Tissue Profiling by MALDI-Tof MS:

Basic Principles From Small Molecules to Proteins

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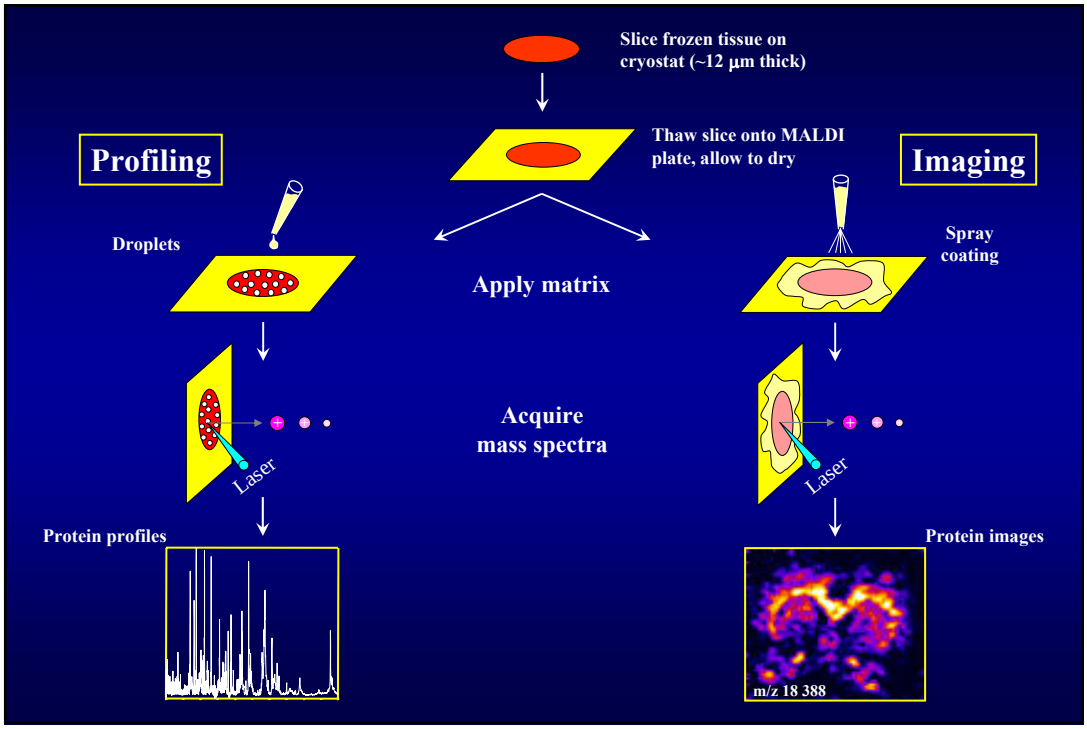
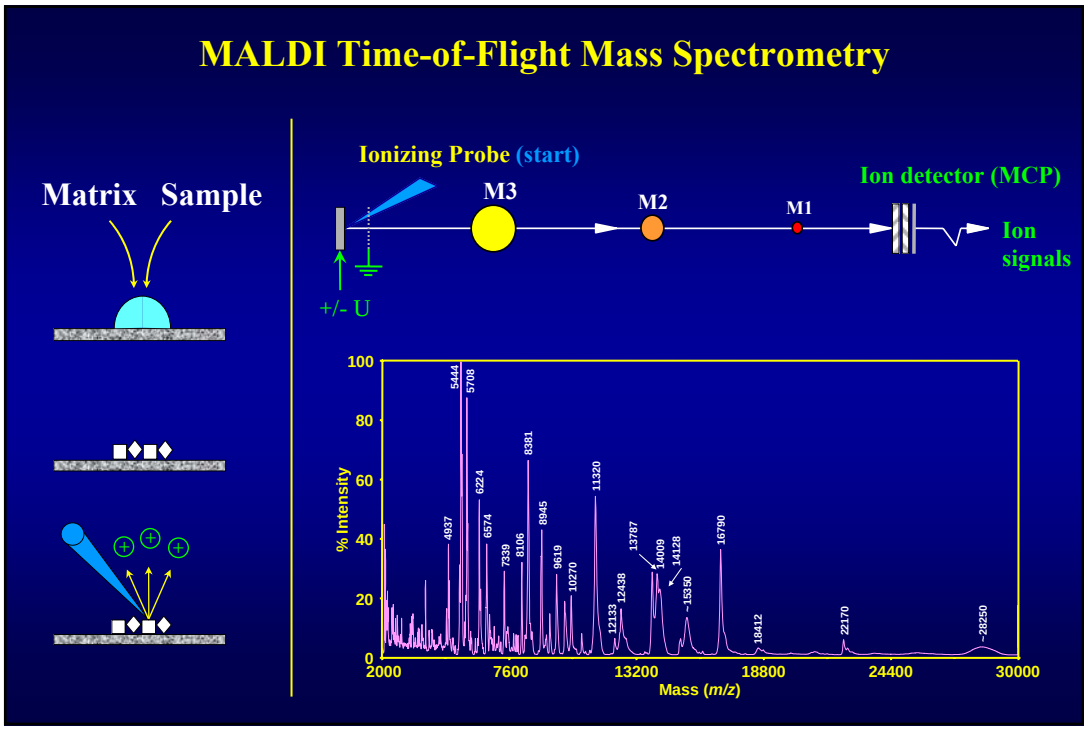
Imaging Mass Spectrometry of Thin Tissue Sections

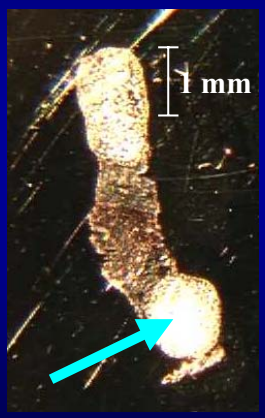
- **To obtain information on the local protein composition at any coordinate on the section**
- **To reconstruct 2-dimensional density maps (or images) for all of the signals detected**
- **To obtain molecular profiles and images indicative of health status**
- **Discovery tool to answer fundamental questions relevant to protein expression in normal and diseased tissues**

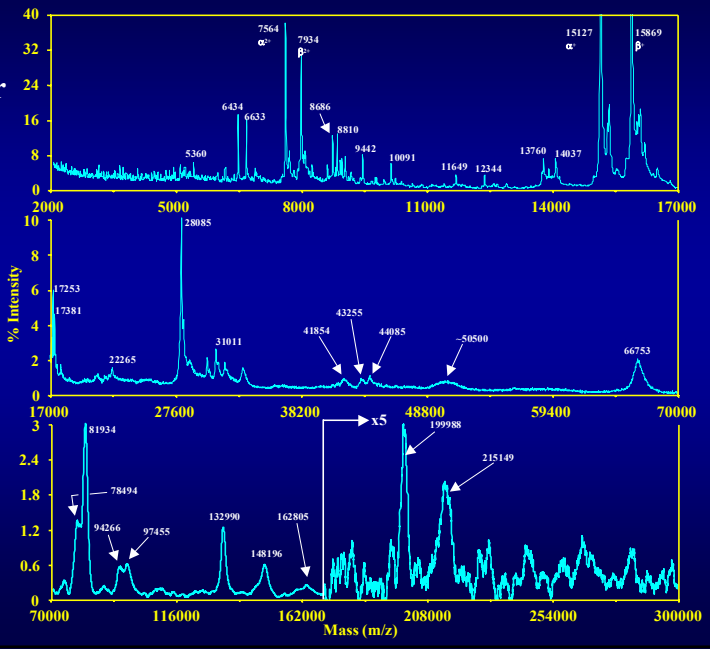

Slice frozen tissue on cryostat (~12 µm thick)
Thaw slice onto MALDI plate, allow to dry
Profiling
Imaging
Droplets
Spray coating
Apply matrix
Acquire mass spectra
Laser
Laser
Protein profiles
Protein images
m/z 18 388

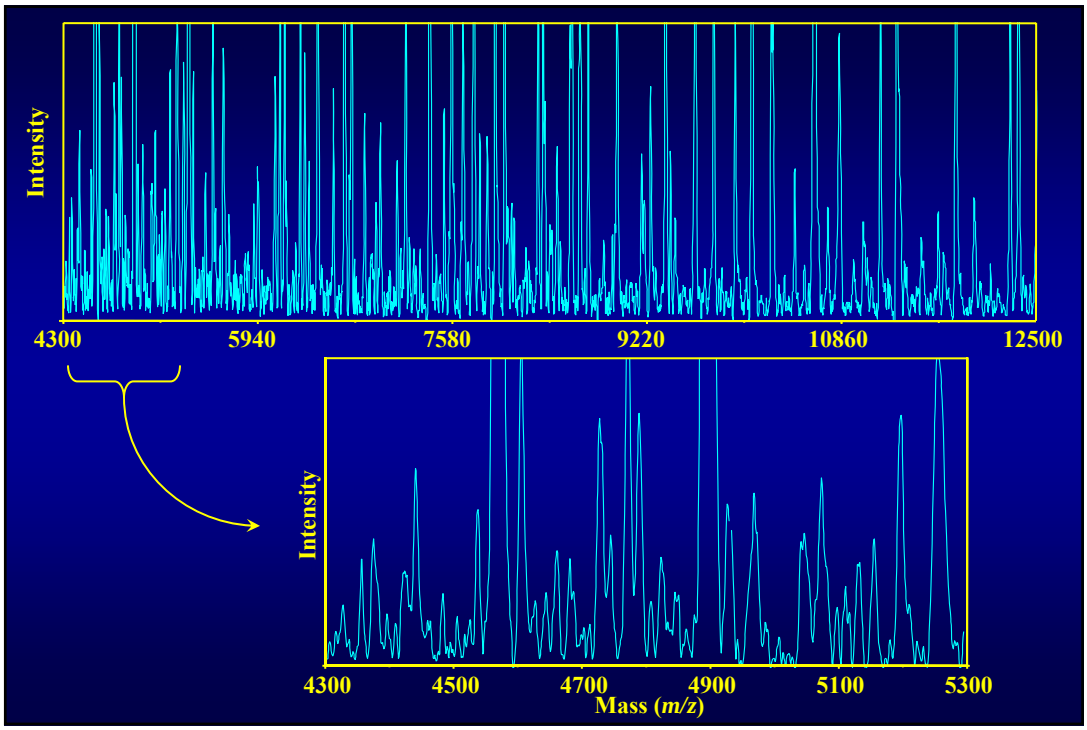

MALDI Time-of-Flight Mass Spectrometry
Matrix Sample
Ionizing Probe (start)
M3
M2
M1
Ion detector (MCP)
Ion signals
+/- U
% Intensity
Mass (m/z)
2000
7600
13200
18800
24400
30000
0
20
40
60
80
100
5444
5708
6224
6574
4937
7339
8106
8381
8945
9619
10270
11320
12133
12438
13787
14009
14128
~15350
16790
18412
22170
~28250

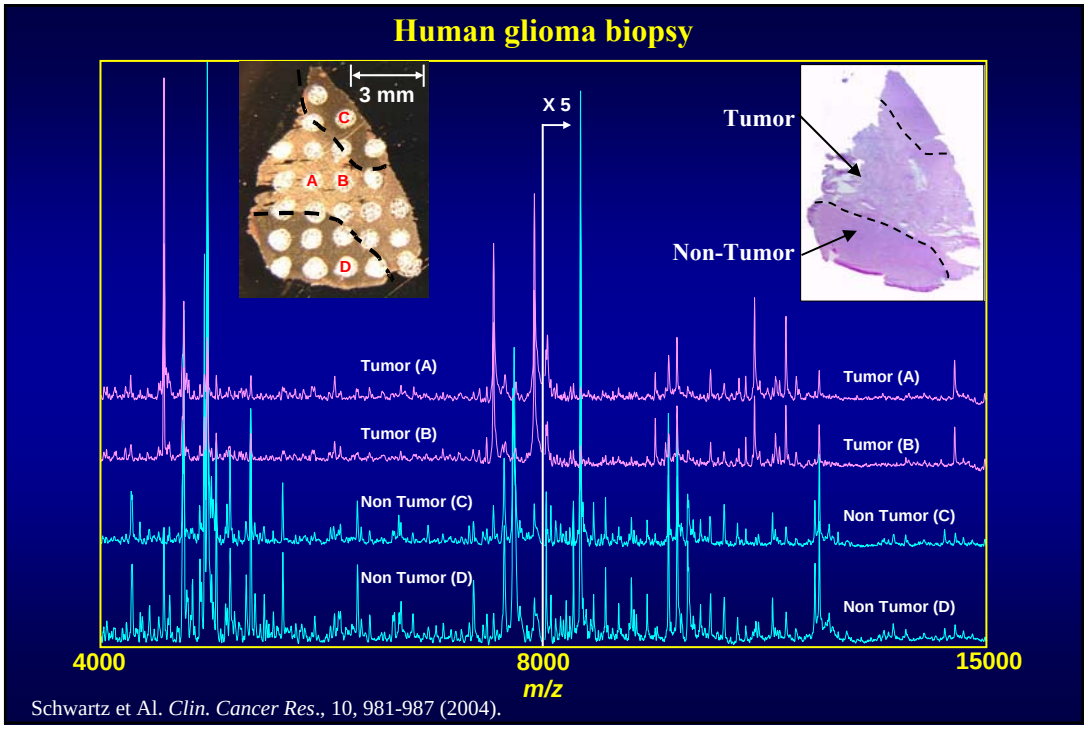
Protein expression profiling by MALDI-MS

Human breast tumor needle biopsy

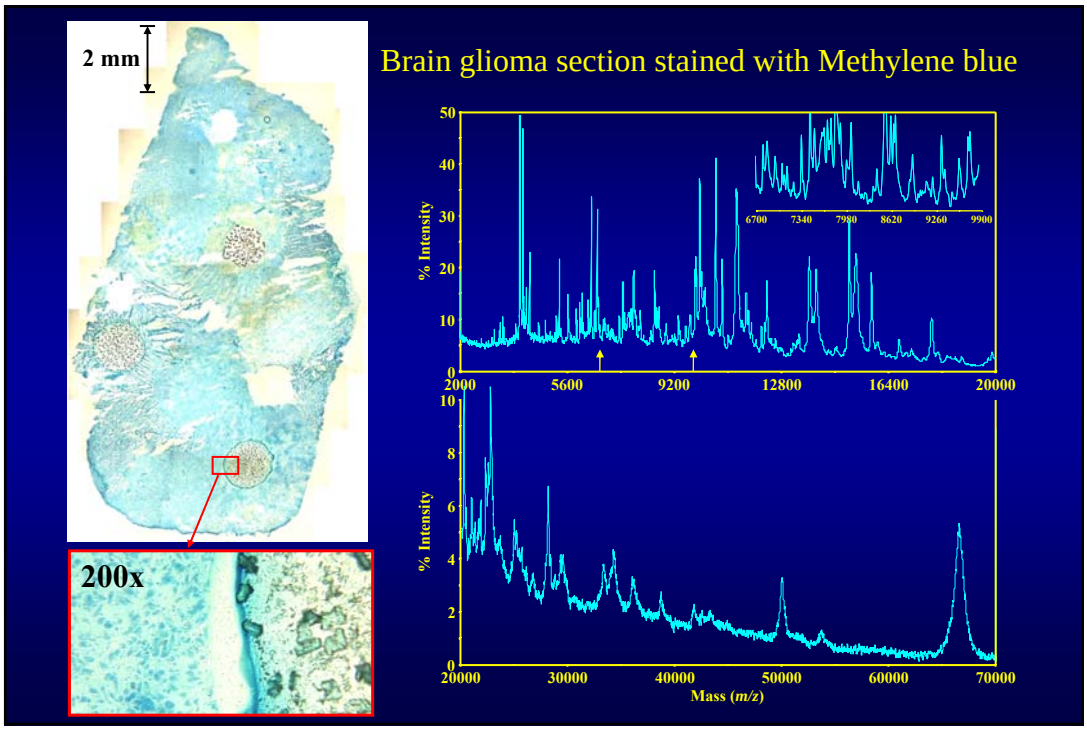




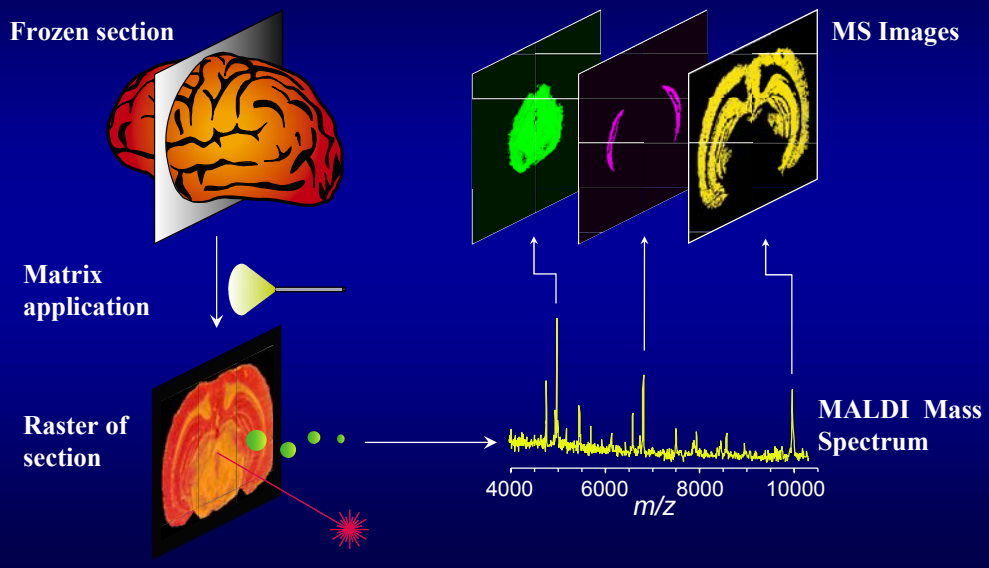




Schwartz et Al. *Clin. Cancer Res*., 10, 981-987 (2004).

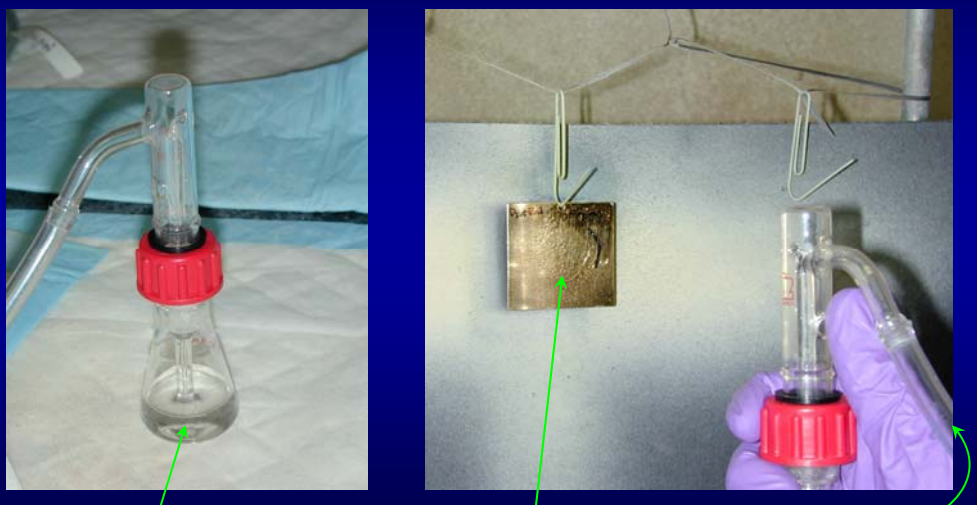


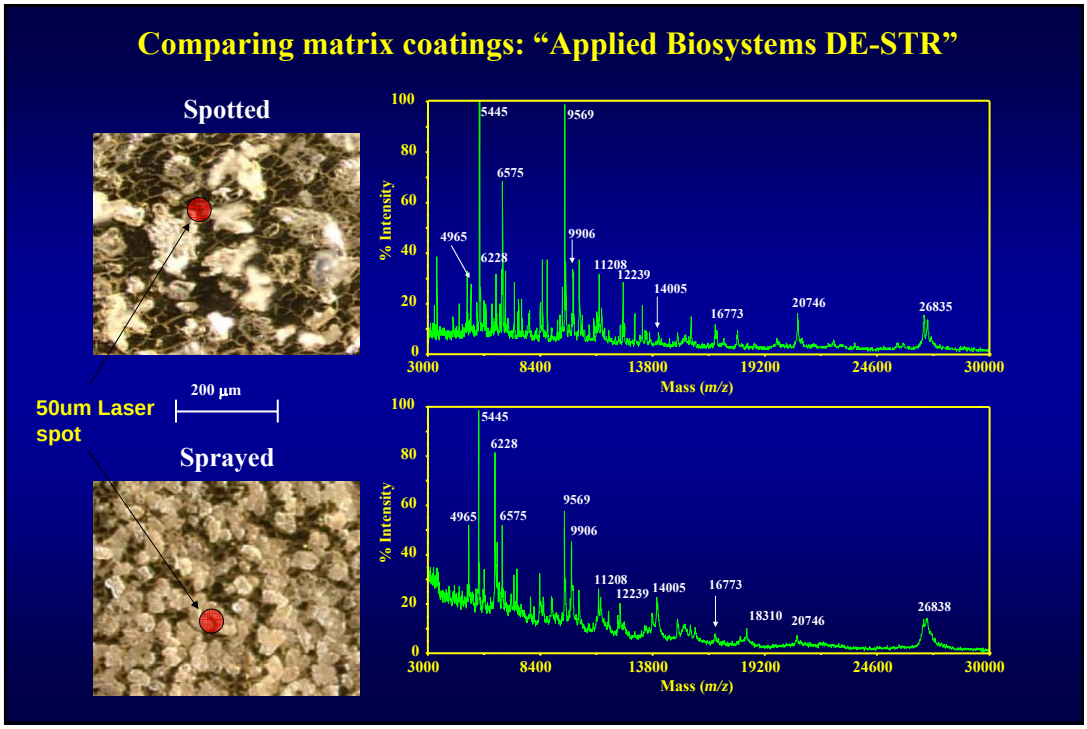
Principle of MALDI MS Imaging

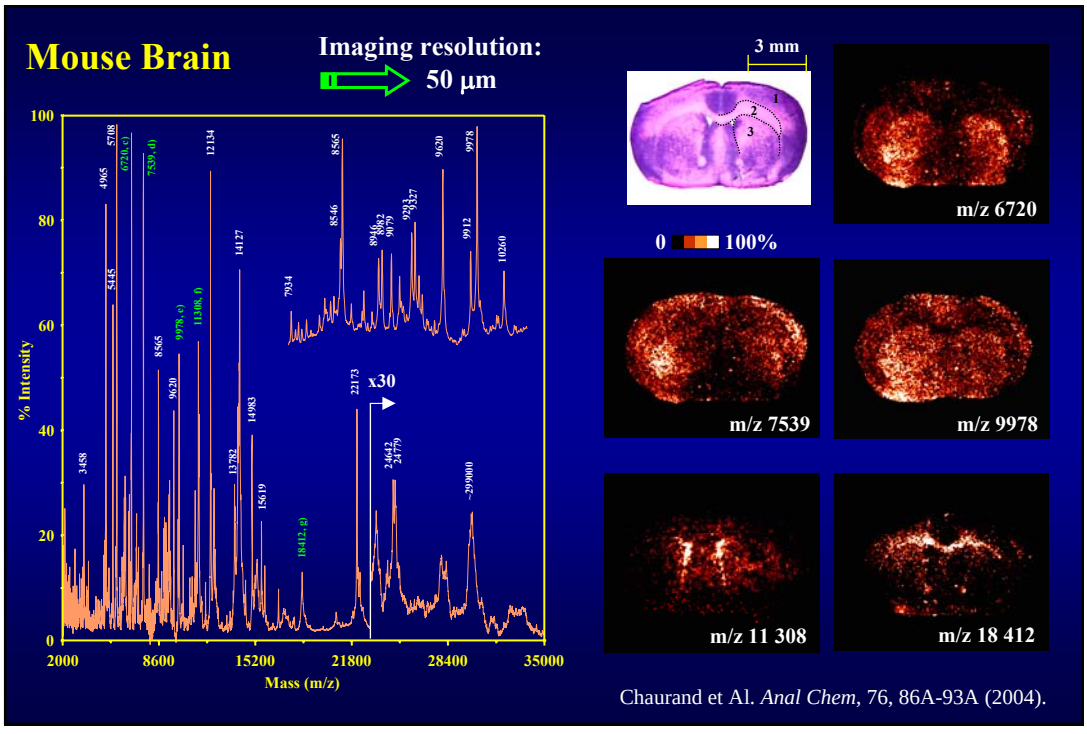


Spray deposition of matrix on tissue sections

Spray nebulizer for TLC plates

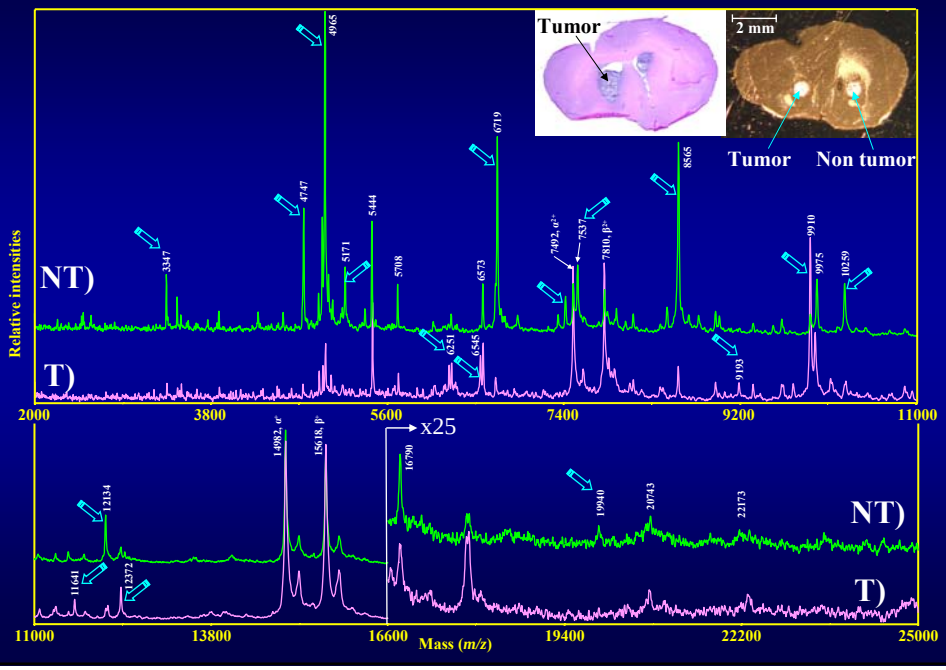




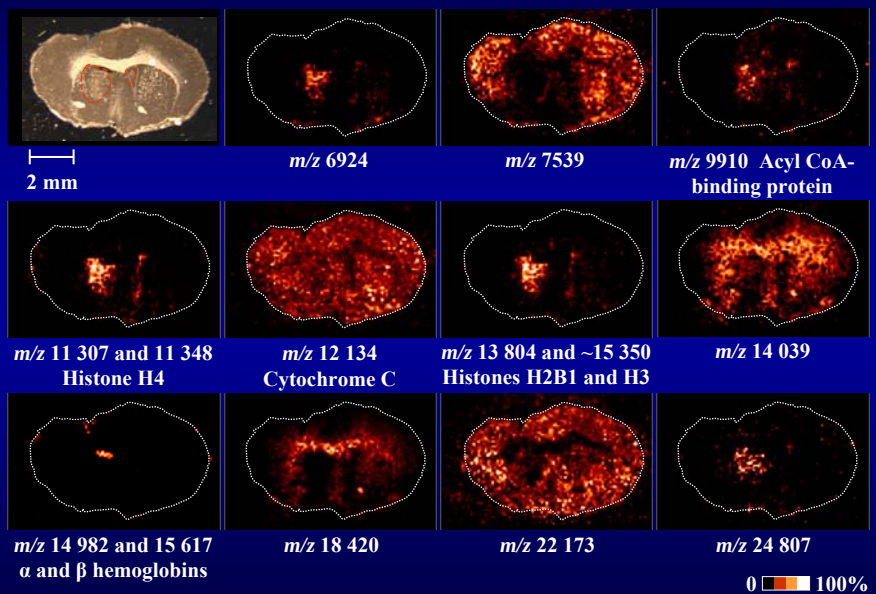


Chaurand et Al. *Anal Chem*, 76, 86A-93A (2004).

Glioma mouse model - intracranial injection of GL261 cancer cells

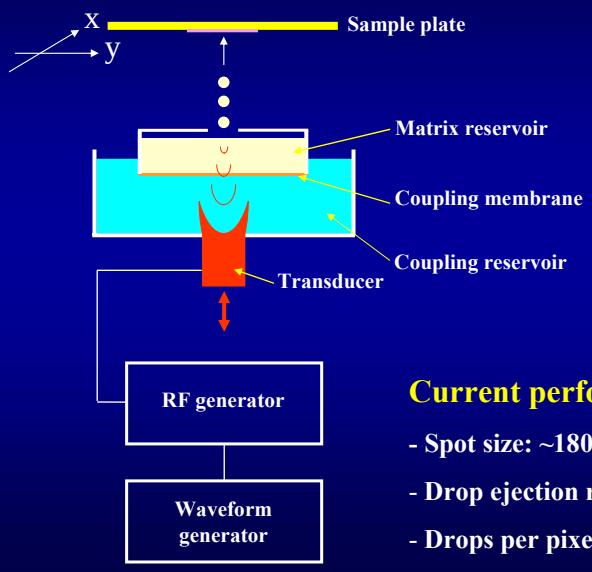


Glioma mouse model. Imaging resolution: 100 µm



Chaurand et Al. *Anal Chem*, 76, 86A-93A (2004).

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



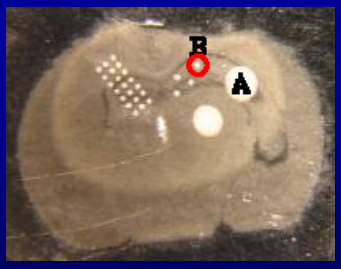
"Ejection of microdroplets"

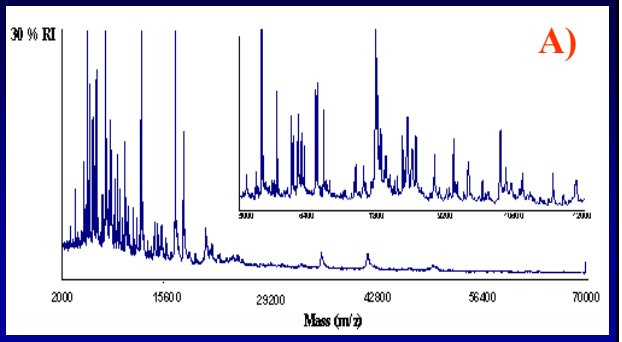
Current performances:

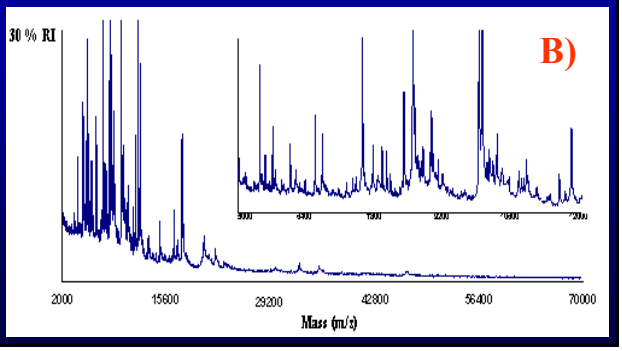
- Spot size: ~180-200 µm
- Drop ejection rate: 10 Hz
- Drops per pixel: 60-80

Manual spotting vs The RapidSpotter

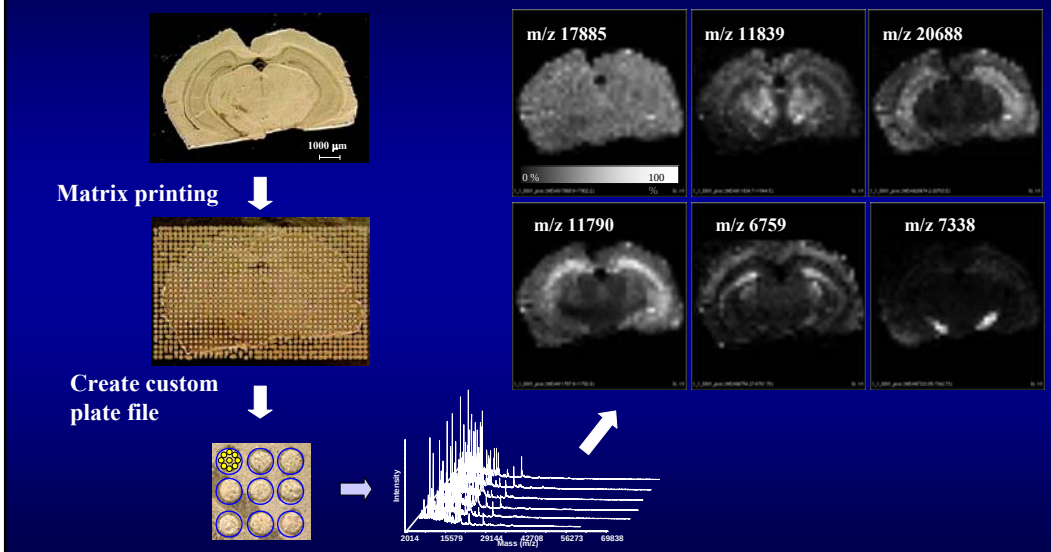
Mouse brain,
Analysis of the corpus callosum

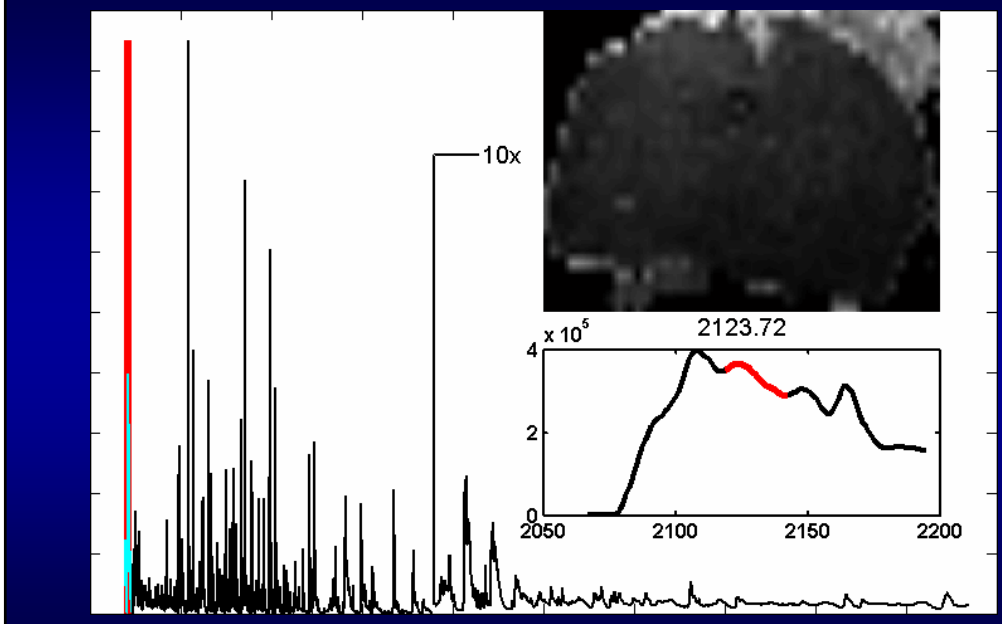




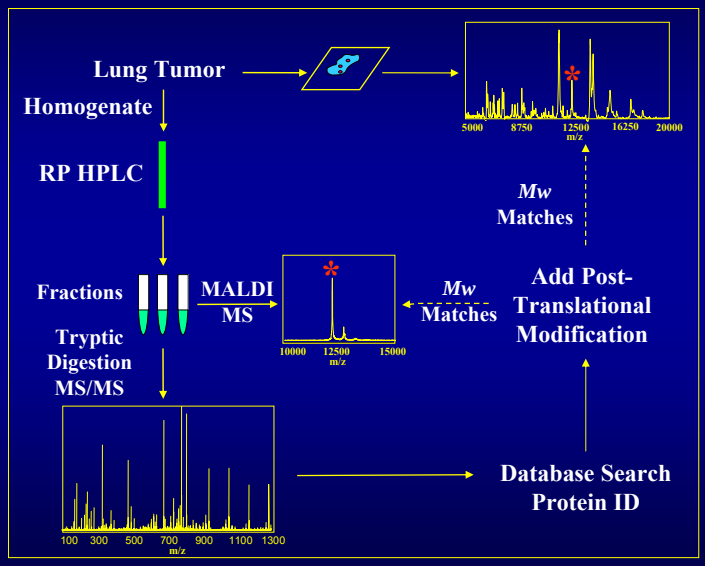


RapidSpotter Imaging of a Mouse Brain Section

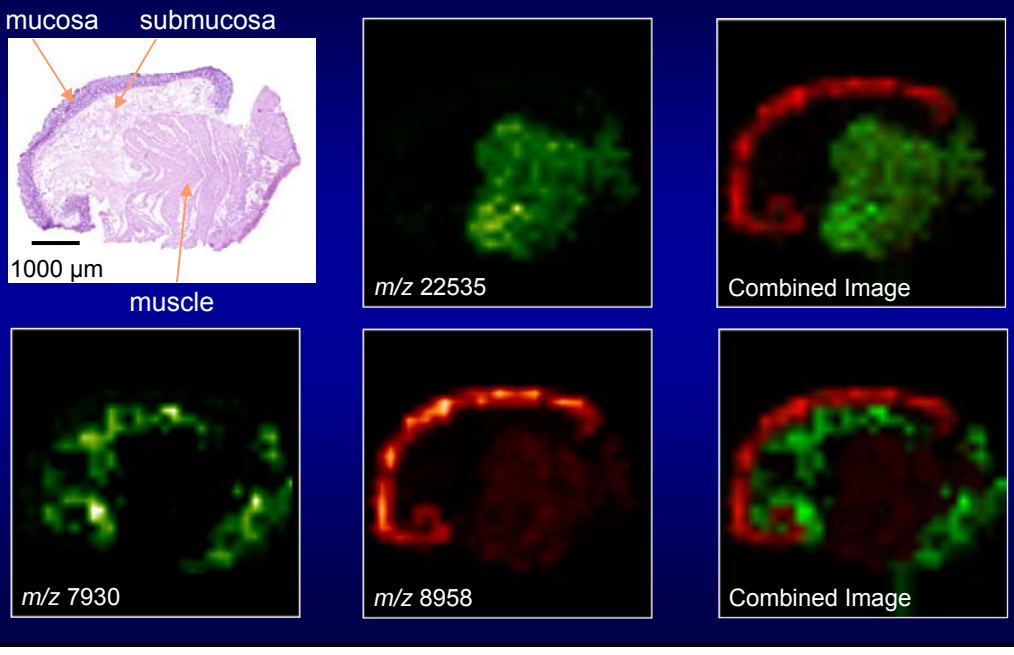




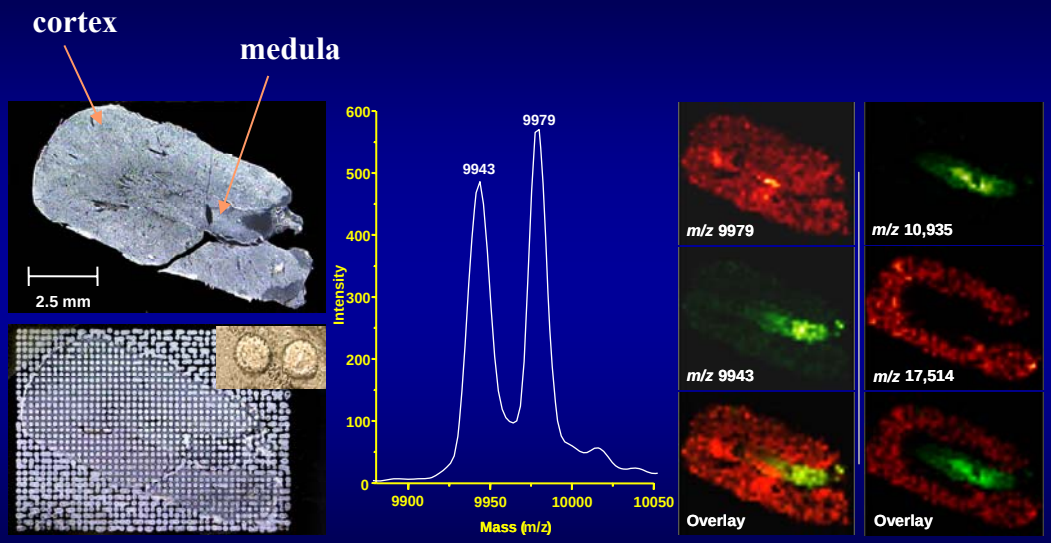
Schematic Representation of Protein Marker Identification



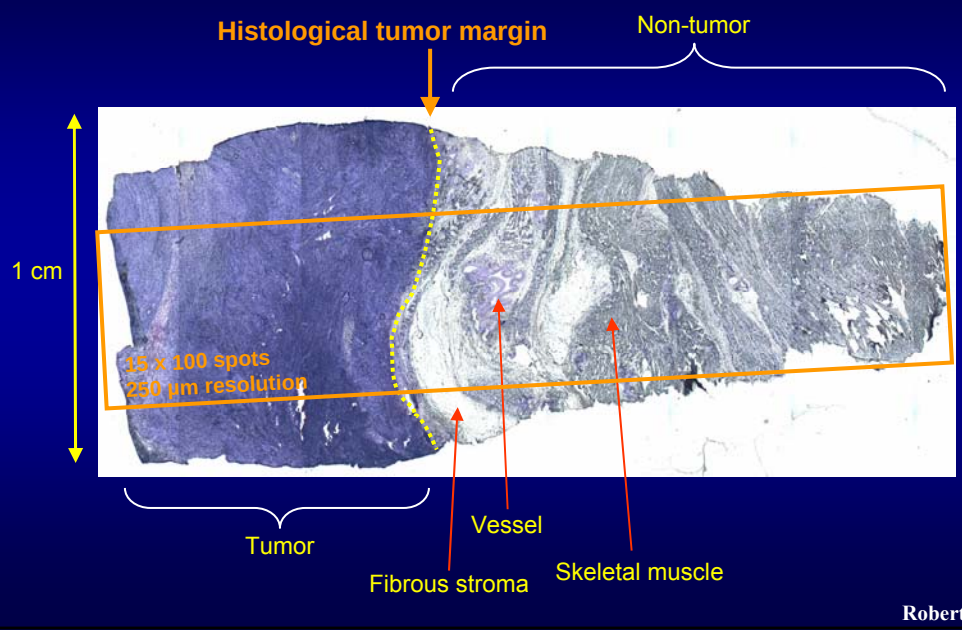
Normal human colon biopsy



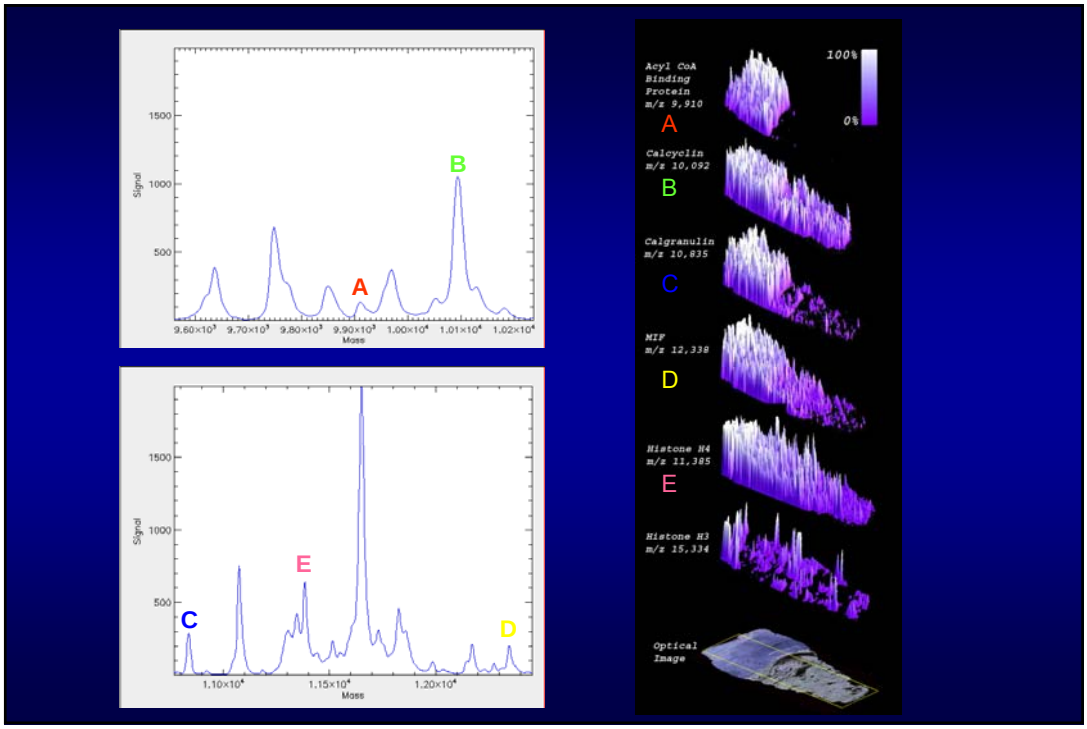
Rat kidney sagittal section

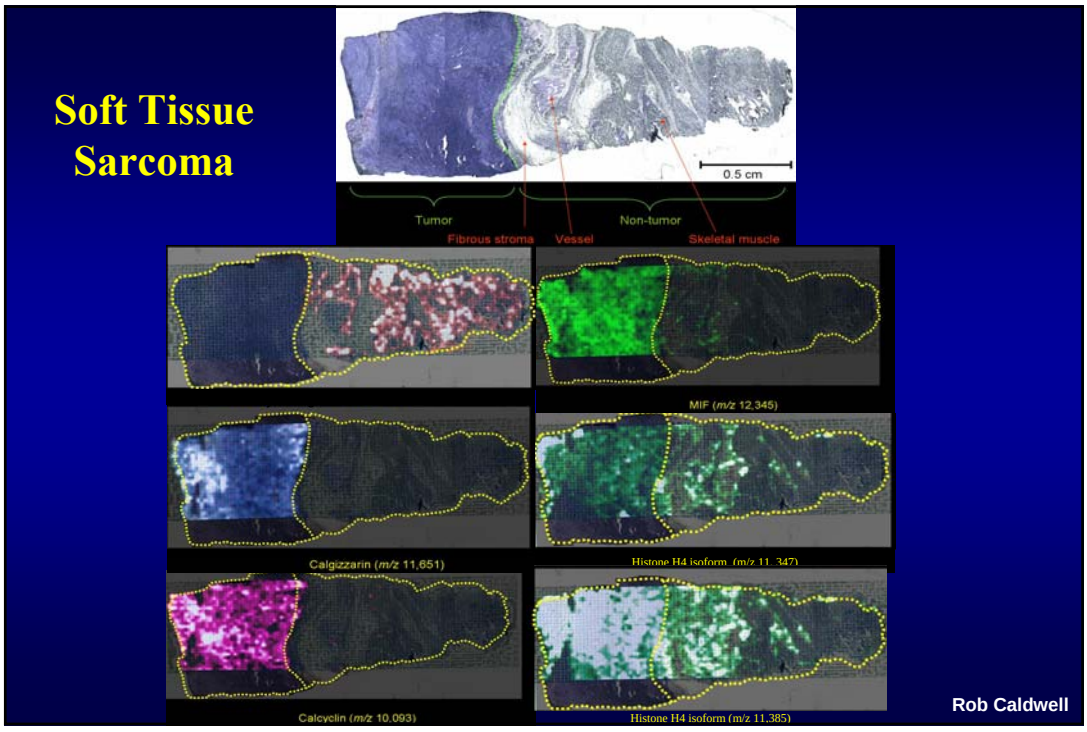


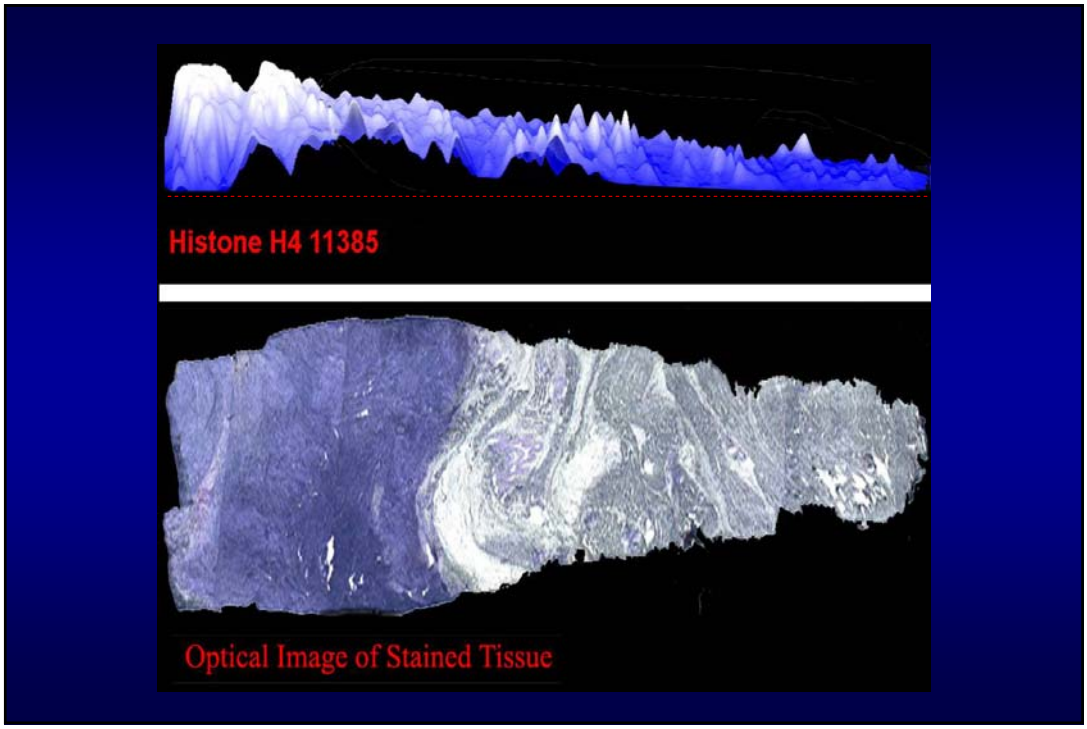
Molecular Determination of Tumor Margins

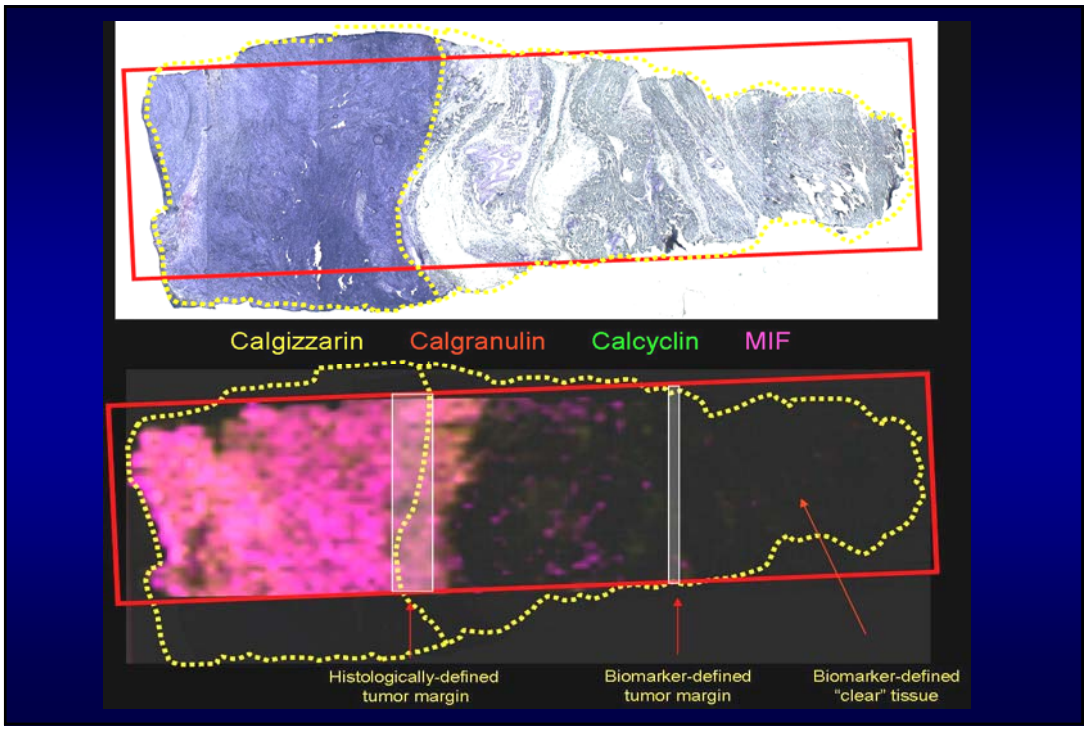


Robert Caldwell,

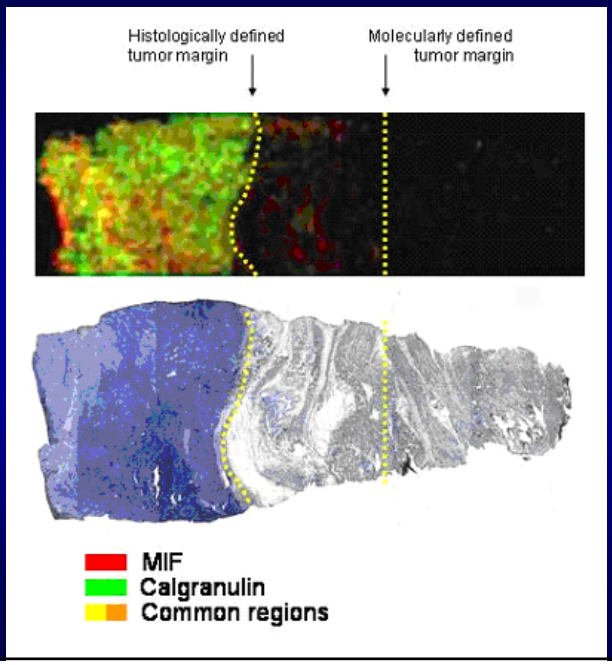

Signal
1500
1000
500
A
B
9.60×10³ 9.70×10³ 9.80×10³ 9.90×10³ 1.00×10⁴ 1.01×10⁴ 1.02×10⁴
Mass
C
E
D
1.10×10⁴ 1.15×10⁴ 1.20×10⁴
Acyl CoA Binding Protein m/z 9,910
Calcyclin m/z 10,092
Calgranulin m/z 10,835
MIF m/z 12,338
Histone H4 m/z 11,385
Histone H3 m/z 15,334
Optical Image
100%
0%


Soft Tissue Sarcoma
0.5 cm
Tumor
Non-tumor
Fibrous stroma
Vessel
Skeletal muscle
MIF (m/z 12,345)
Calgizzarin (m/z 11,651)
Histone H4 isoform (m/z 11,347)
Calcyclin (m/z 10,093)
Histone H4 isoform (m/z 11,385)
Rob Caldwell

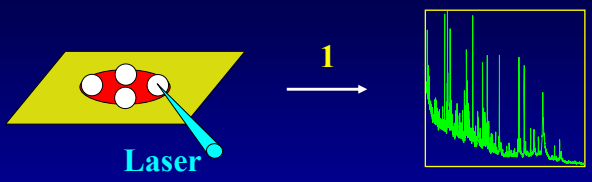

Histone H4 11385
Optical Image of Stained Tissue


Calgizzarin
Calgranulin
Calcyclin
MIF
Histologically-defined tumor margin
Biomarker-defined tumor margin
Biomarker-defined "clear" tissue

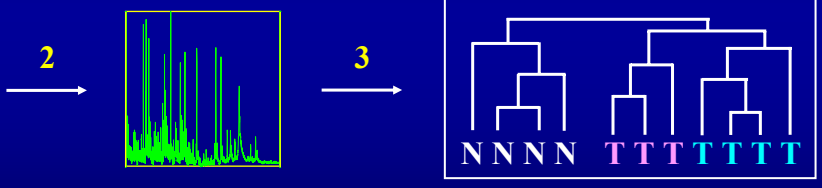
Histological vs. molecular assessment of the tumor margin:



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



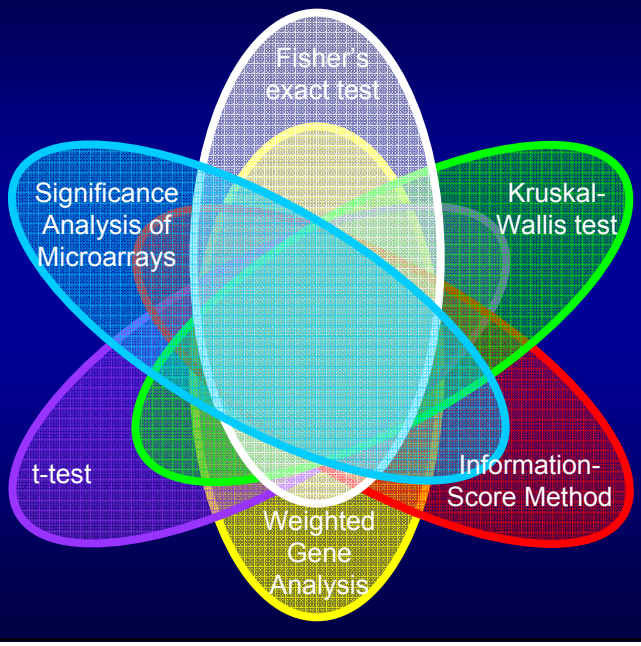
2; Computer algorithms are used to smooth and correct baseline of each spectrum

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

Schwartz SA et Al, Cancer Research, 65(17), 7674-7681 (2005)

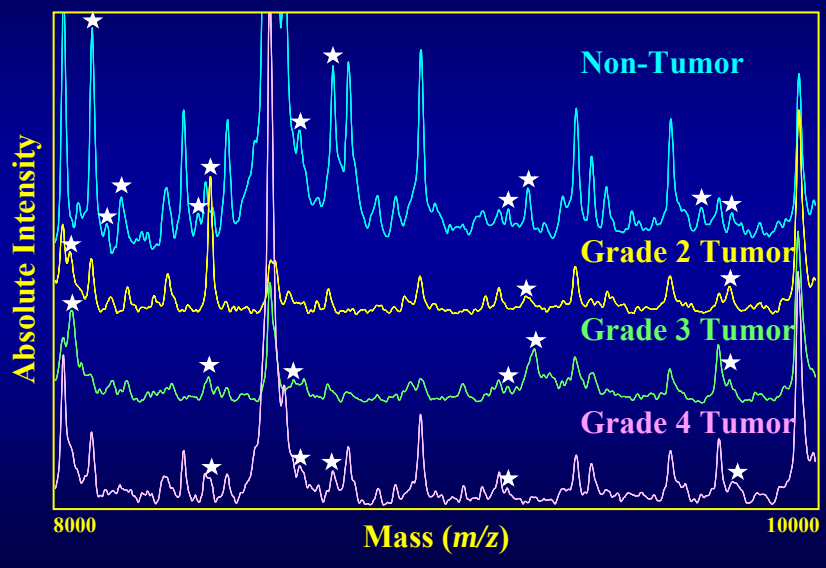
Weighted Flexible Compound Covariate Method

- Pairwise comparisons are performed between groups
- Individual protein signals differentially expressed are selected using 6 independent statistical analysis
- Proteins found in at least 3 analysis were included in the final marker list
- A pathology prediction model is generated using the top significant protein classifiers

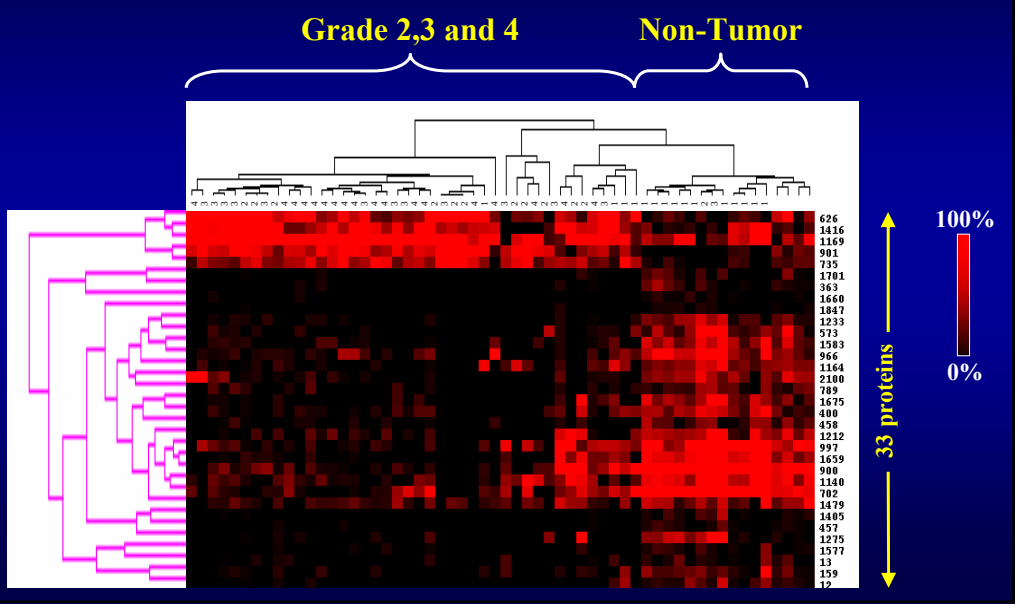


Jason Moore, Yu Shyr
Vanderbilt University

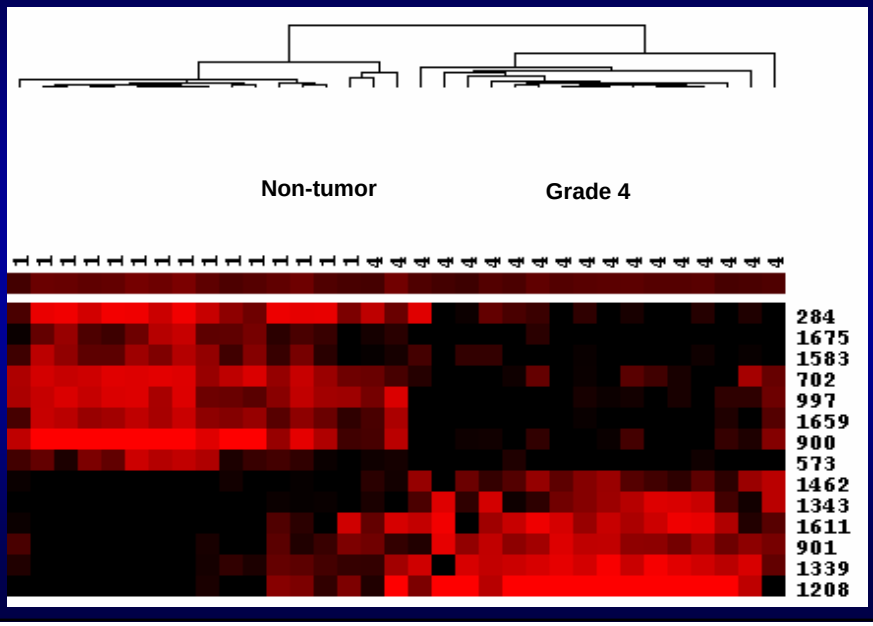
Human glioma protein expression profiling by MALDI-MS

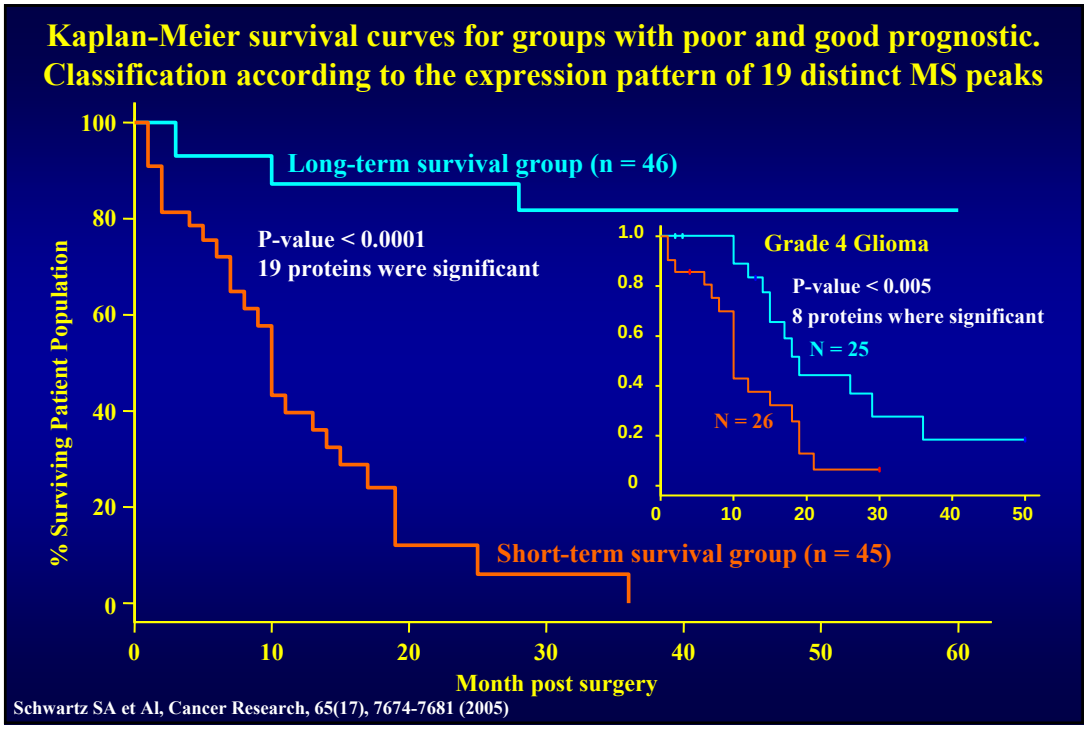


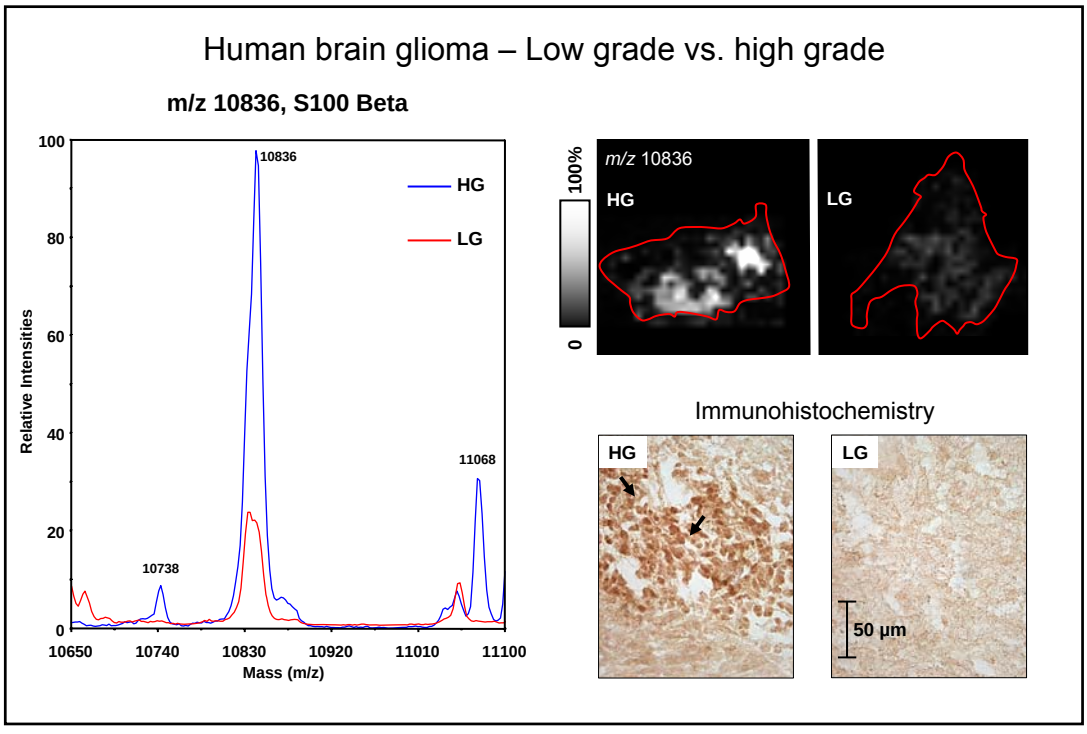
Clustering analysis of proteomic patterns in human glioma
Non-Tumor vs. All Tumor - Training cohort



Clustering analysis of proteomic patterns in human glioma
Non-Tumor vs. Grade 4 - Training cohort




Kaplan-Meier survival curves for groups with poor and good prognostic.
Classification according to the expression pattern of 19 distinct MS peaks
Long-term survival group (n = 46)
P-value < 0.0001
19 proteins were significant
Short-term survival group (n = 45)
% Surviving Patient Population
Month post surgery
Grade 4 Glioma
P-value < 0.005
8 proteins where significant
N = 25
N = 26
Schwartz SA et Al, Cancer Research, 65(17), 7674-7681 (2005)


Human brain glioma – Low grade vs. high grade
m/z 10836, S100 Beta
HG
LG
10836
10738
11068
Relative Intensities
Mass (m/z)
m/z 10836
100%
0
Immunohistochemistry
50 µm

Analysis of drugs in tissue by mass spectrometry

Dose animal
- orally
- i.v.

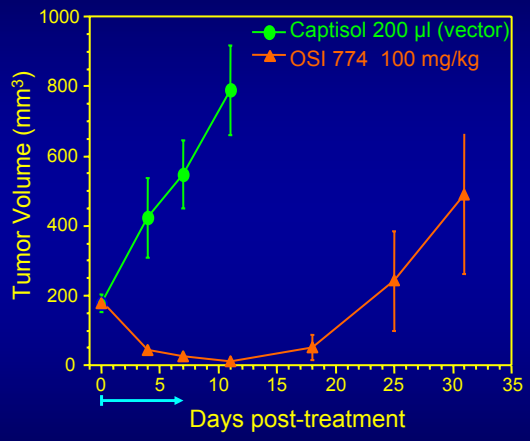
Remove tissue

- Cut frozen slice (12 µm)
- 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

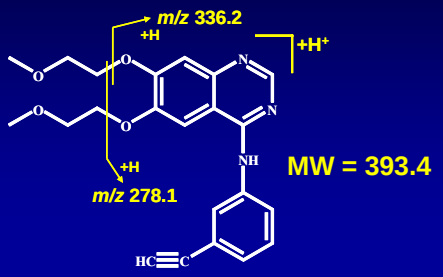
- **MMTV/HER2 cells transplanted in FVB female mice.**
- **Tumor grown to a size of ~200 mm^3.**
- **OSI 774 is an intracellular tyrosine kinase EGF receptor inhibitor.**
- **Administered orally for 1 week**

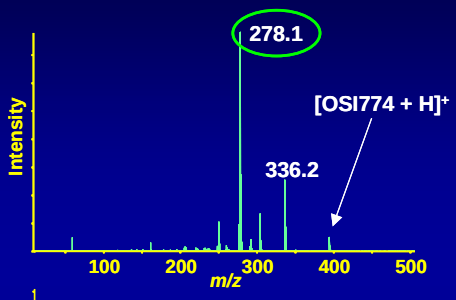


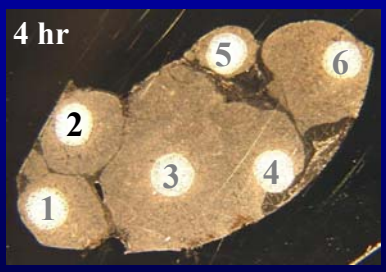
Contributed by M. Sliwkowski (Genentech, Inc.)

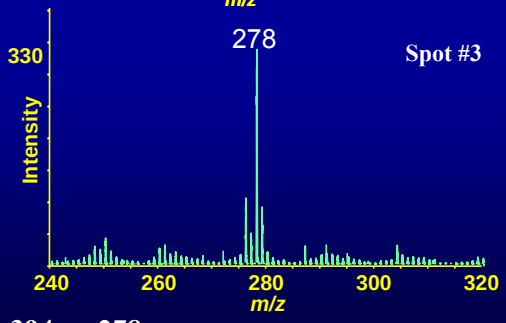
MS/MS analysis of OSI-774 in tumor tissue

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







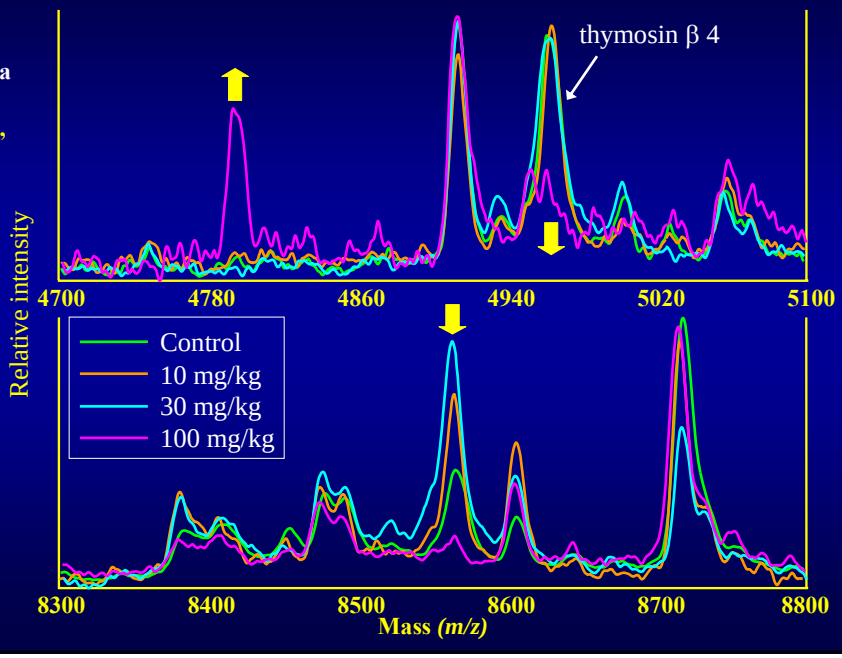


- Monitored CAD transition m/z 394 → 278

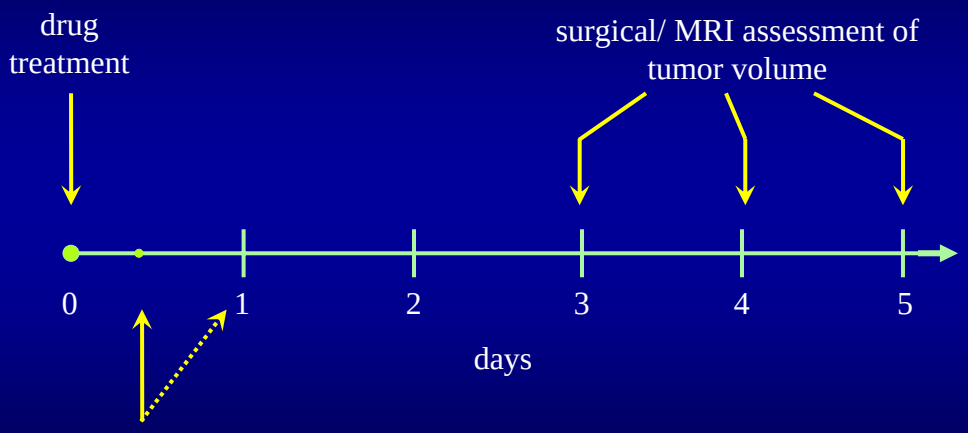
Dose dependence of protein alteration (20 hr after dose)

9 control mice, Av of 54 spectra

3x3 dosed mice, Av of 9 spectra per dose

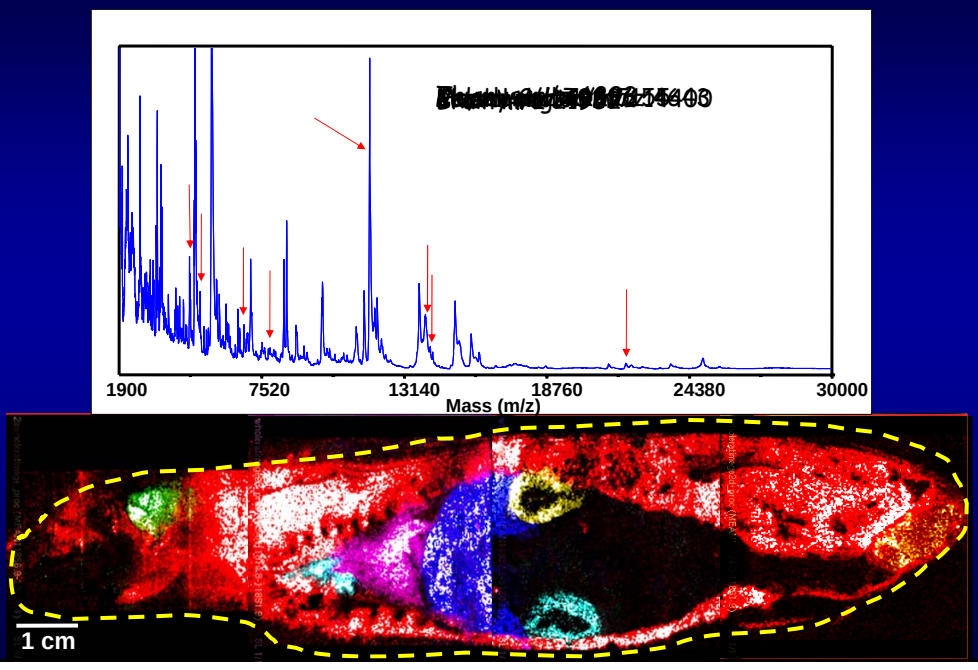


Using Protein Fingerprints to Evaluate Drug Therapy



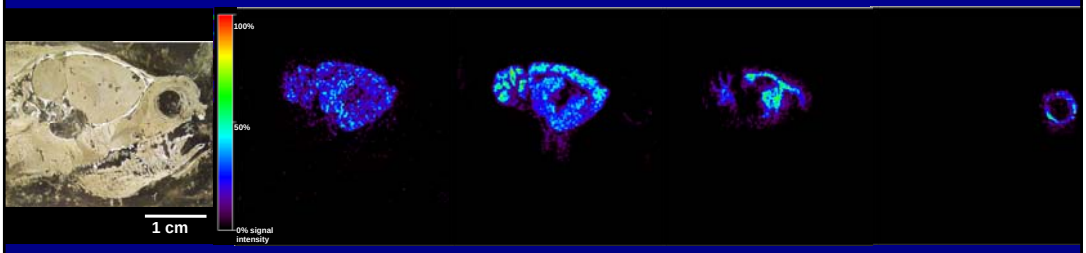
Whole Rat Imaging

Sheerin Khatib-Shahid



Whole Rat Sagittal Section Imaging

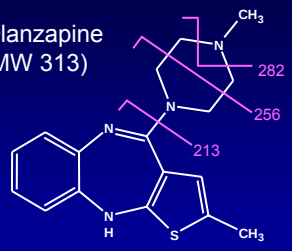
Rat Head Subsection

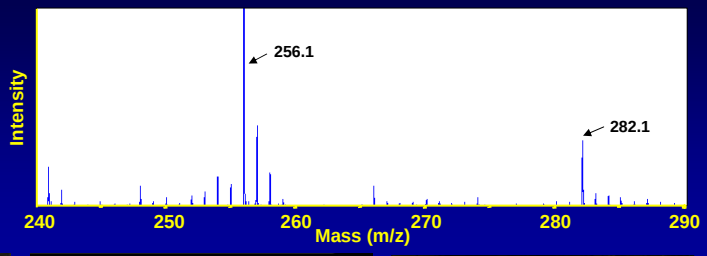


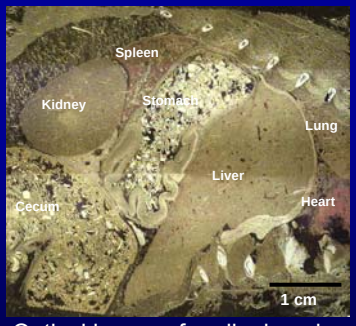
Optical image	Brain	White matter	Corpus Callosum	Eye socket
	m/z 20736	*m/z* 10271	*m/z* 5978	*m/z* 6858

Drug Imaging

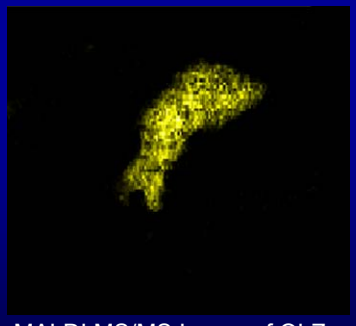
Olanzapine
(MW 313)



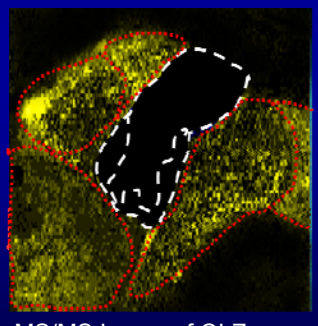




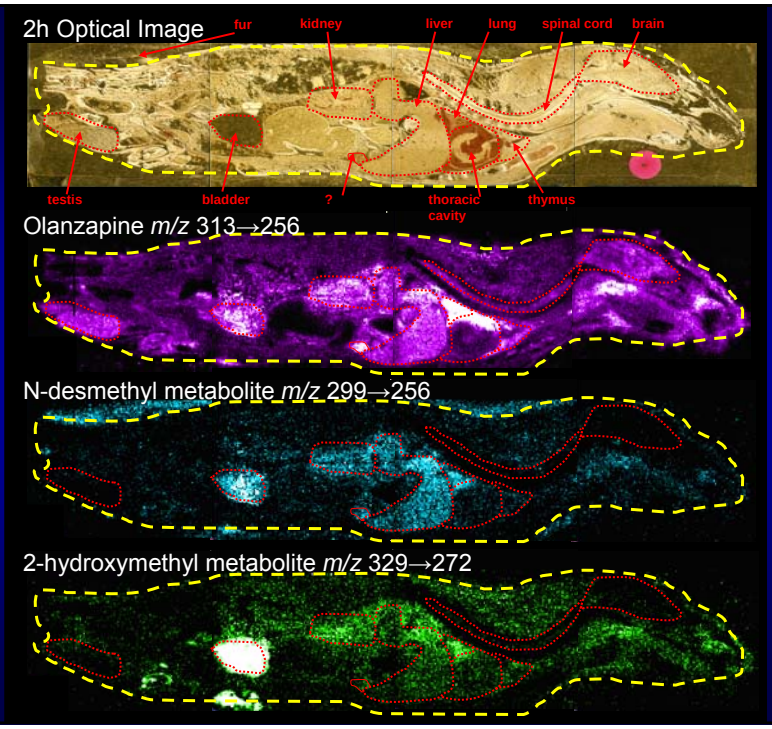
Optical image of orally dosed (8 mg/mL) rat abdomen



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



Areas of Further Development

- Increase number of proteins analyzed to >>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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