

# *Profiling and Imaging Mass Spectrometry*

*Compilation of Work from the Caprioli Lab*

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# Primary Focus of Today's Lecture?

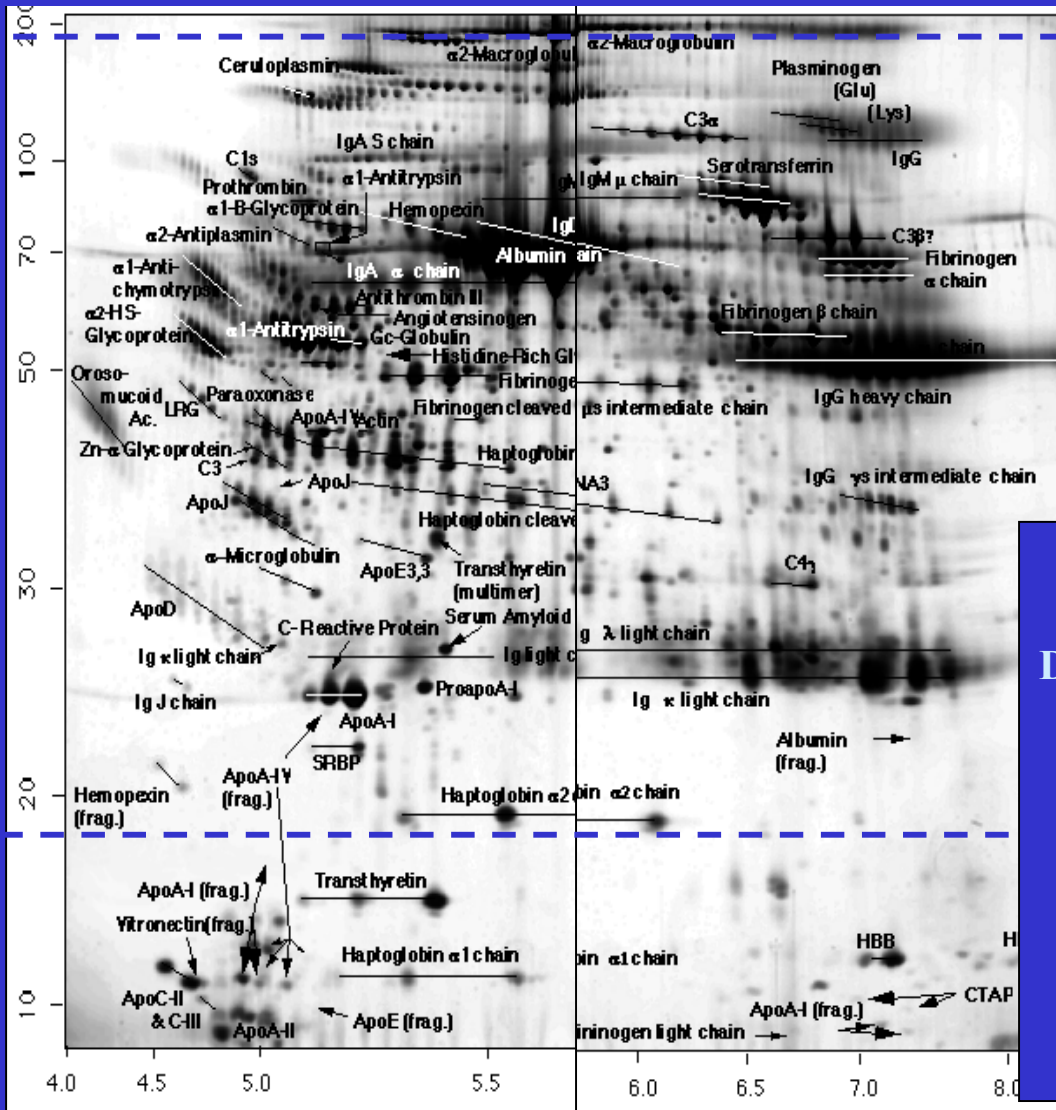
- Brief Overview of Biomarker Discovery for Clinical Applications, Why do we do it, Why do we Often use MALDI-ToF?
- Understanding Advances in MALDI-ToF Driven Profiling of Tissue Sections for BMD.
- How to produce a Mass Image from a Series of Profiles.
- What to Do with All that Data.
- How to ID those Peaks, are they really Proteins?

First off.....

## Why Do Biomarker Discovery?

- To find associations between biological components (i.e. SM, FA's, Proteins) and any clinical endpoint quickly, non-invasively, affordably.
- To non-invasively determine.....
  - Pathologic Changes (i.e. early detection of cancer)
  - Aggressiveness/ Stage of Disease
  - Predicting Rx Response
  - Drug Target Discovery
  - Mechanistic Studies (Systems Biology)
- The Potential Clinical Impact is Tremendous!!

# Can 2D PAGE Get Us There?



Primarily HMW  
Proteins!

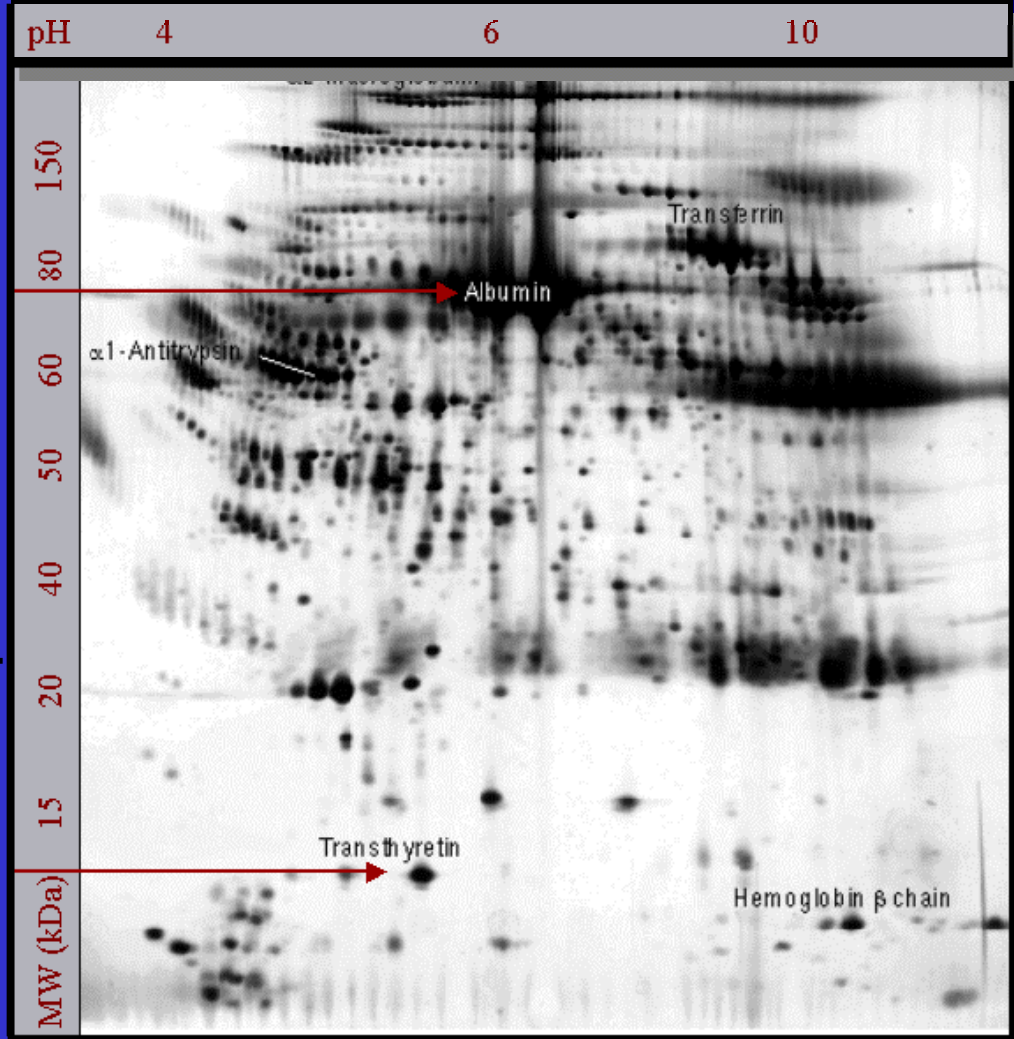
**Serum Proteome**  
Anderson, PNAS, 1977  
Direct Analysis ~1000 Protein Spots

↓ 25yrs later

Pieper, Proteome, 2003  
Affinity, SCX, Size Exclusion  
Yielded 74 Fractions - 2D  
3700 Proteins Spots  
327 Distinct Proteins

# What About MALDI-ToF for Biomarker Discovery?

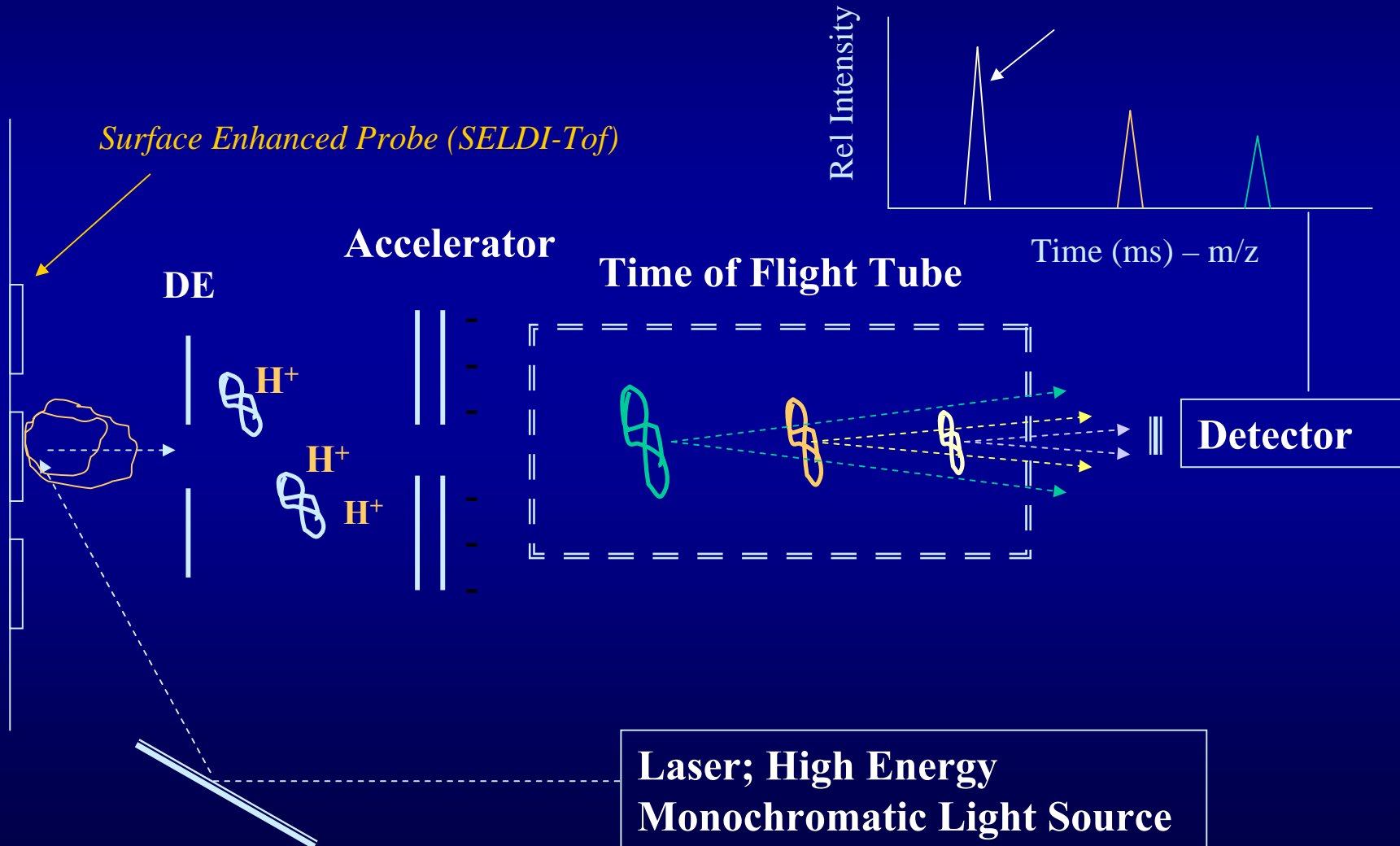
2D PAGE, ID by MALDI-ToF



Primarily LMW Proteins!

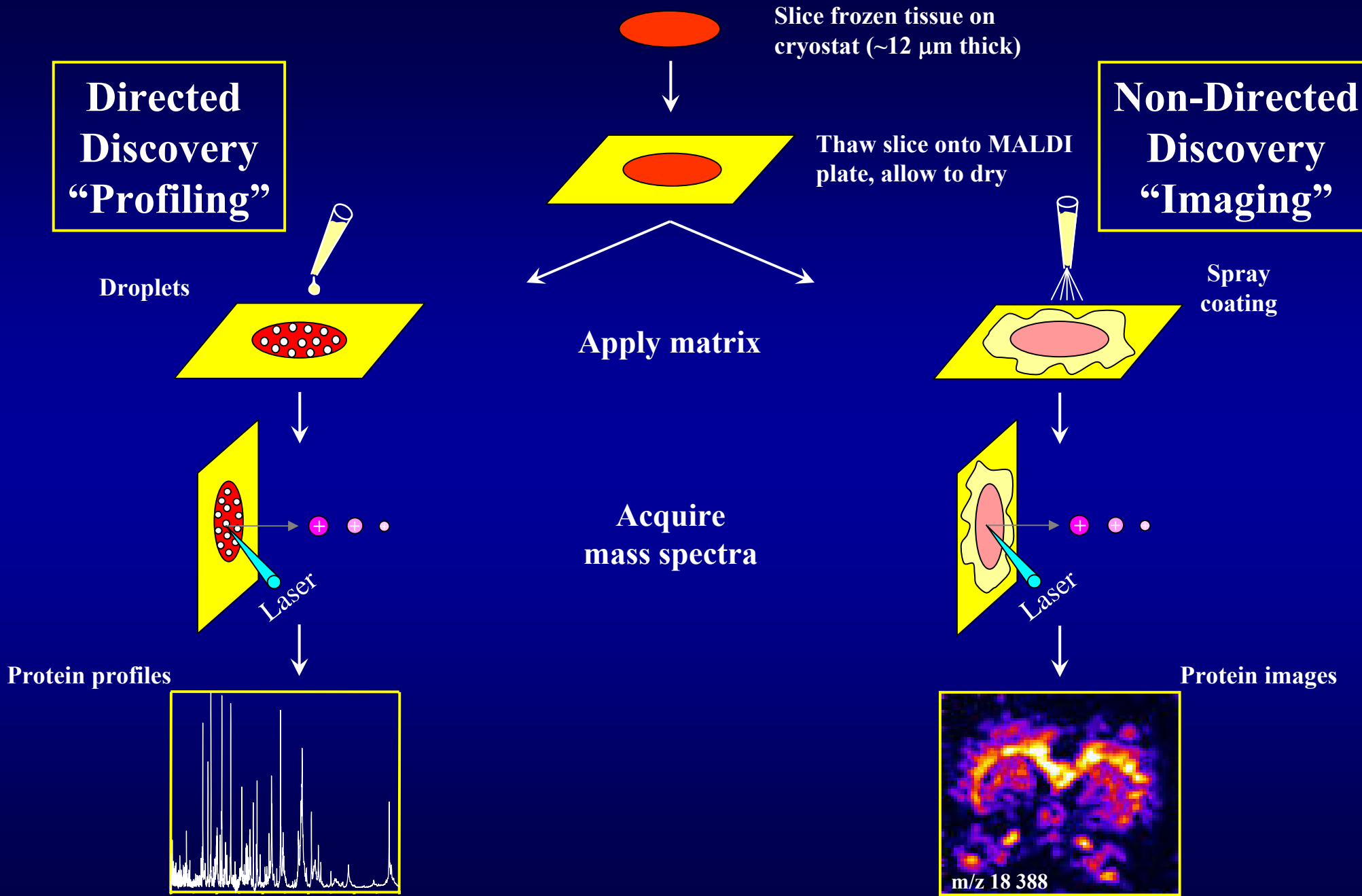
# A Closer Look?

## Matrix Assisted Laser Desorption Ionization (MALDI)- Time of Flight (ToF) Mass Spectrometry



# Imaging Mass Spectrometry of Thin Tissue Sections

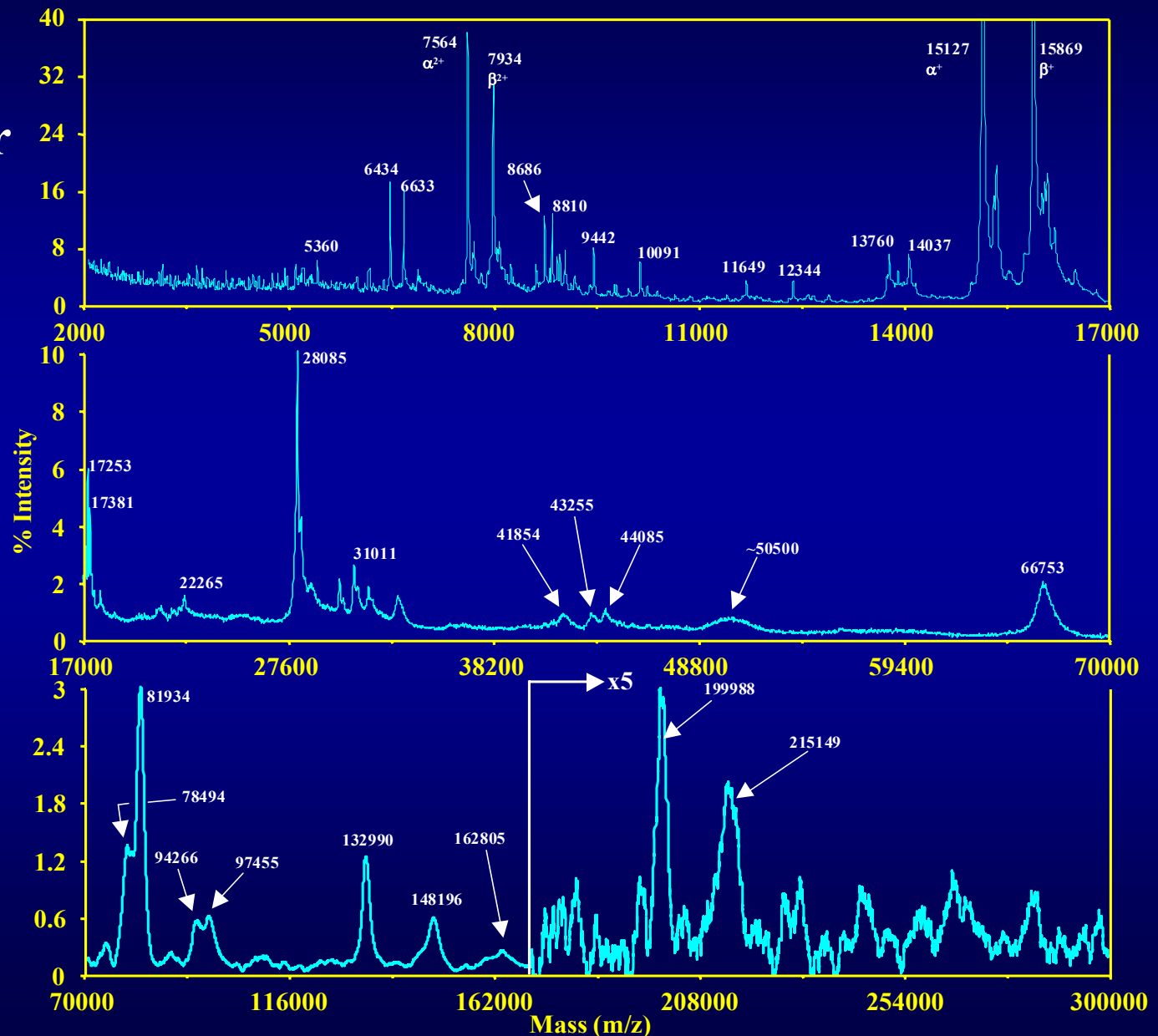
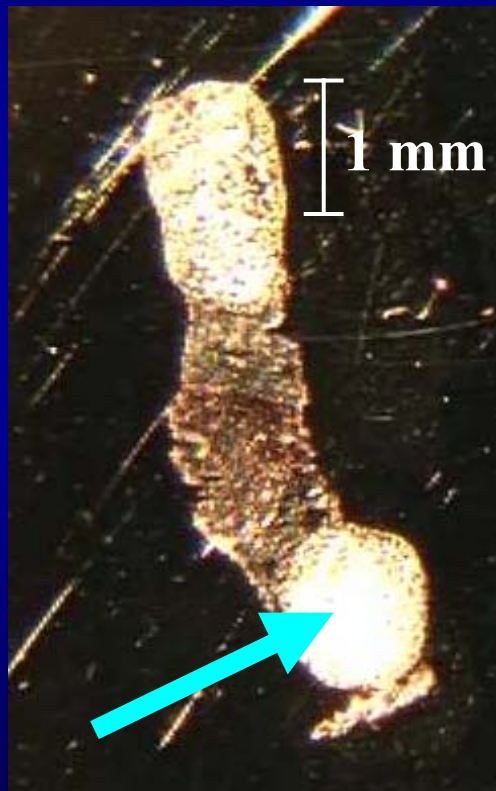
1. To obtain information on the **local protein composition** at any coordinate on the section
2. To **reconstruct 2-dimensional density maps** (or images) for all of the signals detected
3. To **extract molecular information** from grouped profiles and images indicative of health status
4. To be used in “**directed**” and “**non-directed**” discovery; to answer fundamental questions relevant to protein expression in normal and diseased tissues



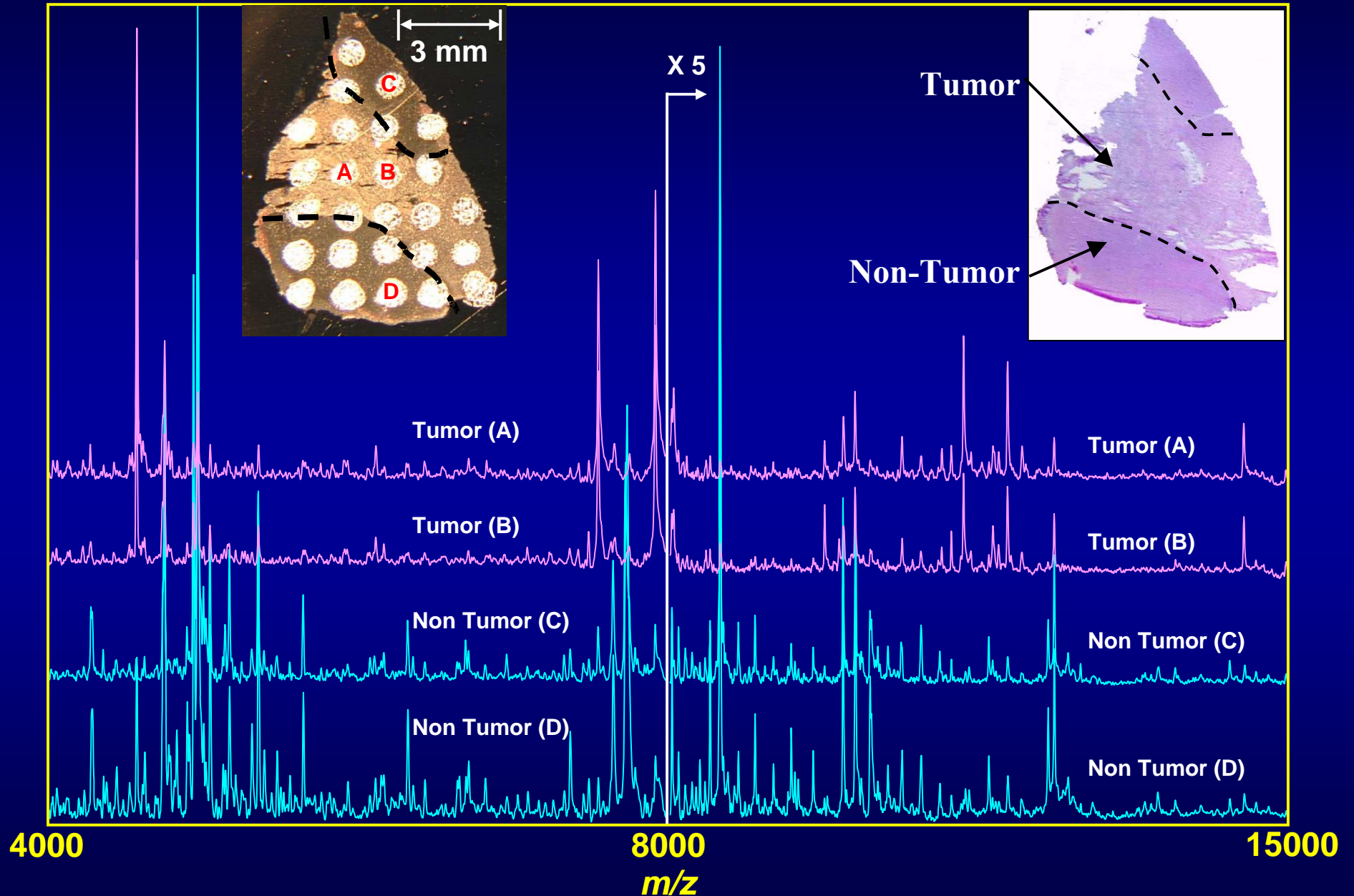


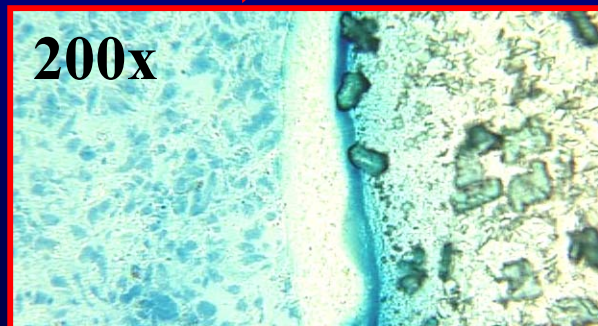
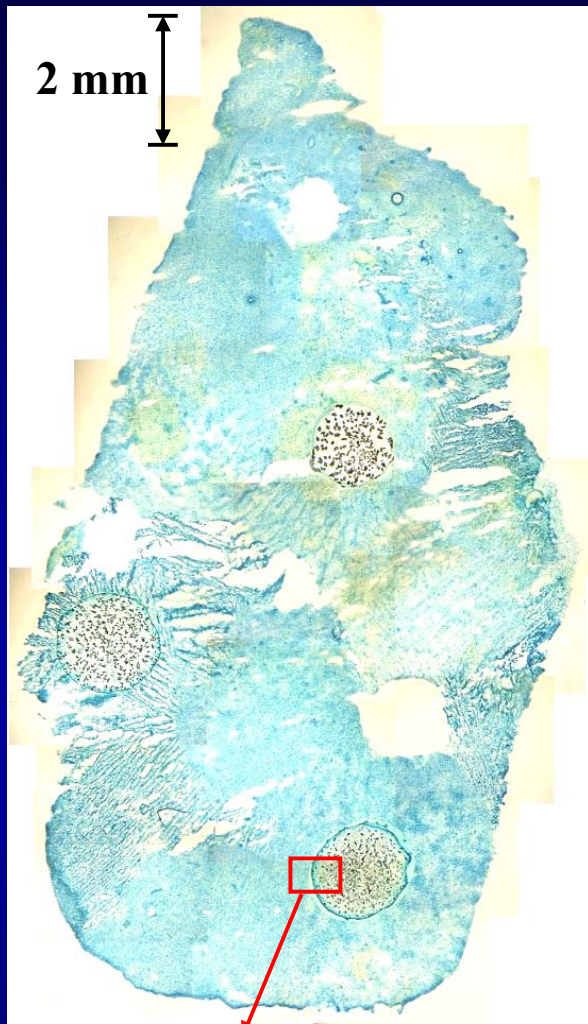
# Protein expression profiling by MALDI-MS

Human breast tumor  
needle biopsy

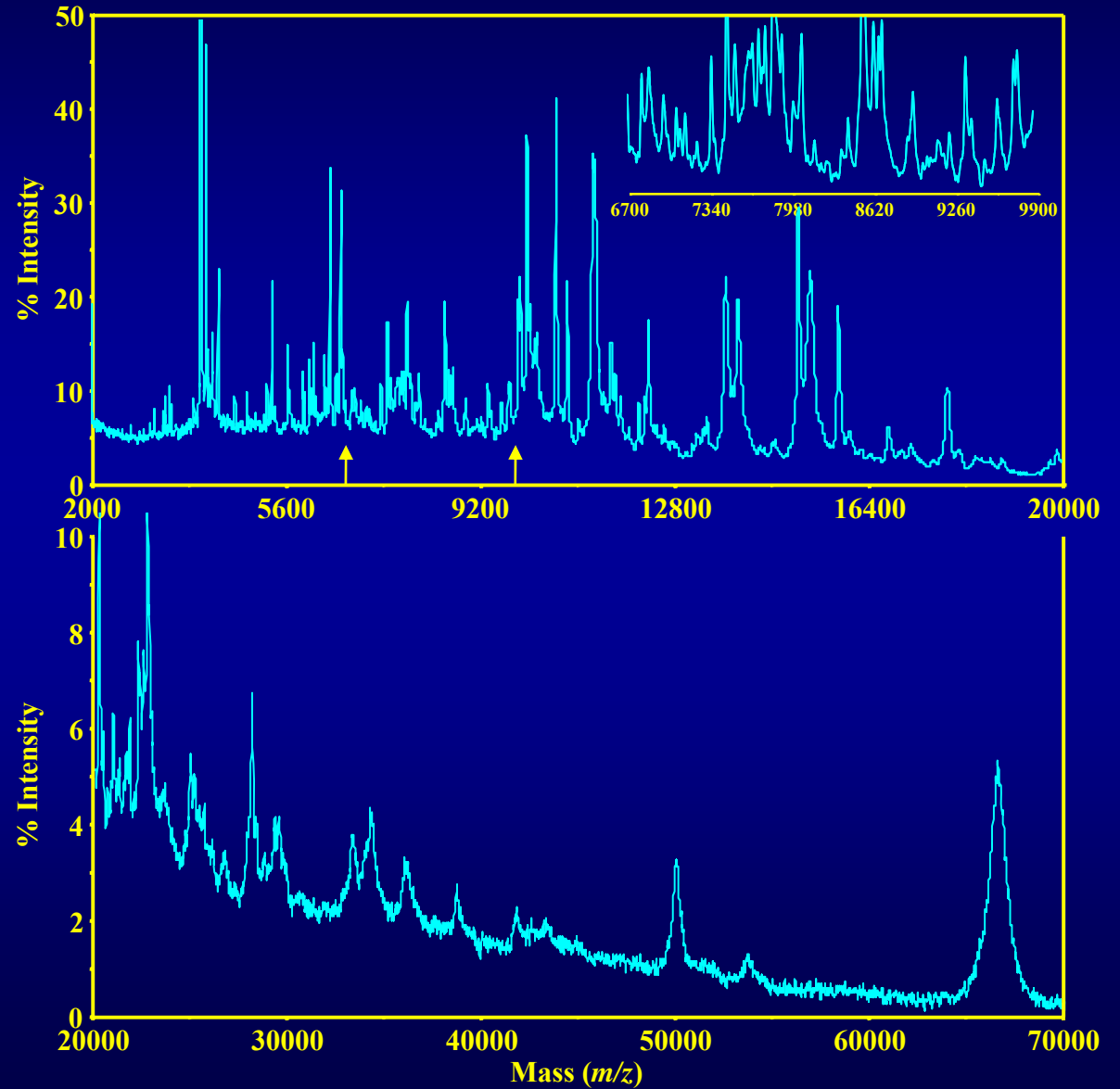


# Human glioma biopsy

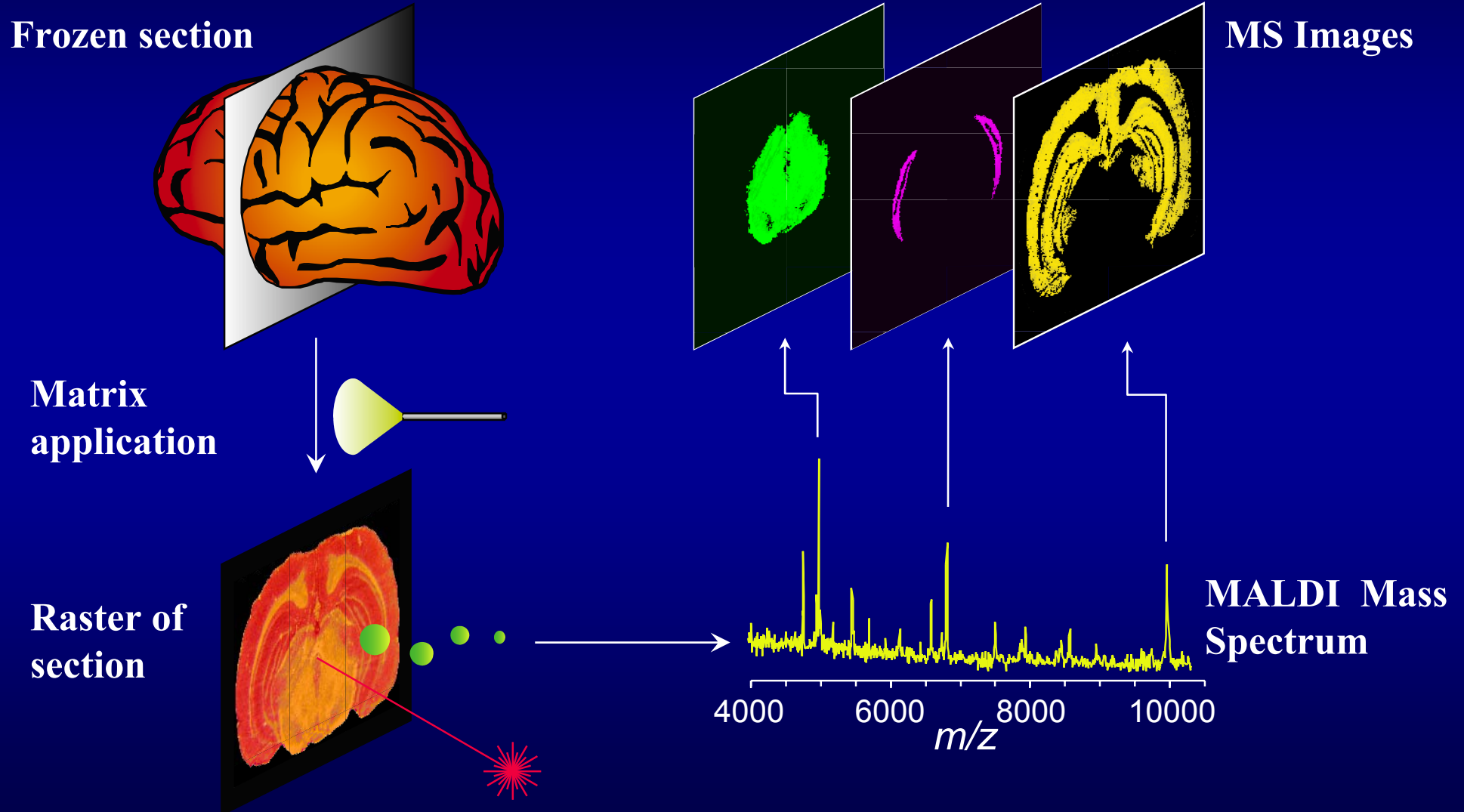




Brain glioma section stained with Methylene blue



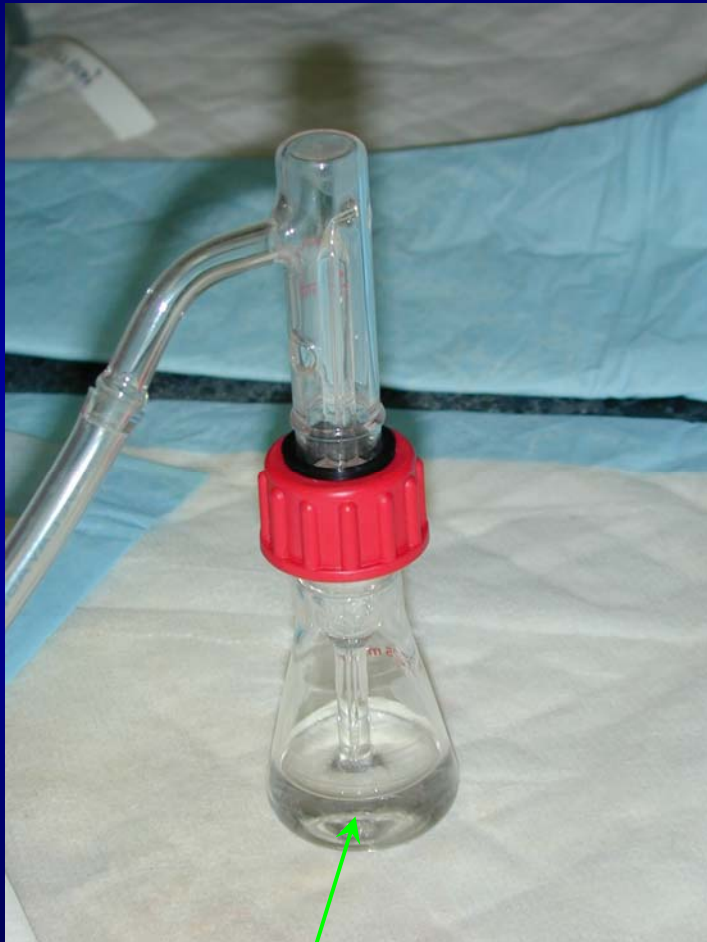
# Principle of MALDI MS Imaging



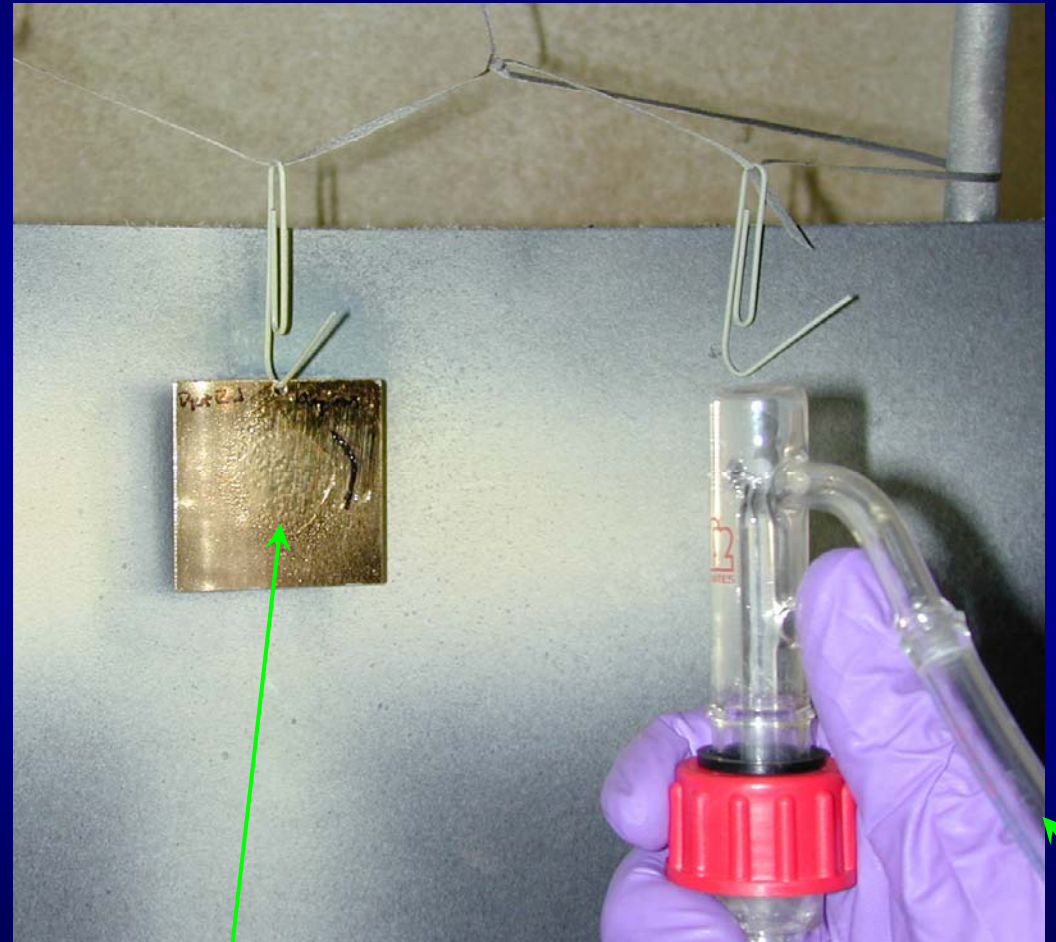


# Spray deposition of matrix on tissue sections

Spray nebulizer for TLC plates



Matrix solution

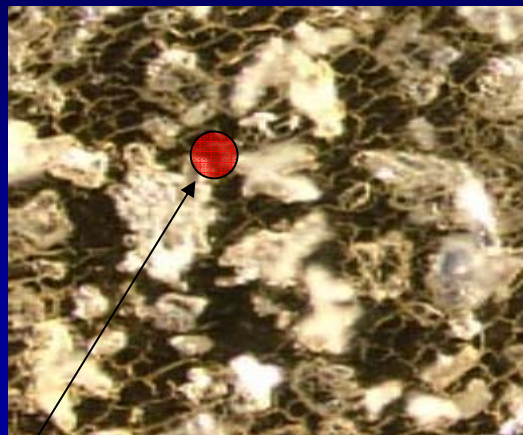


Tissue section

Nitrogen

# Comparing matrix coatings: “Applied Biosystems DE-STR”

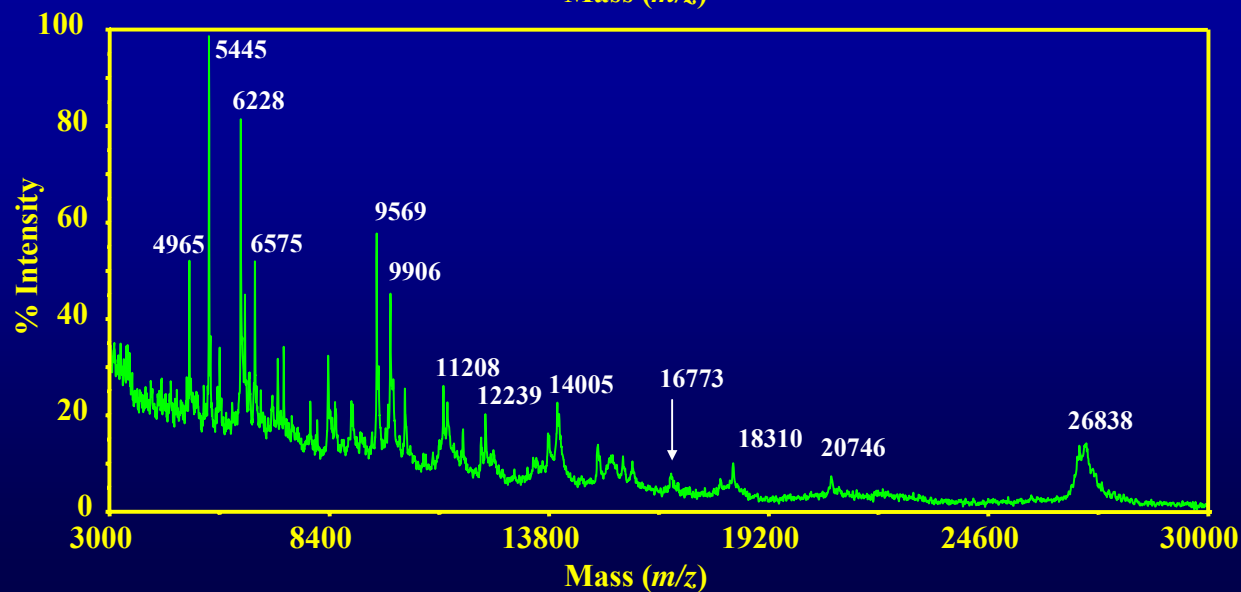
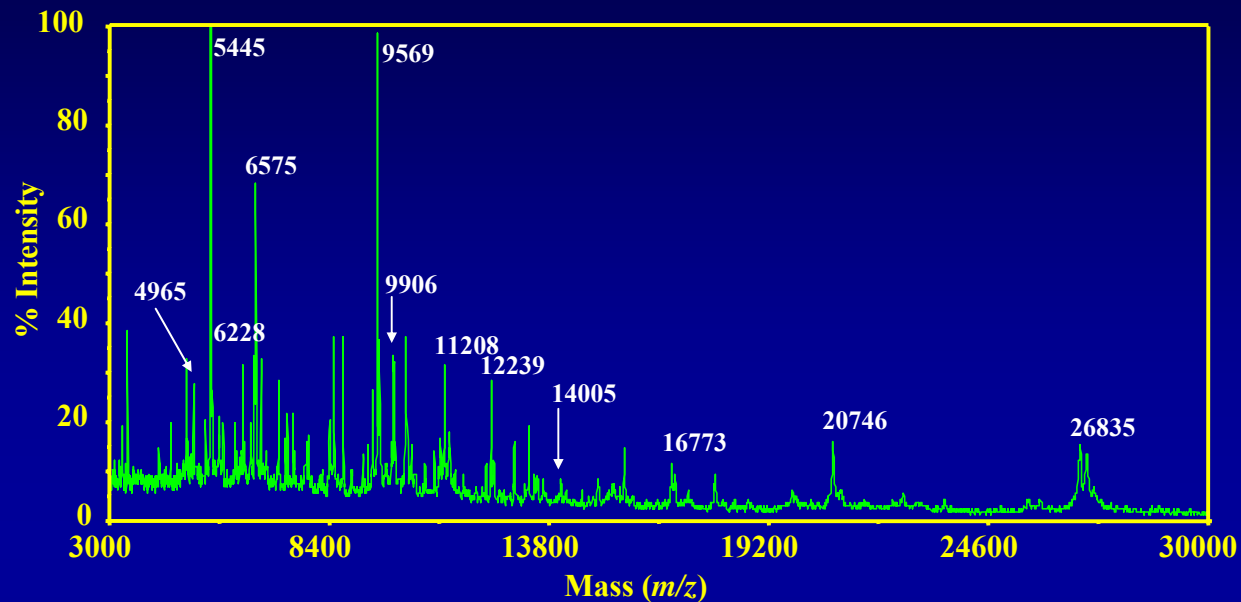
Spotted



200 μm

50um Laser spot

Sprayed

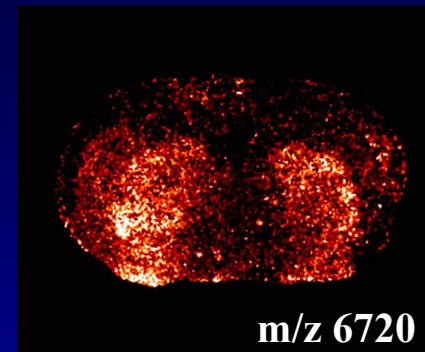
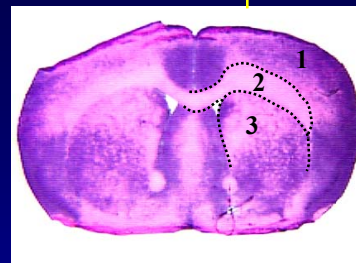


# Mouse Brain

Imaging resolution:

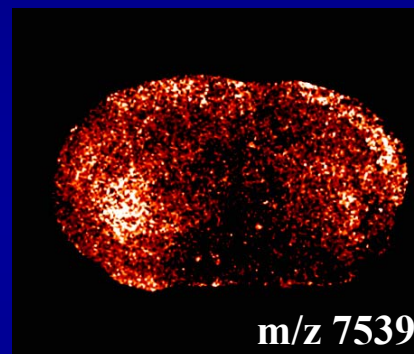
50  $\mu\text{m}$

3 mm

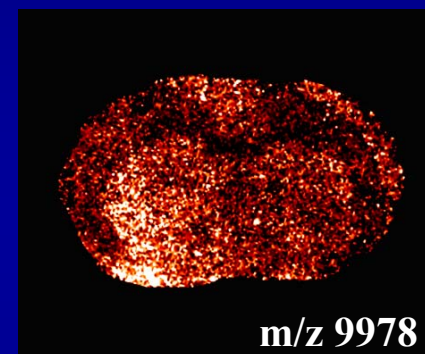


m/z 6720

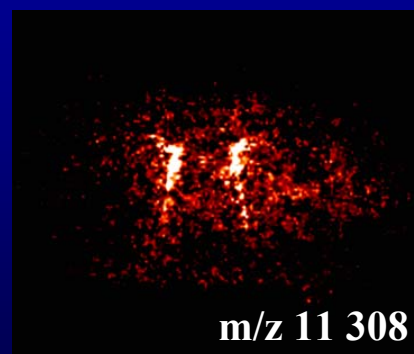
0 100%



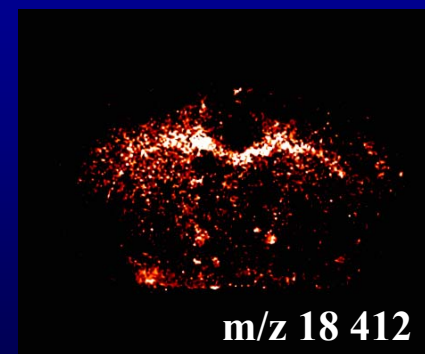
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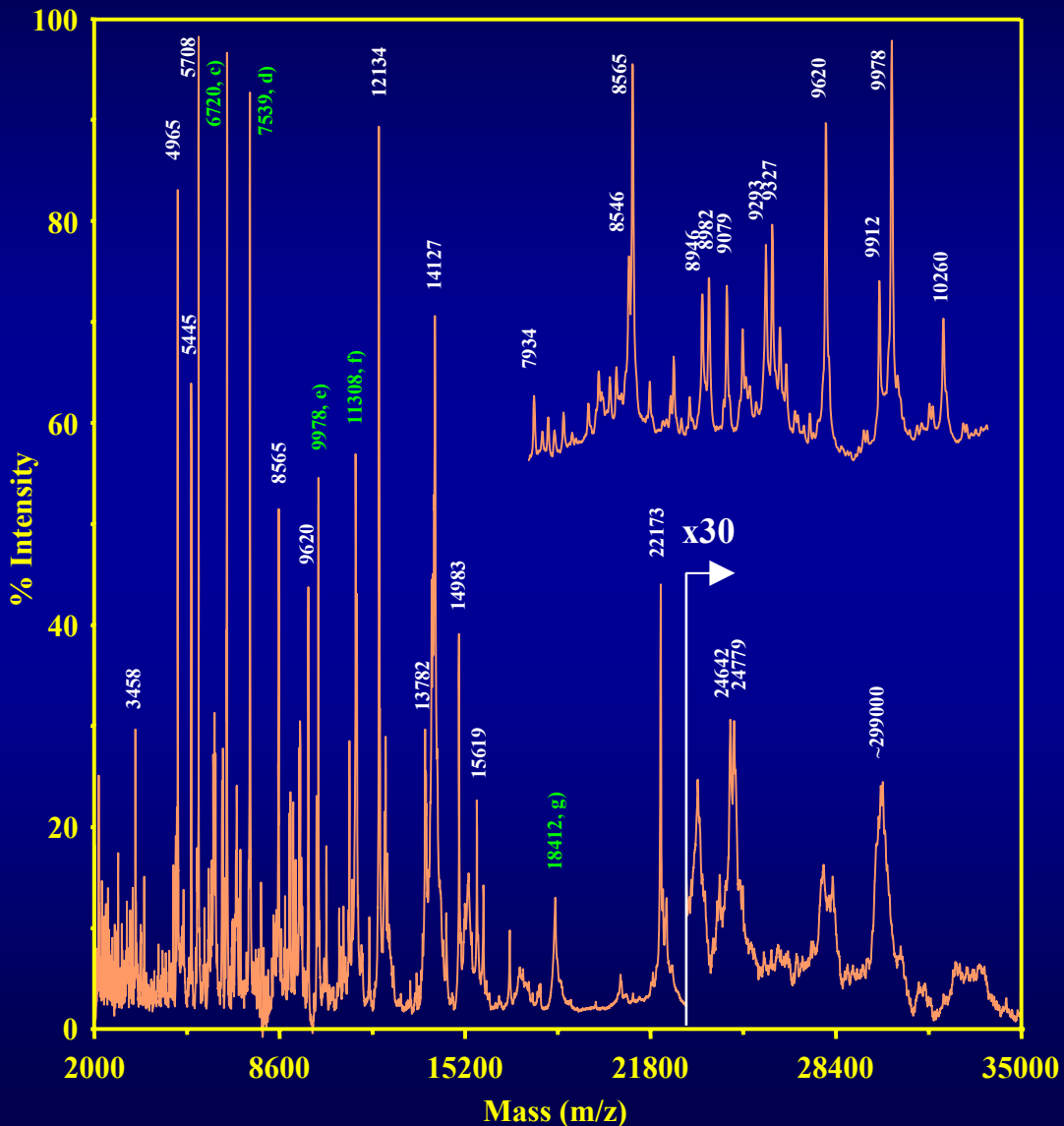
m/z 9978



m/z 11 308

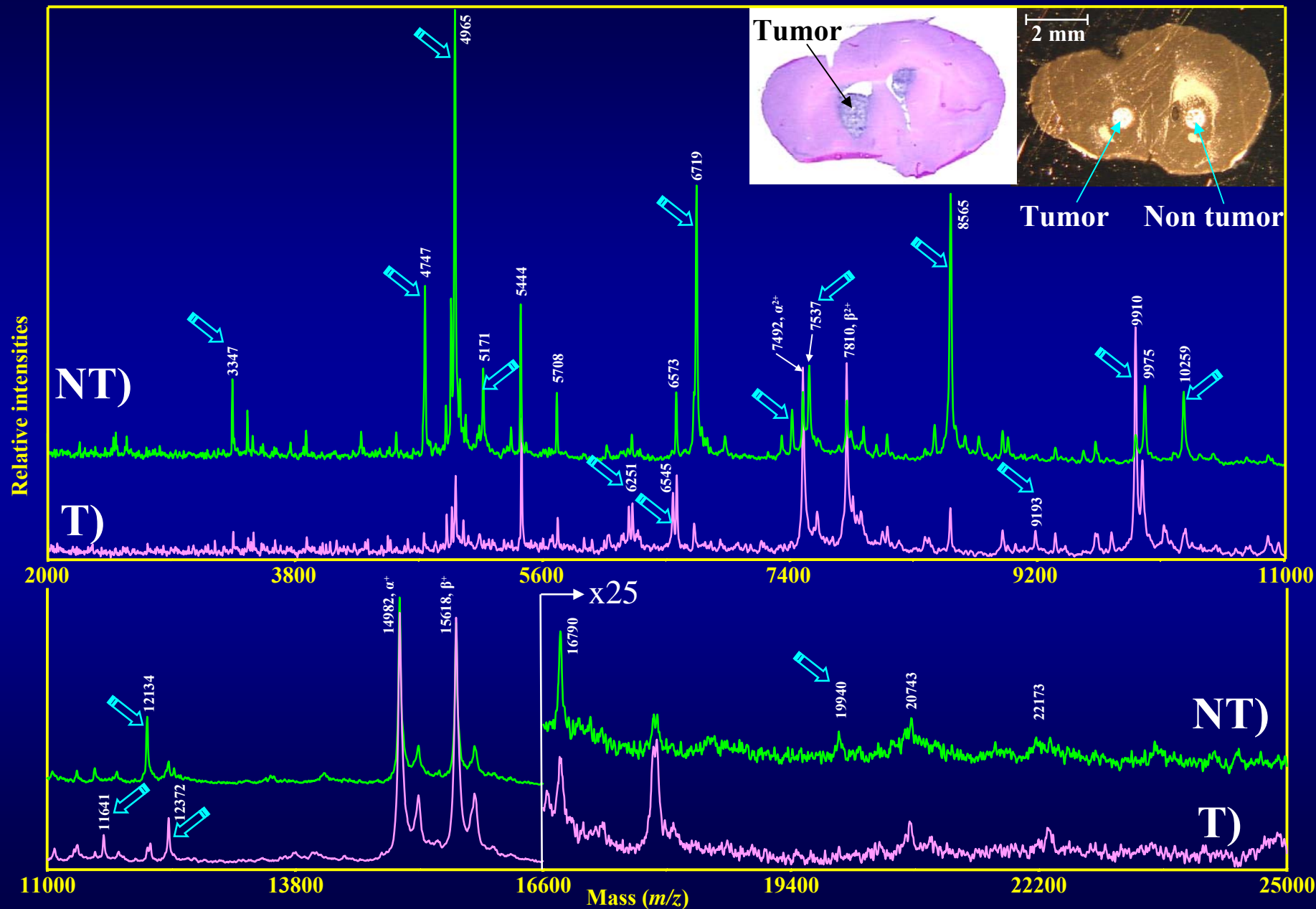


m/z 18 412



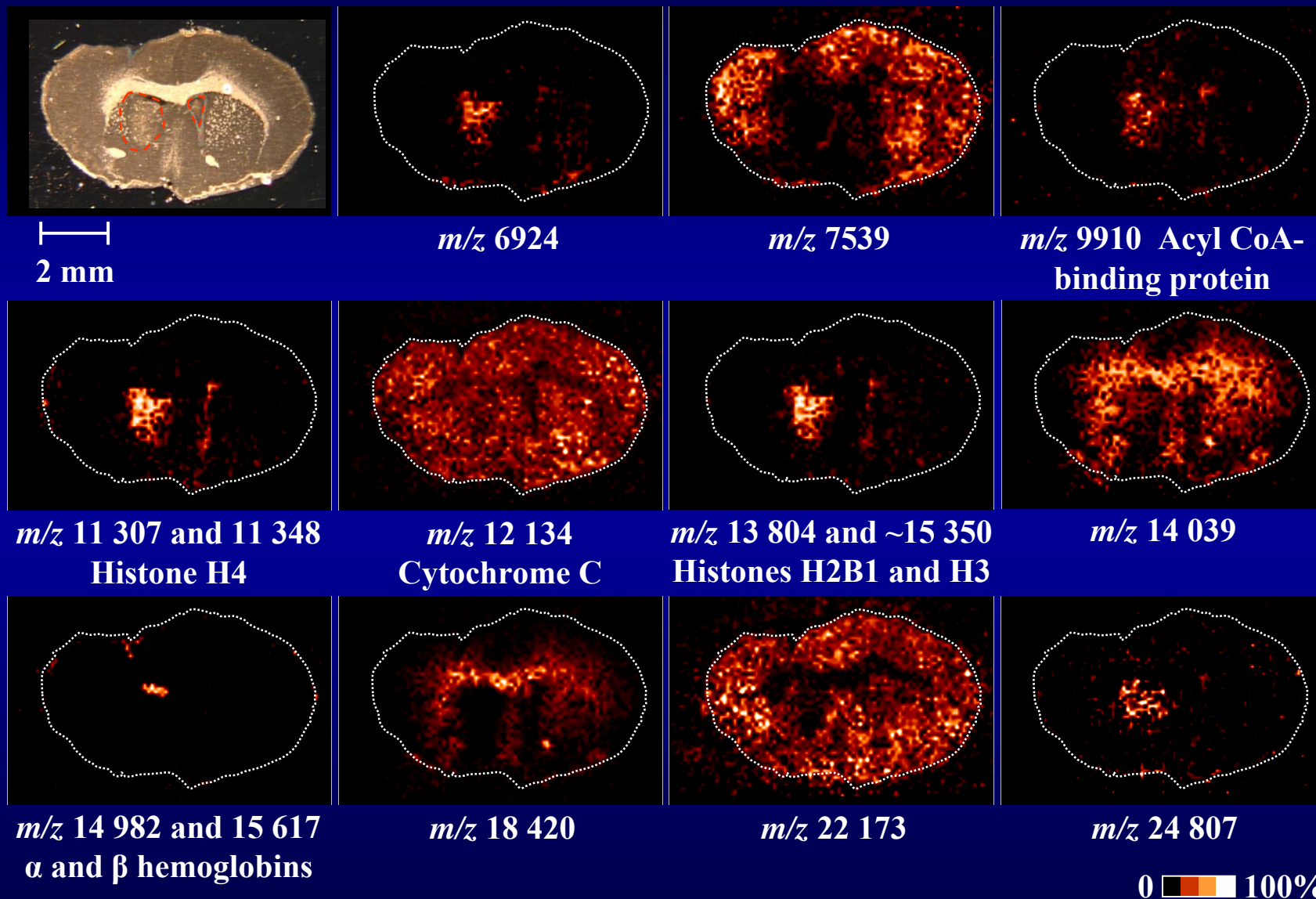


# Glioma mouse model - intracranial injection of GL261 cancer cells





# Glioma mouse model. Imaging resolution: 100 $\mu\text{m}$



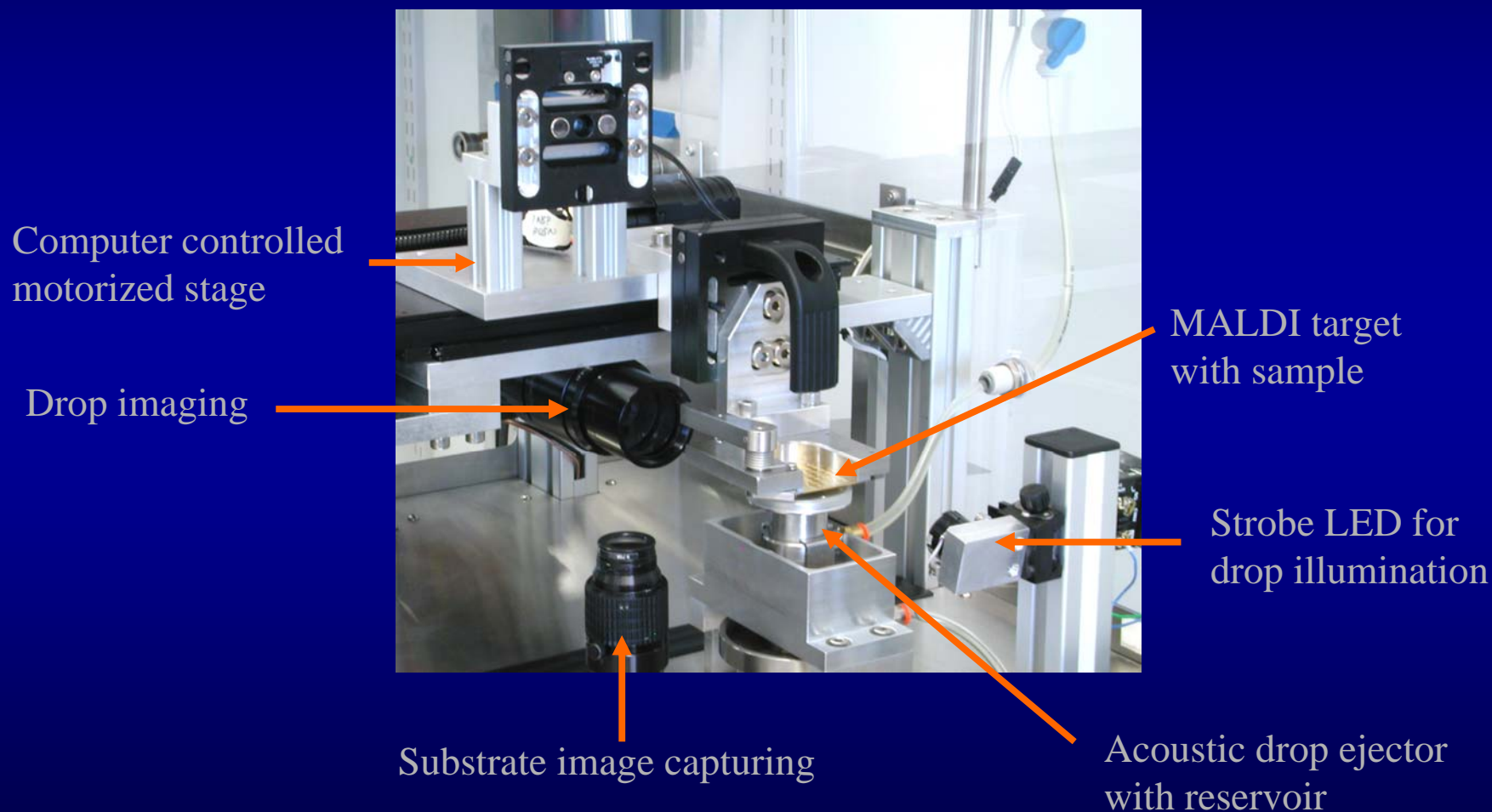
# Tissue Profiling – Which Approach?

## Matrix Deposition Variables:

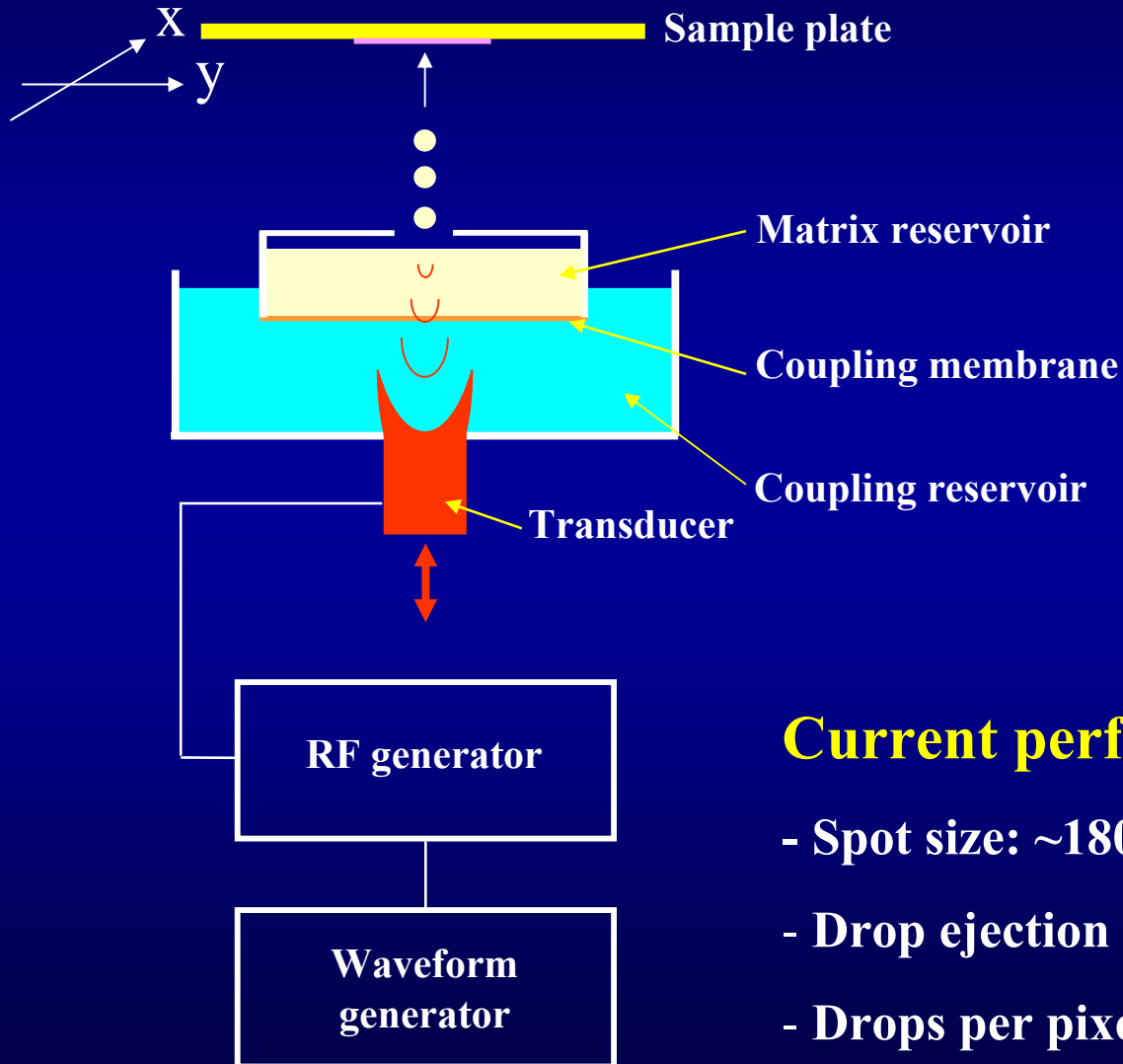
	Time Demand	Repr.	Inherent BG	Spot Res.	Laser Dependent Spot Size
Manual Spotting	seconds	variable	N	>1mm	Y
Robotic Spotting	minutes	good	N	~200 $\mu$	Y
Laser Capture	hours	good	Y	~7 $\mu$ to 100 $\mu$	N

Current Laser Spot Size for Old STR: 25 $\mu$  x 50 $\mu$

# The RapidSpotter



# Acoustic Drop Ejection technology provides a nozzle-free means of generating microdroplets on-demand



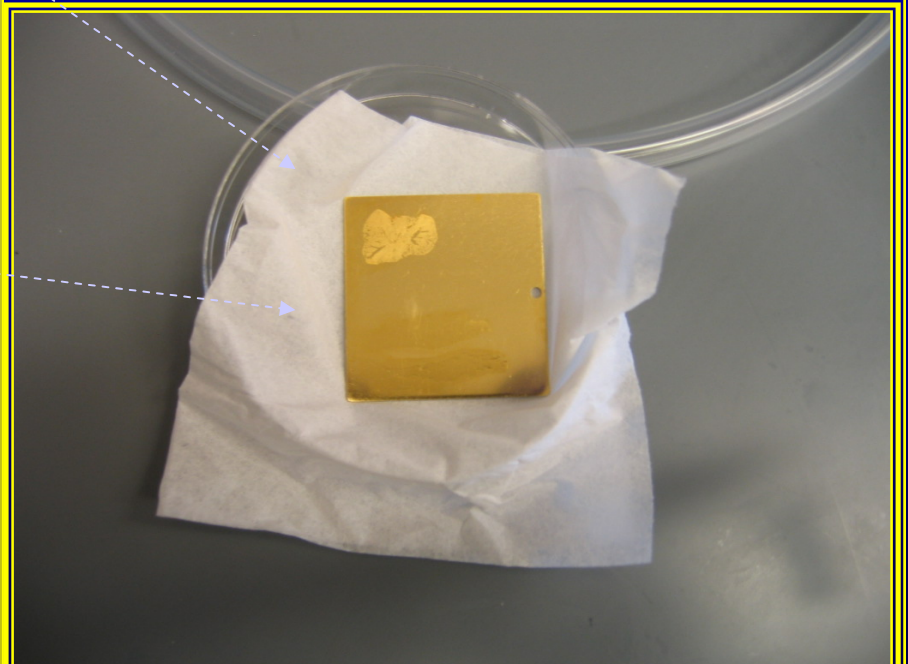
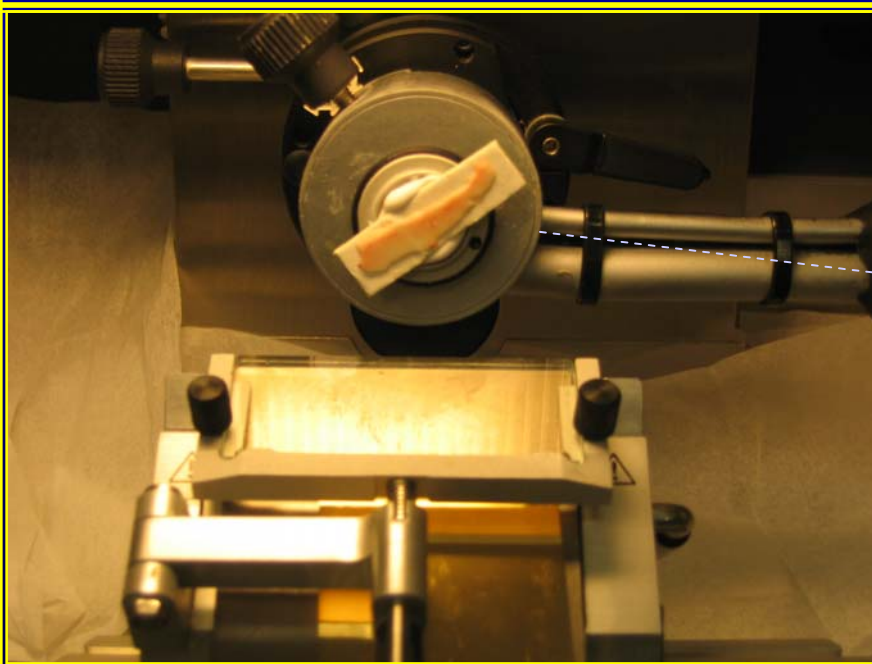
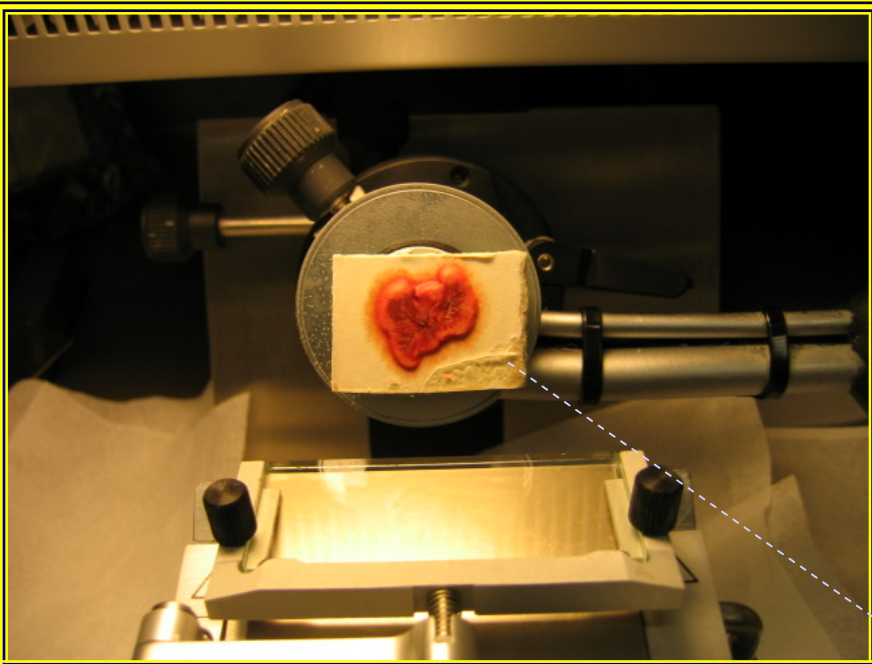
“Ejection of microdroplets”



## Current performances:

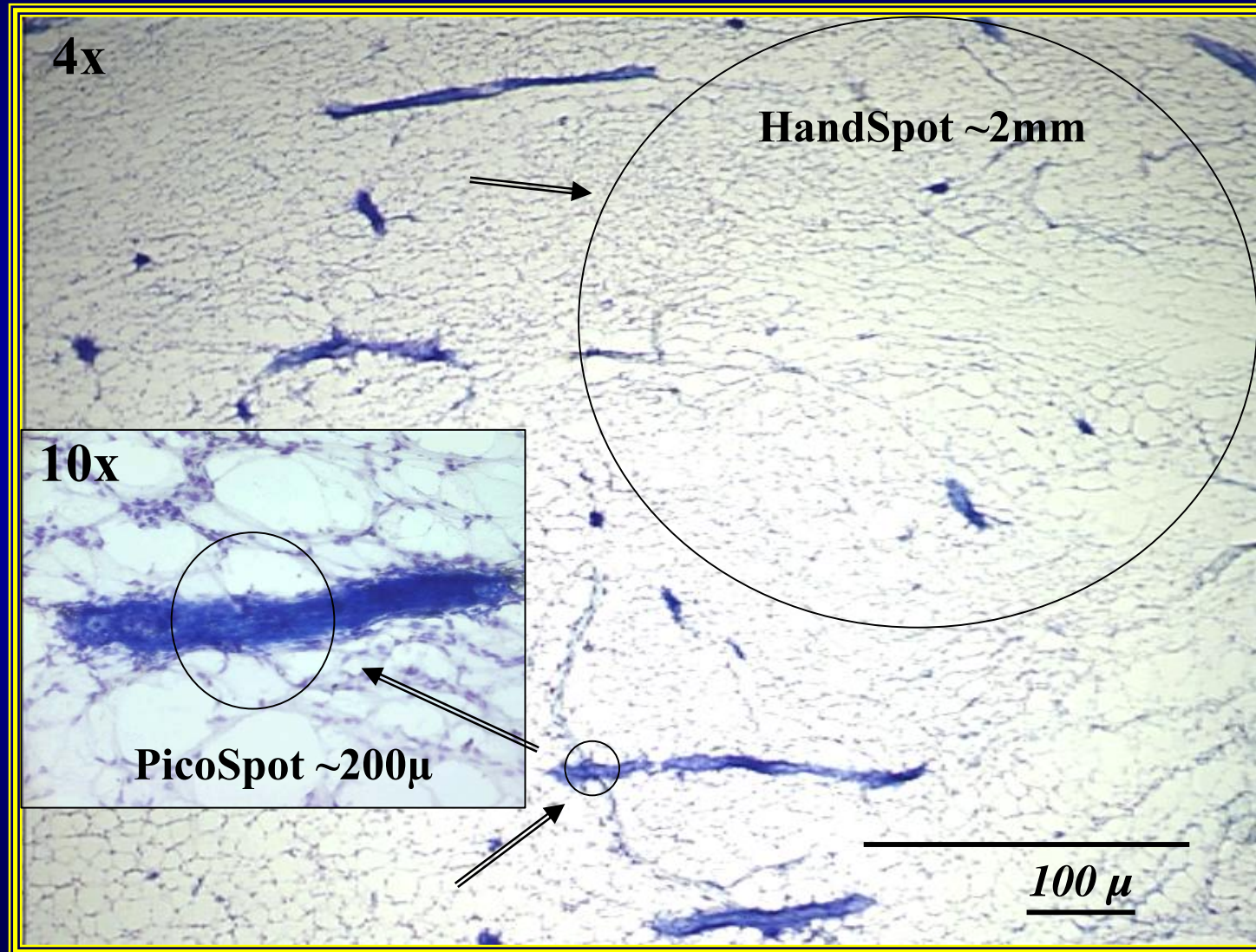
- Spot size:  $\sim 180\text{-}200\ \mu\text{m}$
- Drop ejection rate: 10 Hz
- Drops per pixel: 60-80

## Tissue Sectioning for Protein Profiling

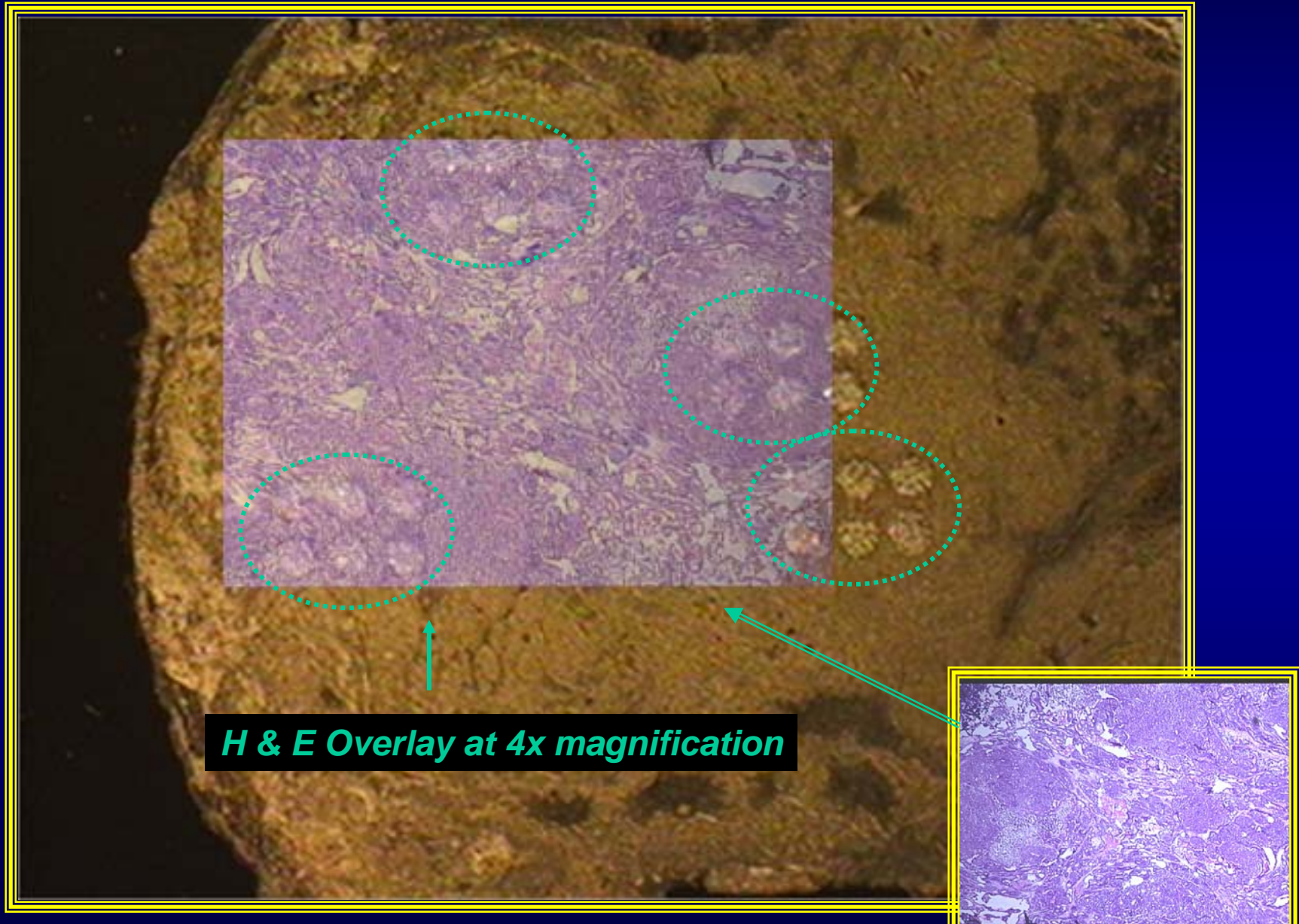




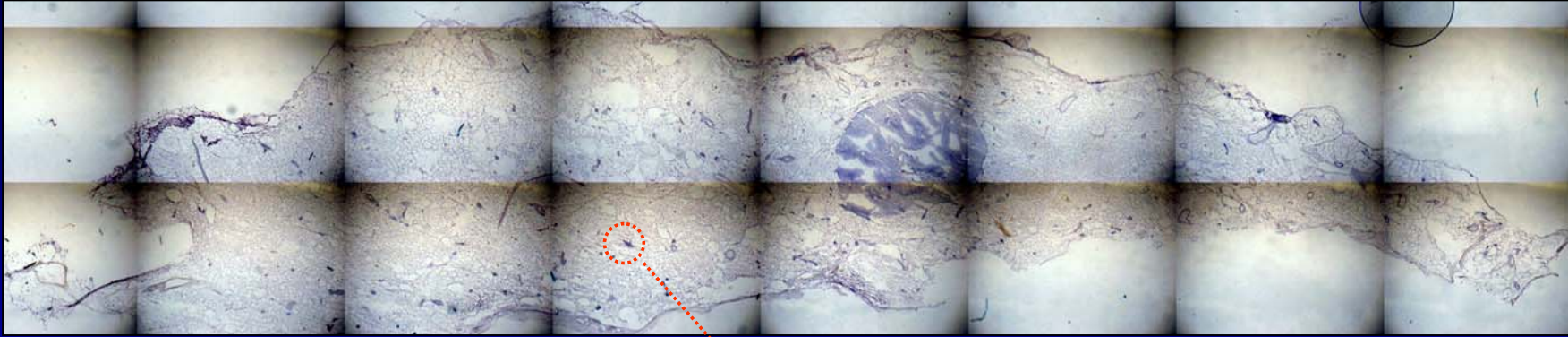
# Hand Spotting Vs. Pico Spotting Vs. LCM



# H & E Overlay's are Used to Determine Regions of Interest

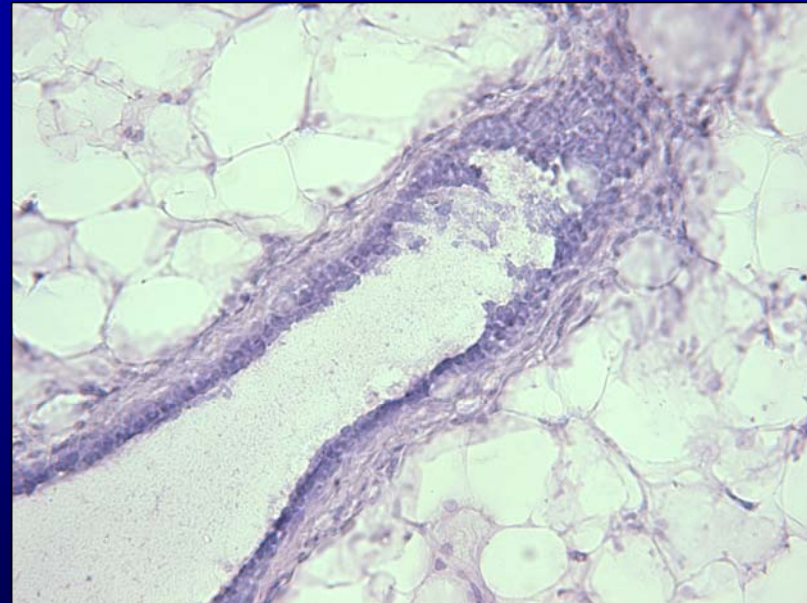






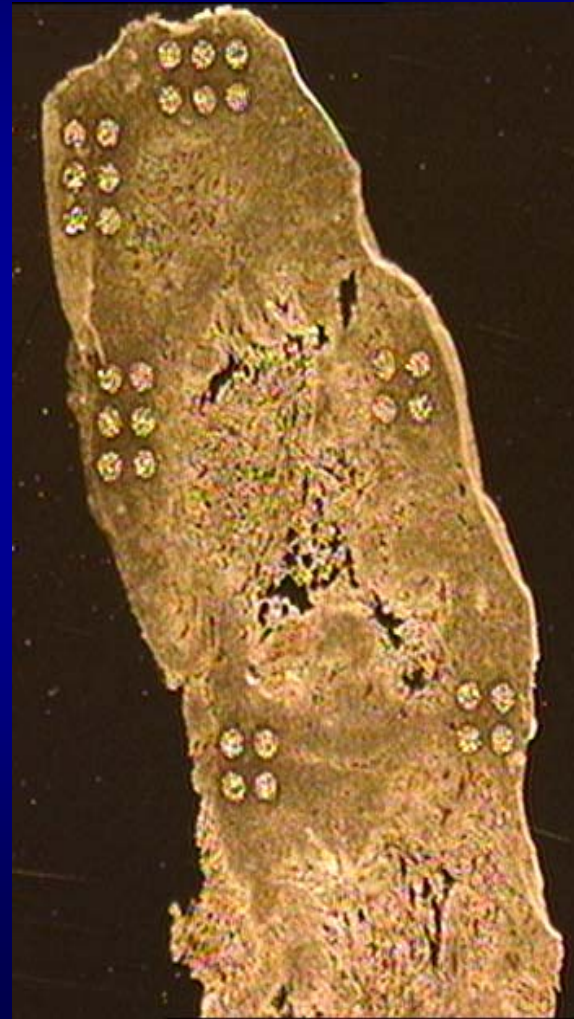
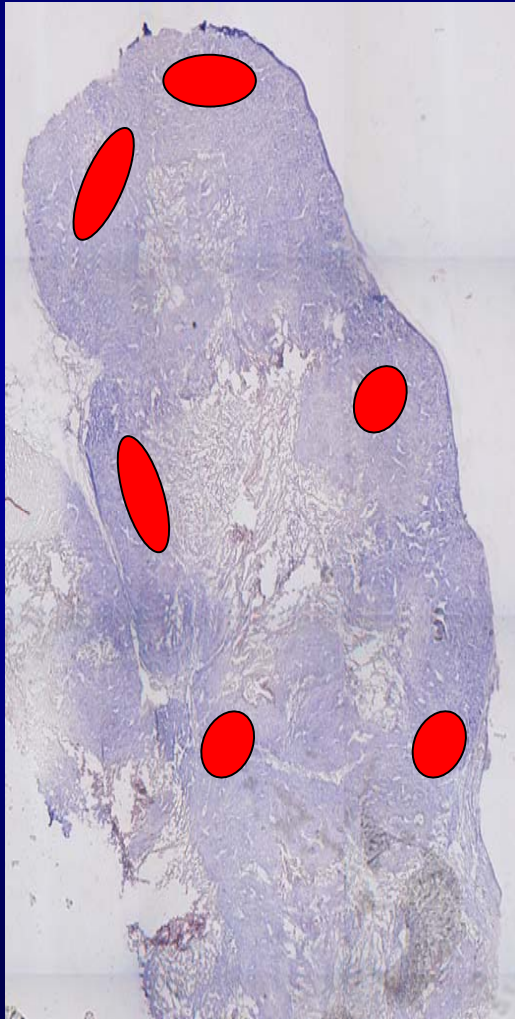
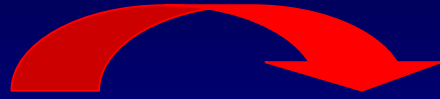
Whole Mouse Mammary Gland

Seamless High Resolution  
Imaging of Histological Slides

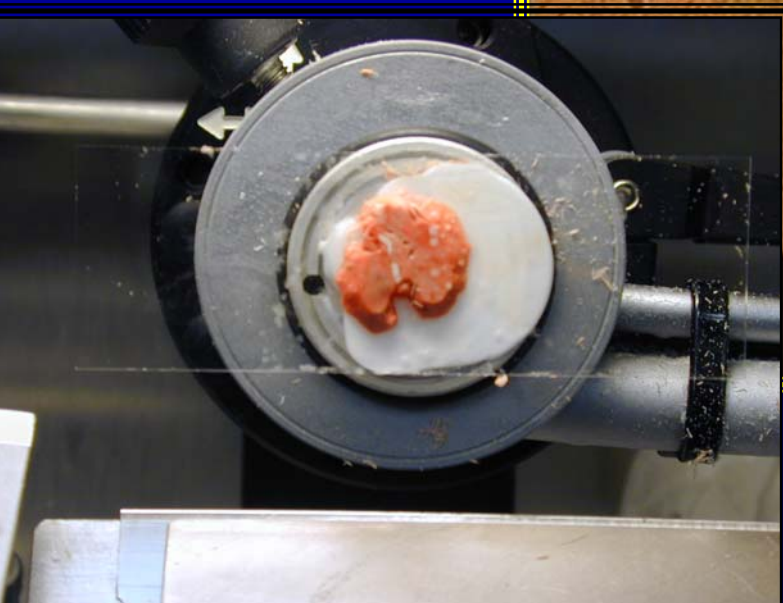




# Histology Directed Matrix Deposition



# Whole Lung Sections





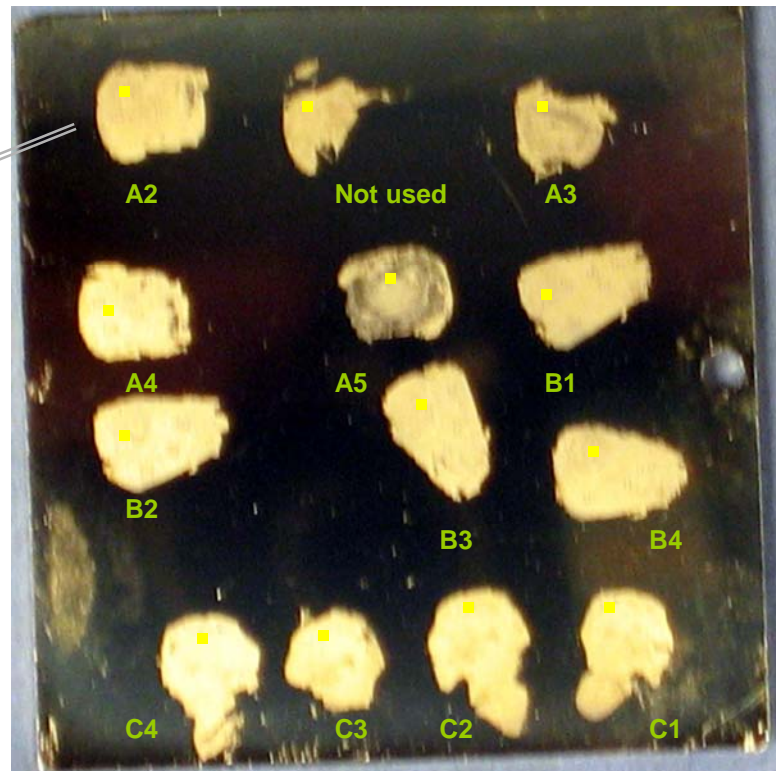
# Automating – “Whole Plate Profiling”

6X15dps was found to be optimal for normal glands and tumors of the breast. Spot Sets can be made to coincide with specific histologic features for each section over the entire plate.

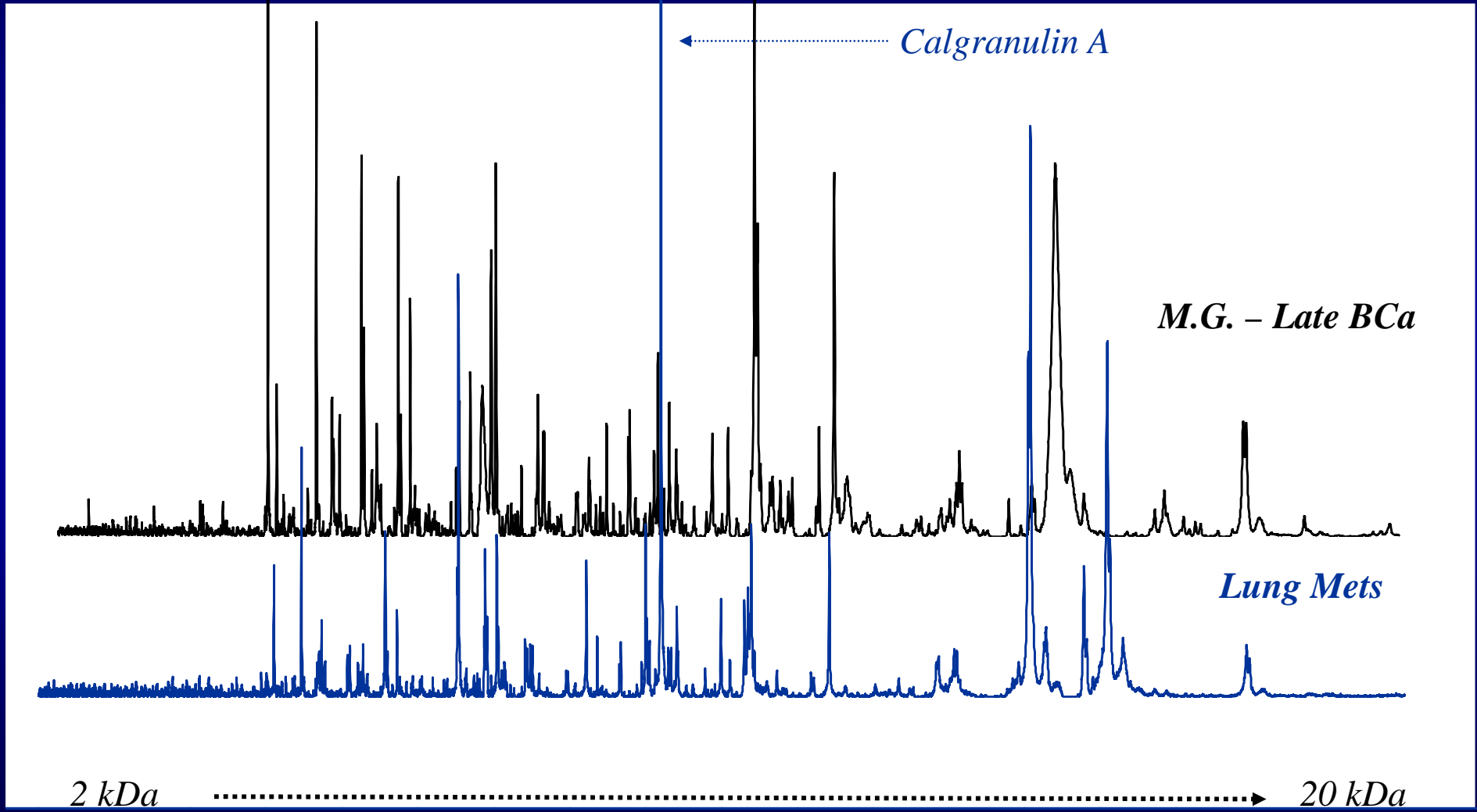
A 12 point calibration can correct for plate placement errors from the PicoSpotter to the STR.

## MALDI-ToF Plate

### Rapid Spotting Over Entire Plate

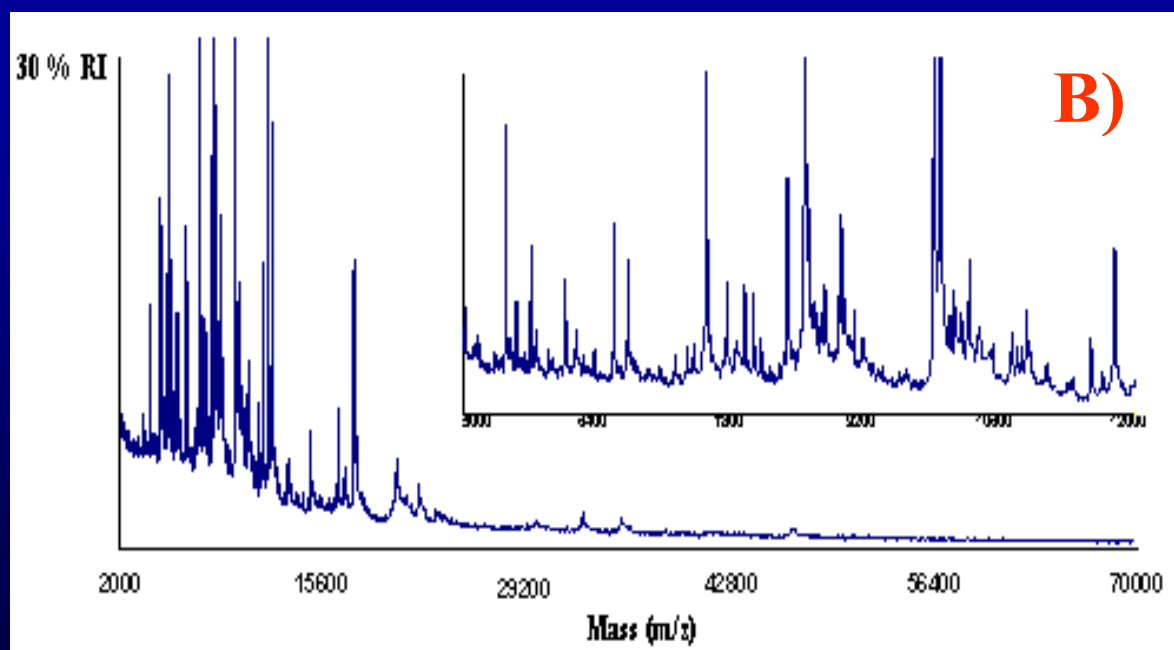
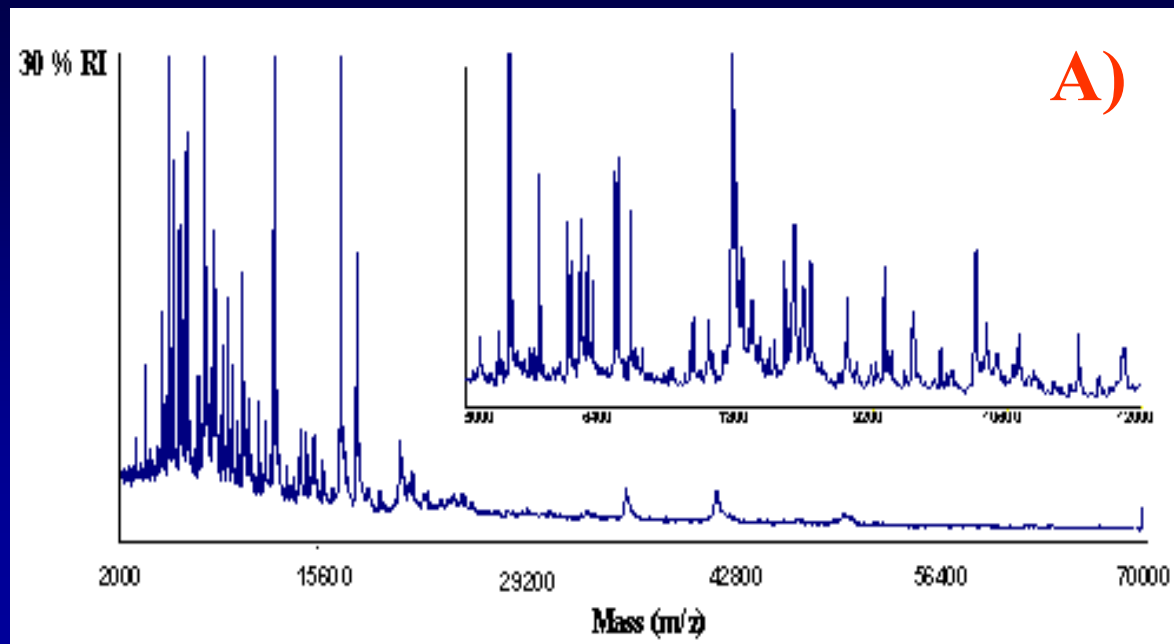
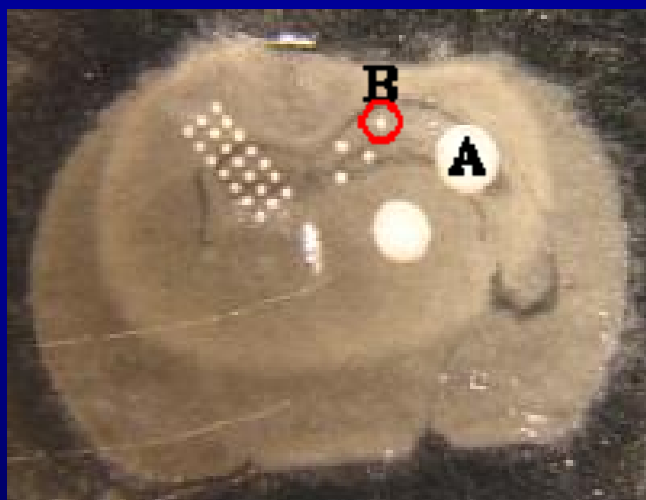


# Results from Mass Profiling Tissue Sections Lung Mets Vs. Late Carcinoma

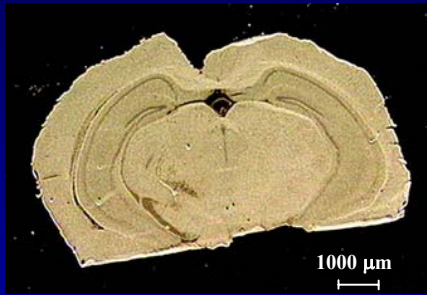


# Manual spotting vs The RapidSpotter

Mouse brain,  
Analysis of the corpus callosum



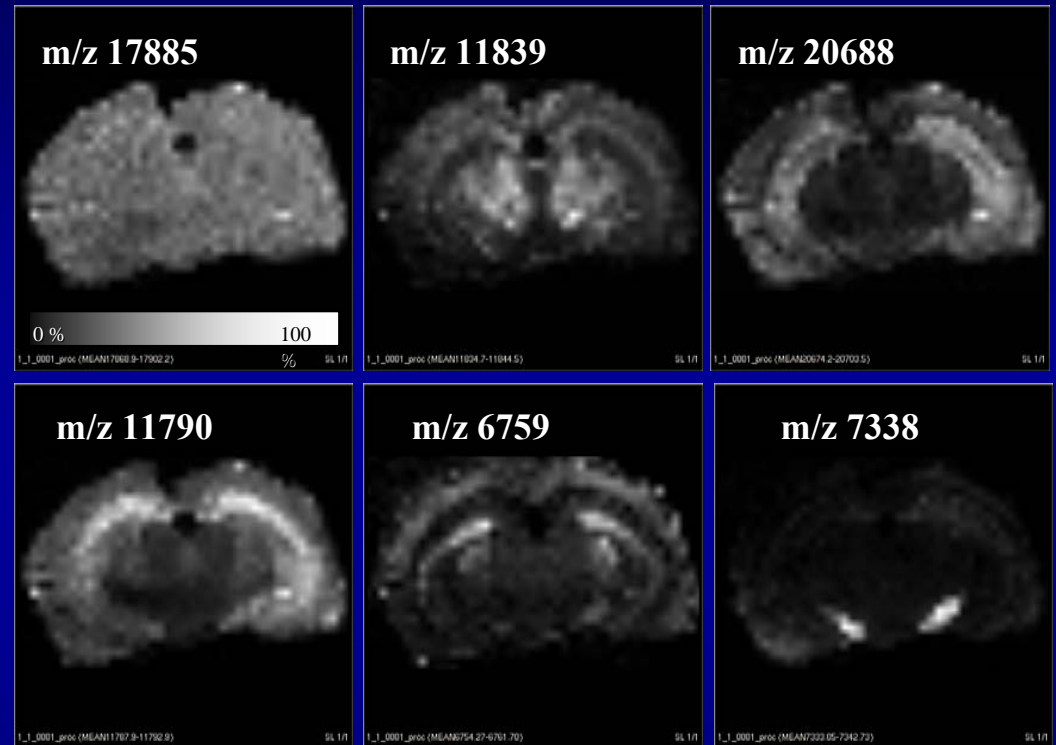
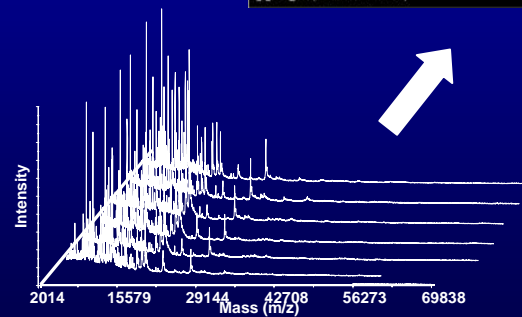
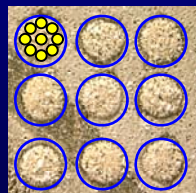
# RapidSpotter Imaging of a Mouse Brain Section



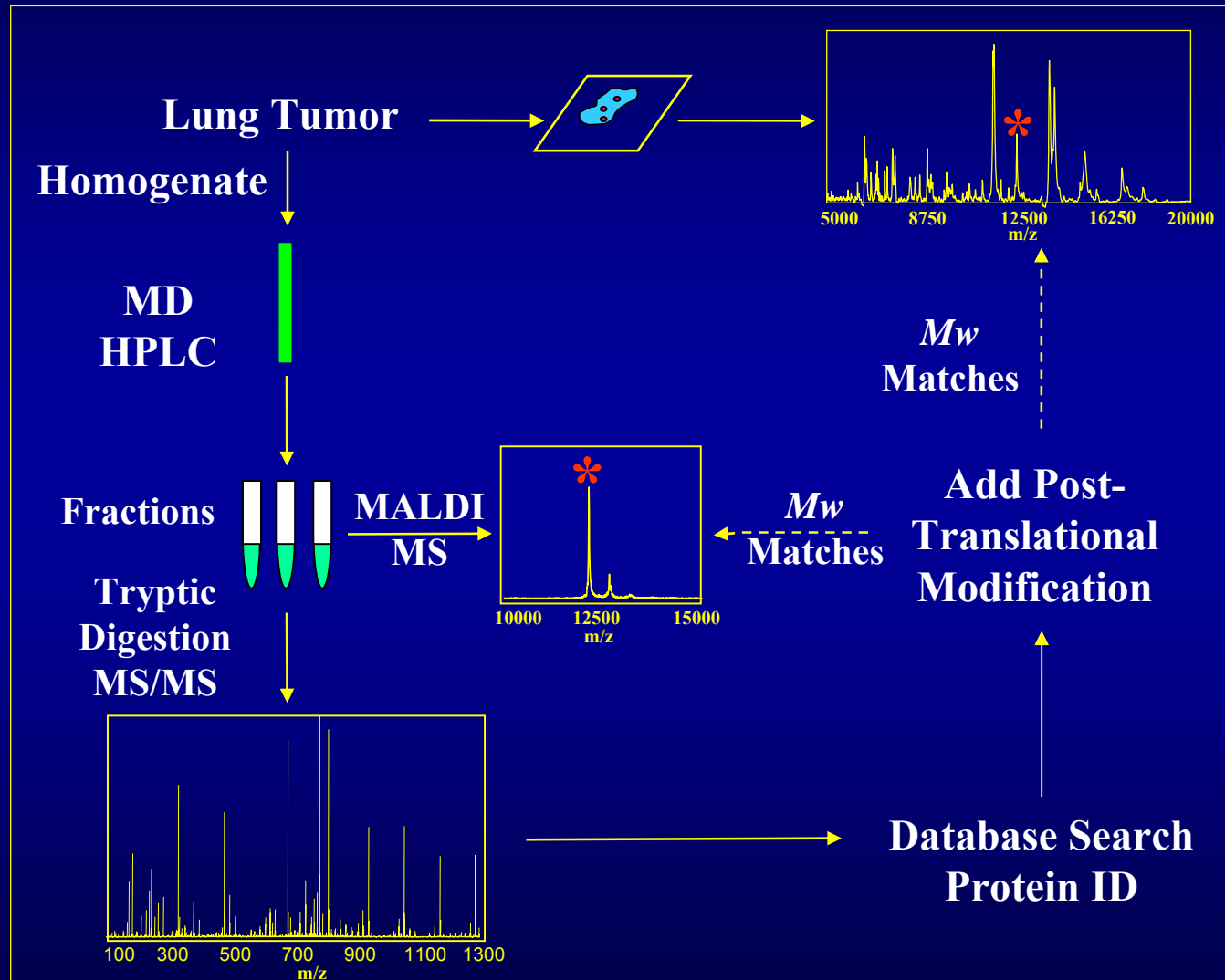
Matrix printing



Create custom  
plate file

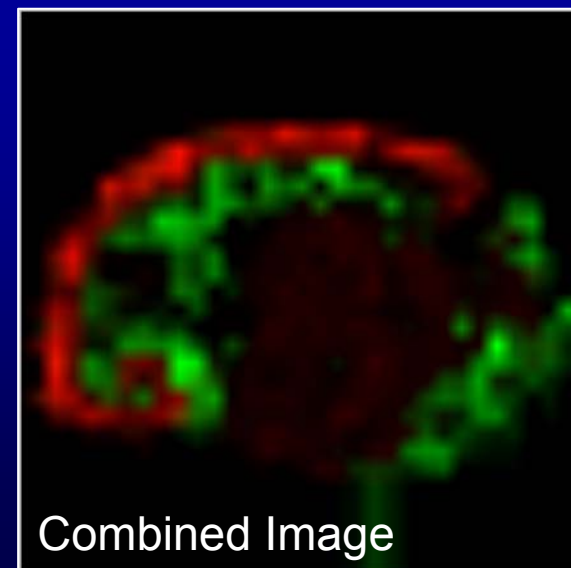
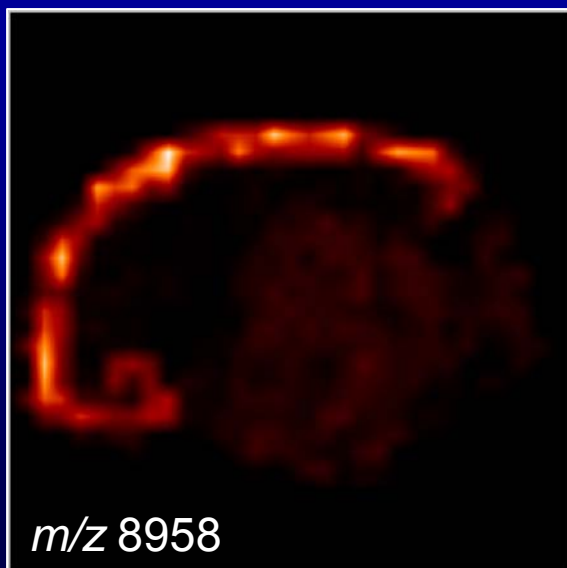
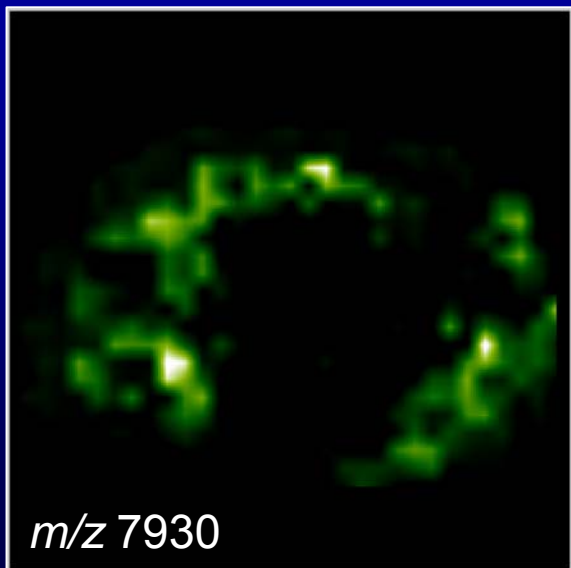
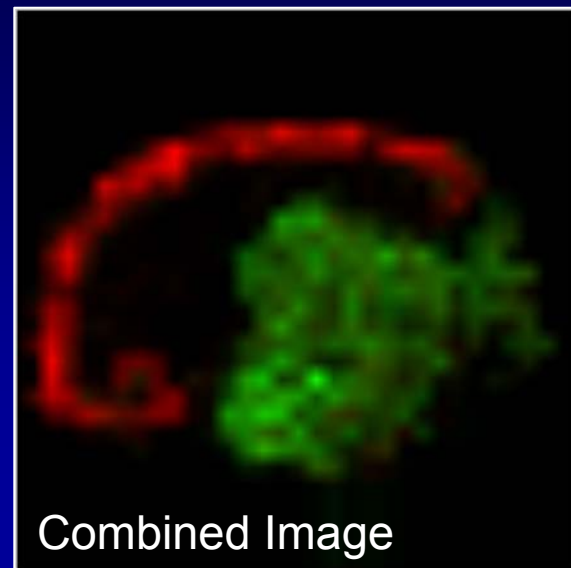
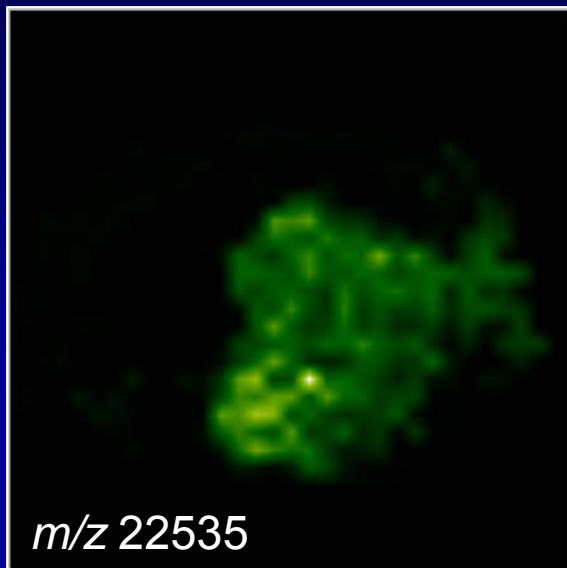
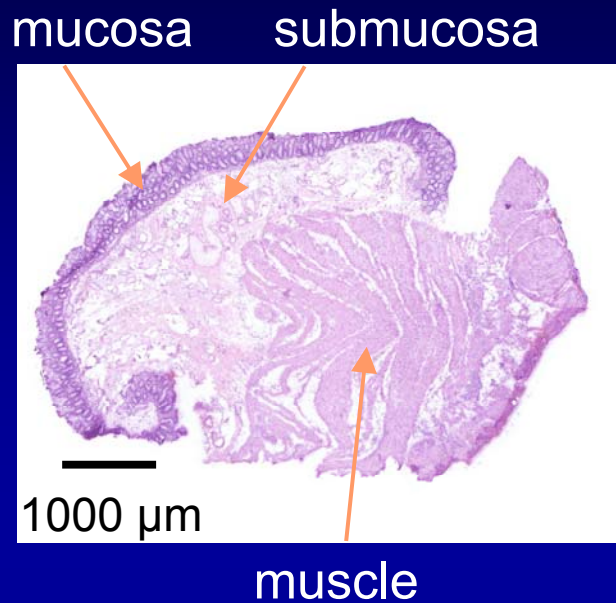


# Schematic Representation of Protein Marker Identification





# Normal human colon biopsy

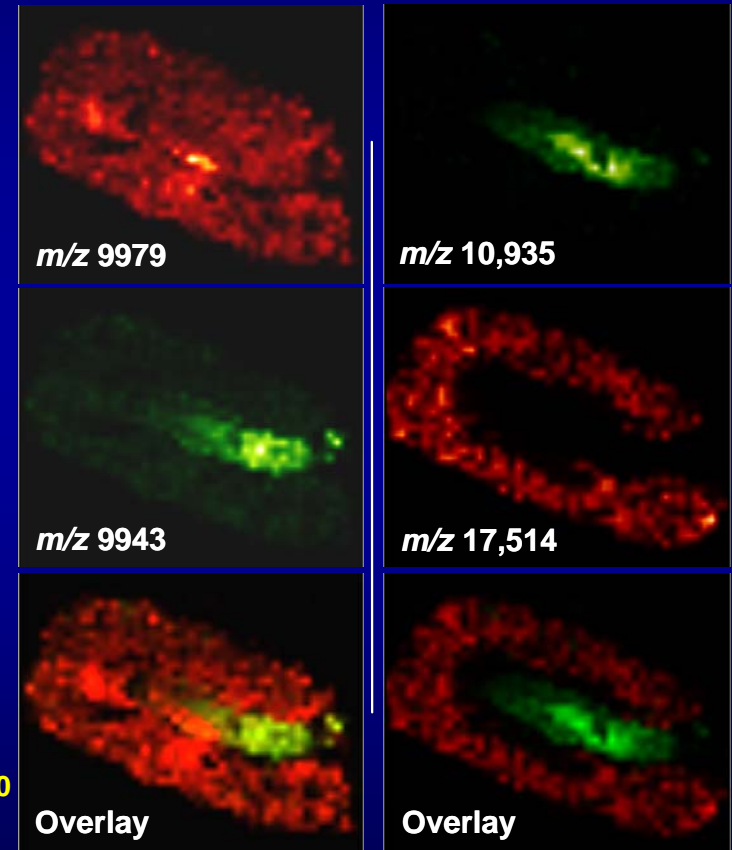
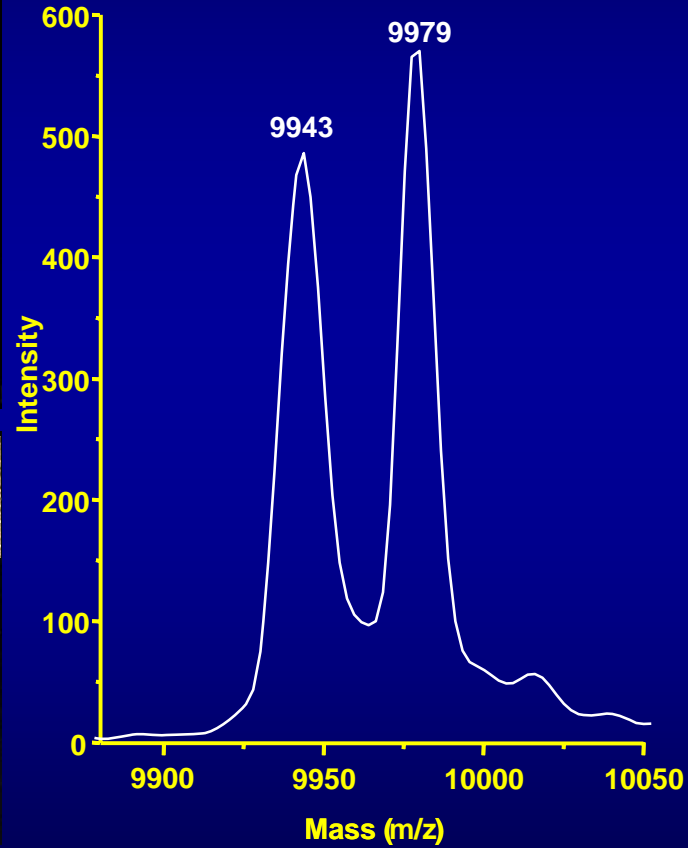
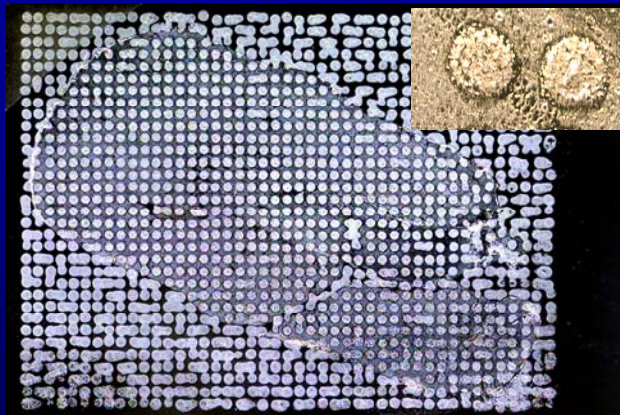
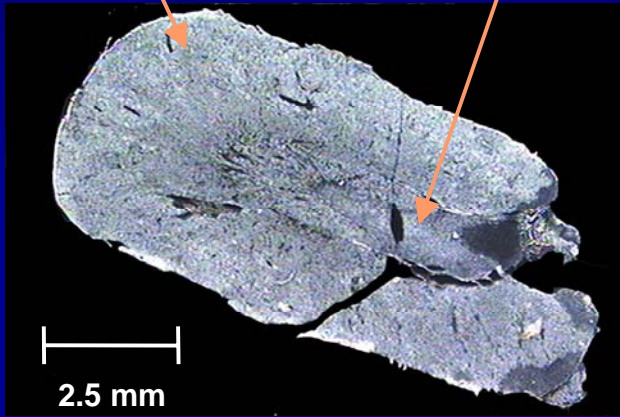




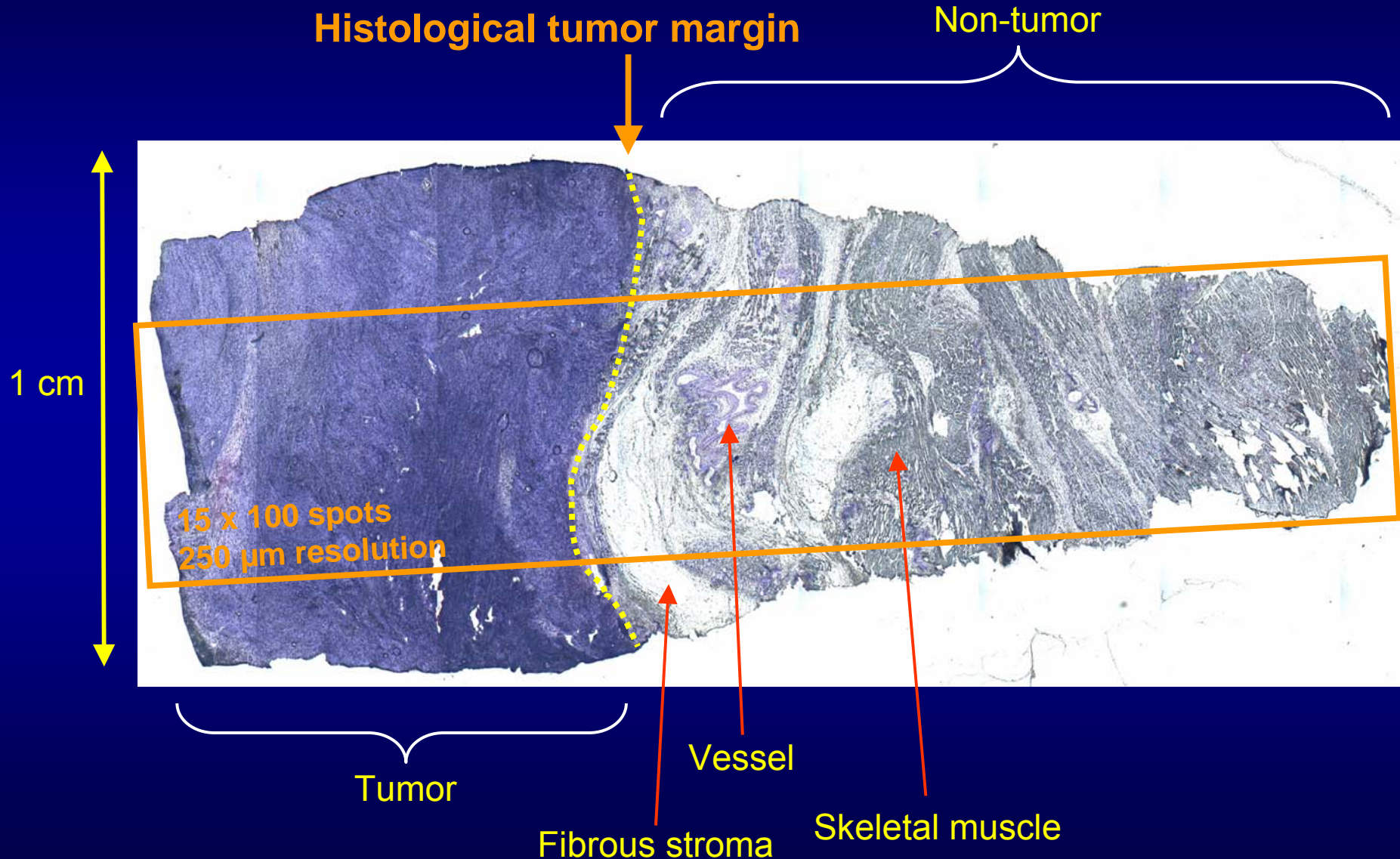
# Rat kidney sagittal section

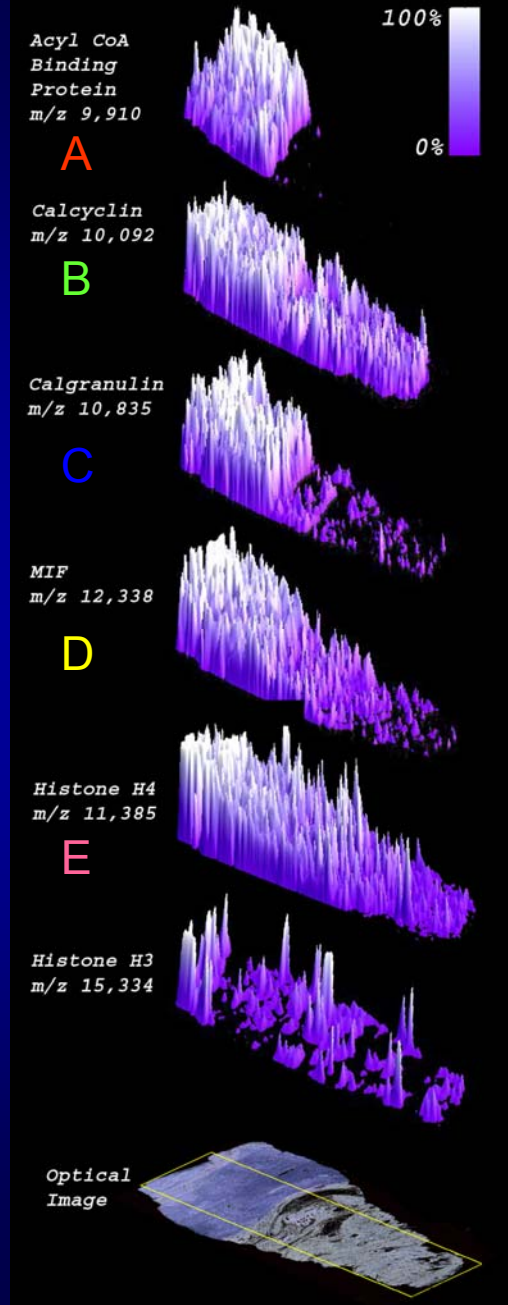
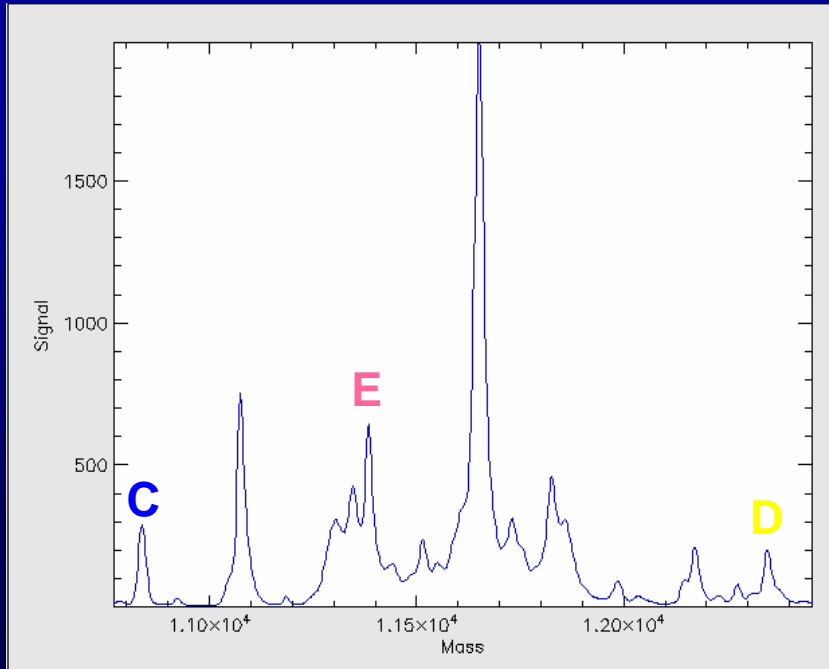
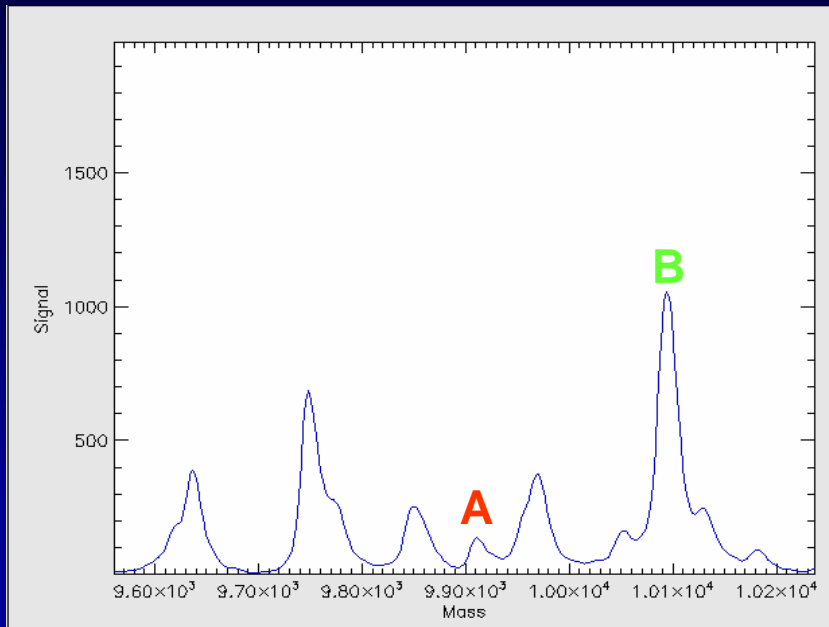
cortex

medulla

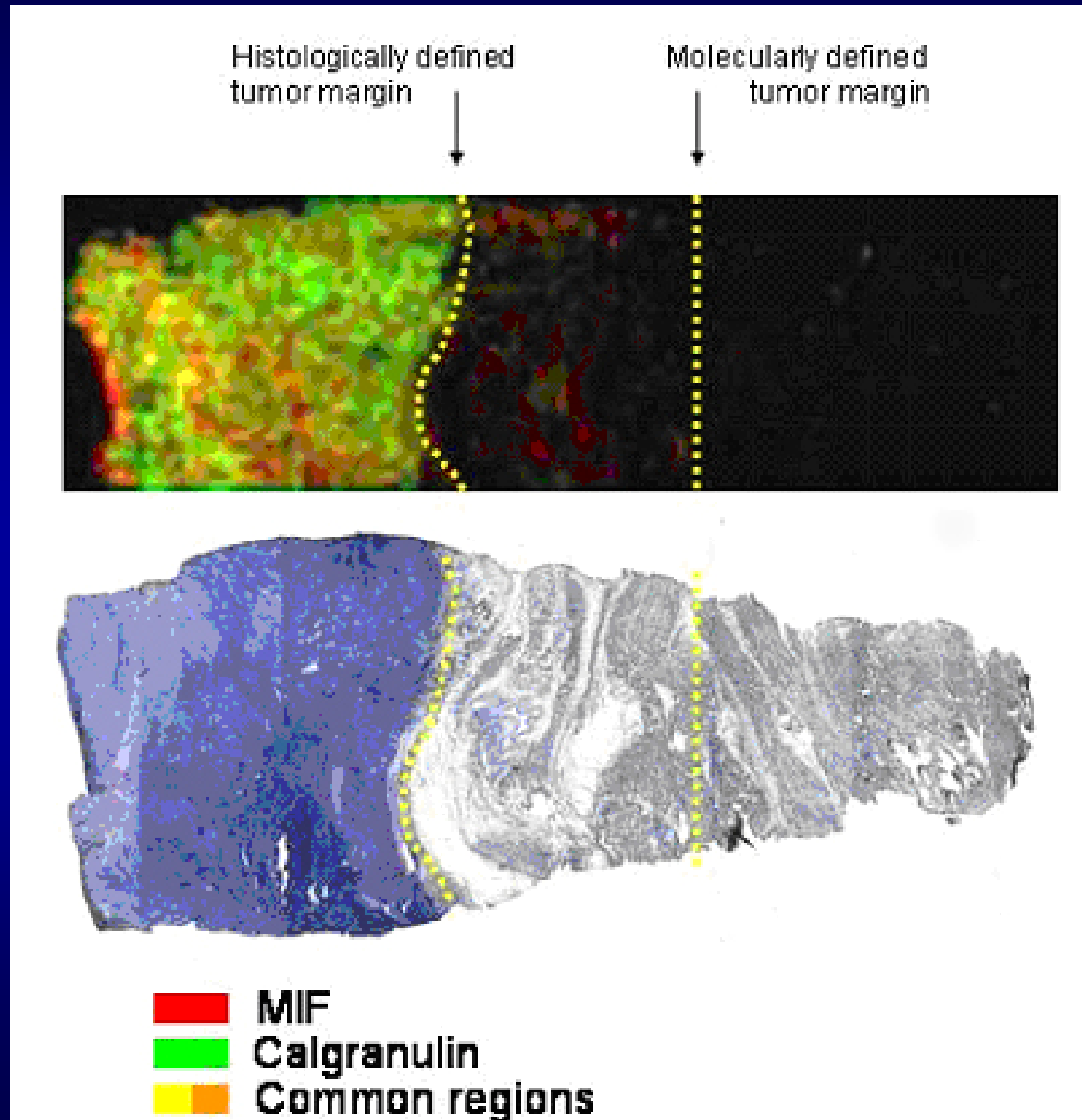


# Molecular Determination of Tumor Margins



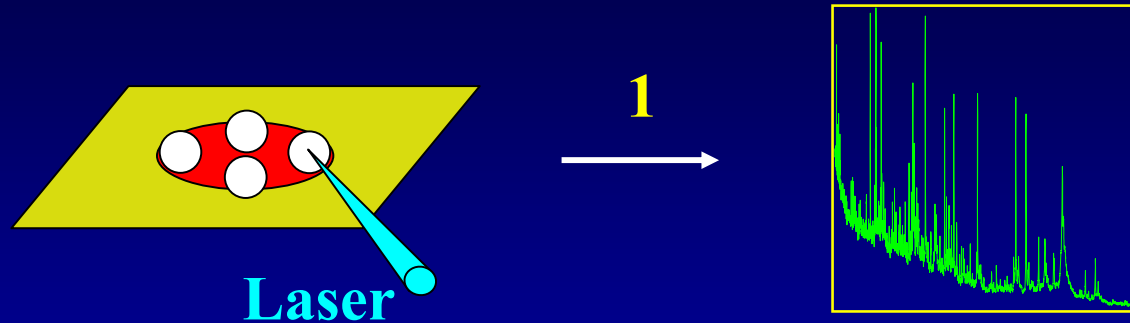


# Histological vs. molecular assessment of the tumor margin:

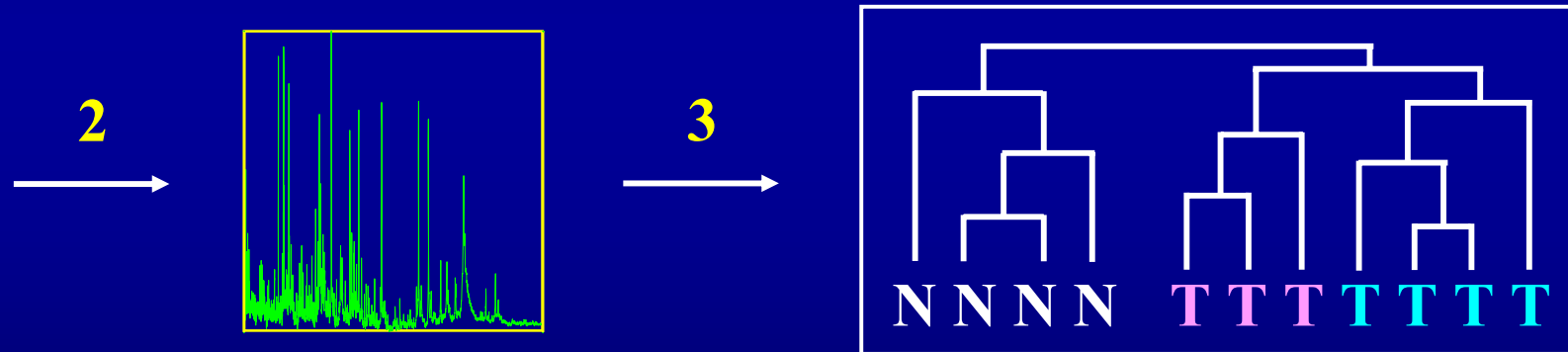




# Brain Cancer protein expression profiling by MALDI-MS



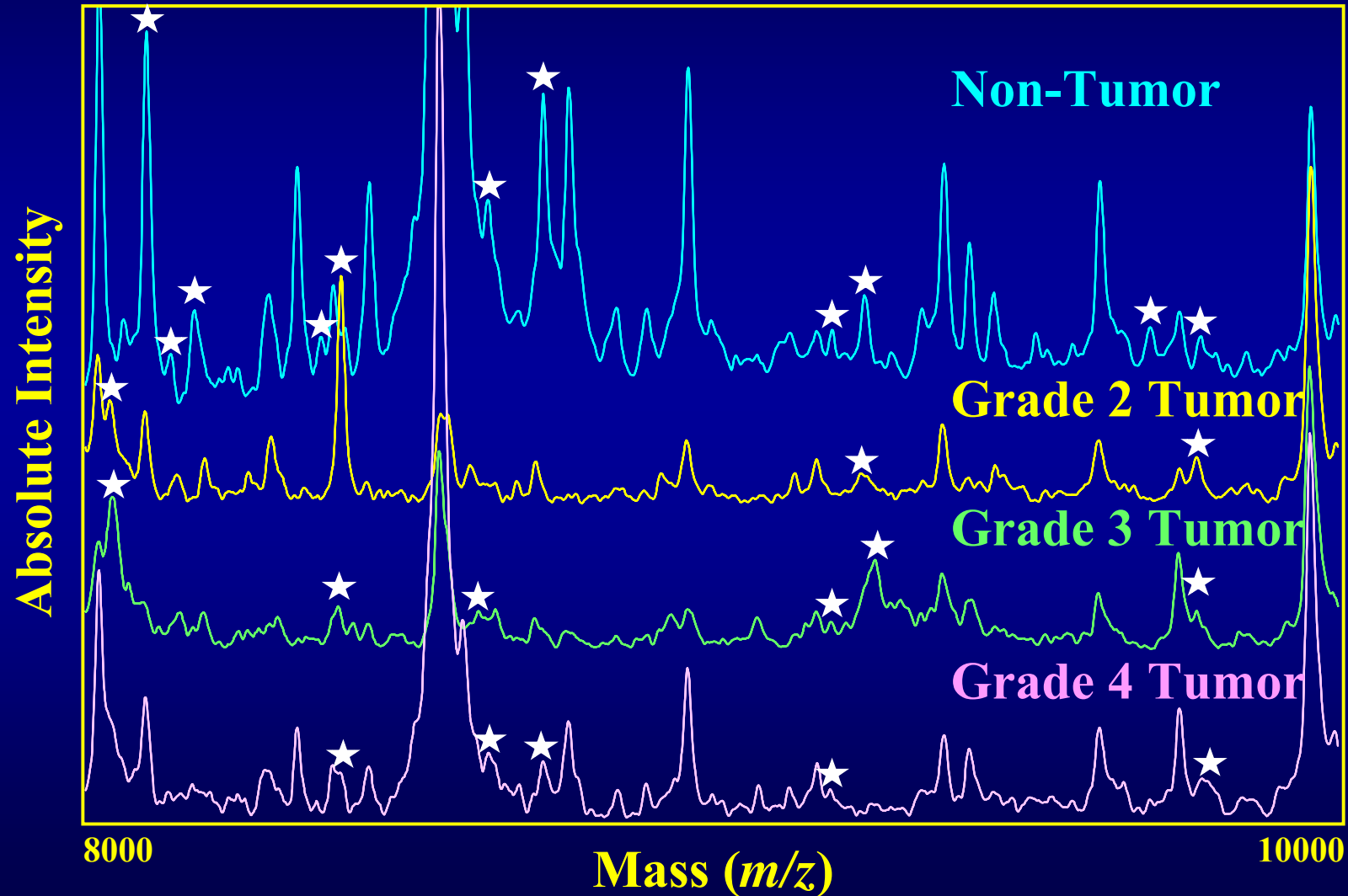
**1; Matrix droplets are regionally deposited on the sample. Each spot area is analyzed using MALDI-MS**



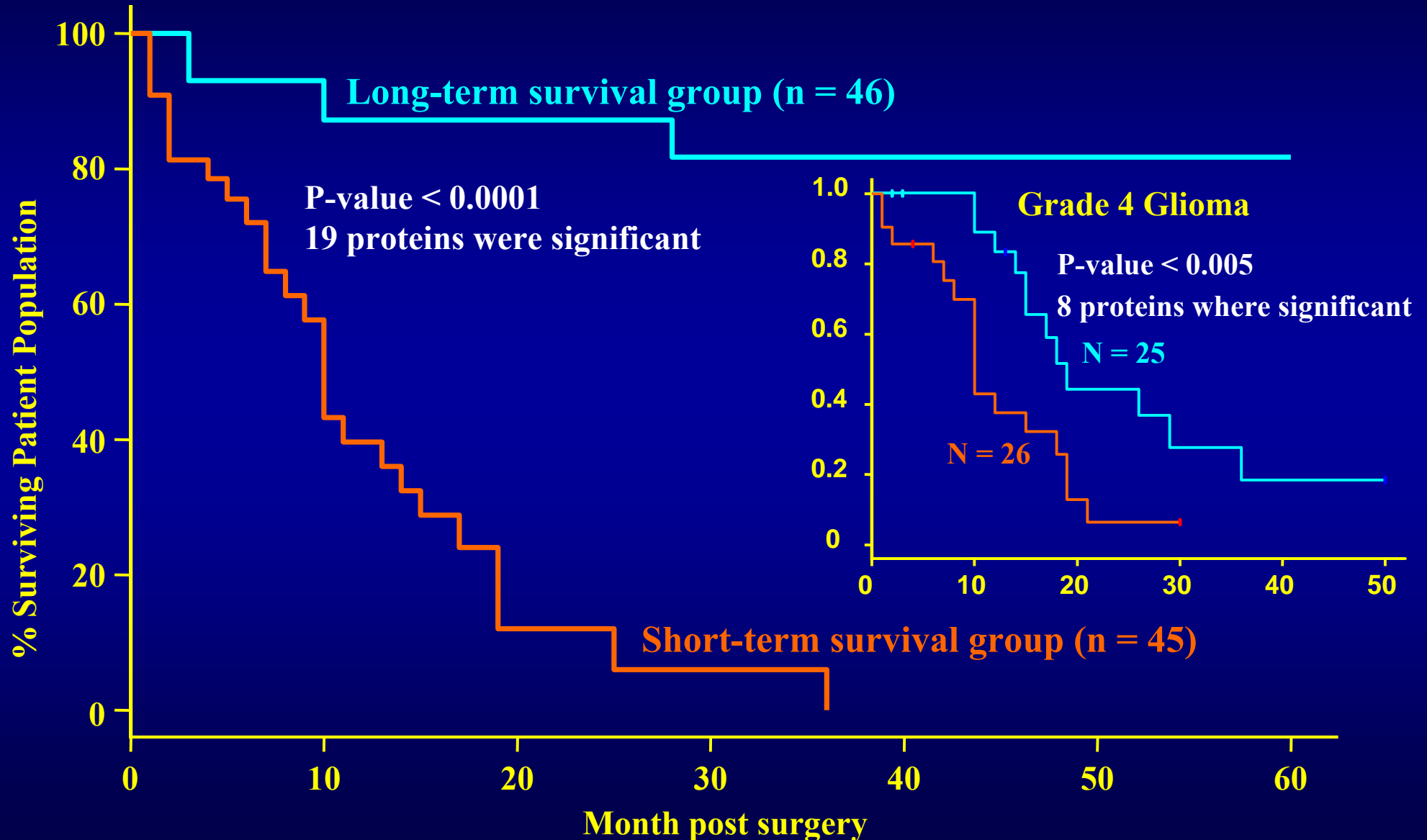
**2; Computer algorithms are used to smooth and correct baseline of each spectrum**

**3; Hierarchical cluster analysis of human brain cancer and normal brain spectra**

# Human glioma protein expression profiling by MALDI-MS

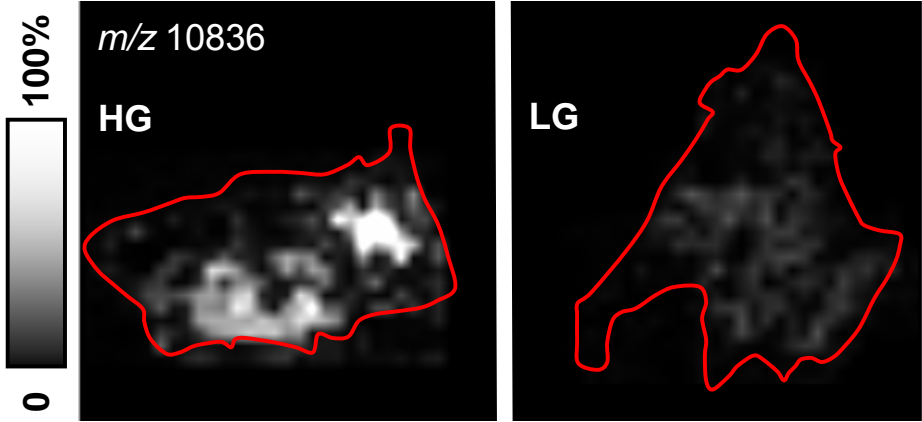
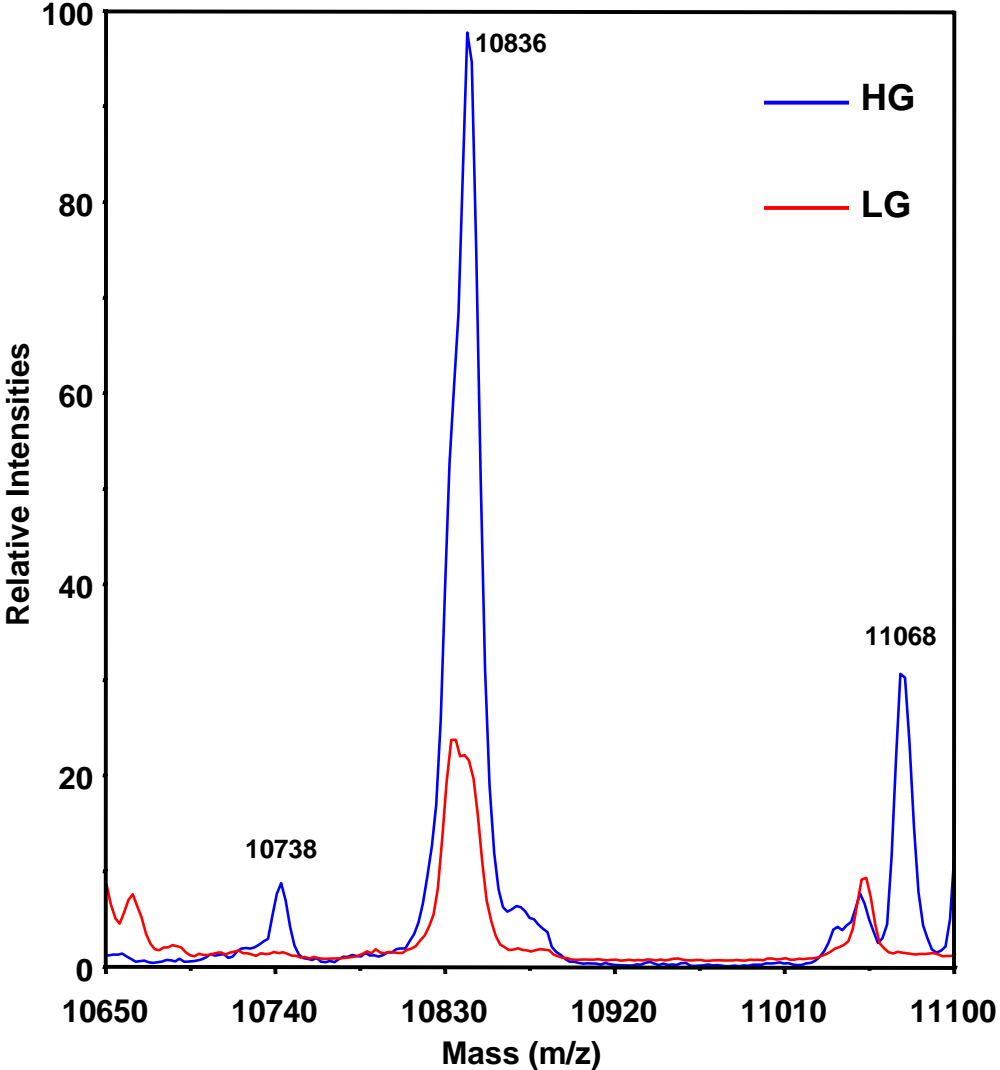


# Kaplan-Meier survival curves for groups with poor and good prognostic. Classification according to the expression pattern of 19 distinct MS peaks

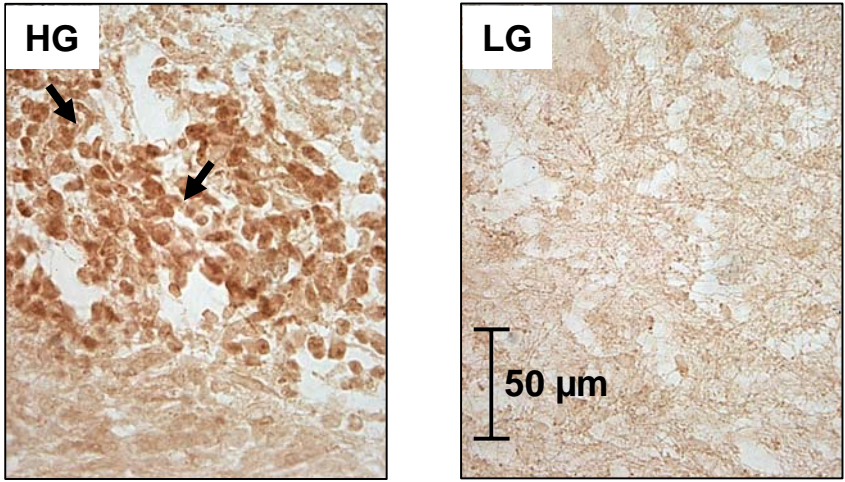


# Human brain glioma – Low grade vs. high grade

**m/z 10836, S100 Beta**



## Immunohistochemistry





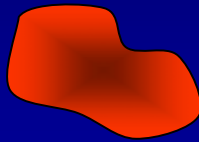
# Proteins are OK.....

## But What About Drug Distribution/ Metabolism?

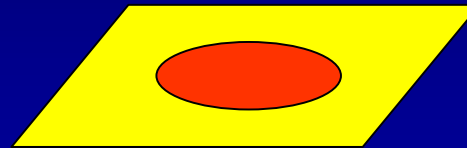


Dose animal

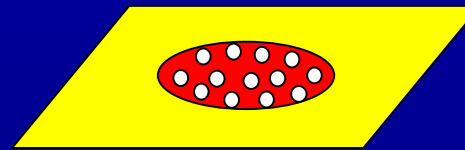
- ◆ orally
- ◆ i.v.



Remove  
tissue



- ◆ Cut frozen  
slice (12  $\mu\text{m}$ )



- ◆ Apply  
matrix



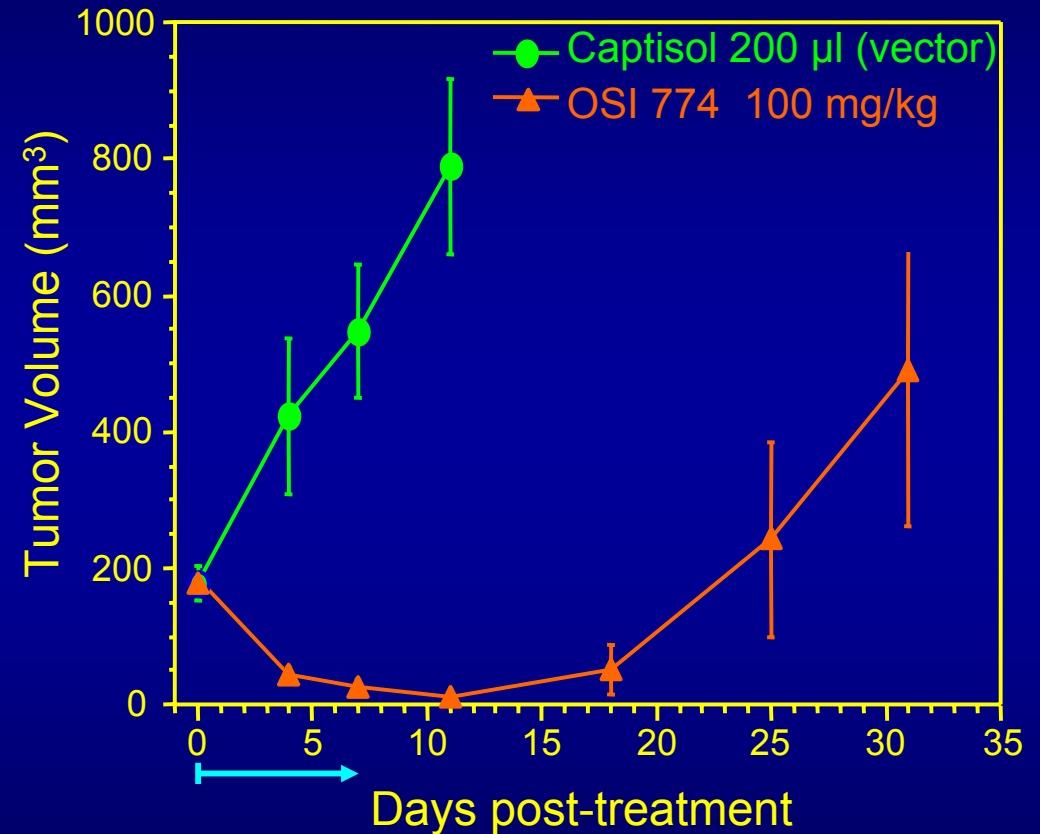
- ◆ Analyze by:  
MALDI MS and  
MALDI MS/MS

Reyzer ML et al, J Mass Spectrom, 38, 1081-1092 (2003)

Reyzer ML et al, Cancer Res 64, 9093-9100 (2004)

# MMTV/HER2 transgenic mouse mammary tumors

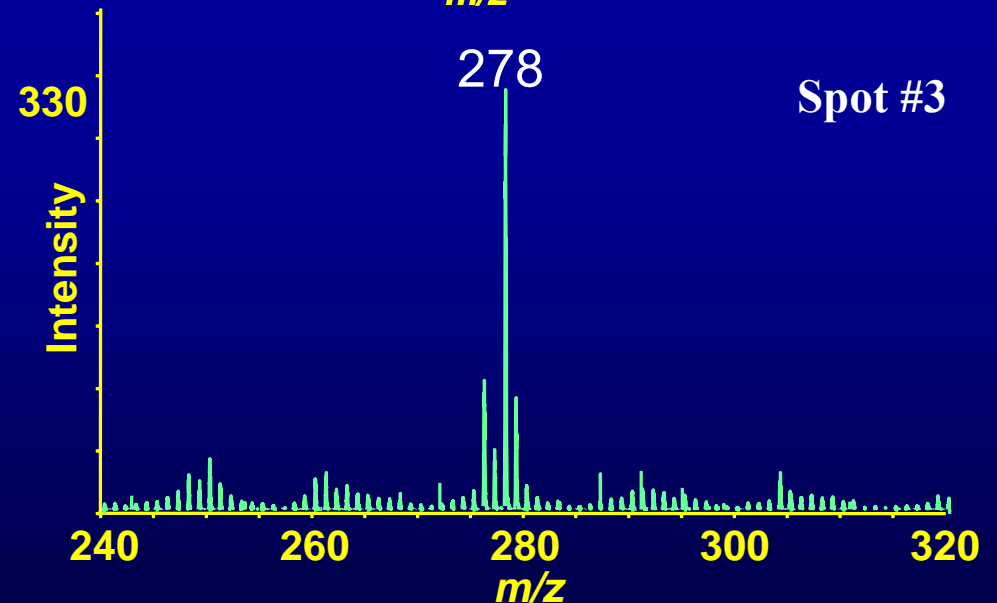
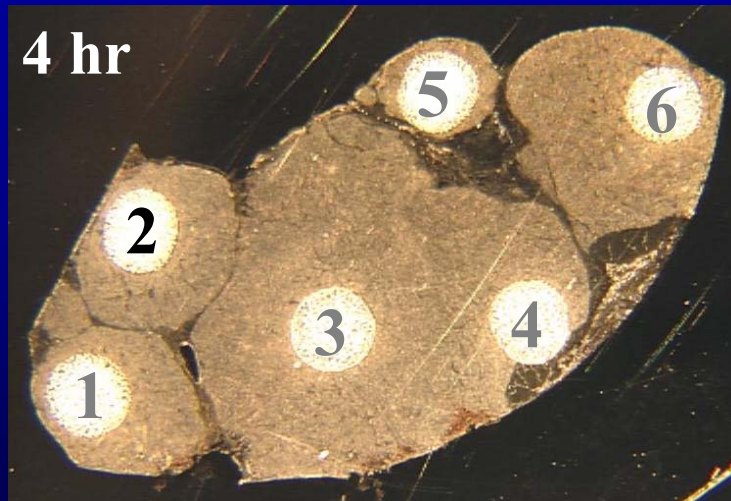
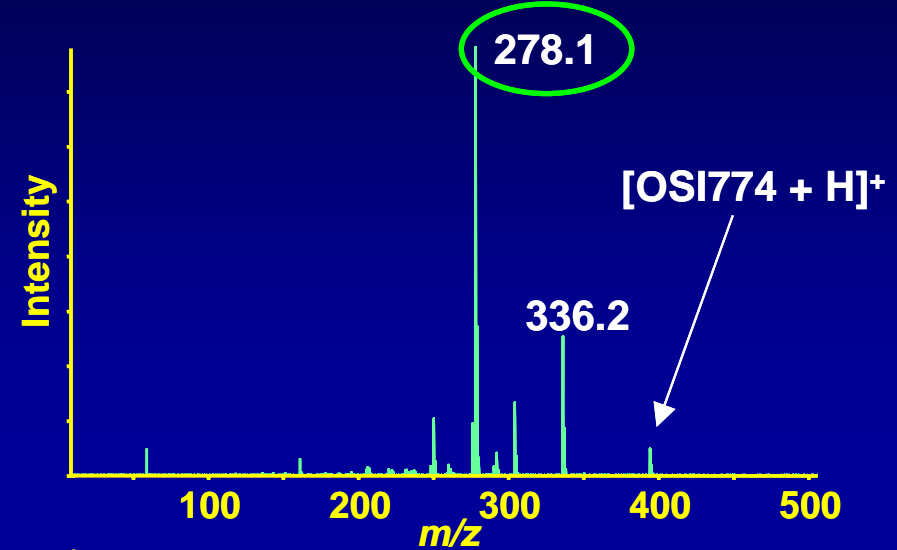
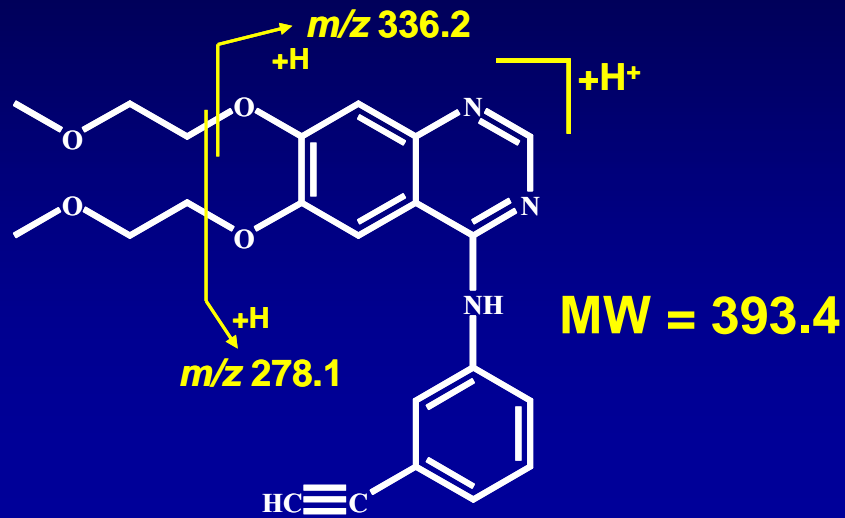
- MMTV/HER2 cells transplanted in FVB female mice.
- Tumor grown to a size of ~200 mm<sup>3</sup>.
- OSI 774 is an intracellular tyrosine kinase EGF receptor inhibitor.
- Administered orally for 1 week



Contributed by M. Sliwkowski (Genentech, Inc.)

# MS/MS analysis of OSI-774 in tumor tissue

Tumors removed after a single 100 mg/kg dose of OSI-774

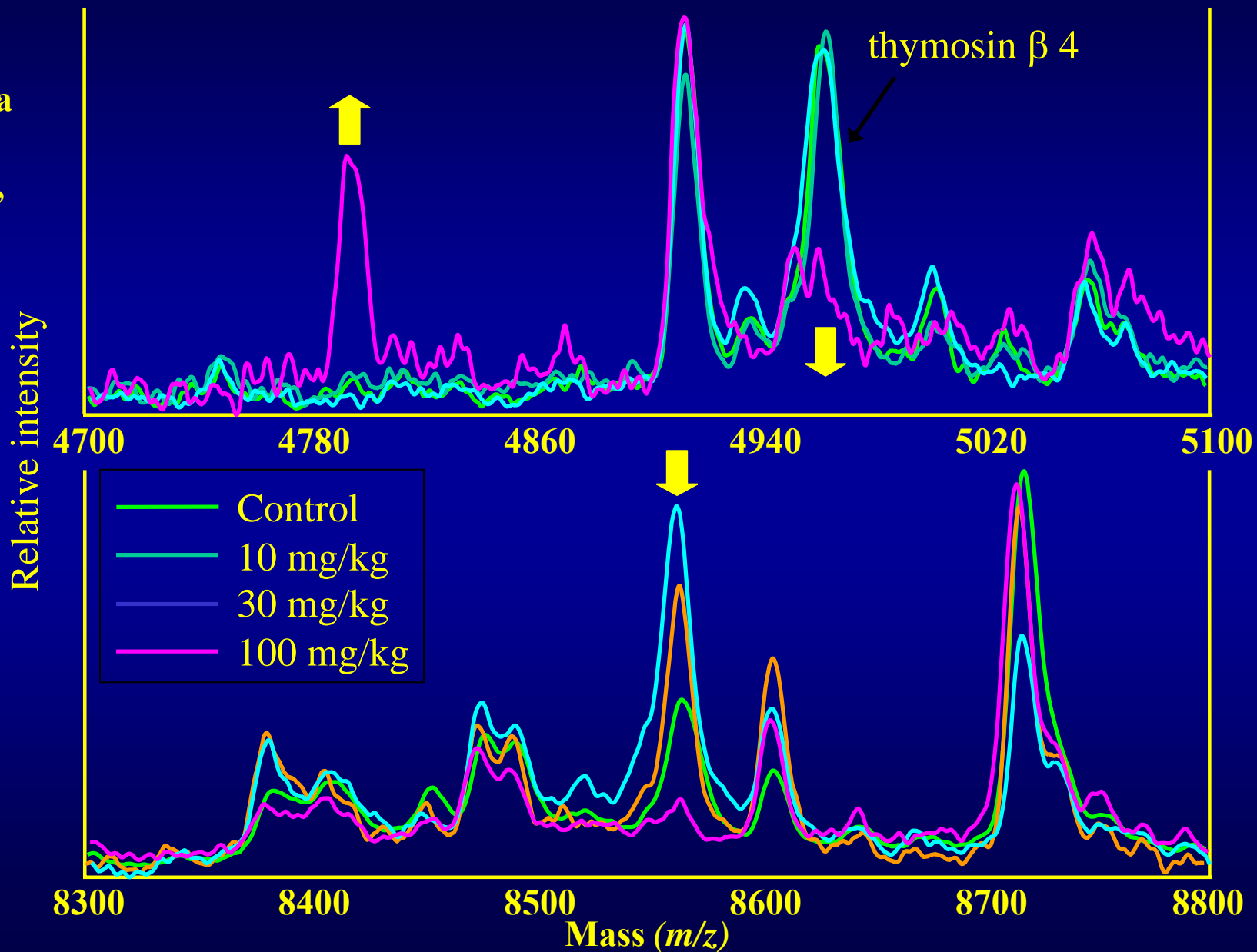


◆ Monitored CAD transition  $m/z$  394  $\rightarrow$  278

# Dose dependence of protein alteration (20 hr after dose)

9 control mice,  
Av of 54 spectra

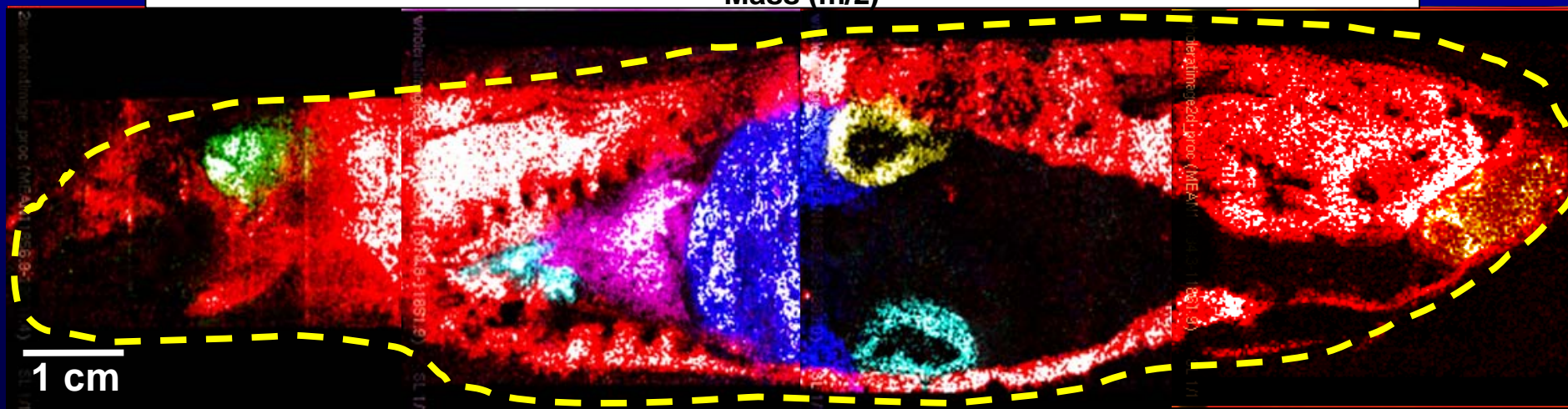
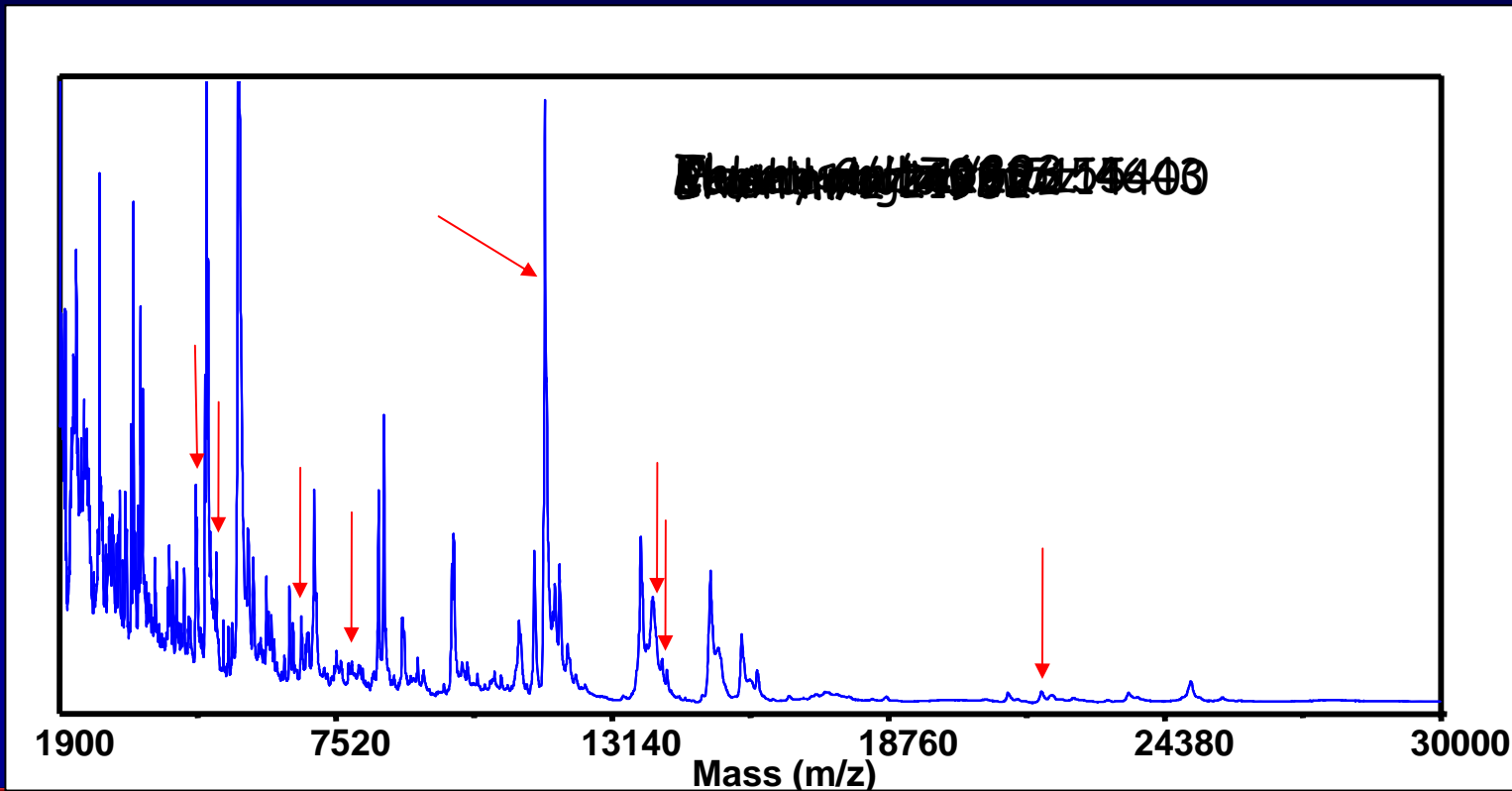
3x3 dosed mice,  
Av of 9 spectra  
per dose





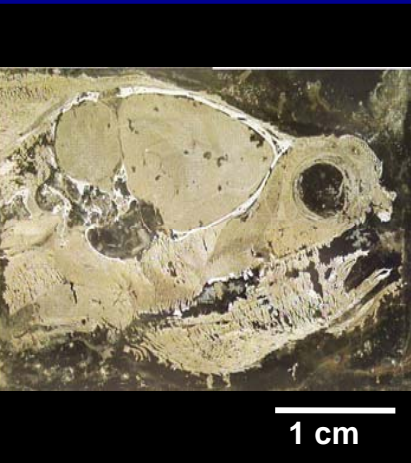
# Whole Rat Imaging

Sheerin Khatib-Shahid

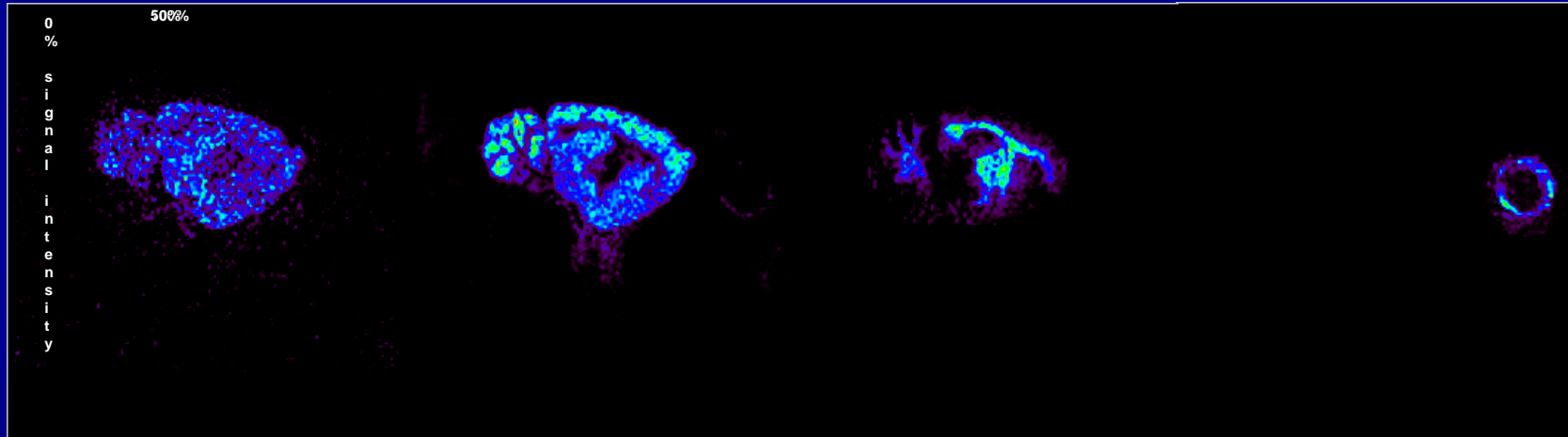


# Whole Rat Sagittal Section Imaging

## Rat Head Subsection



Optical image



Brain  
 $m/z$  20736

White matter  
 $m/z$  10271

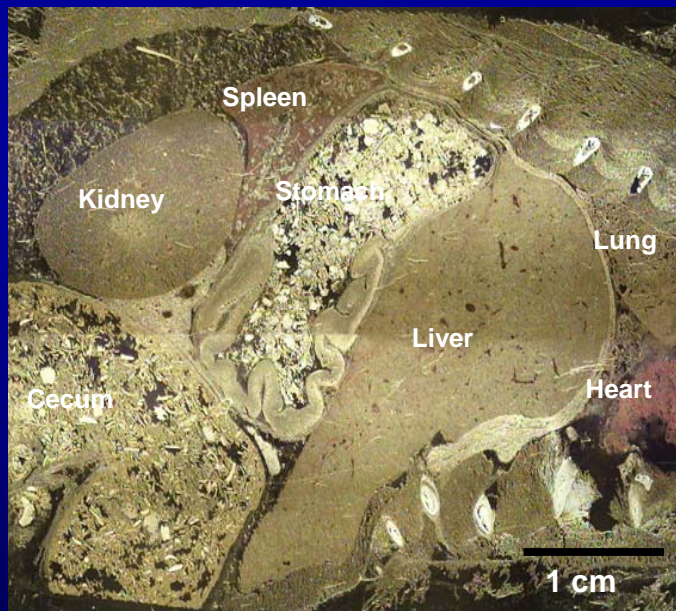
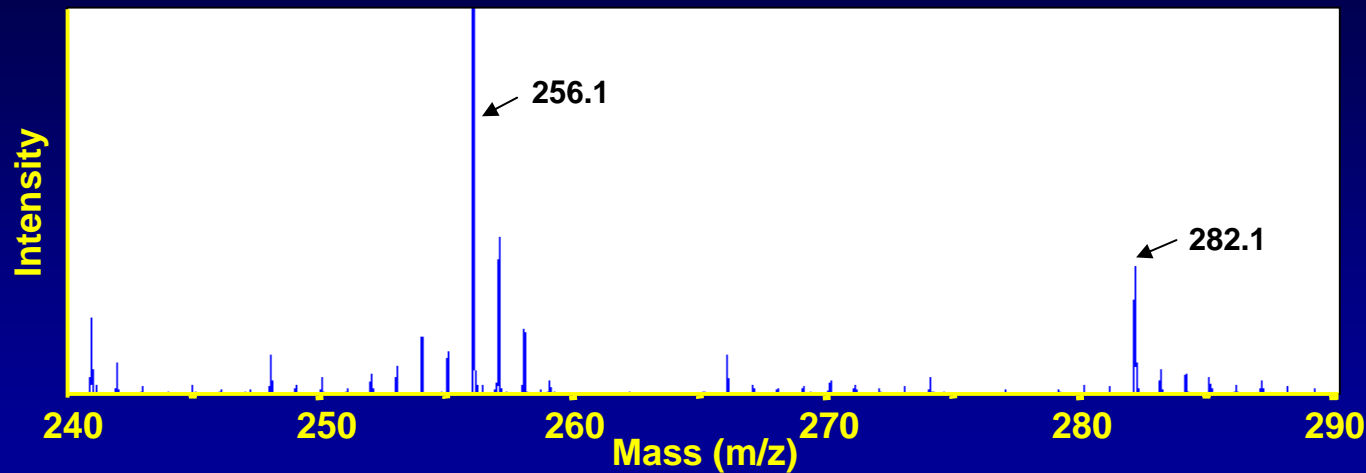
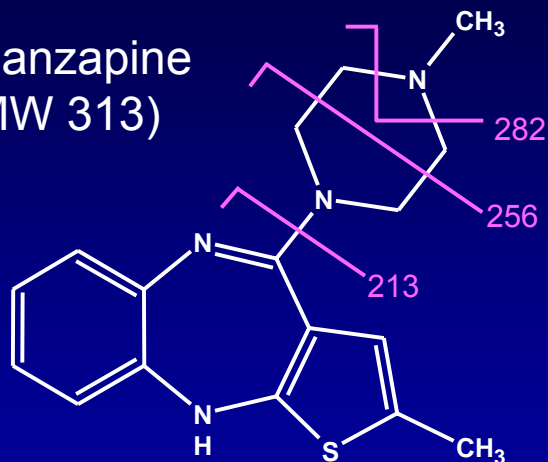
Corpus Callosum  
 $m/z$  5978

Eye socket  
 $m/z$  6858

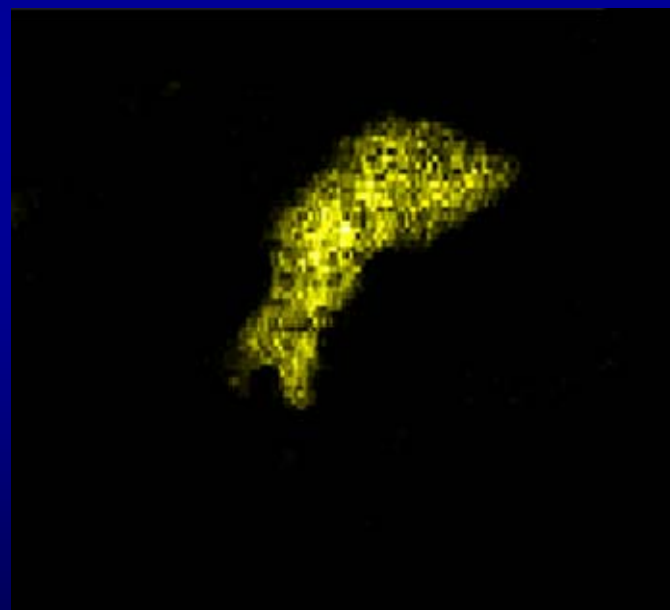
Work by Sheerin Khatib-Shahid

# Drug Imaging

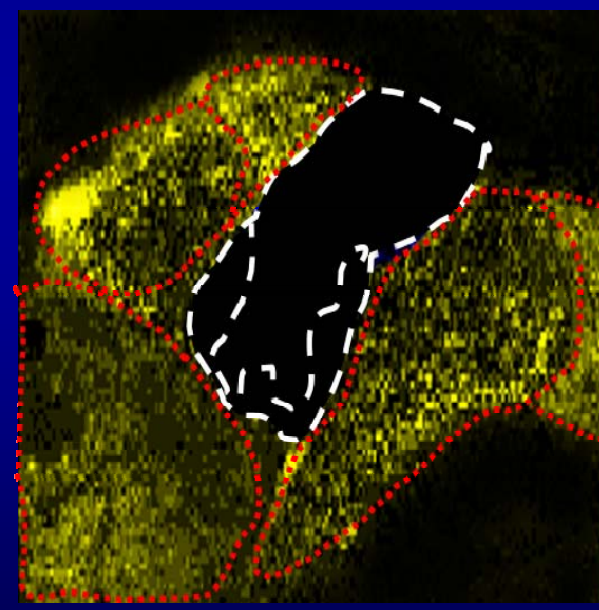
Olanzapine  
(MW 313)



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



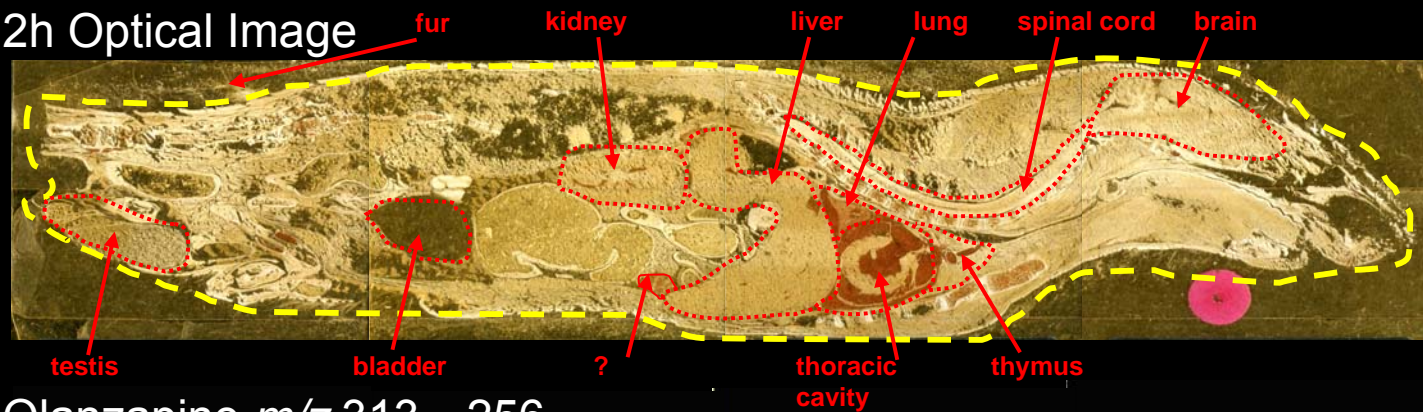
MALDI MS/MS Image of OLZ  
( $m/z$  256) in stomach 2 hours  
post-dose



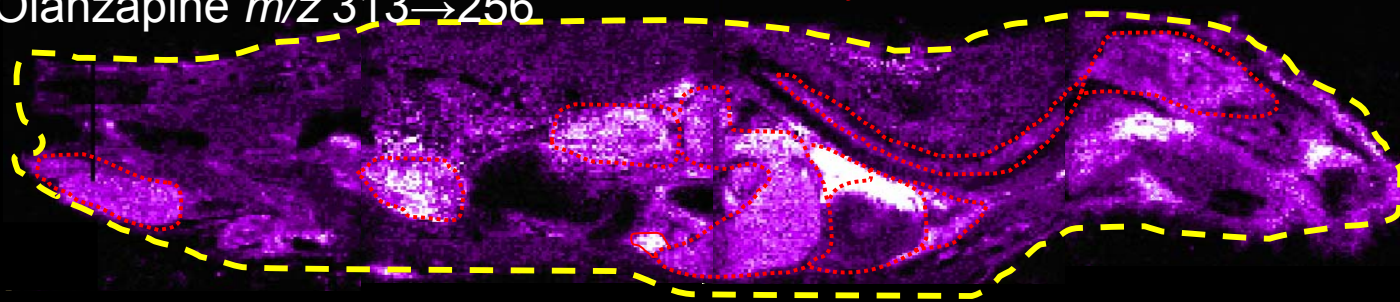
MS/MS Image of OLZ,  
10x signal intensity with  
stomach signals masked



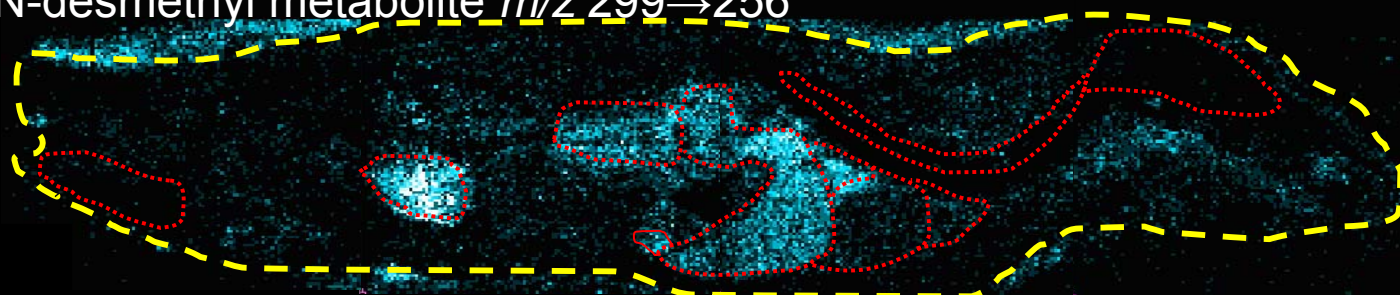
2h Optical Image



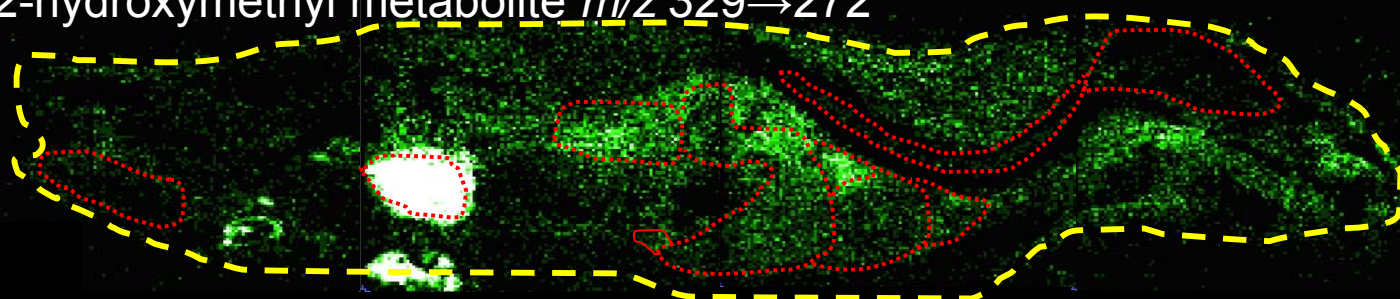
Olanzapine  $m/z$  313  $\rightarrow$  256



N-desmethyl metabolite  $m/z$  299  $\rightarrow$  256



2-hydroxymethyl metabolite  $m/z$  329  $\rightarrow$  272





## **Areas of Further Development**

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

# Tissue Profiling/Imaging MS

## Conclusions

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

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