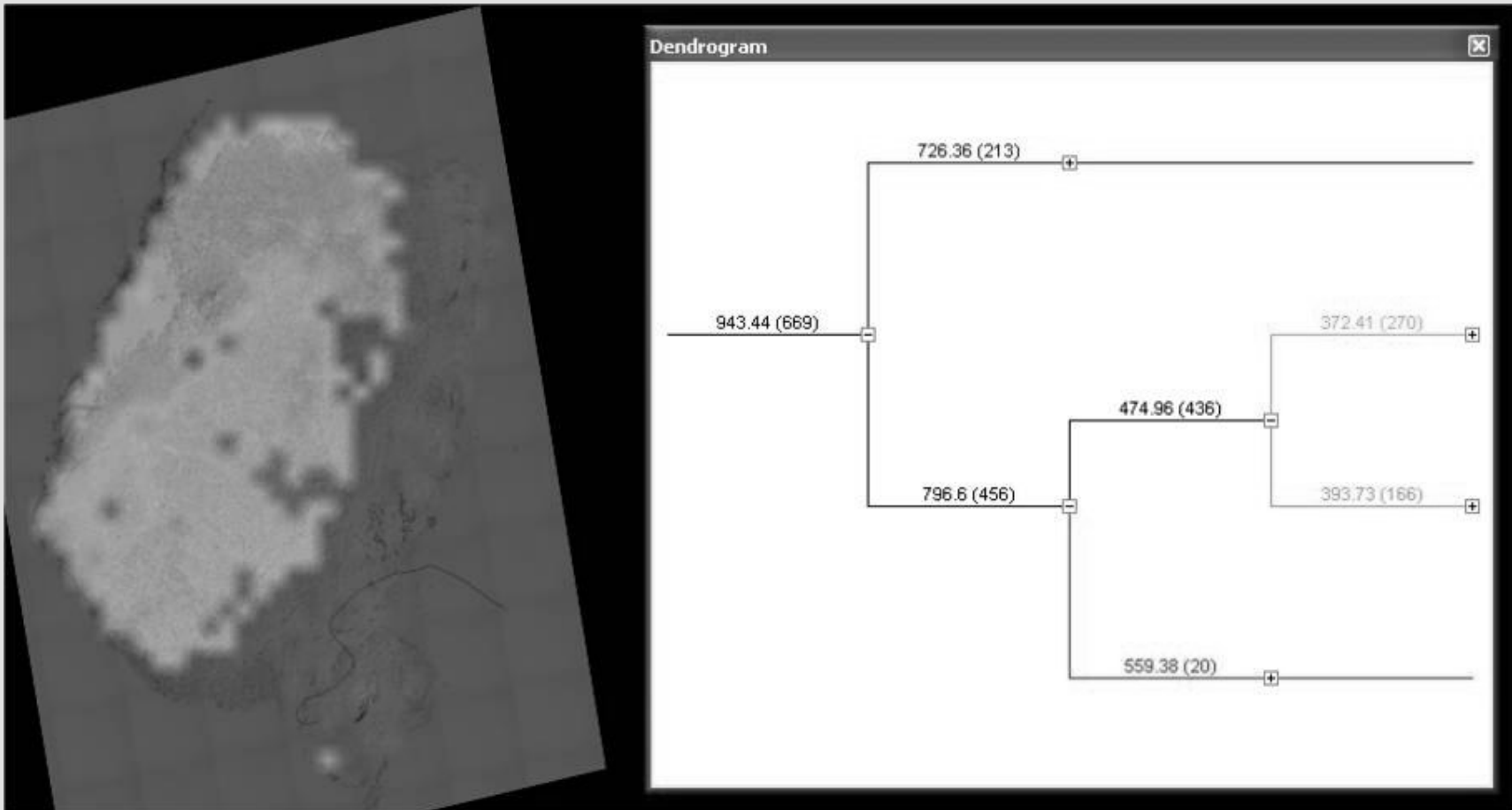


Each point represents one peak. The distance from (0/0) indicates the contribution to variation. Peaks with high variation can be selected on the MALDI image.



**Staining with the selected markers.** Correlation with histology can be examined

# Hierarchical clustering

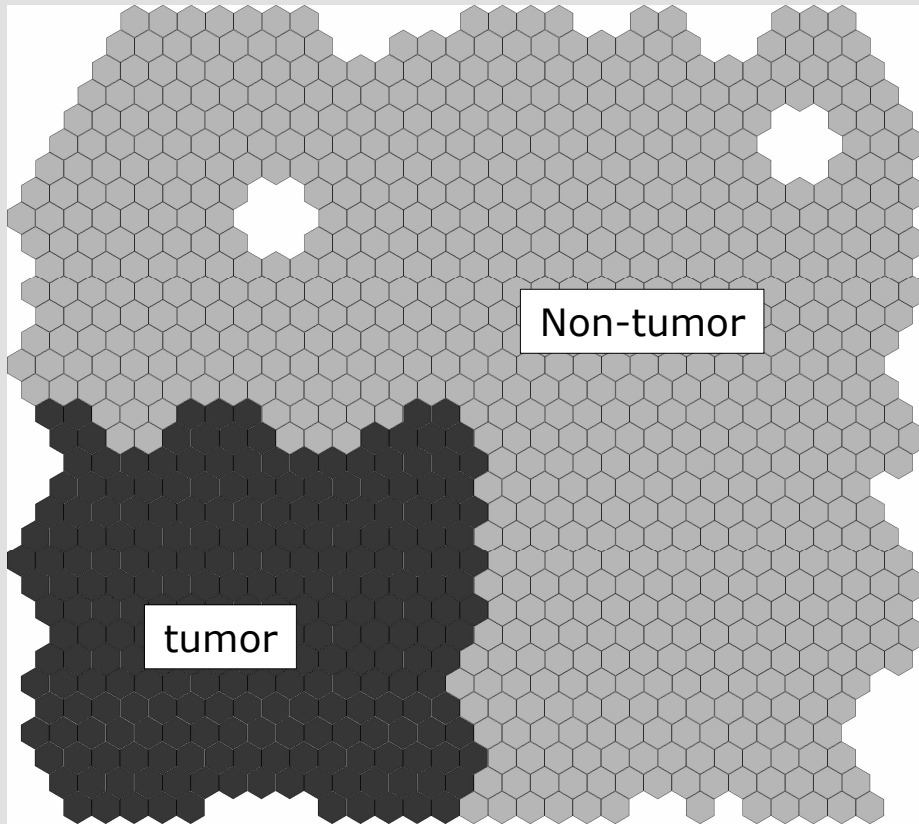


## Applications of MALDI Tissue Imaging

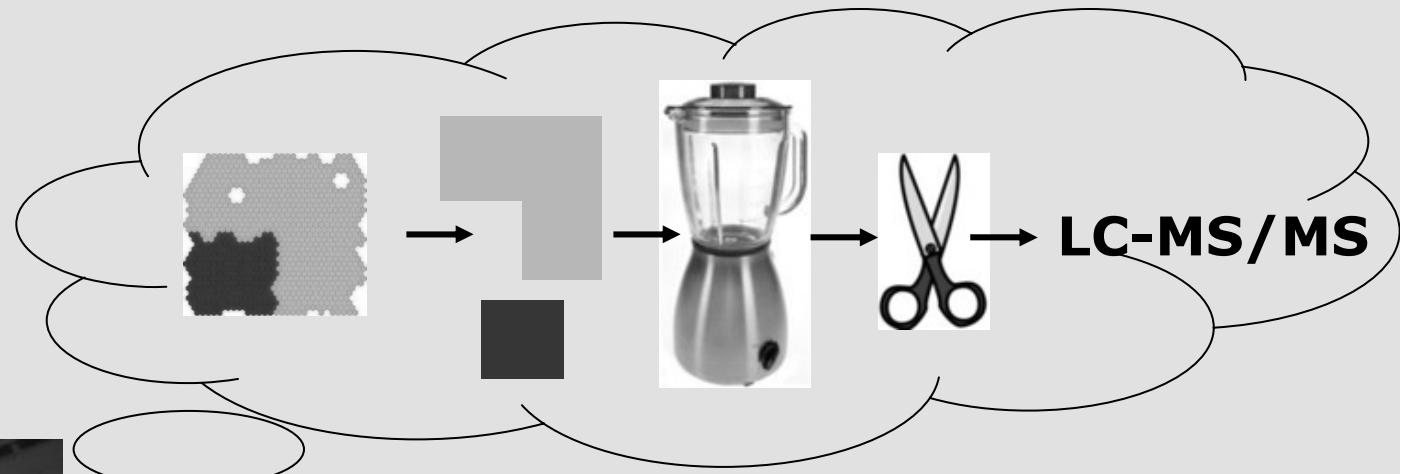
- **Peptide/Protein Biomarkers and Diagnostics  
= Molecular Histology**
- **Drug distributions + Metabolites**



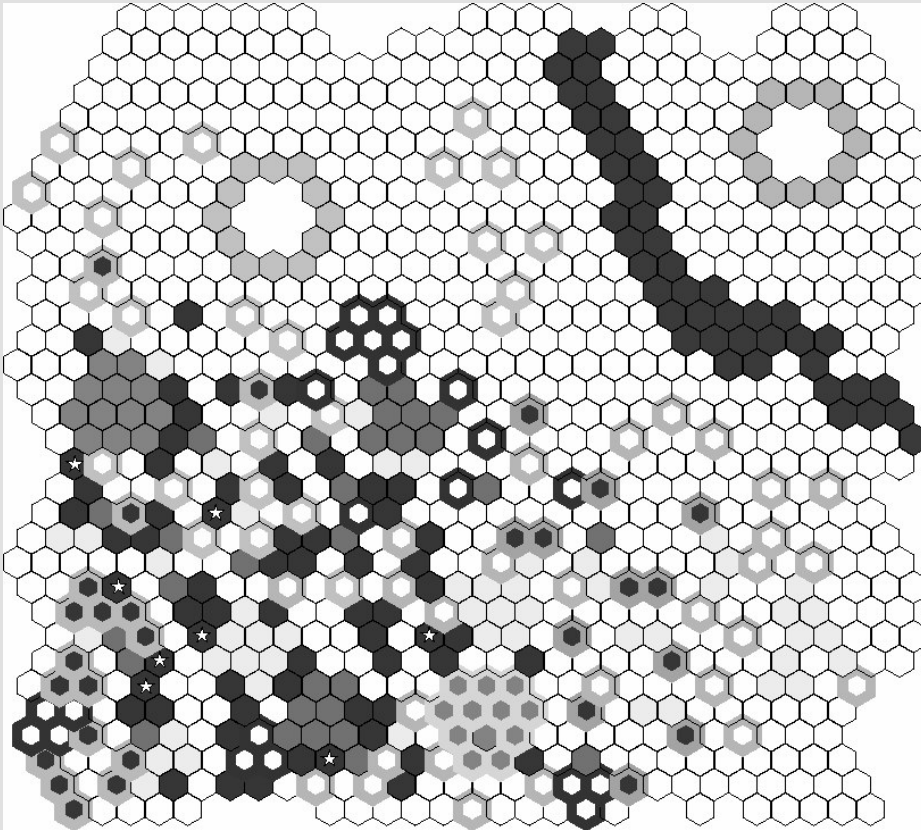
# Simple Minded View of: TUMOR!



# „Proteomics“ thinking



# Towards a More Realistic Understanding of: TUMOR



**Invasive tumor cells**

**Carinoma in situ (early stage tumor cells)**

**Neoplastic cells (pre-tumor cells)**

**Epithelial cells**

**Lymphocyte infiltration**

**Inflammation**

**Connective Tissue**

**Tumor cells are not homogenous:  
tumor stem cells (\*)**

**Different stages**

**Different clones**

**The surrounding of the tumor  
(stroma) influences the tumor:**

**tumor microenviement**

# The Right Understanding of Tumors



**tumors are highly heterogenous on a cellular level and highly dynamic**

**Some of the information can be seen in the microscopy of a stained section („conventional histology“)**

**Some information can only be revealed by molecular techniques. This information may be highly relevant for treatment, prognosis...**

## **Invasive tumor cells**

**Carinoma in situ (early stage tumor cells)**

**Neoplastic cells (pre-tumor cells)**

**Epithelial cells**

**Lymphocyte infiltration**

**Inflammation**

**Connective Tissue**

**Tumor cells are not homogenous:  
tumor stem cells (\*)**

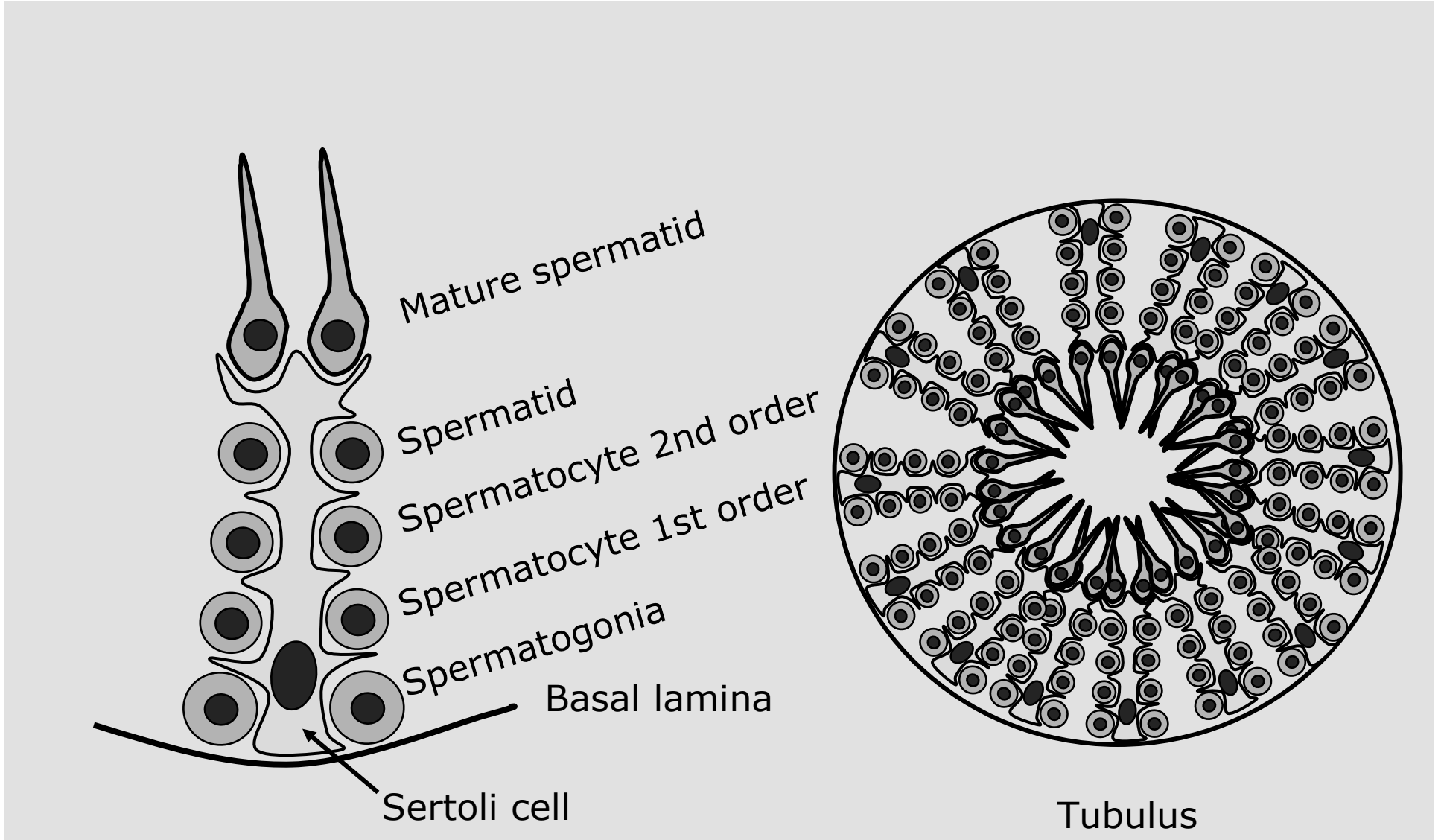
**Different stages**

**Different clones**

**The surrounding of the tumor (stroma) influences the tumor:**

**tumor microenviement**

# Spermatogenesis and Tubular Morphology



# Rat Testis: MALDI Imaging and H&E Staining from the Same Section

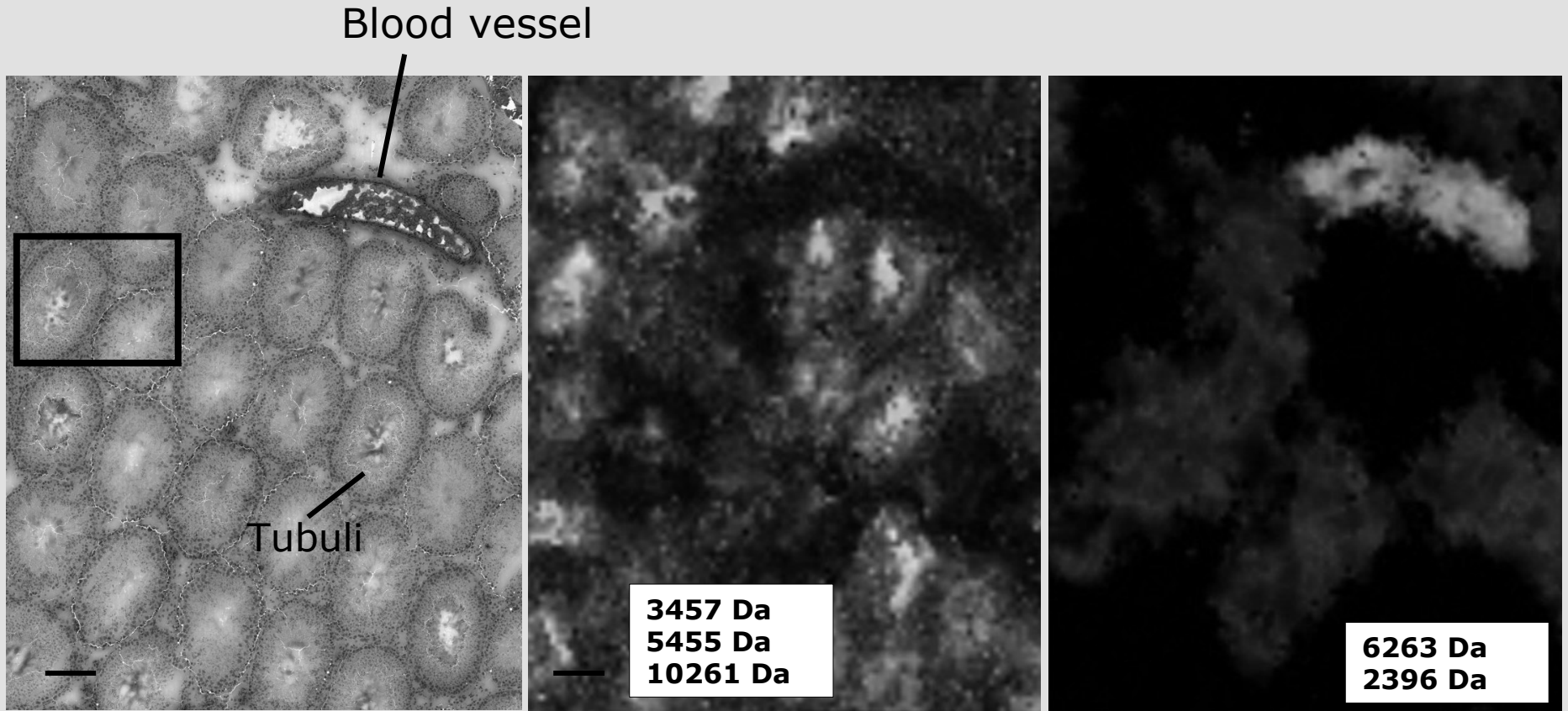


Image Resolution: 20  $\mu\text{m}$

H&E and MALDI Images obtained from same section!

# H&E Stain of Tubuli from Rat Testis

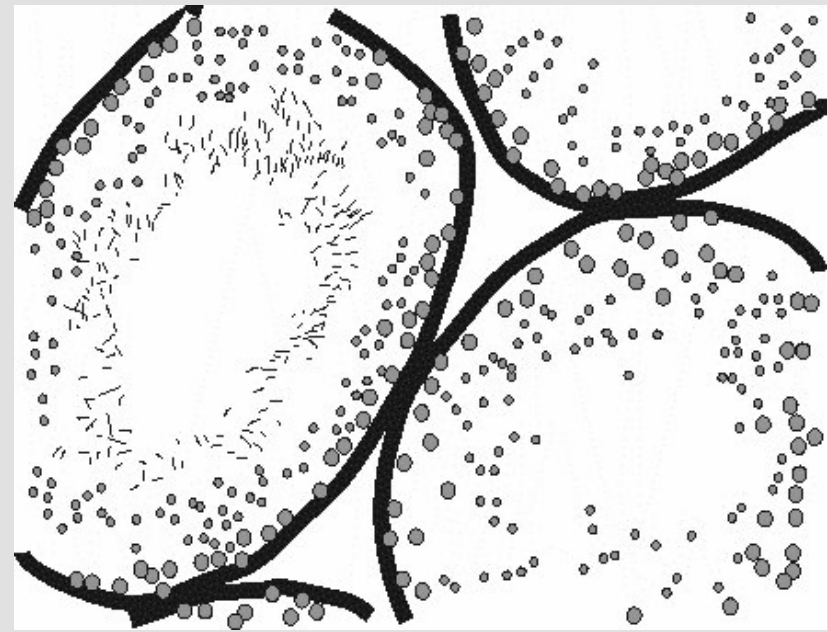
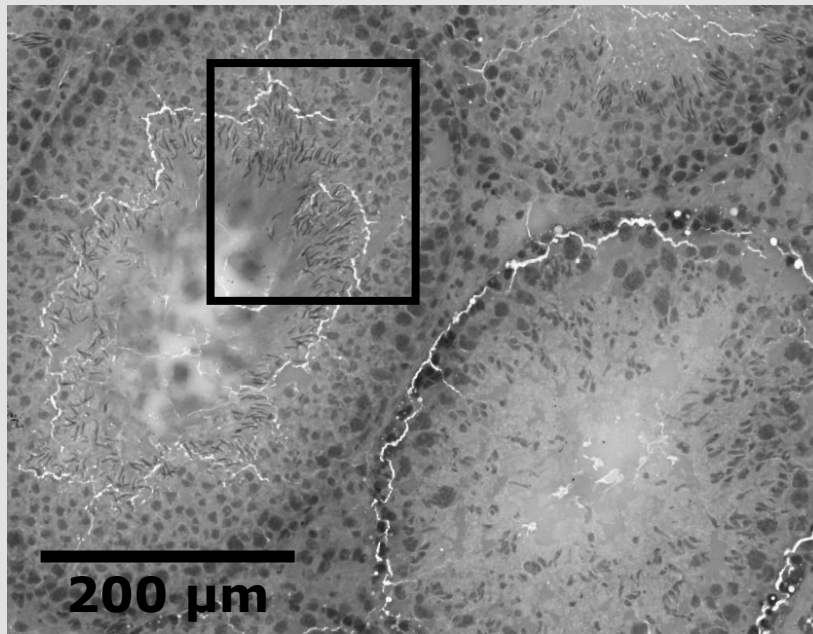
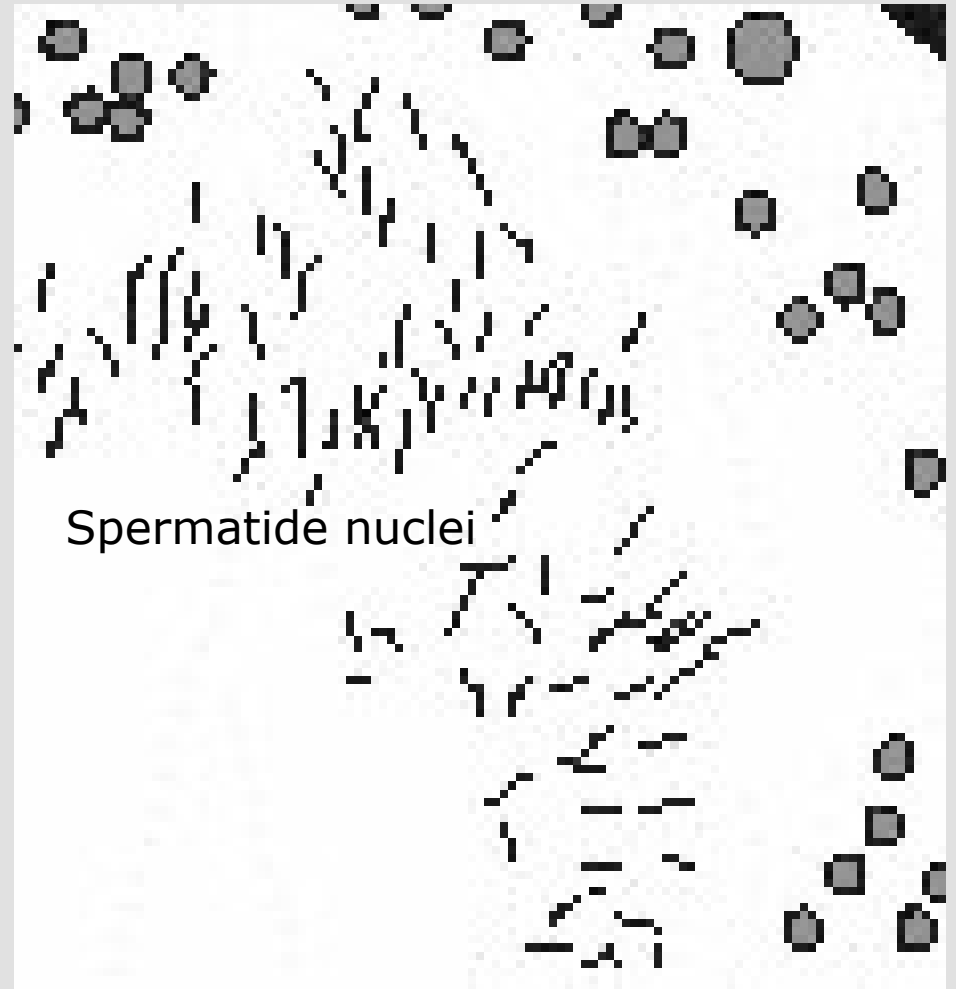
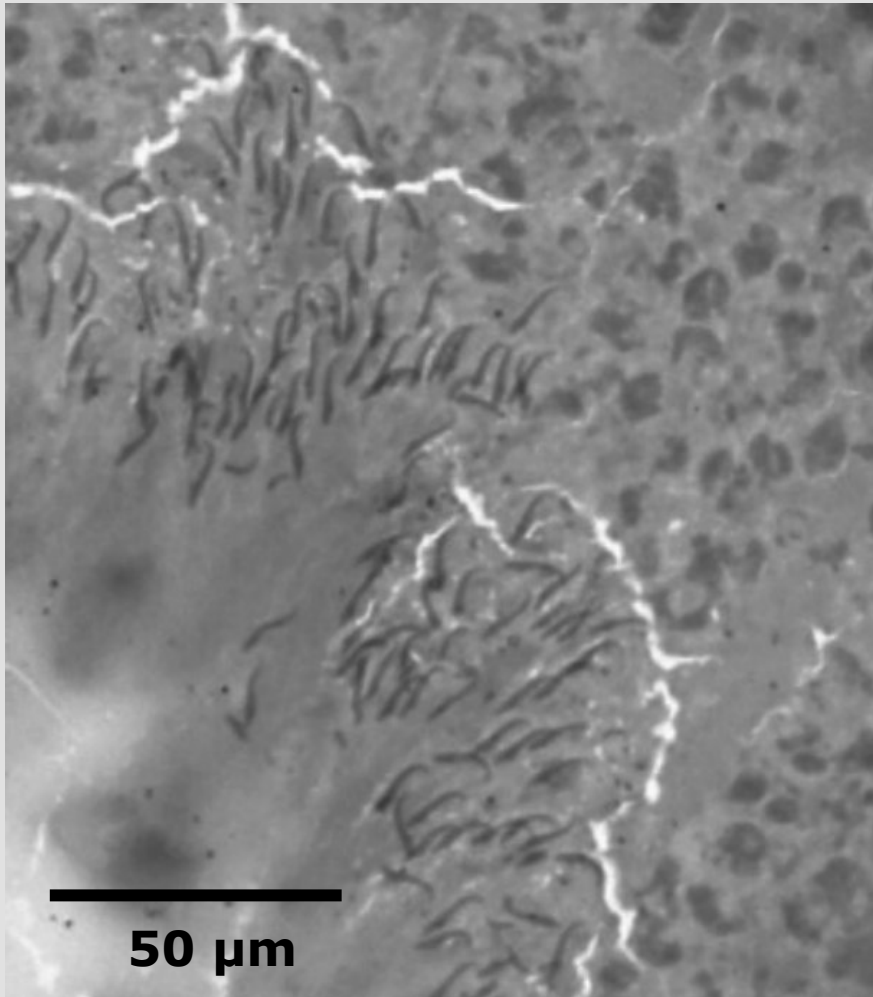


Image Resolution: 20 μm

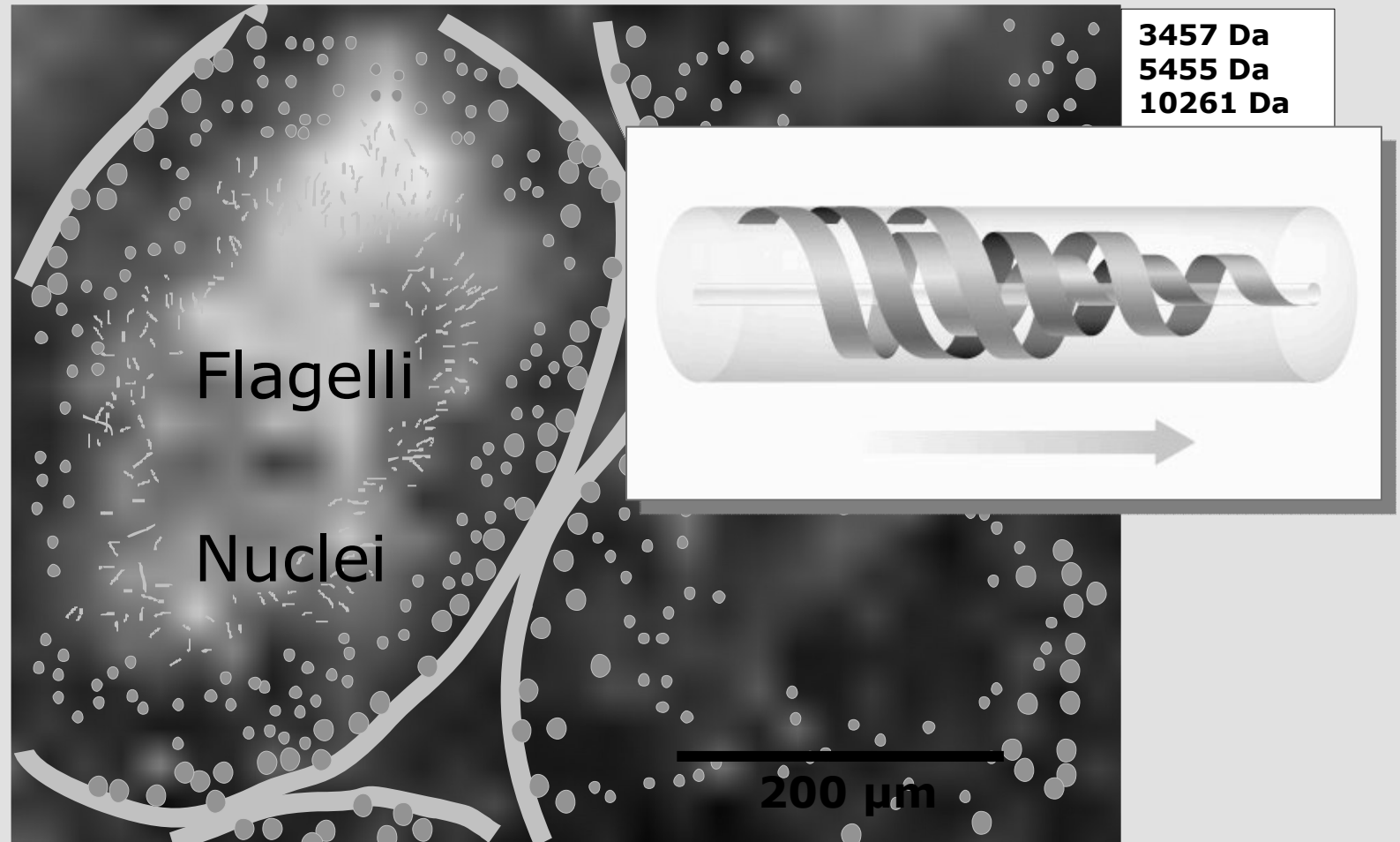
# H&E Stain of Tubuli from Rat Testis





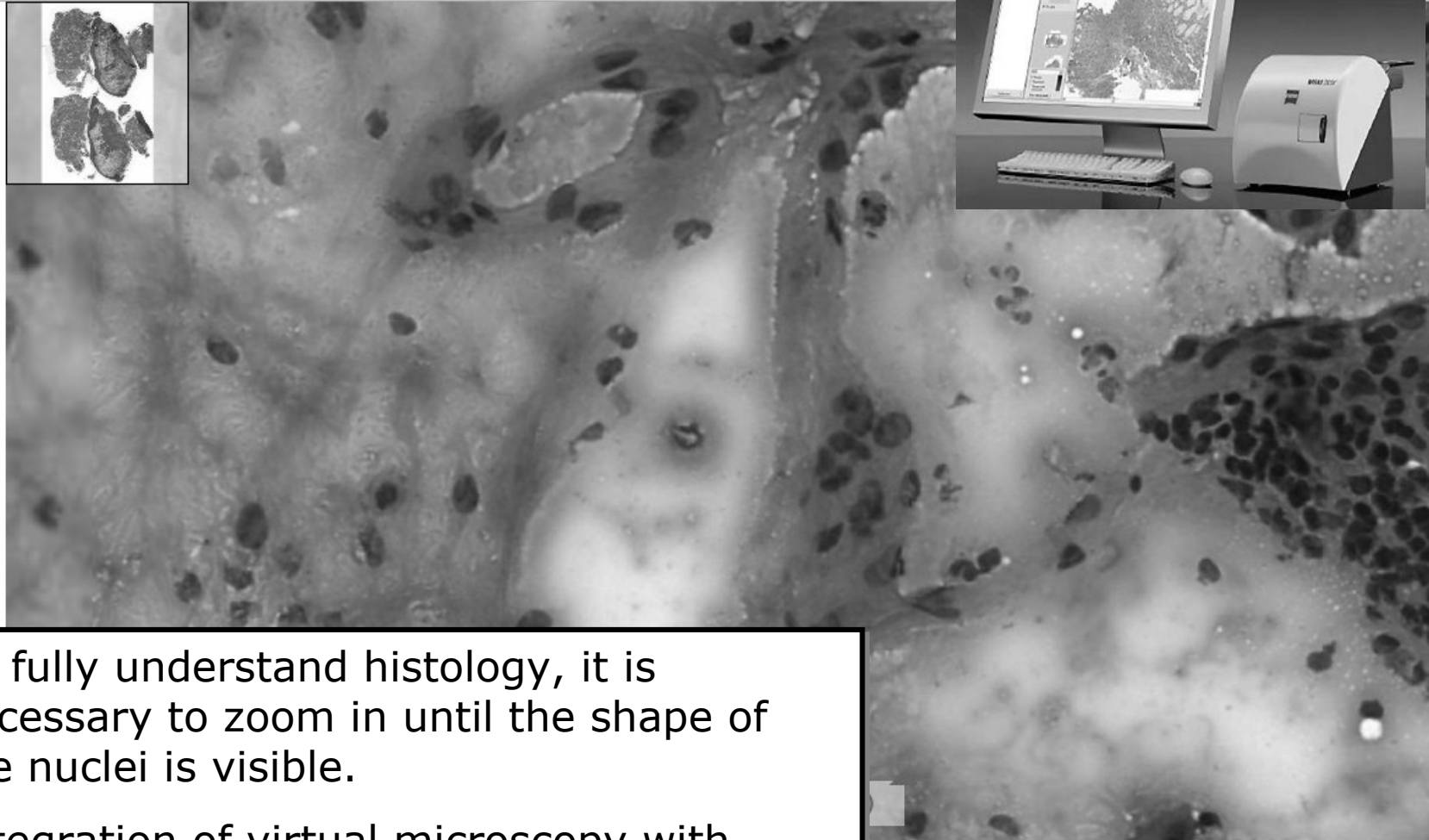
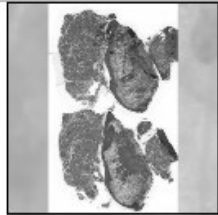
# Molecular Correlation with Tubular Structure

20  $\mu\text{m}$  Lateral Resolution



- **Tubuli filled with mature spermatides can be specifically visualized by high resolution MALDI imaging.**
- **Several proteins indicate the maturation process in these respective tubuli.**

# Virtual Microscopy on Zeiss Mirax Scanner - the GoogleEarth Approach to Molecular Histology

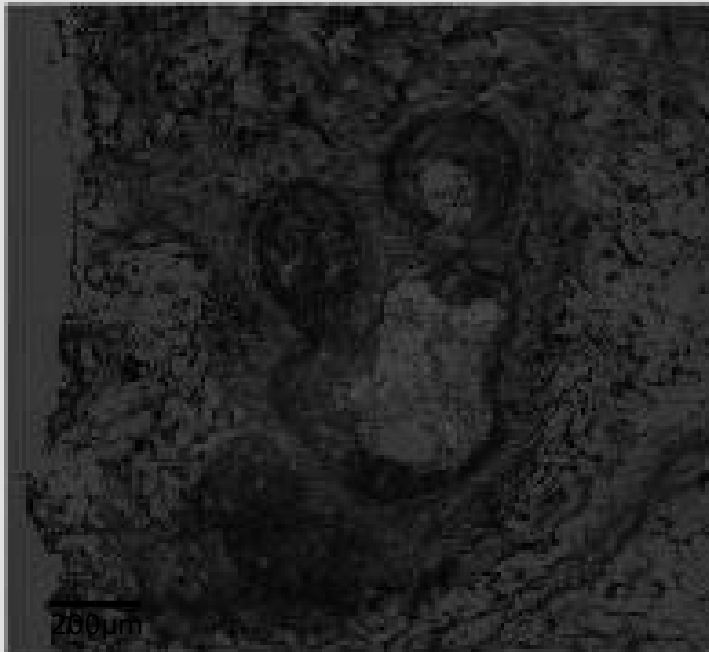


To fully understand histology, it is necessary to zoom in until the shape of the nuclei is visible.

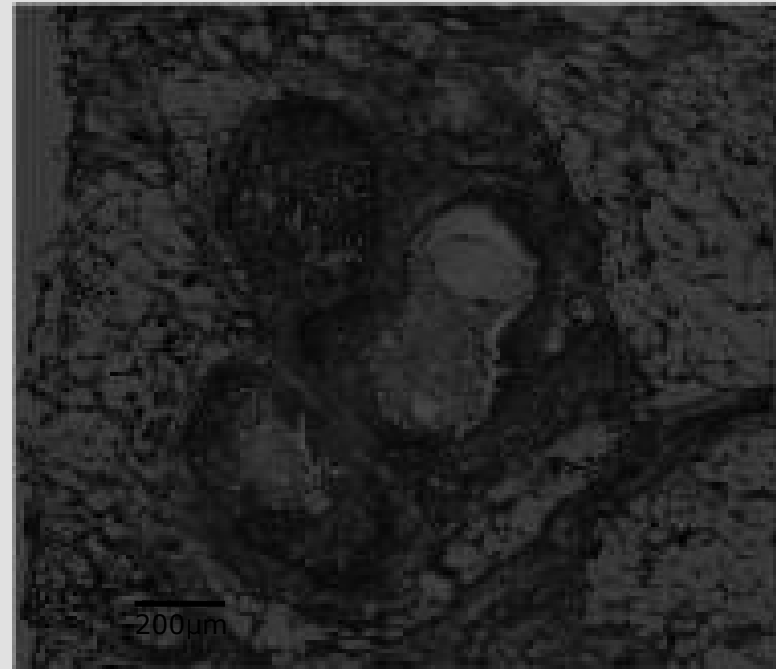
Integration of virtual microscopy with MALDI imaging will accelerate the field.

# Correlation of MALDI Image with Histology

**MALDI Image and H&E Stain, breast cancer  
on serial section**

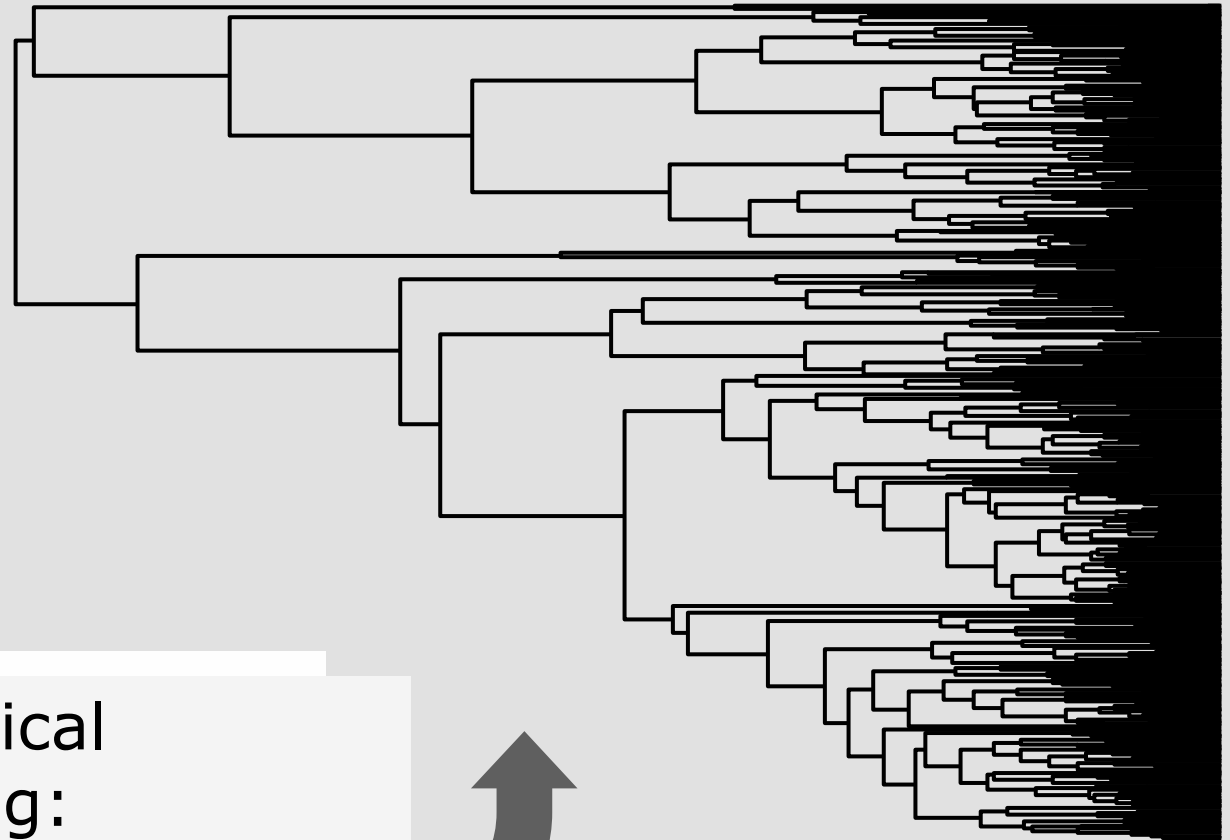
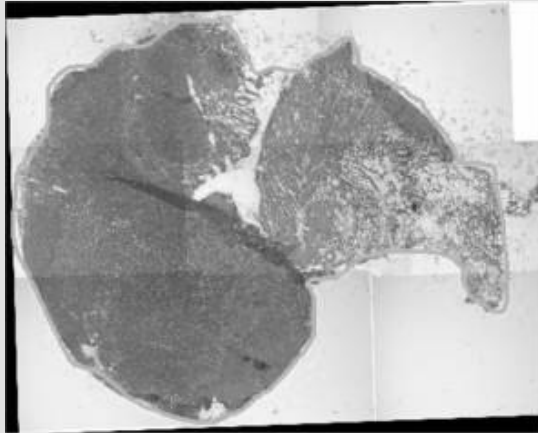


**on same section**



**Same section** allows to match MALDI image and tissue post-staining micro-photographies

# Hierarchical Clustering of Imaging Spectra



Hierarchical  
clustering:  
Unsupervised  
classification

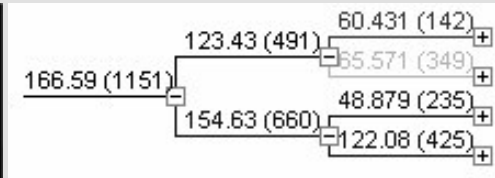
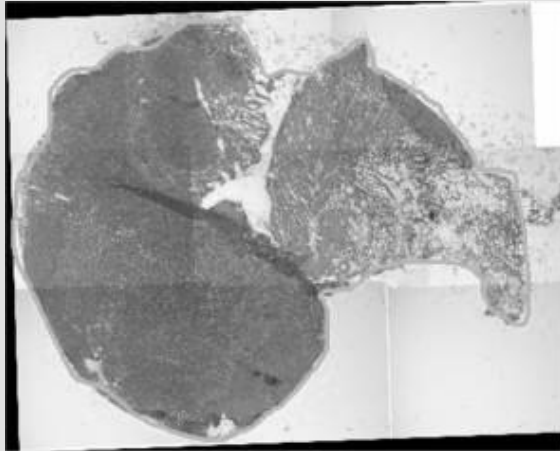


4000 6000 8000 10000  
nm

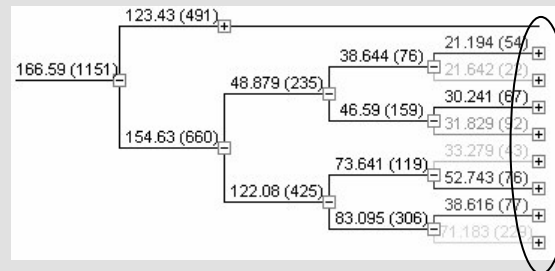
# Unsupervised Detection of Tissue Types

*human breast cancer biopsy*

C



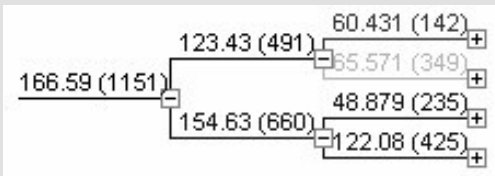
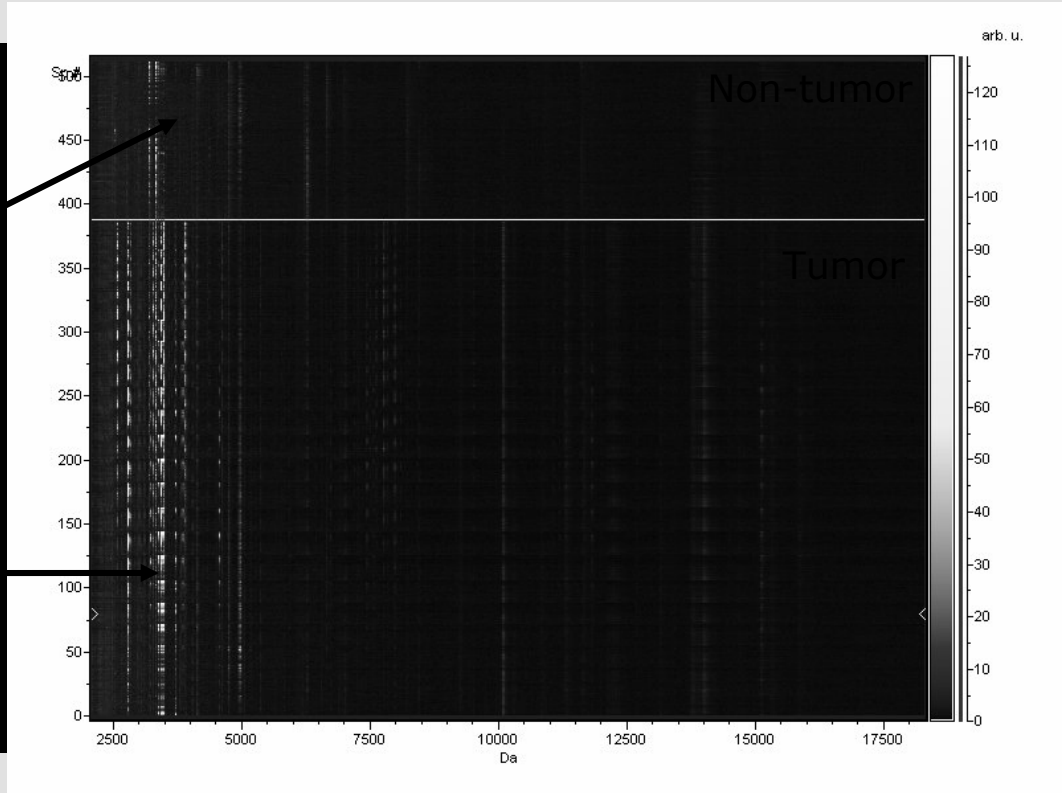
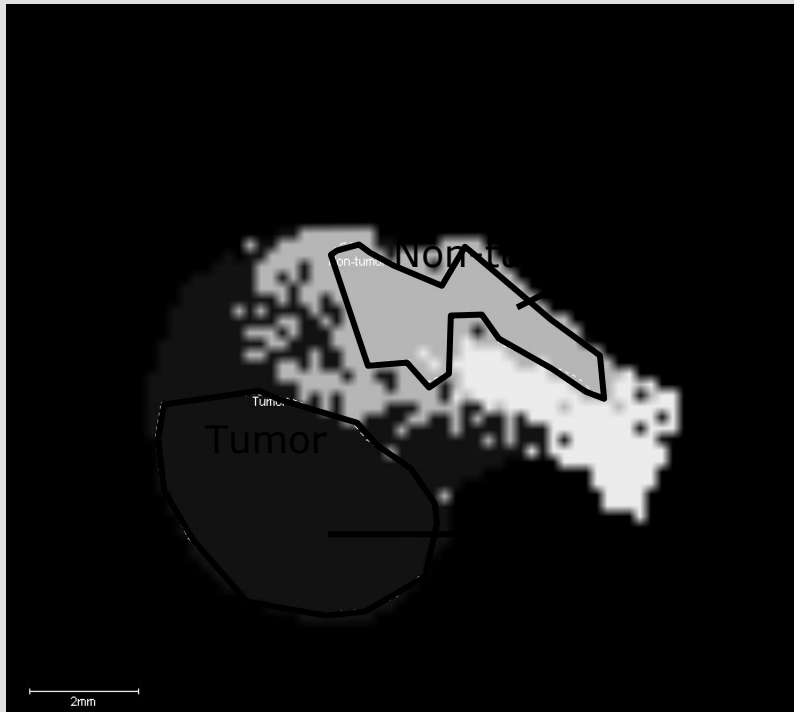
Blue = Tumor



Different Clones?  
Different metabolism?  
Random differences?

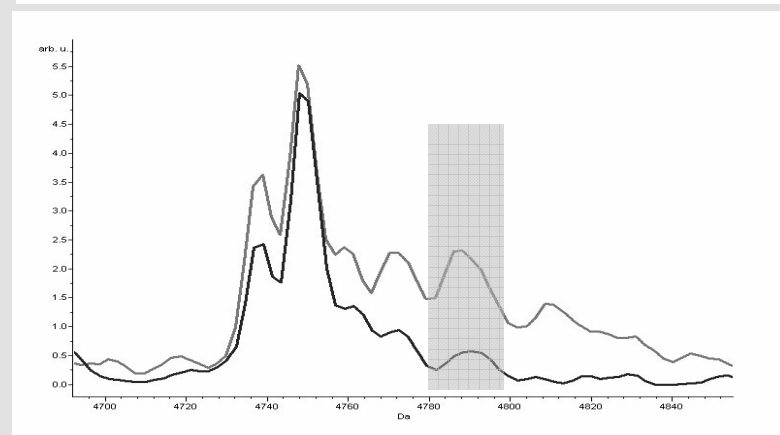
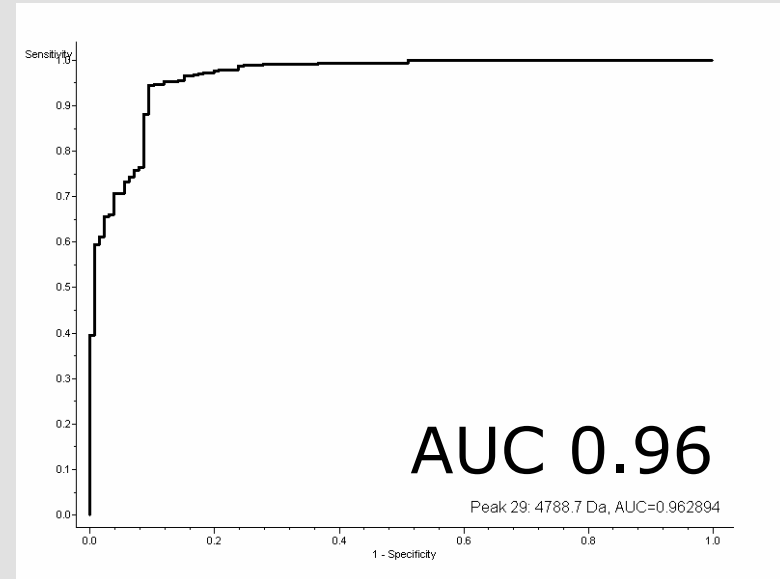
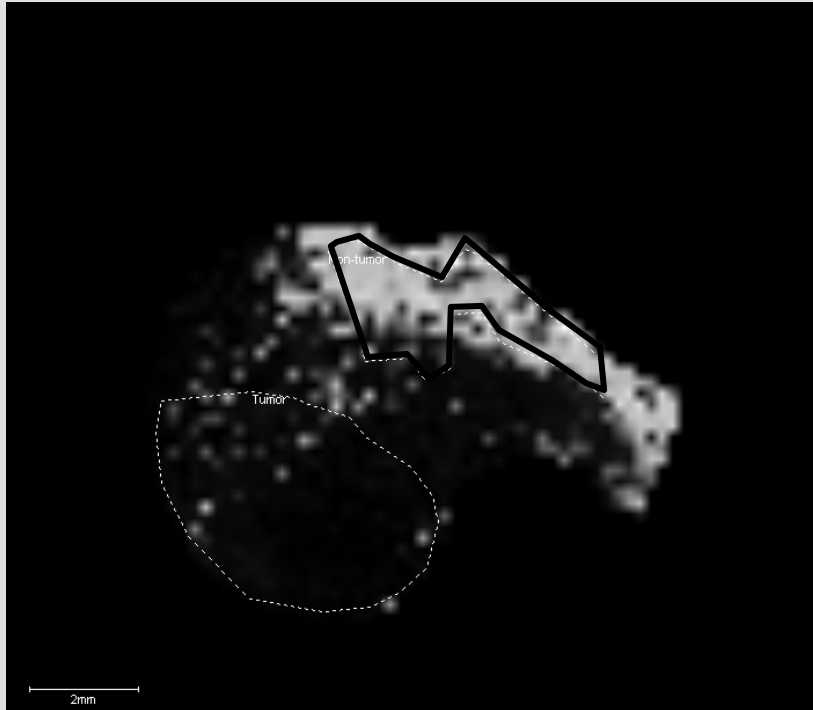
**>LCM>Protein ID**

# From Clusters to Correlate Molecular Species

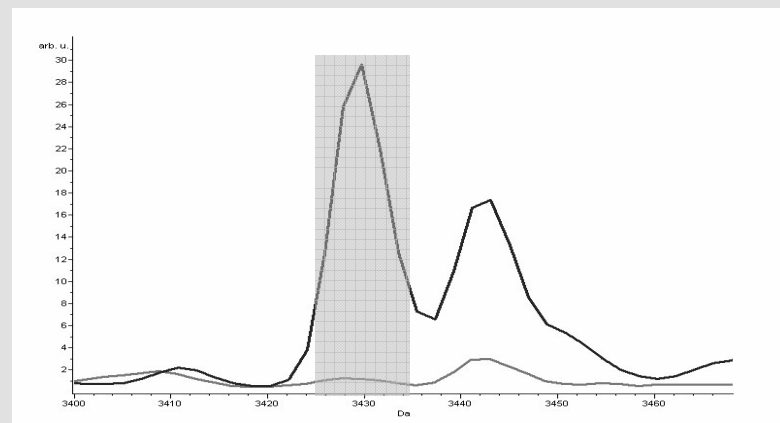
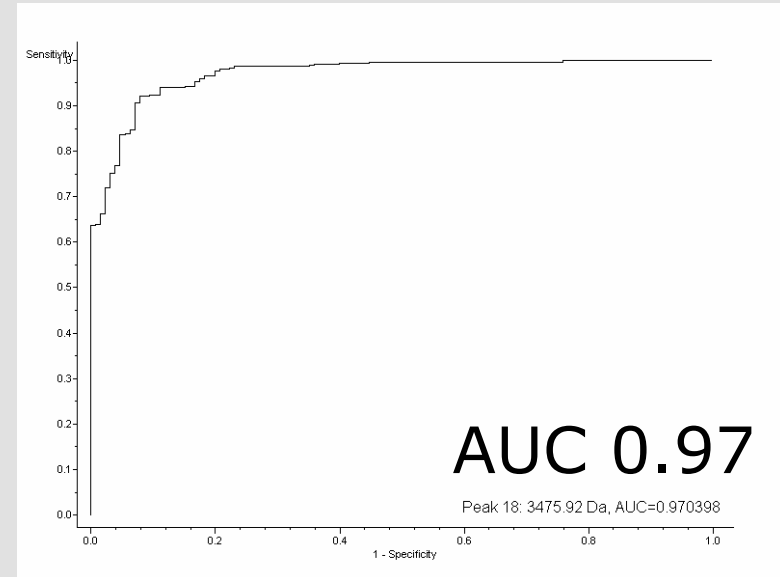
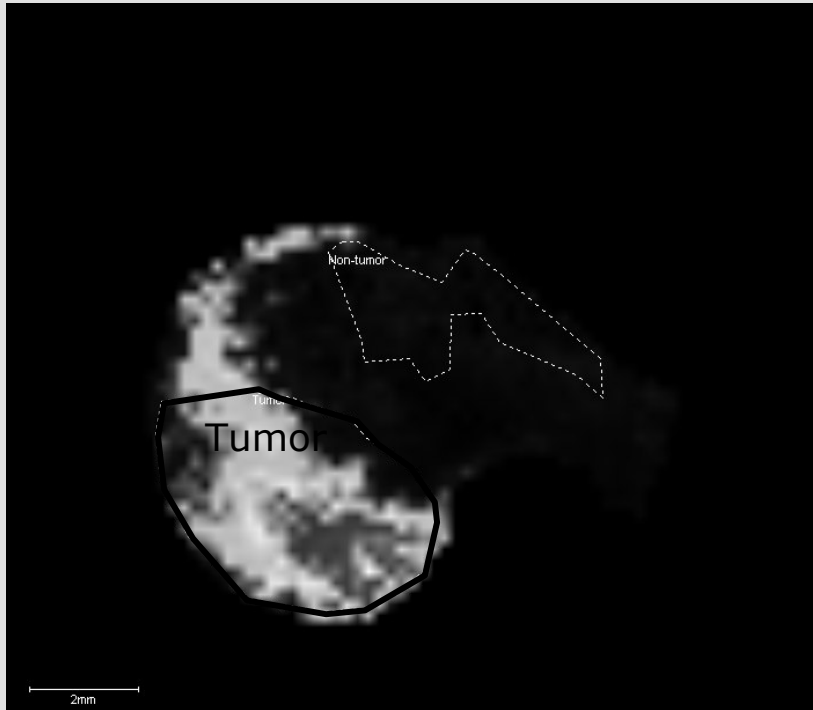


Gel-like representation of 500 spectra from tumor and control

# m/z 4788: Specific for **Non-Tumor** Tissue

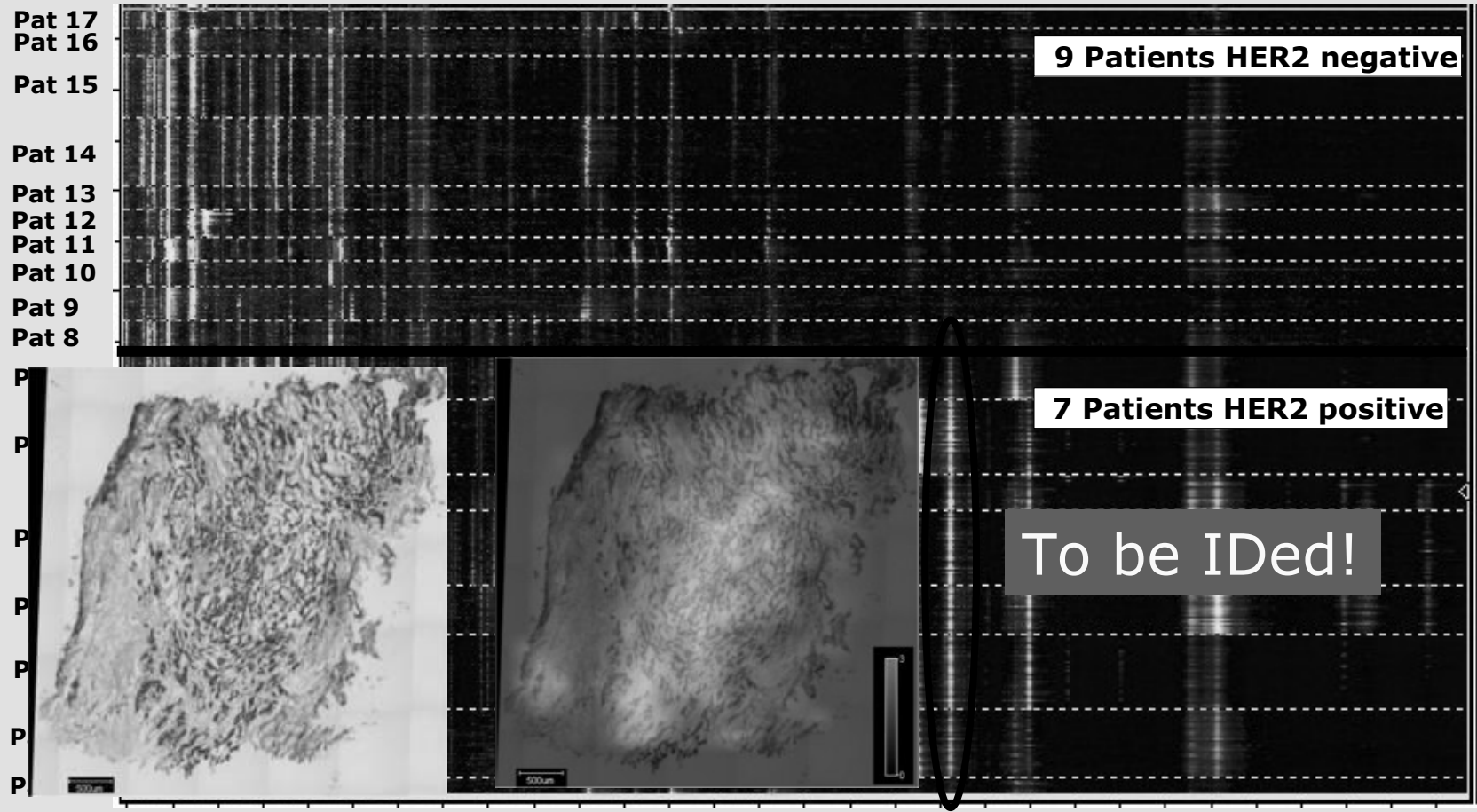


# m/z 3429: Potential **Tumor** Marker

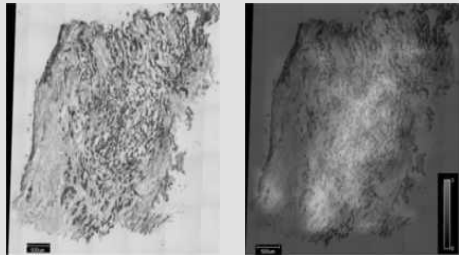




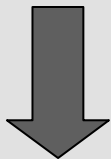
# HER-2 Positive Breast Cancer: Biomarker ID



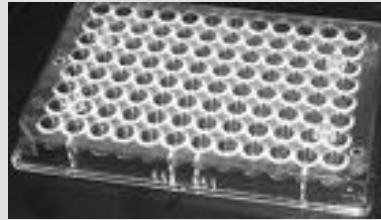
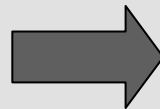
# From Tissue to Spectrum: Biomarker Top-Down ID Workflow



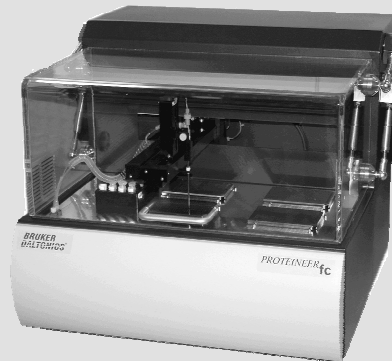
MALDI image & Histological info  
→ Potential biomarker m/z



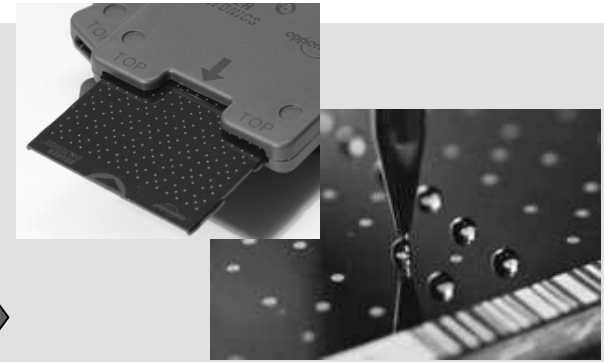
Tissue lysis / diafiltration



Fractions in 96-well plate  
4,0µl/well (2x)



Offline-LC separation with mRP  
High Recovery Protein Column



LC-MALDI separation on  
PAC Target (0.5 µl/spot)  
for peak localization

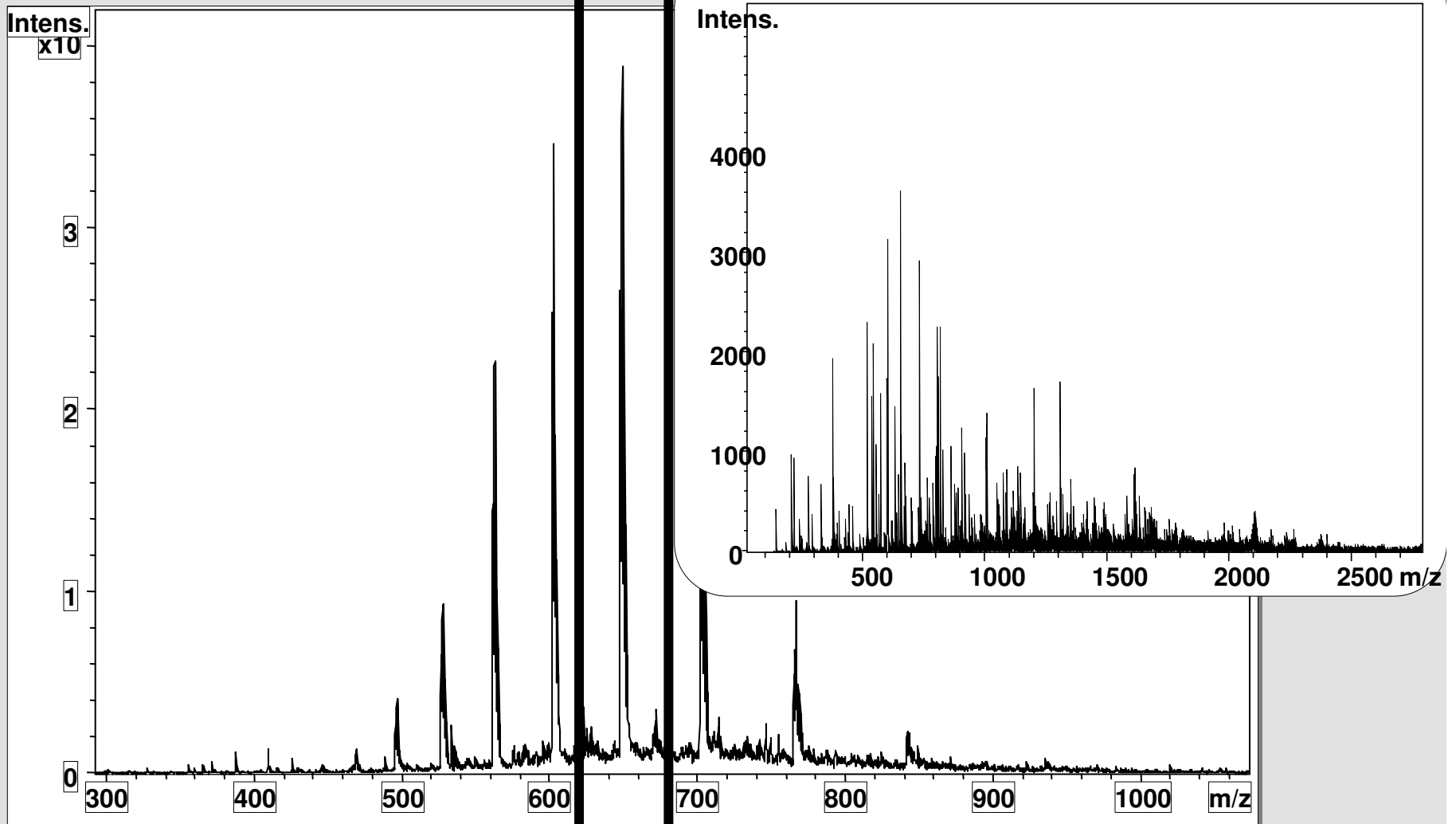


**amazon spherical trap**

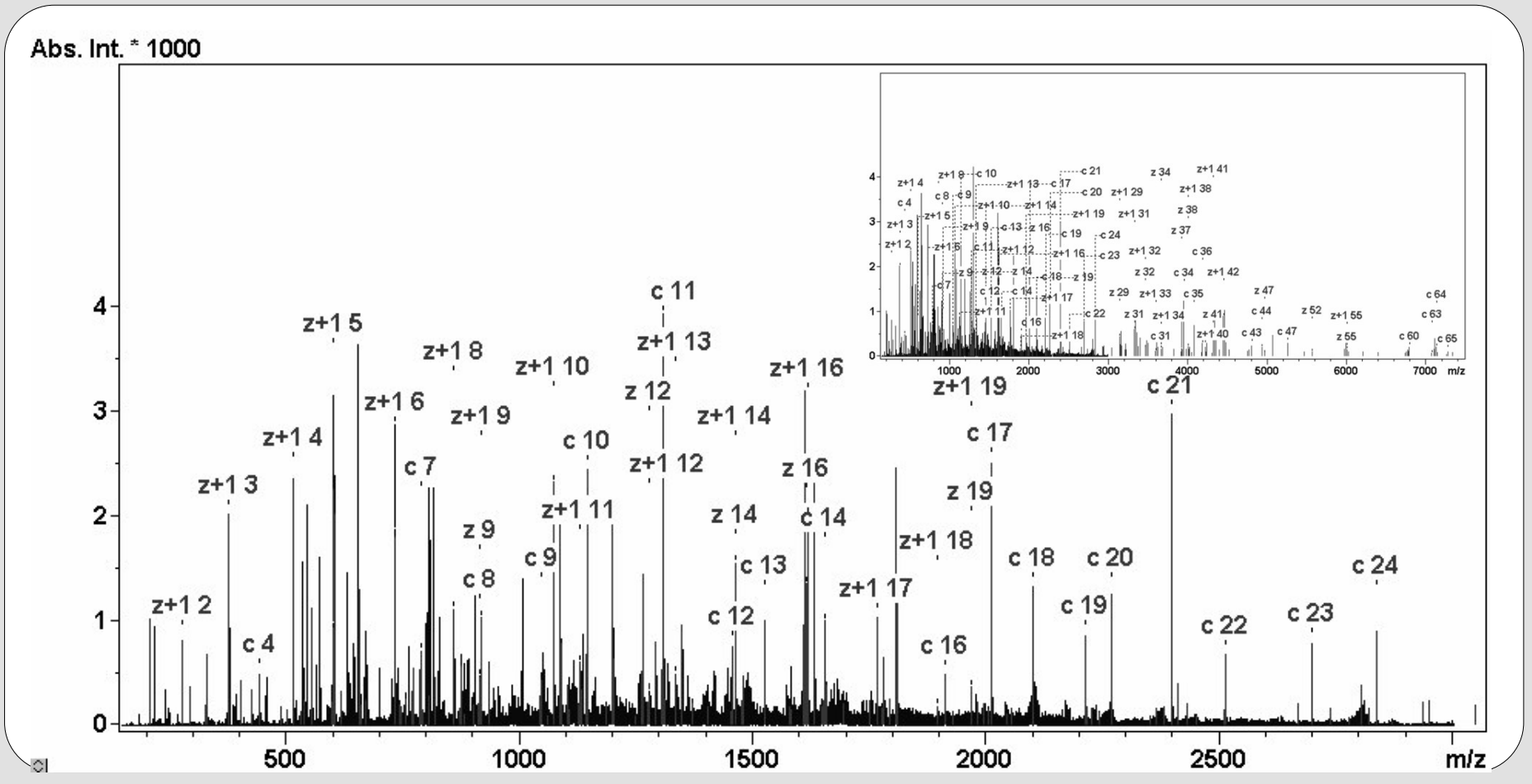


3 combined fractions  
measured with **ETD/PTR**

# ESI-Trap MS Spectrum of Marker and ETD/PTR Top-Down Sequence Analysis



# Top-Down ID of Marker Candidate with Mascot: Score 113



Patent pending

# solarix

**Ultimate Mass Accuracy**

**Unbeaten Resolution**

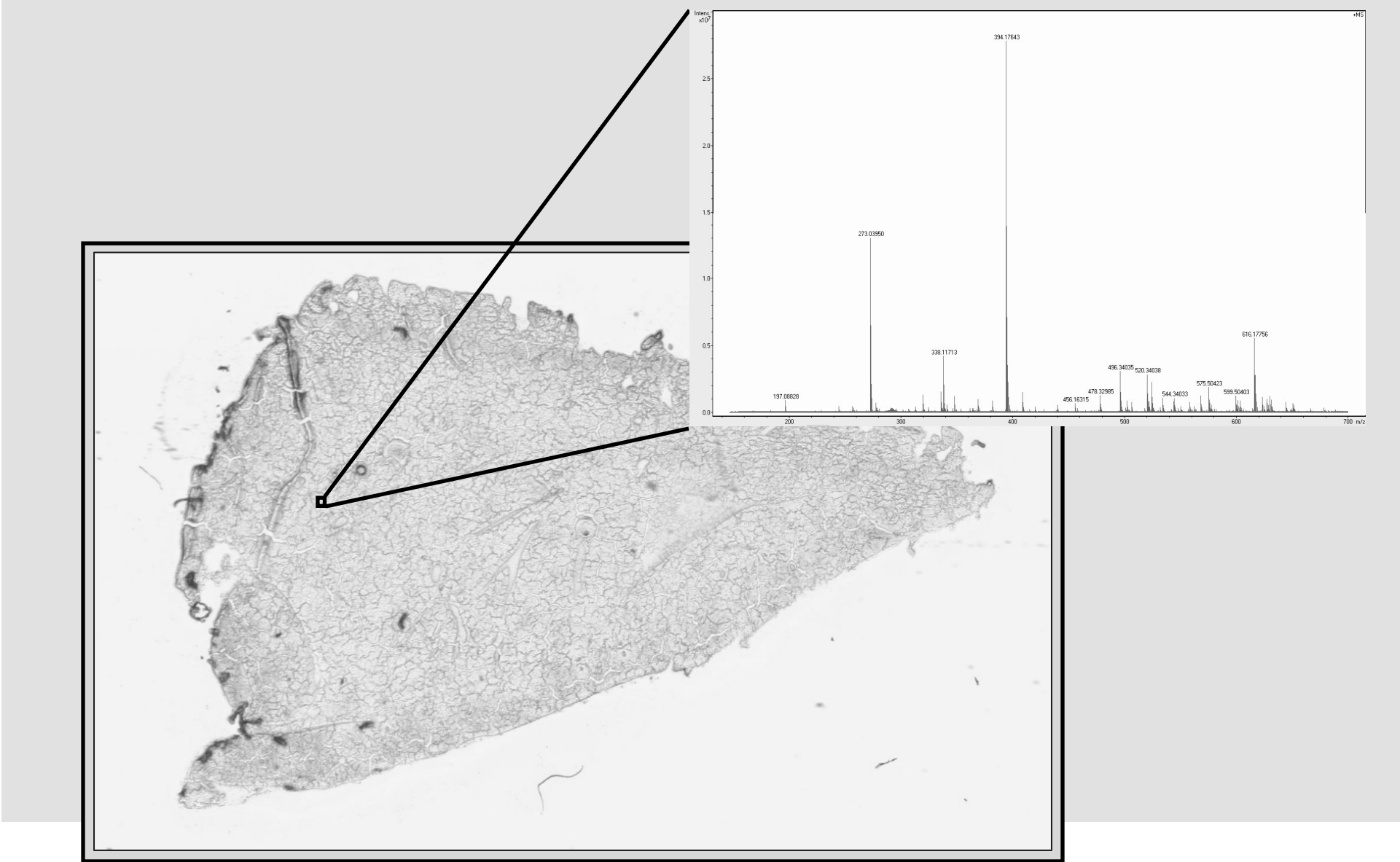
**DualSource for  
ESI and MALDI,  
Cocurrent ESI & MALDI  
operation**

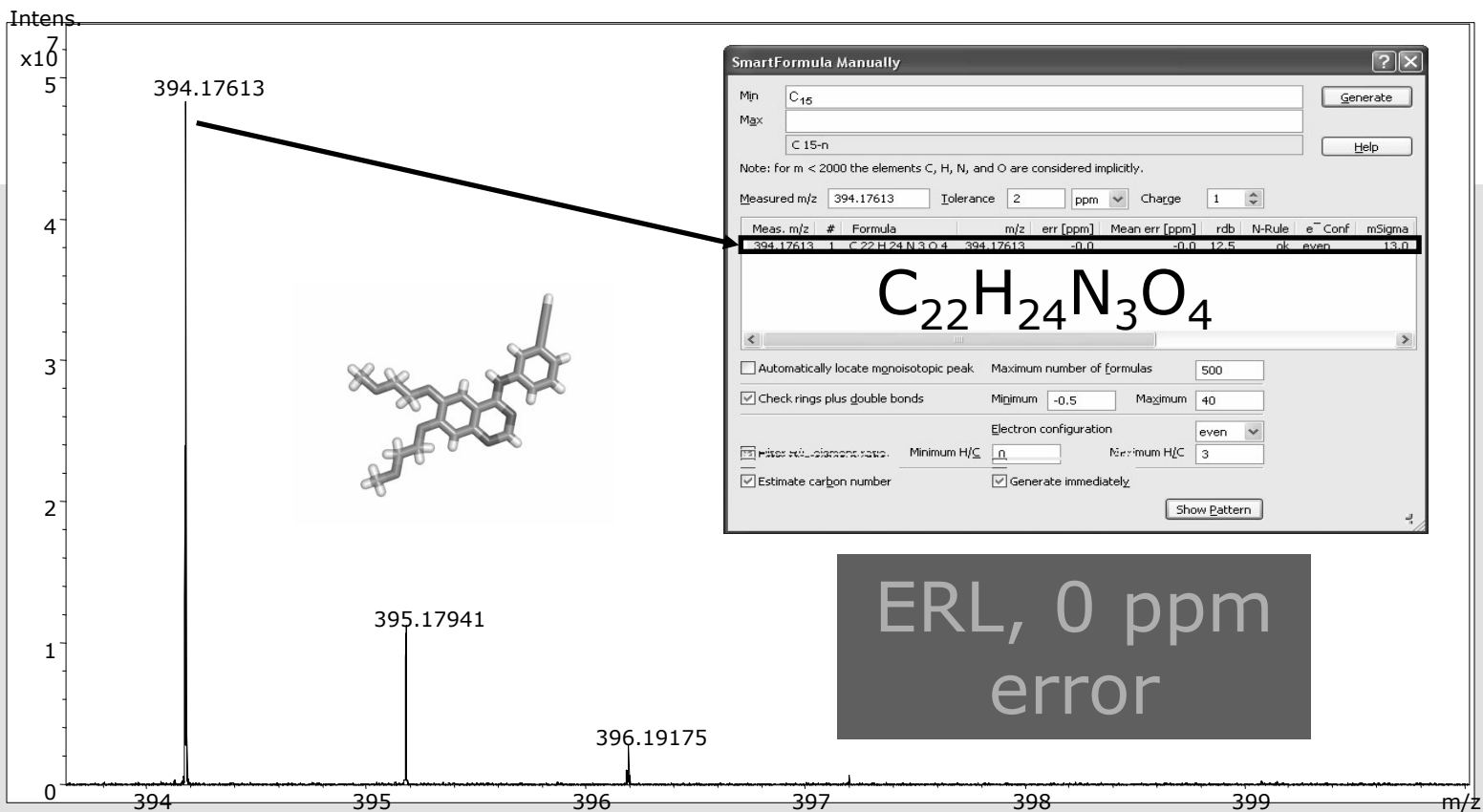
**ECD and ETD**

**CASI for low abundant  
species**



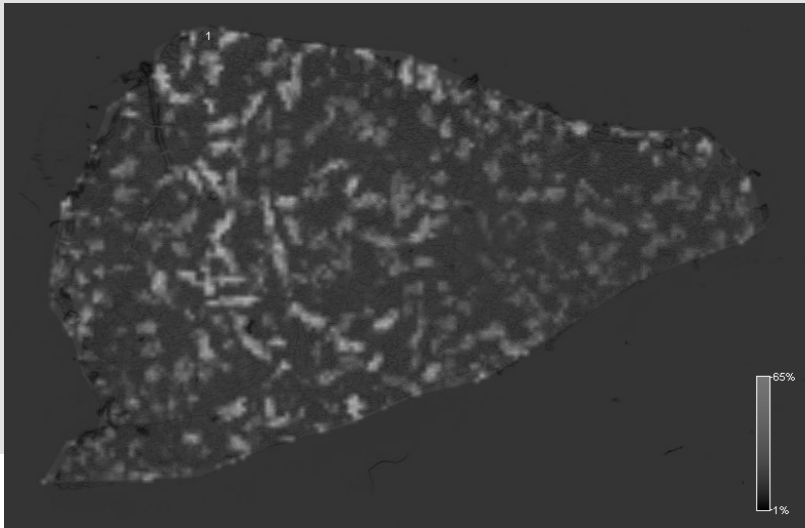
# Single MS spectrum obtained from liver tissue section

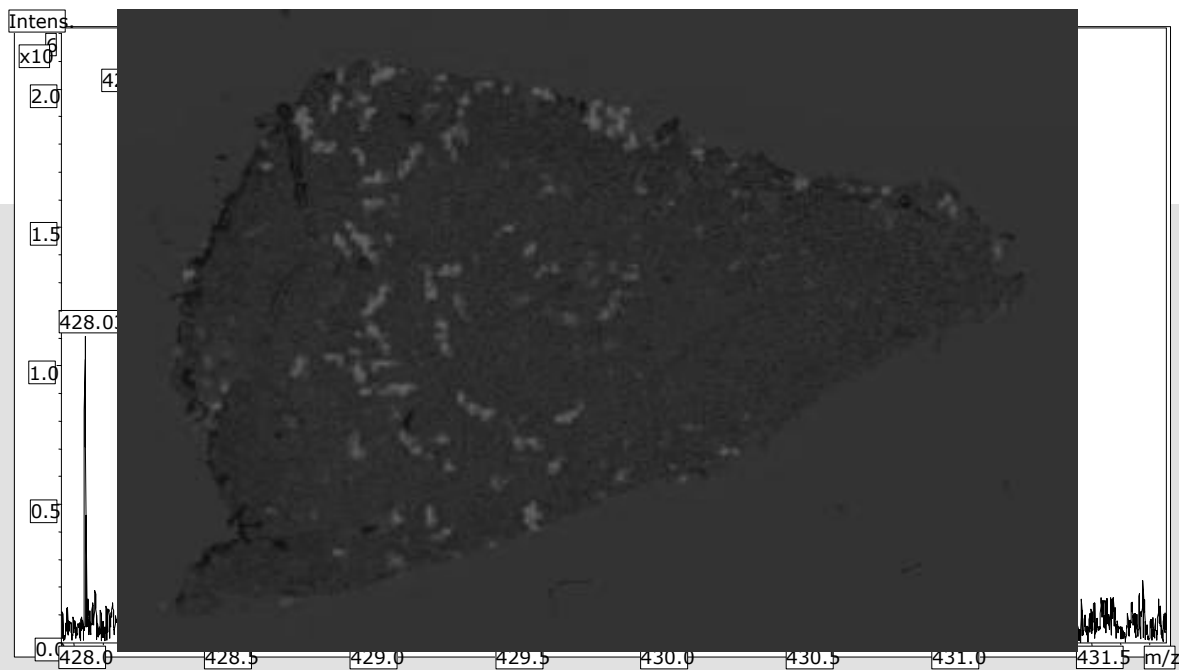




ERL, 0 ppm error

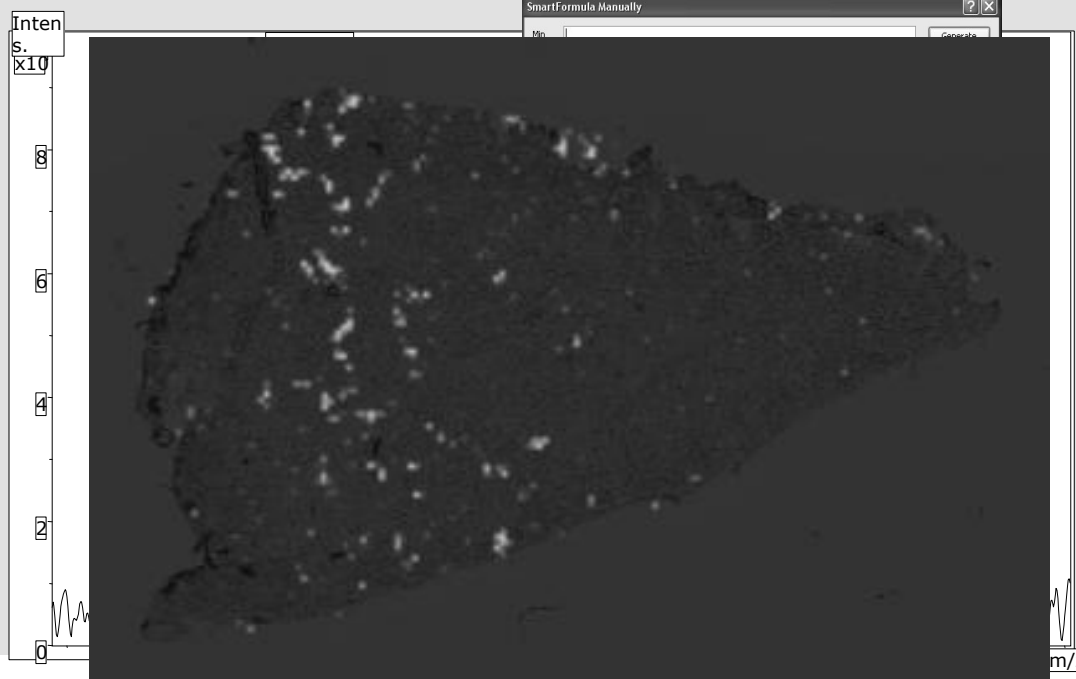
**Presence of erlotinib in liver section confirmed by SmartFormula**





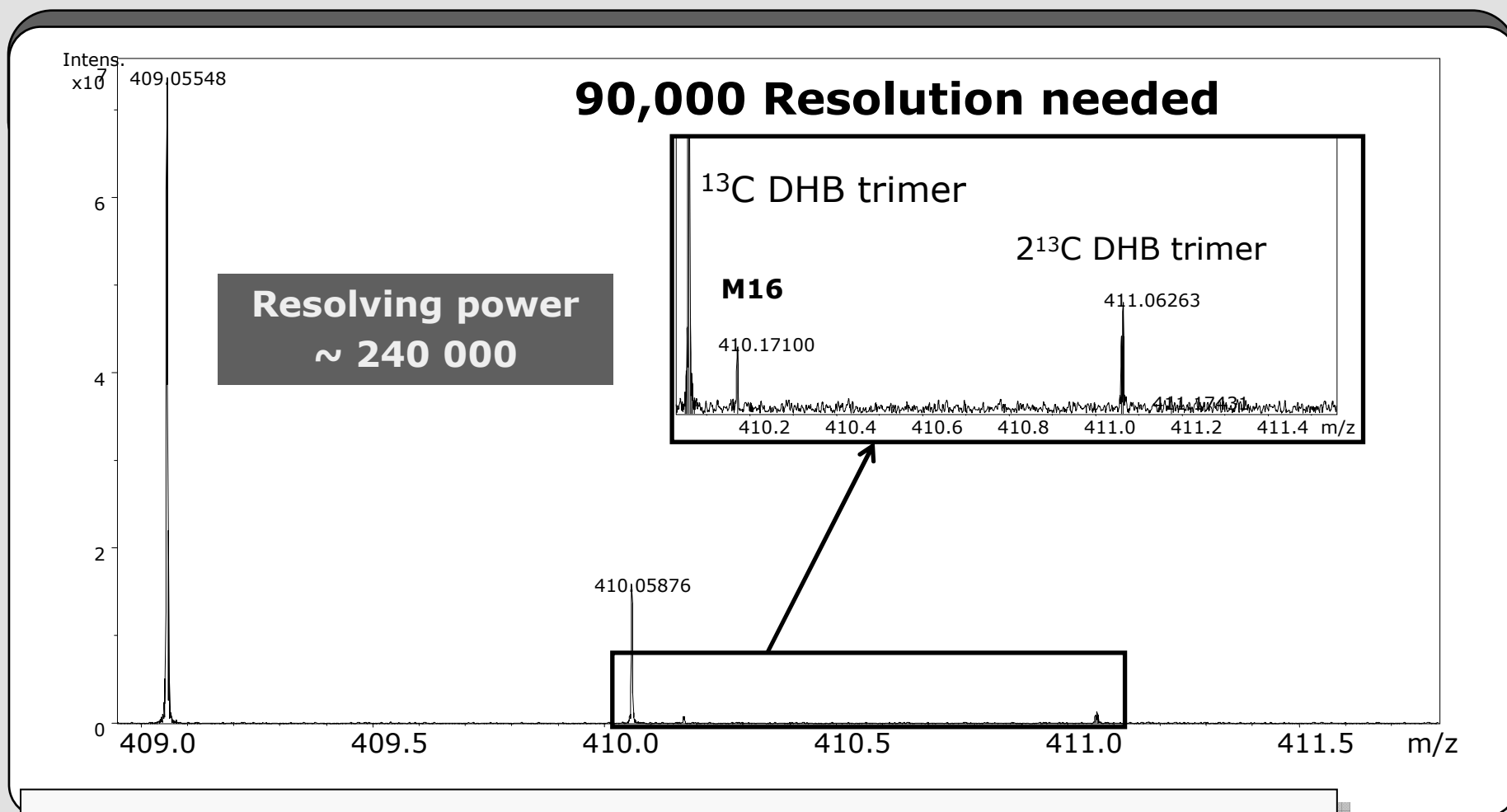
**M6—second most abundant first generation metabolite (pink)**

**M2—most abundant second generation metabolite (yellow)**



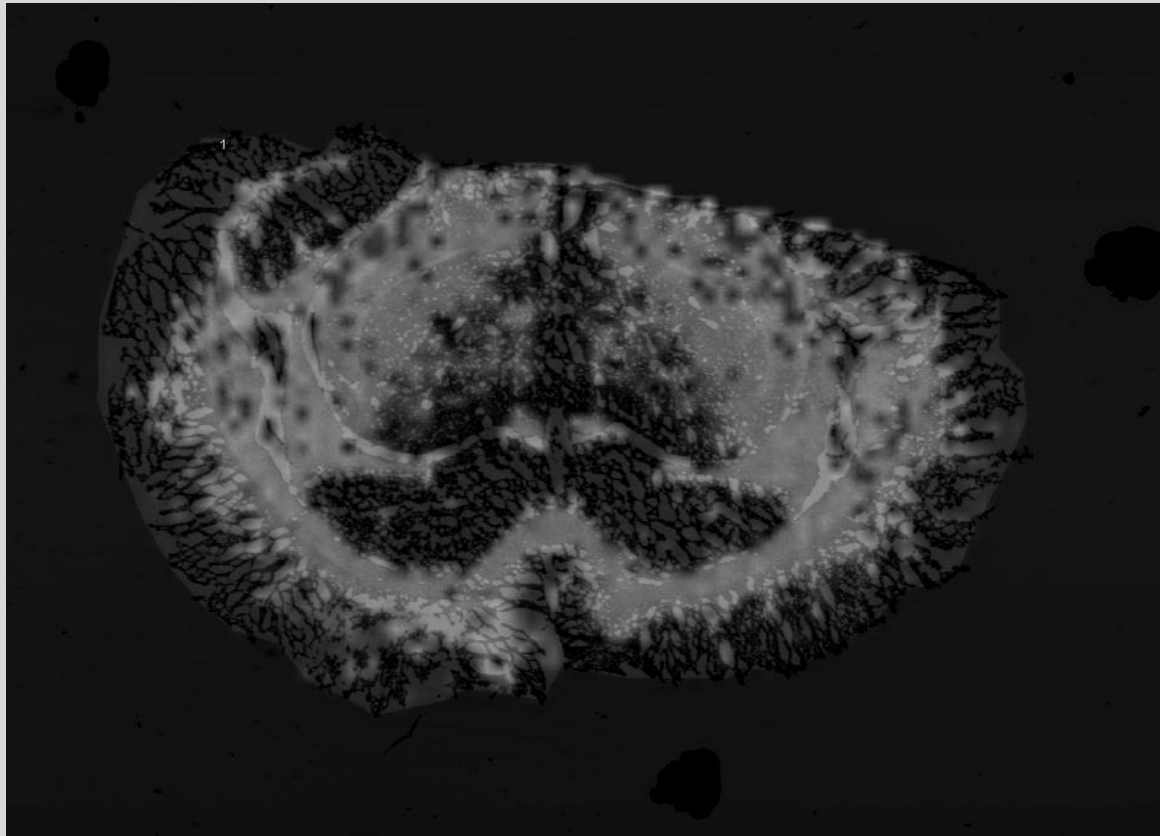


M16 detected even in the presence of nearly isobaric  $^{13}\text{C}$  peak from DHB matrix trimer.



**PMM-171, Kellersberger; Easterling; Kesari; Sauvageot; J. Agar; N. Agar**

# Identification of lipid markers in microwave vs. control rat brain tissue preparation

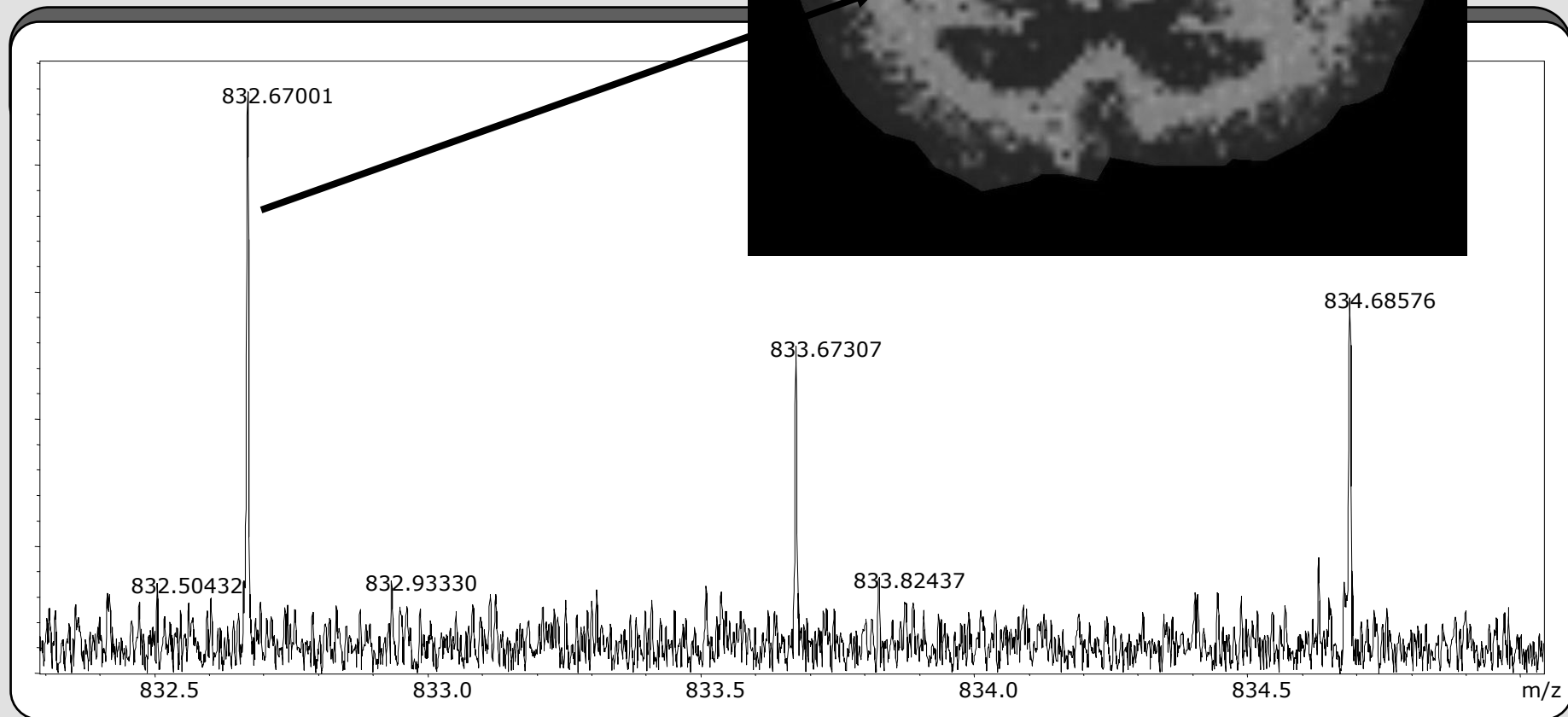
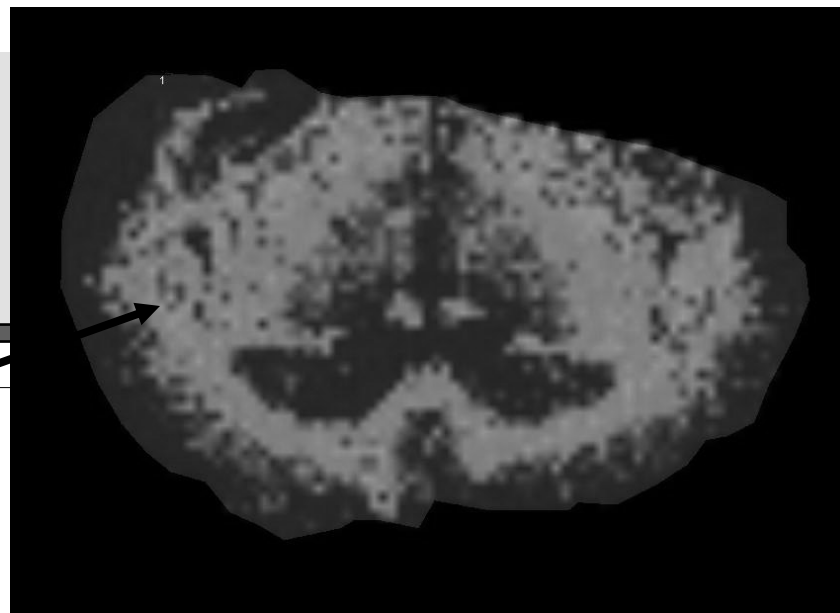


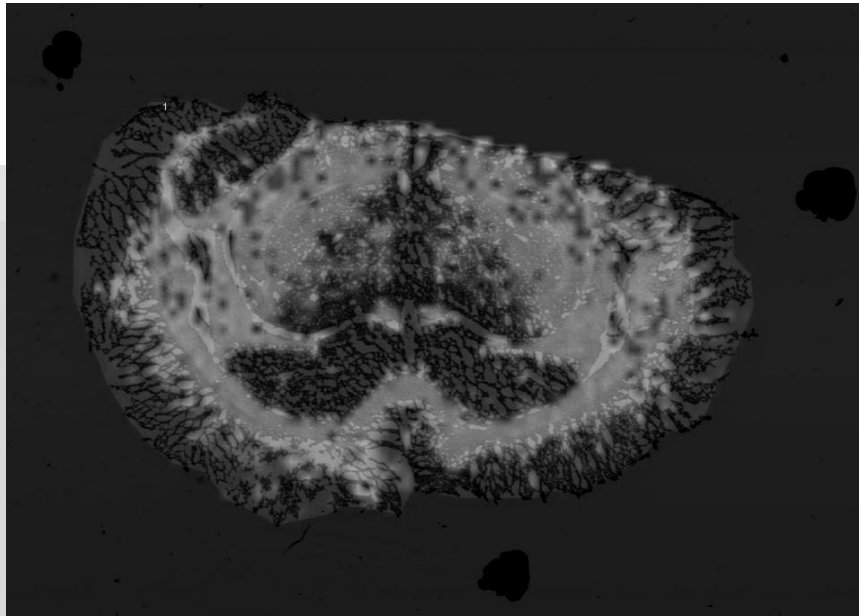
m/z 832.670 in microwaved rat brain, heatmap view

Samples courtesy of  
Dr. Erol Gulcicek  
Dr. Raimund Herzog  
Dr. Tu Lam  
Yale University  
Keck Laboratory

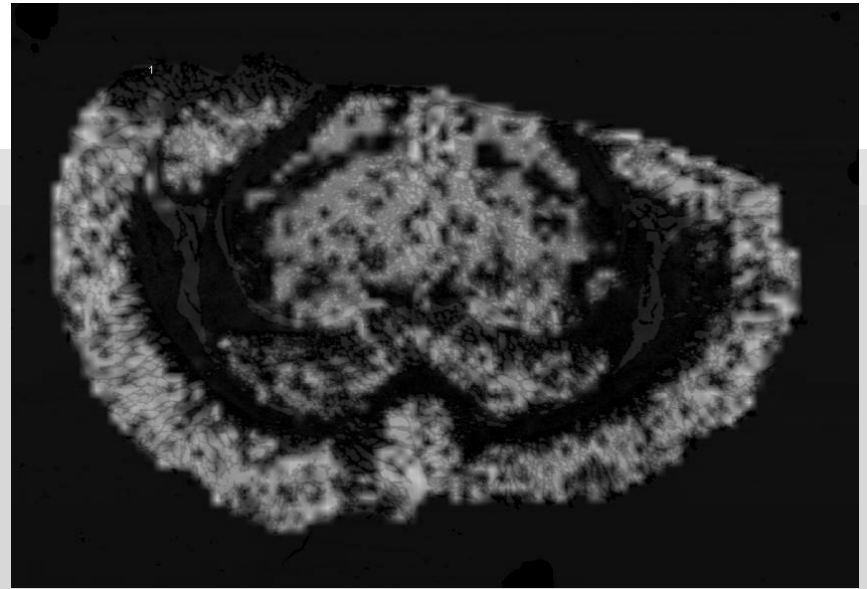


**Spectrum of  $m/z$   
832.670 in microwaved  
brain. Resolving power  
( $m/\Delta m$ ) =  $\sim 185\ 000$**

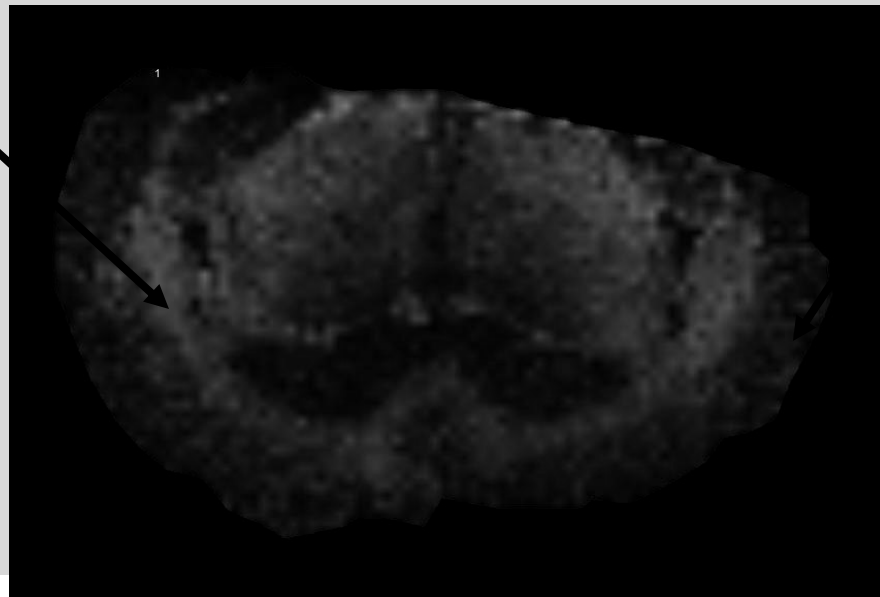




m/z 832.67 tracks to white matter



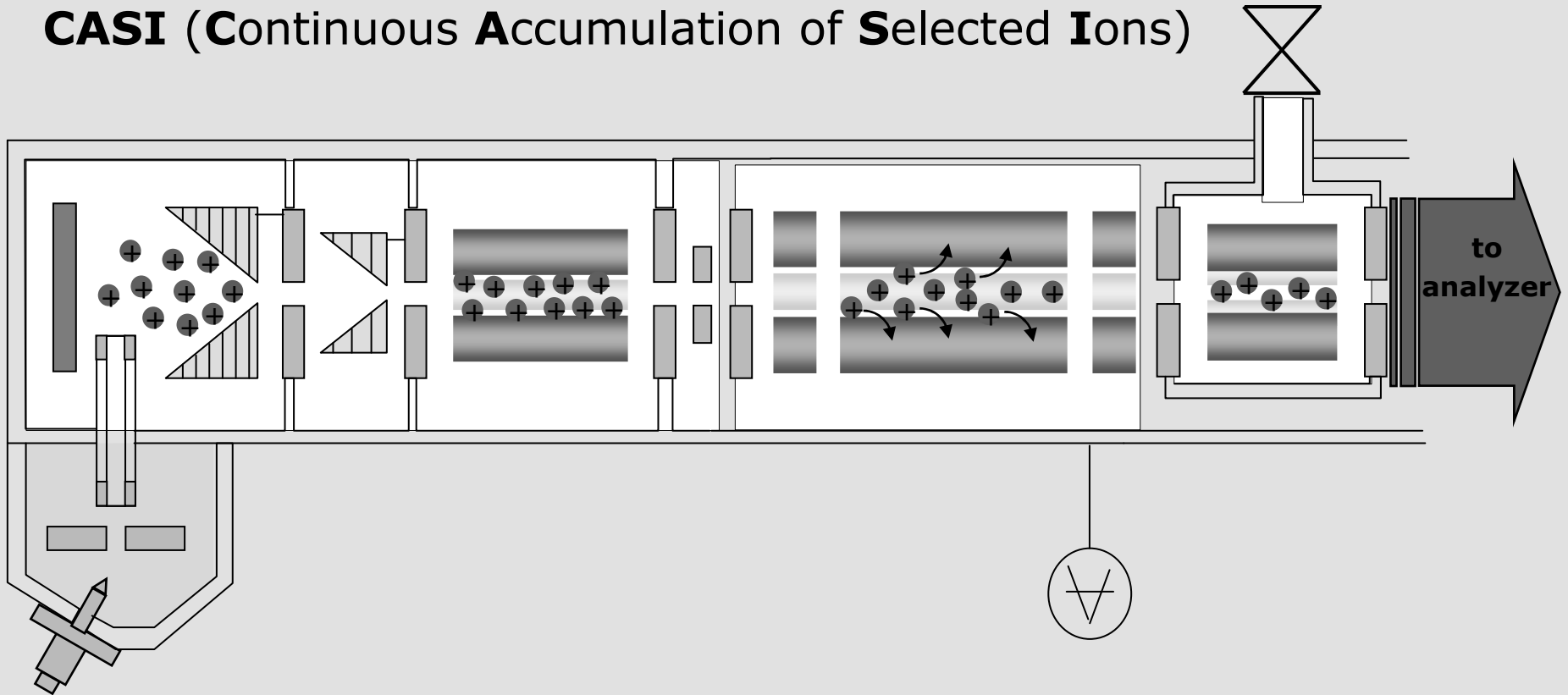
m/z 868.47 tracks to gray matter



# solarix

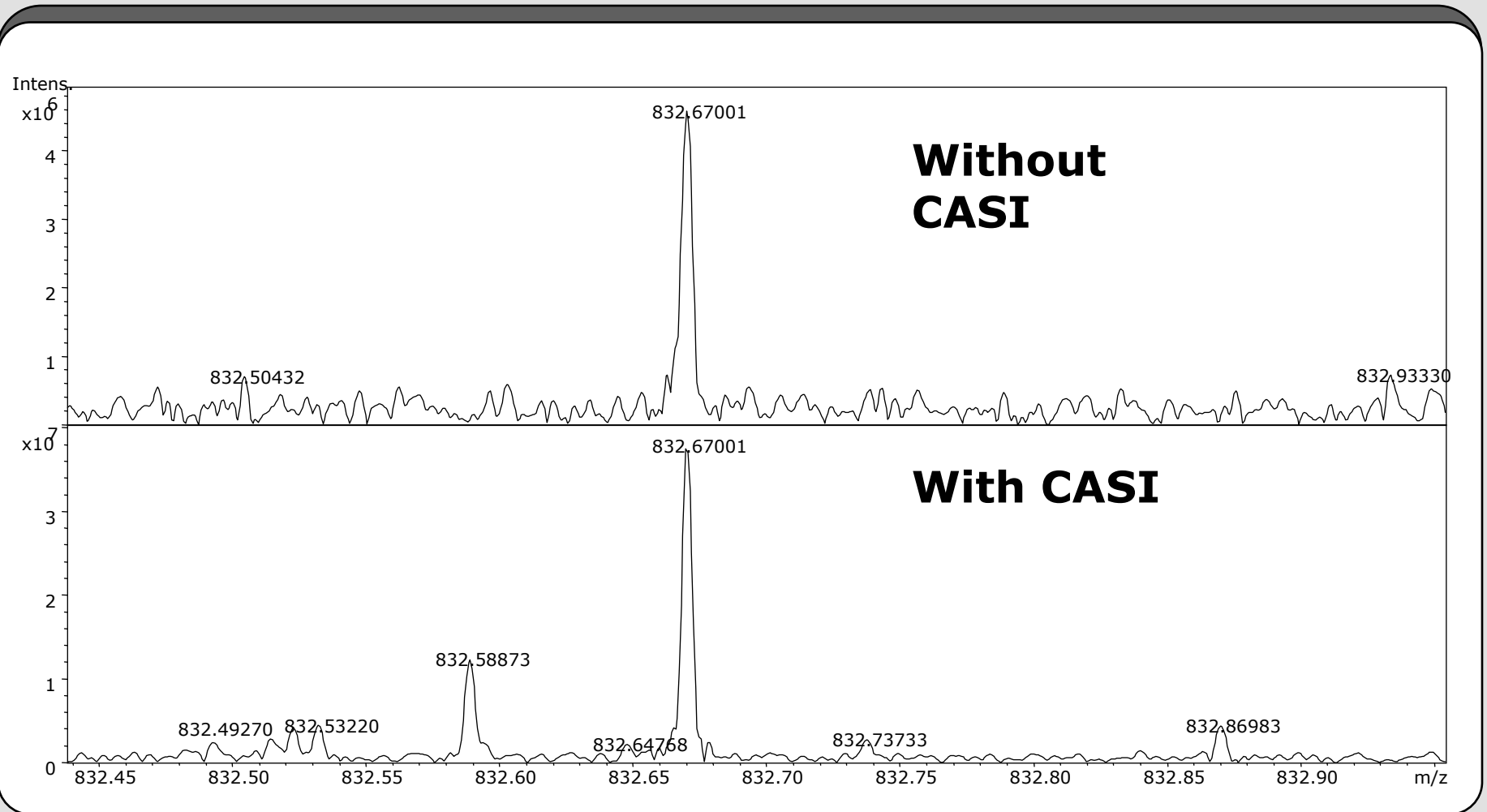
Petroleomics and MALDI Imaging

**CASI** (Continuous **A**ccumulation of **S**electe**d** **I**ons)

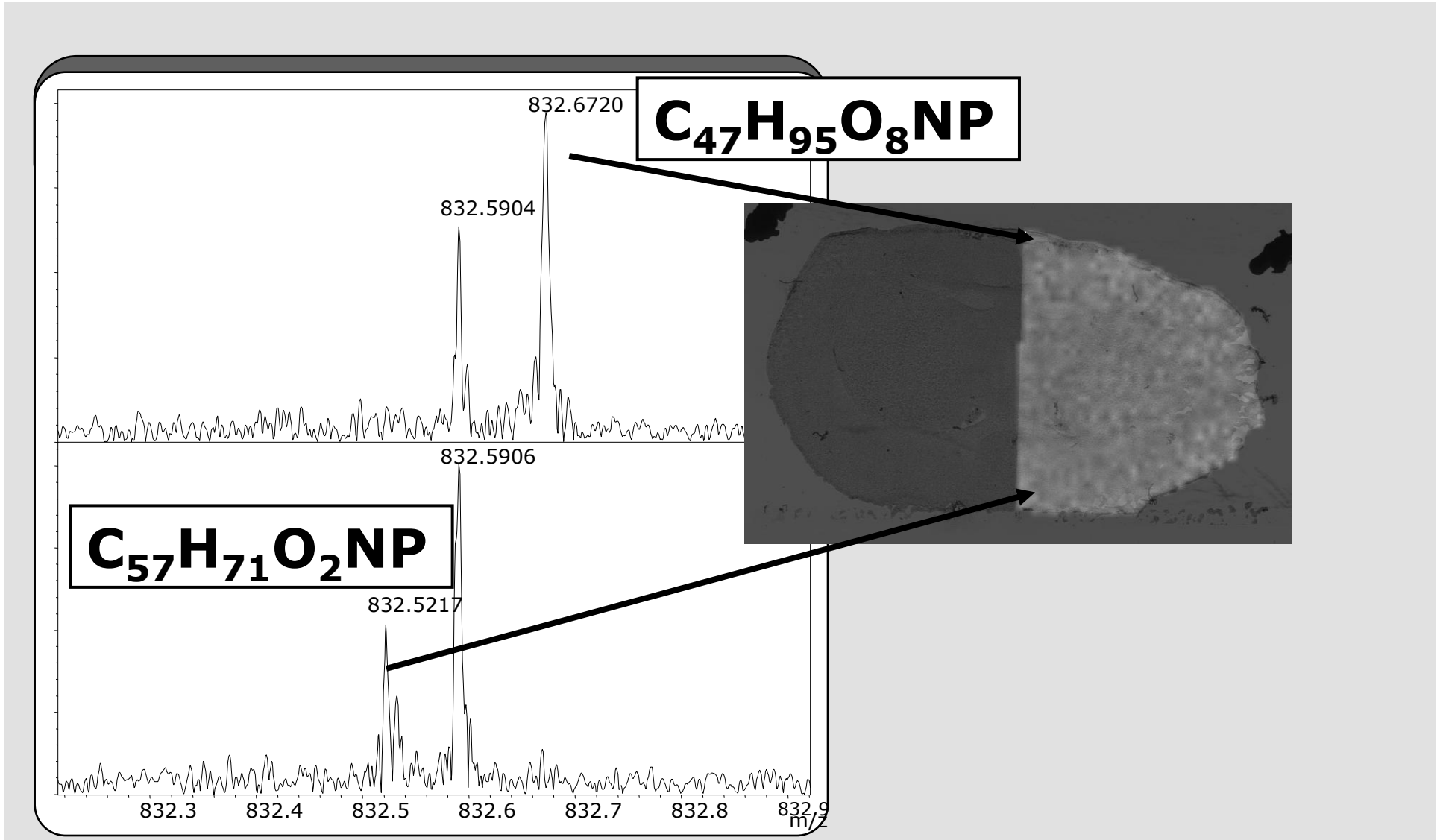


CASI (Continual Accumulation of Selected Ions) enrichment of a narrow mass range results in observation of lower abundance species

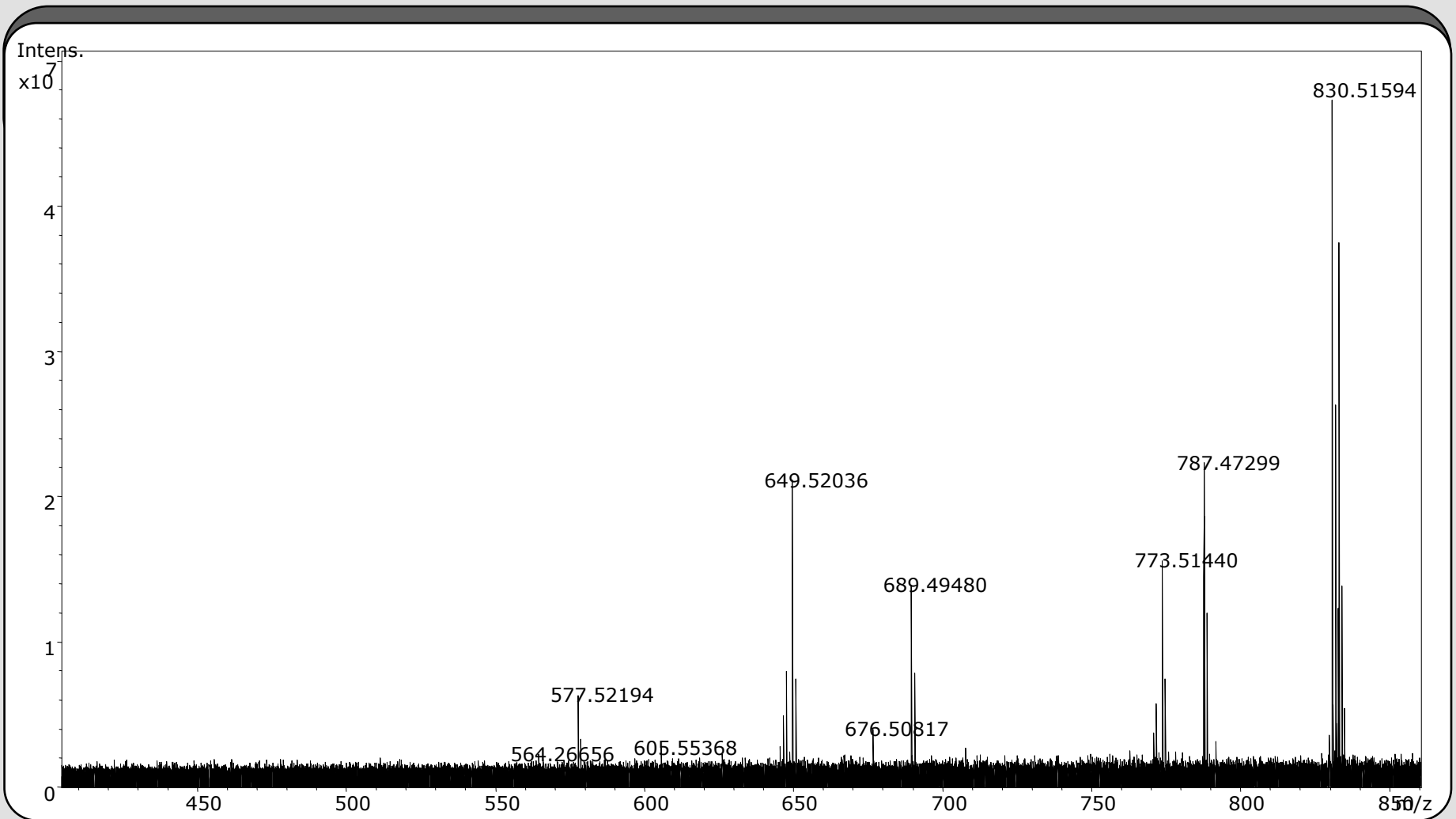
200 $\mu$ m



Control brain sections show different distributions of 832.670 and 832.579



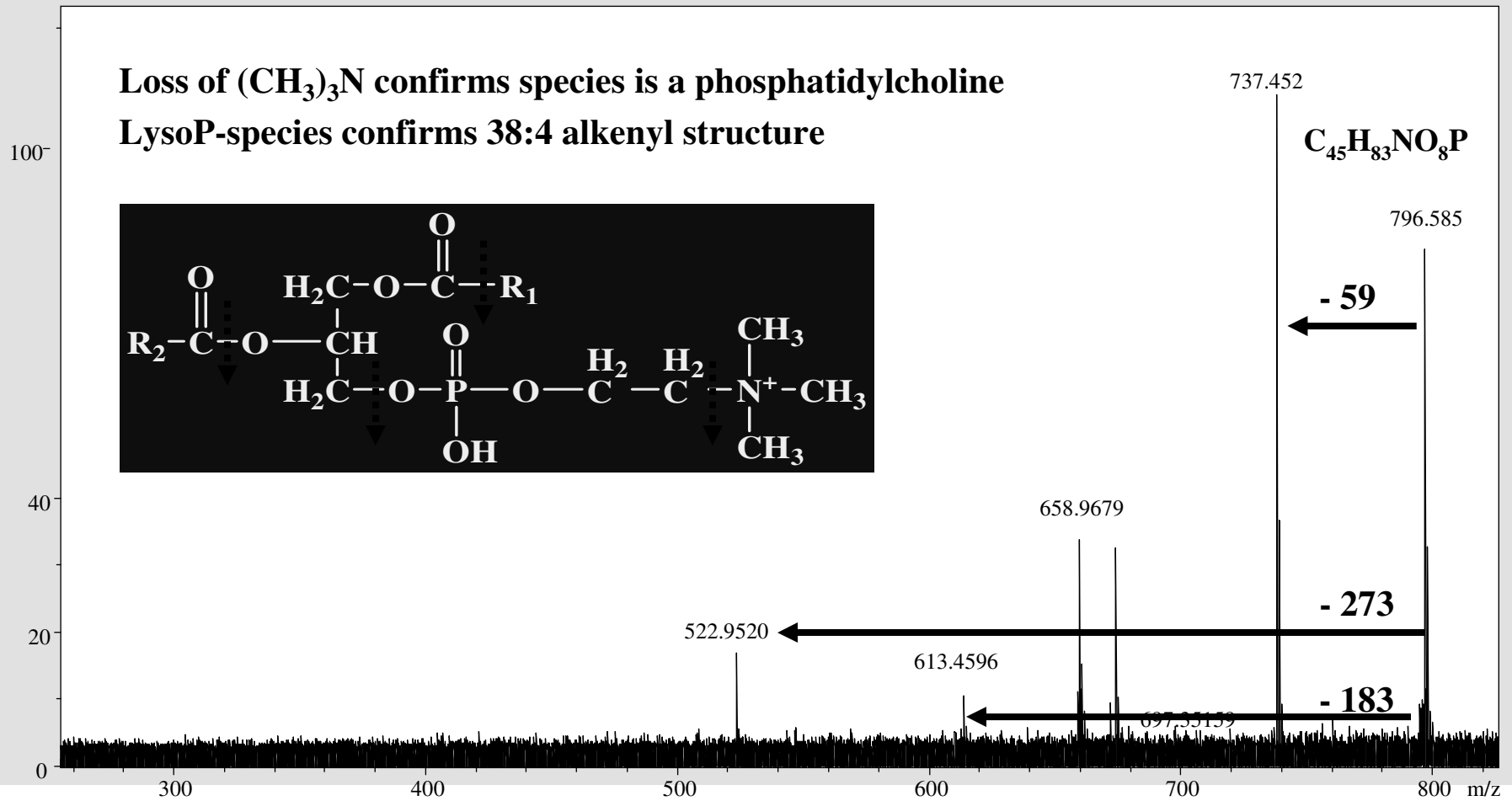
# QCID of species at m/z 832.670



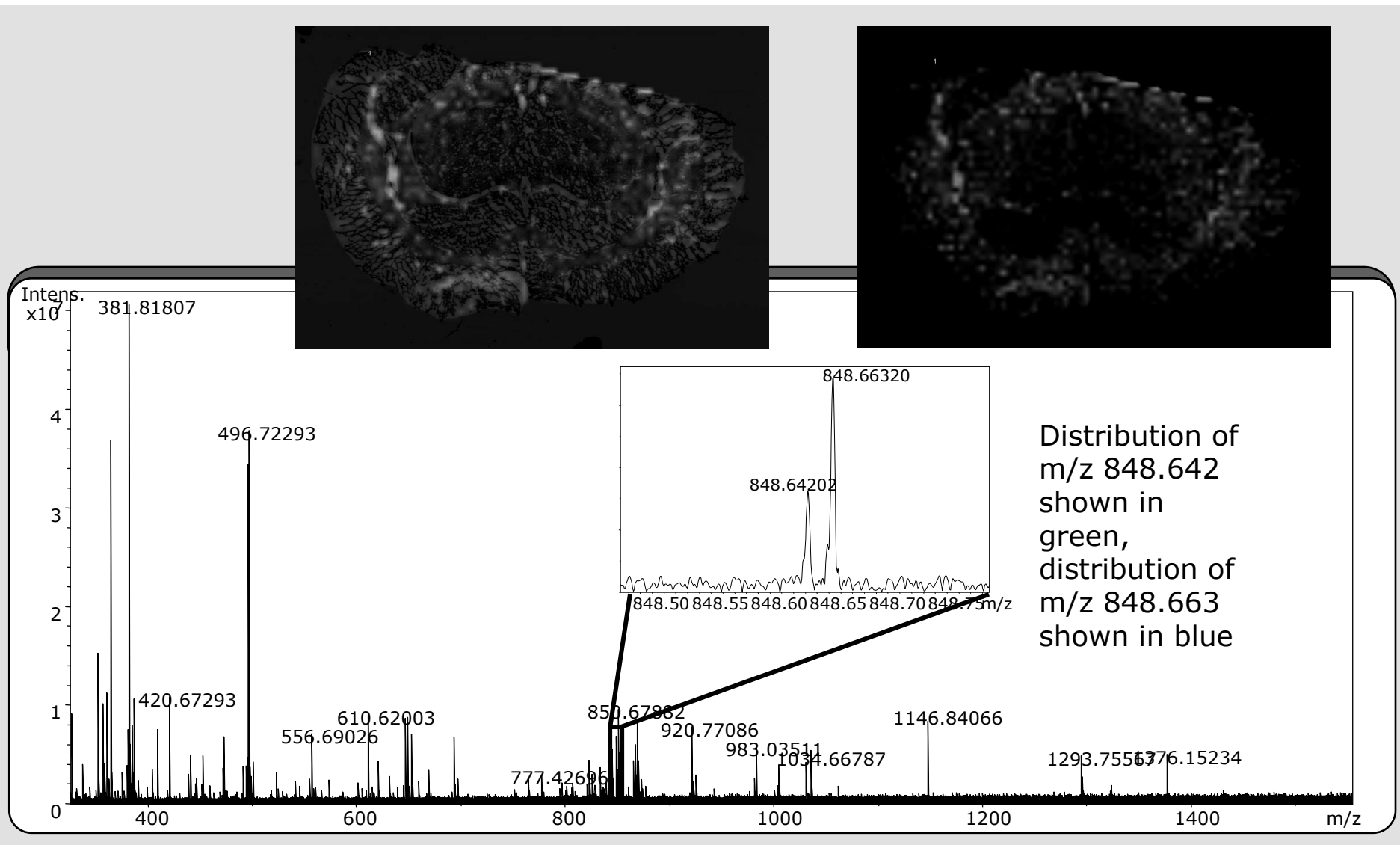


# Biomarker Identification Using MALDI-FTMS/MS

MS/MS fragmentation pattern in combination with accurate mass and isotope ratio measurement (SmartFormula3D) identifies biomarker at  $m/z$  796.5 as PCe 38:4



Nearly isobaric species identified ( $\Delta m = 0.021$  Da).  $m/\Delta m = \sim 220\ 000$ .  
Data from microwaved rat brain.



Thank you!



Axel Walch GSF, Munich  
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