

# High Performance MALDI MS, MS/MS, and Multiplexed MS/MS Tissue Imaging

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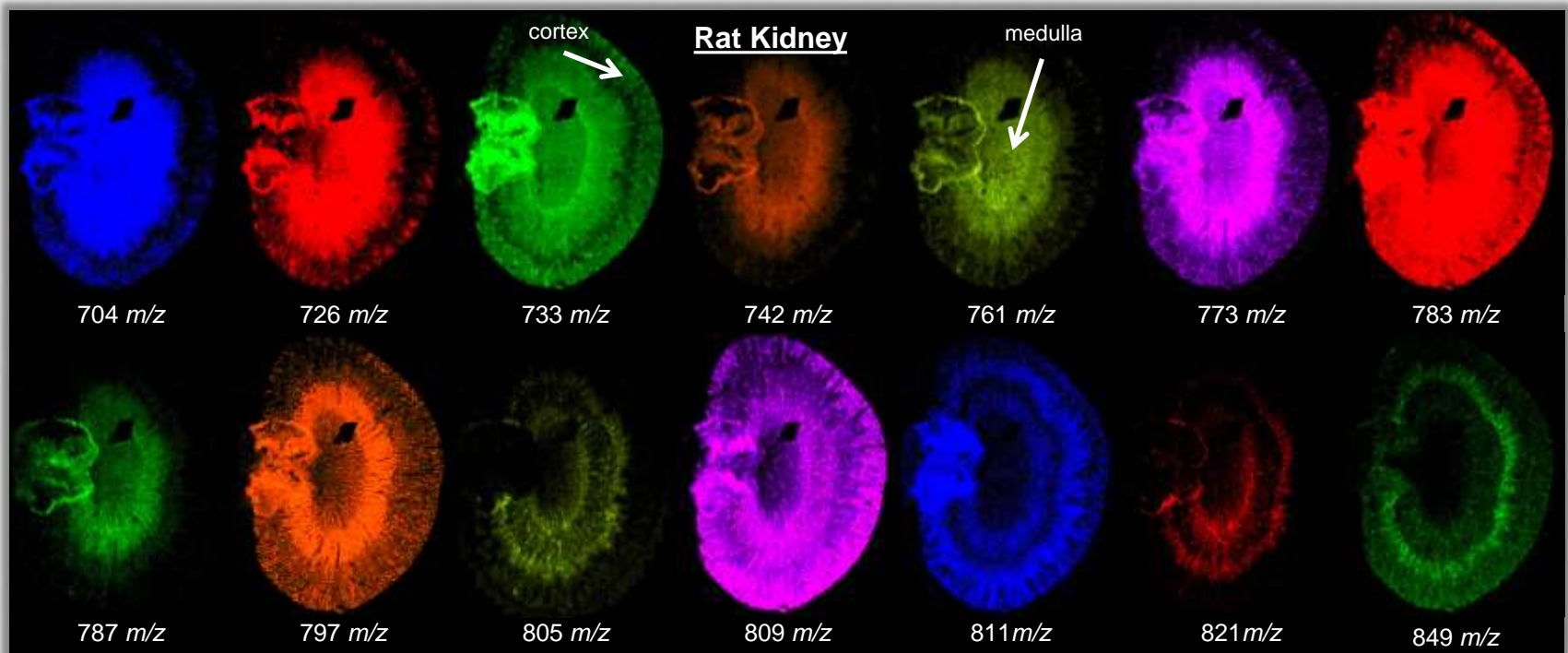
**MSACL 2014 US**

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# Why Imaging Mass Spectrometry?

IMS combines molecular specificity with location



*The multiplexed nature of MS analysis allows for the parallel acquisition of many different molecular signals, each which can be reconstructed to give a molecular picture.*

# Imaging Mass Spectrometry

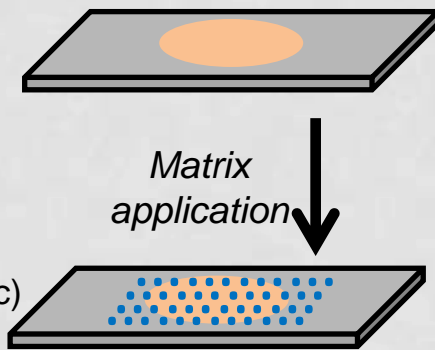
## Sample Preparation

### Matrix

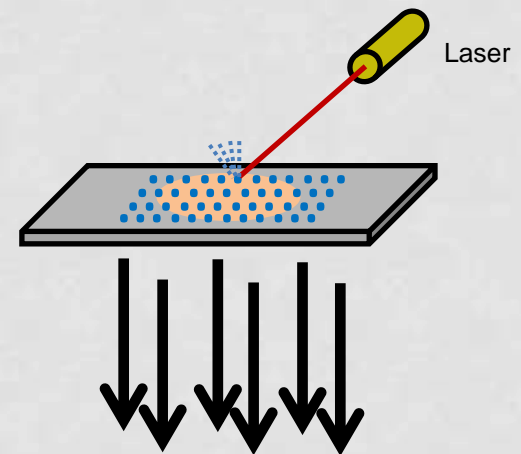
SA – proteins/peptides  
DHB –proteins/peptides  
CHCA- lipids/peptides  
DAN– lipids

### Application Method

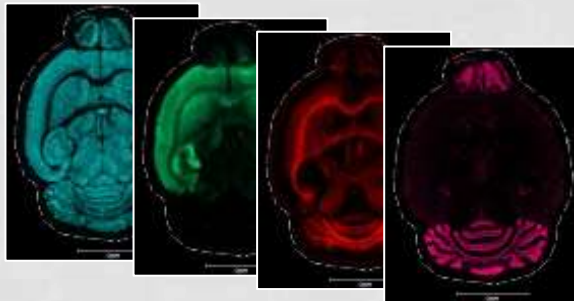
Spraying (manual, robotic)  
Sublimation  
Spotting (robotic)



## Matrix-Assisted Laser Desorption/Ionization

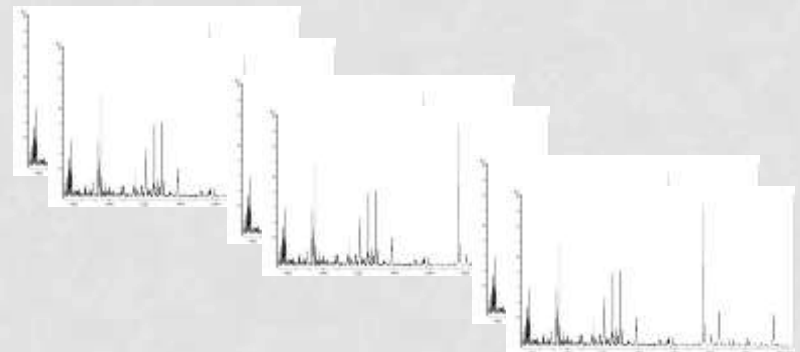


## Lipid & Protein Ion Images



*Images for individual m/z values integrated over all pixels*

## Data Processing



*MS spectrum for each x, y coordinate (pixel)*

# IMS Performance Considerations

## Traditional MS Figures of Merit

- Mass resolving power:  $m/\Delta m$
- Mass accuracy: difference between measured mass and exact mass
- Sensitivity: overall response of the instrument for a given analyte
- Dynamic range: range of detectable signals
- MS/MS capabilities: ability to perform fragmentation experiments

## Special IMS Considerations

- Spatial resolution: distance between two neighboring pixels on the sample surface
- Throughput (acquisition rate): number of spectra acquired per unit time
- Data/file sizes: storage costs and processing abilities

# MALDI Imaging Platforms

## Next Generation MALDI TOF

SimulTOF 200 Combo & 300 Tandem

## MALDI TOF

Bruker Daltonics Autoflex II

## LIT

Thermo LTQ XL

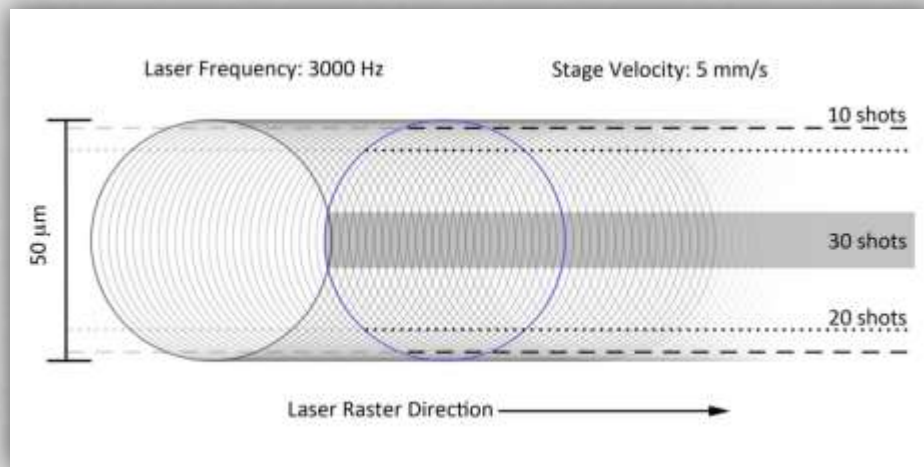
## FT-ICR

Bruker 9.4 T FTMS

## IM Q-TOF

Waters Synapt G-2

- High mass resolution ( $m/\Delta m = 20,000$ )
- High repetition rate laser (5 kHz Nd:YLF)
- High digitizer acquisition rate (50-100 pixels/sec)
- Continuous laser raster sampling
- MS/MS



$$\text{Lateral Spatial Resolution} = H \cdot A \left( \frac{v_{\text{stage}}}{f_{\text{rep}}} \right)$$

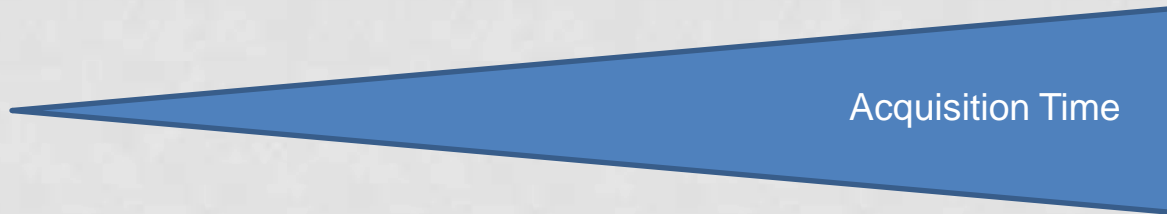
# Performance Tradeoffs

Low Spatial  
Resolution  
( $>100\ \mu\text{m}$ )



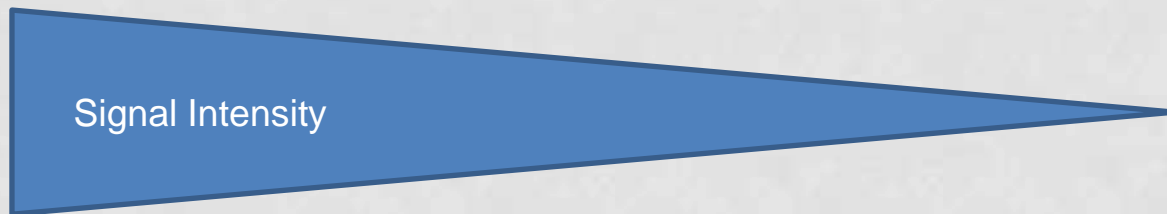
High Spatial  
Resolution  
( $<10\ \mu\text{m}$ )

Minutes



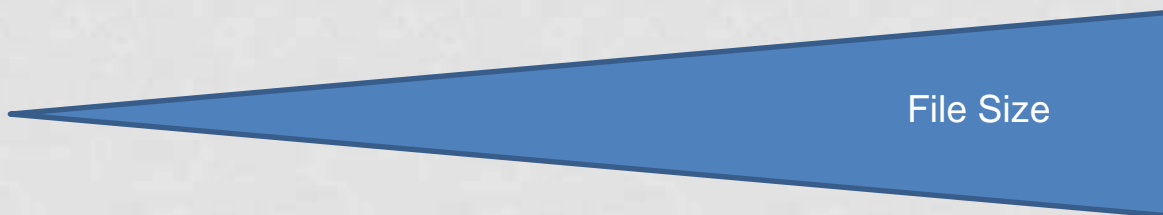
Hours

$1 \times 10^8$



$1 \times 10^5$

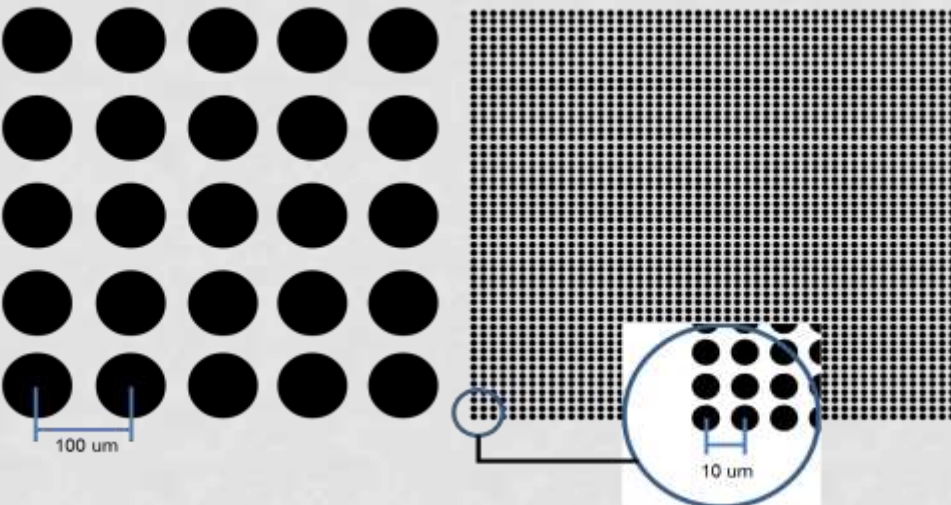
$<5\ \text{GB}$



$> 10\text{GB}$

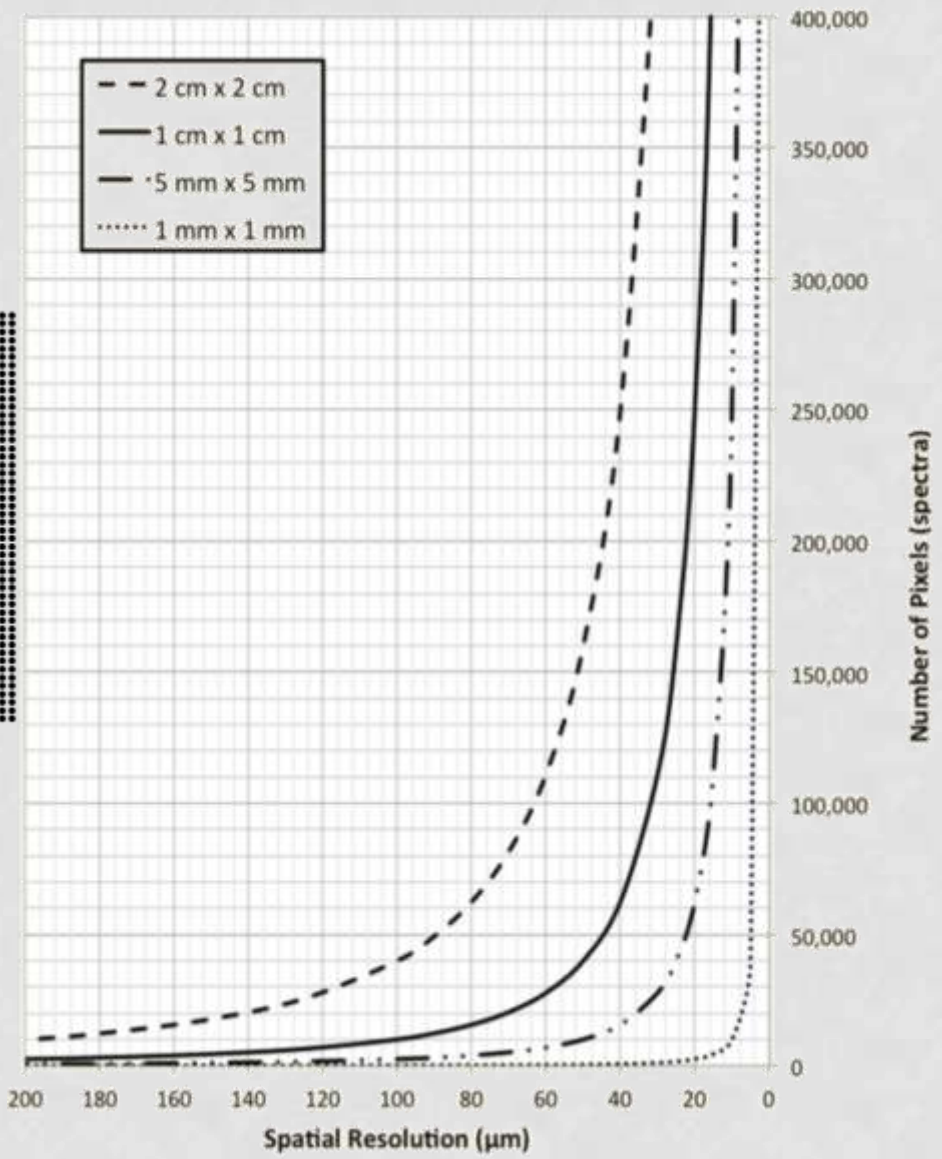
# #Pixels = f(Spatial Resolution, Area)

High-spatial resolution and/or large area IMS experiments can require prohibitive analysis times.



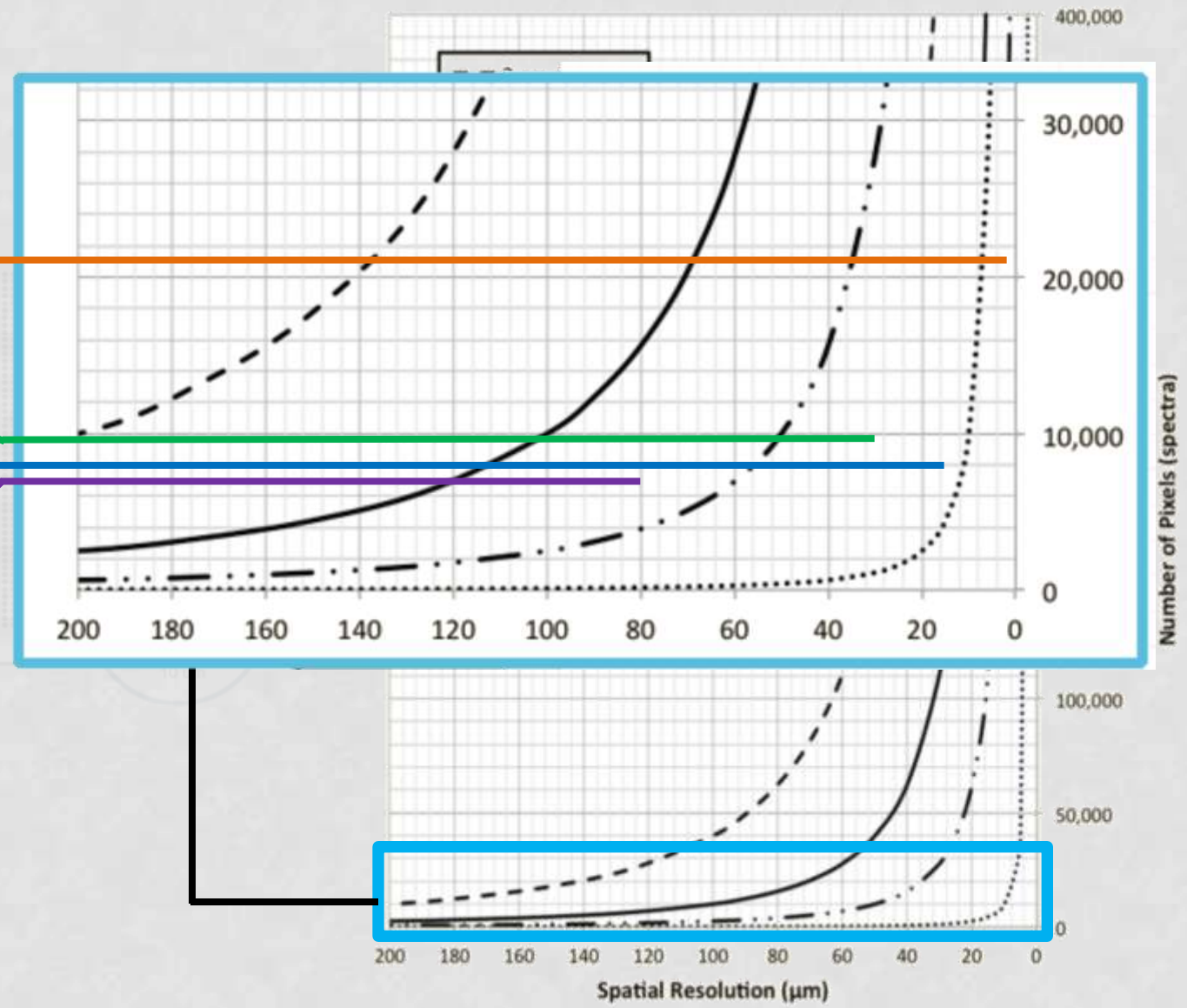
25 pixels

2,500 pixels



# Pixel Size: The Importance of Speed

~21,000 pixels/3hr MALDI TOF  
~9,800 pixels/3hr LIT  
~7,700 pixels/3hr FT-ICR  
~7,200 pixels/3hr IM Q-TOF





# High Speed MALDI TOF

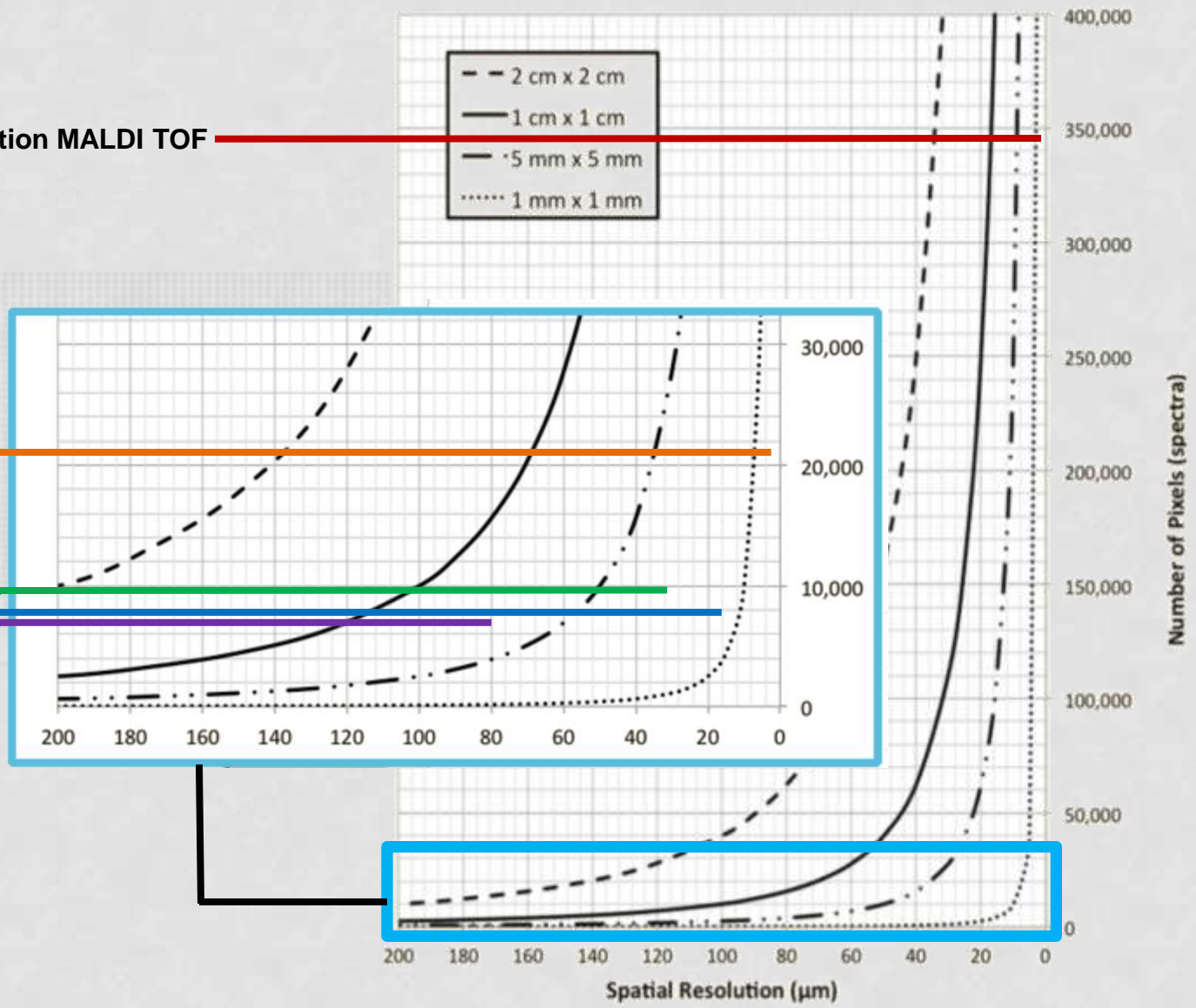
~345,000 pixels/3hr **Next Generation MALDI TOF**

~21,000 pixels/3hr **MALDI TOF**

~9,800 pixels/3hr **LIT**

~7,700 pixels/3hr **FT-ICR**

~7,200 pixels/3hr **IM Q-TOF**



# MS Protein Imaging

**Sample:** +Mouse Kidney (infected w/ *S. aureus*)

**Lateral Spatial Resolution:** 25  $\mu\text{m}$

**Vertical Step:** 50  $\mu\text{m}$

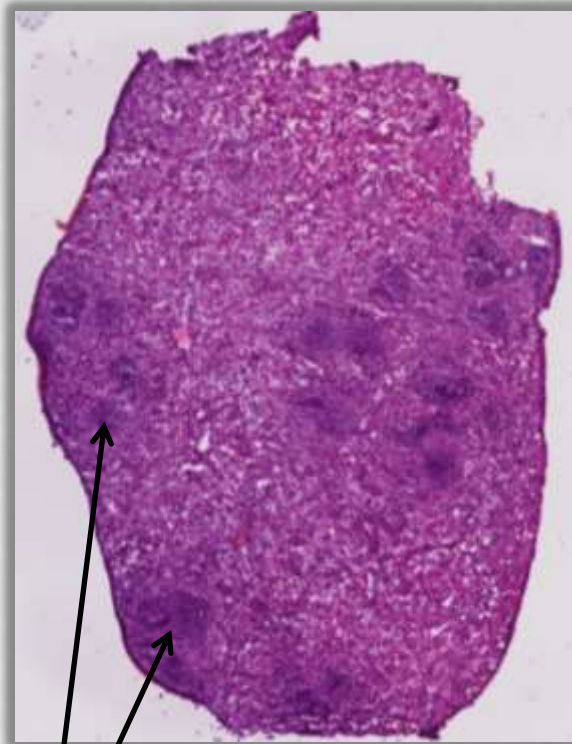
**Wash, Matrix:** Carnoy's, CHCA/DHB mix

**Laser Rep. Rate:** 1000 Hz

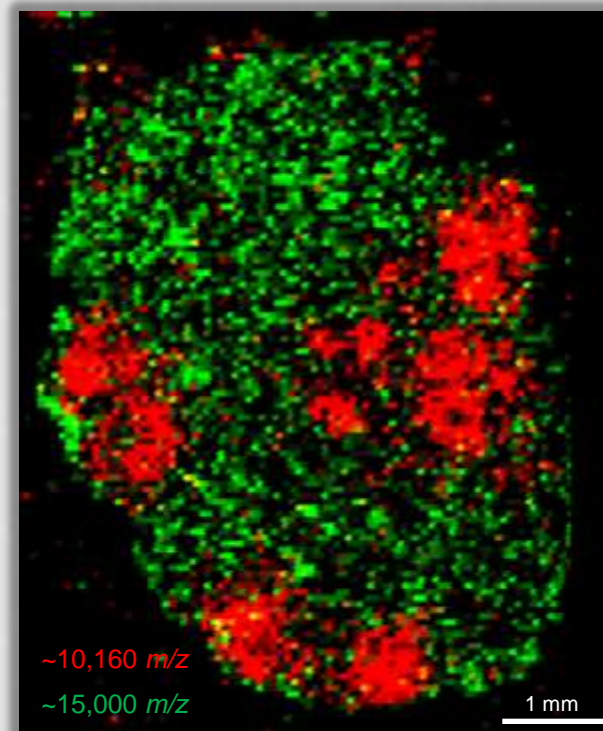
**Acquisition Rate:** 20 pixels/second

**Pixels:** 15,940 pixels

**Analysis Time:** ~20 minutes



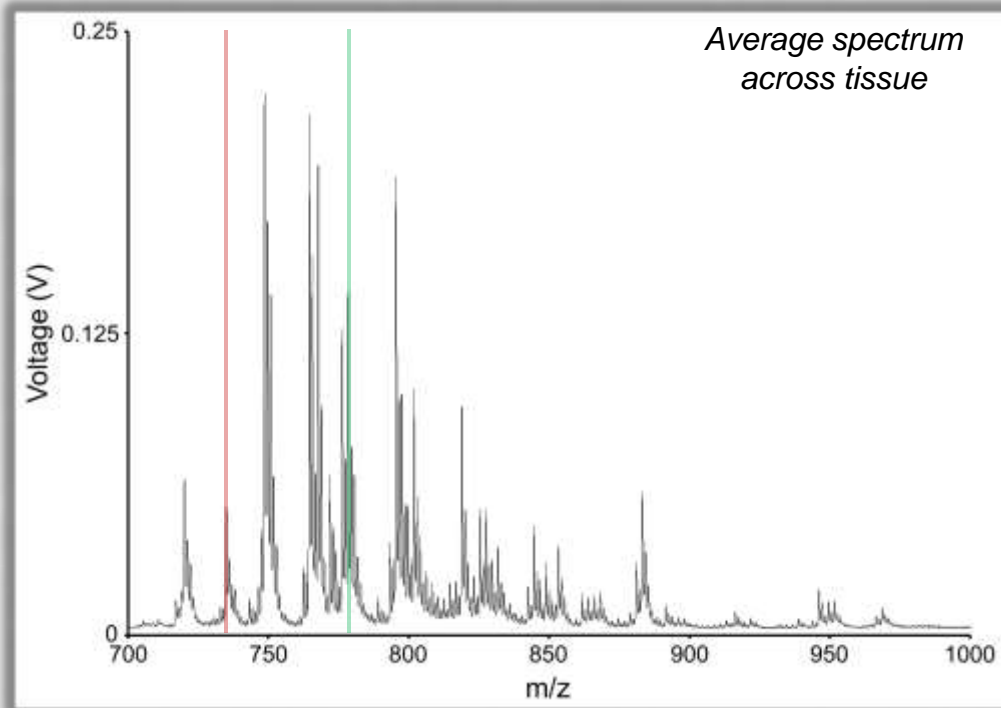
Abscesses



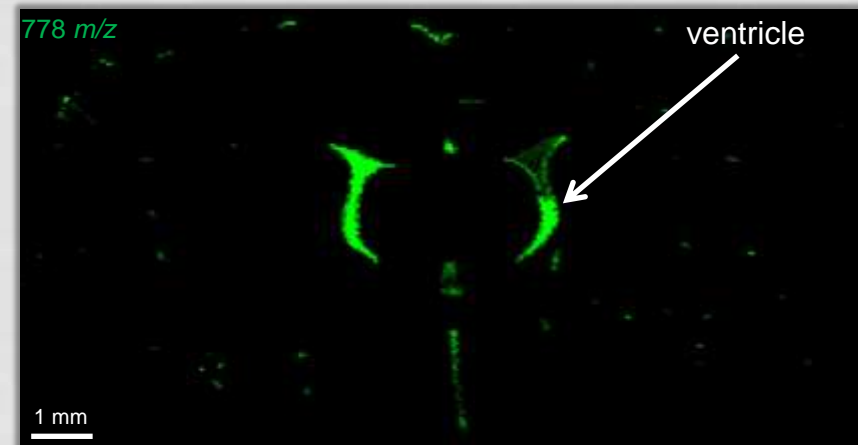
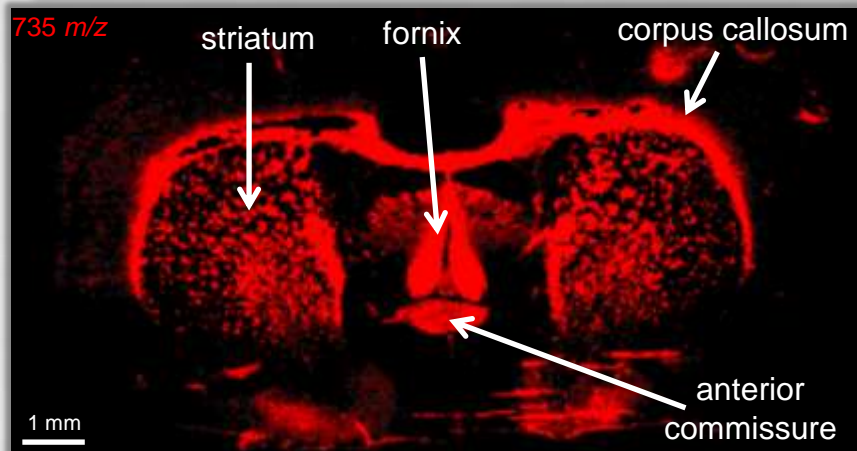
**Red:** S100A8 protein

**Green:** Hemoglobin alpha chain

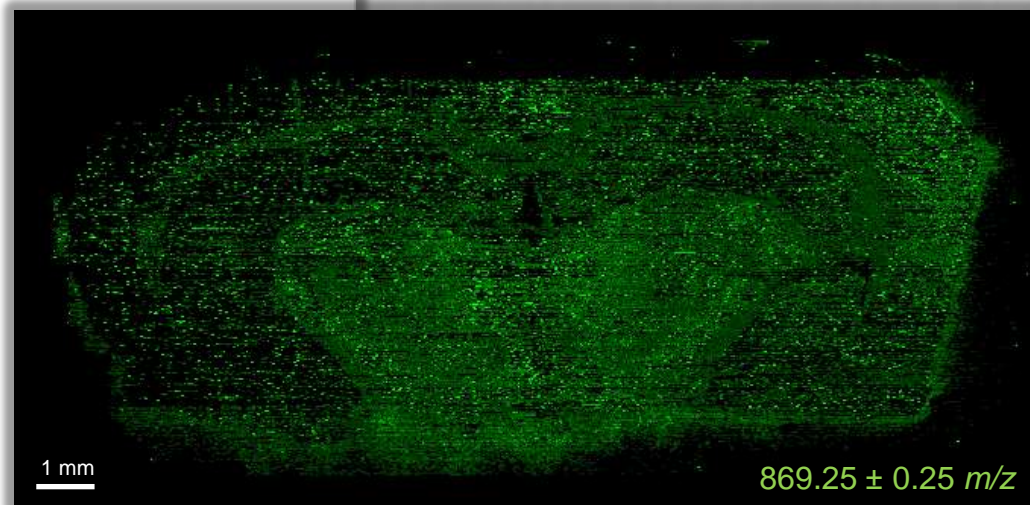
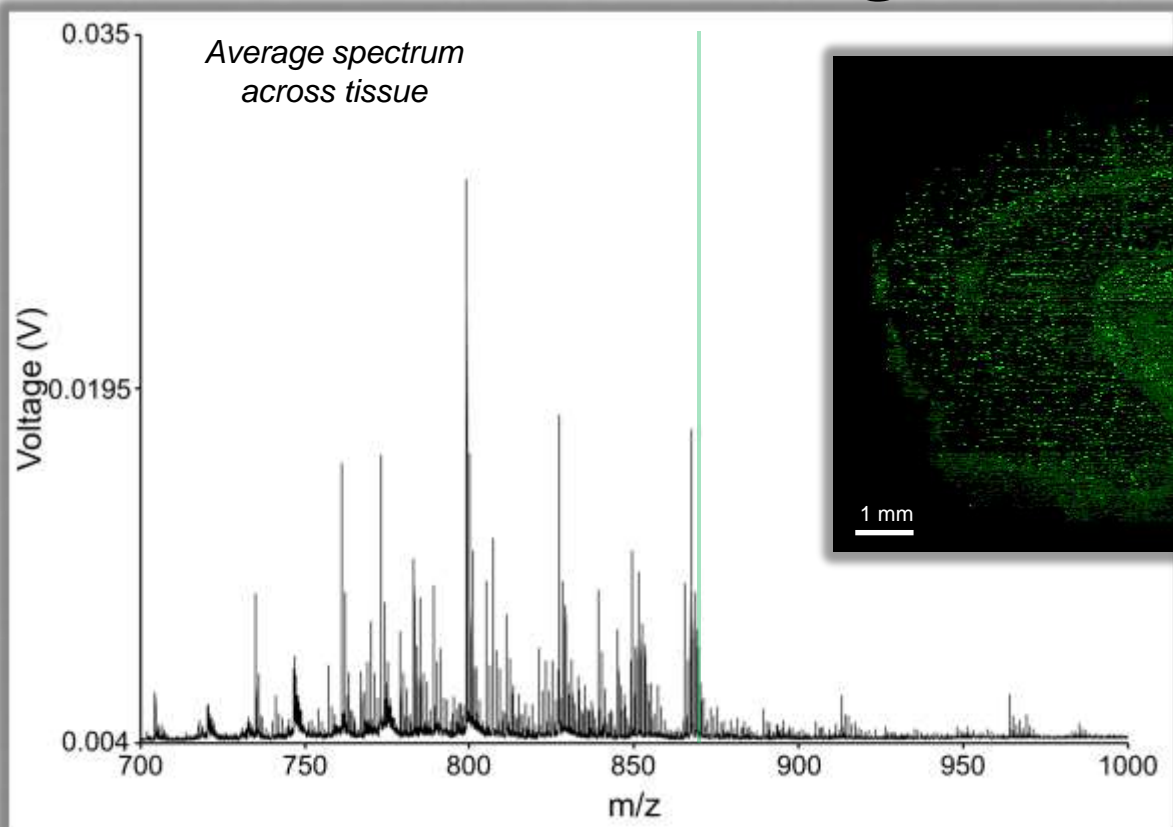
# MS Lipid Imaging



**Sample:** +Rat Brain  
**Lateral Spatial Resolution:** 50  $\mu\text{m}$   
**Vertical Step:** 50  $\mu\text{m}$   
**Matrix:** DAN  
**Laser Rep. Rate:** 1000 Hz  
**Acquisition Rate:** 20 pixels/second  
**Pixels:** 39,073 pixels  
**Analysis Time:** ~40 minutes



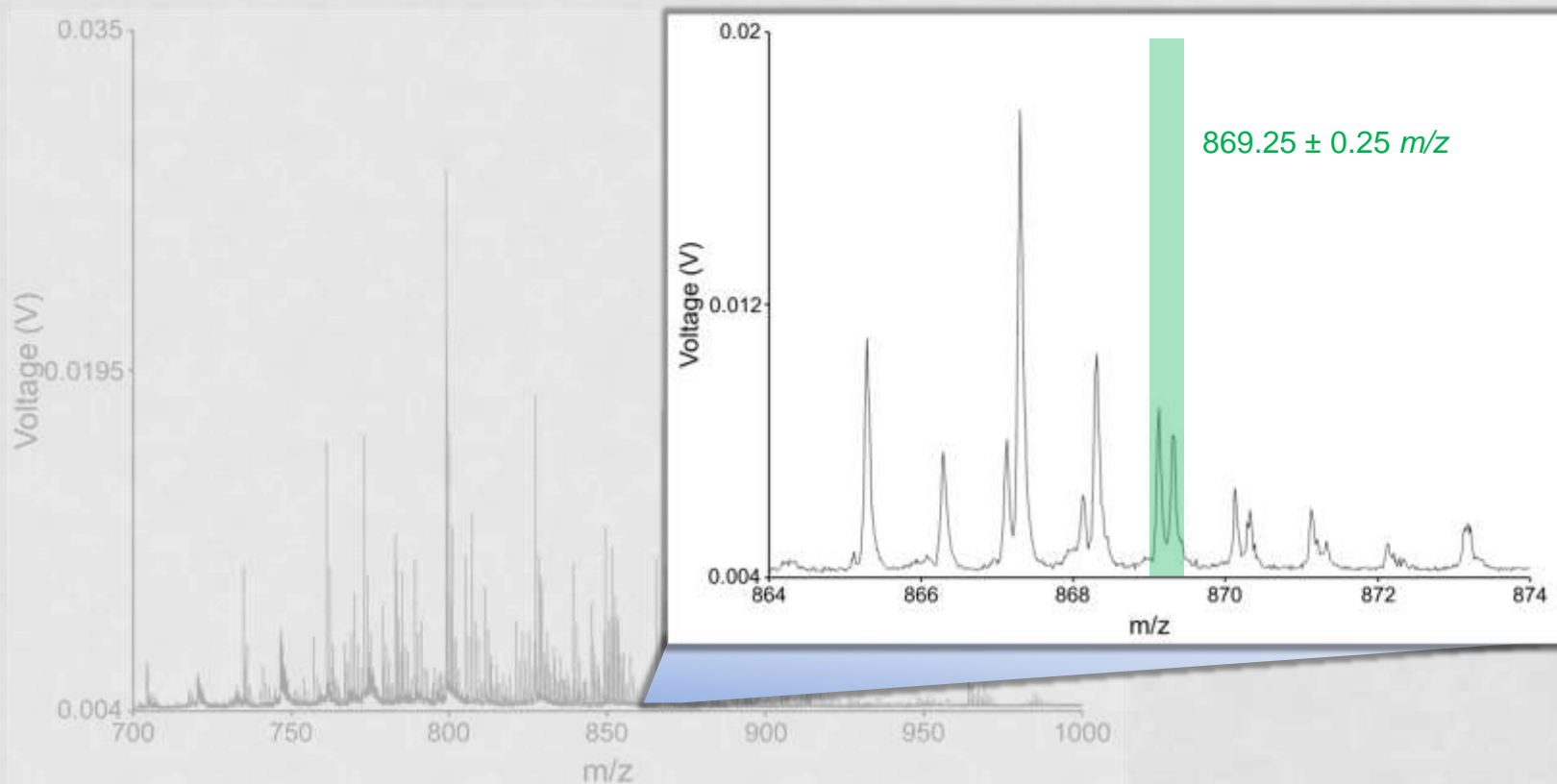
# The Need for High Mass Resolution



**Sample:** +Rat Brain (lipids)  
**Lateral Spatial Resolution:** 25  $\mu\text{m}$   
**Vertical Step:** 25  $\mu\text{m}$   
**Matrix:** CHCA

**Laser Rep. Rate:** 5000 Hz  
**Acquisition Rate:** 100 pixels/second  
**Pixels:** 178,154 pixels  
**Analysis Time:** ~90 minutes

# The Need for High Mass Resolution

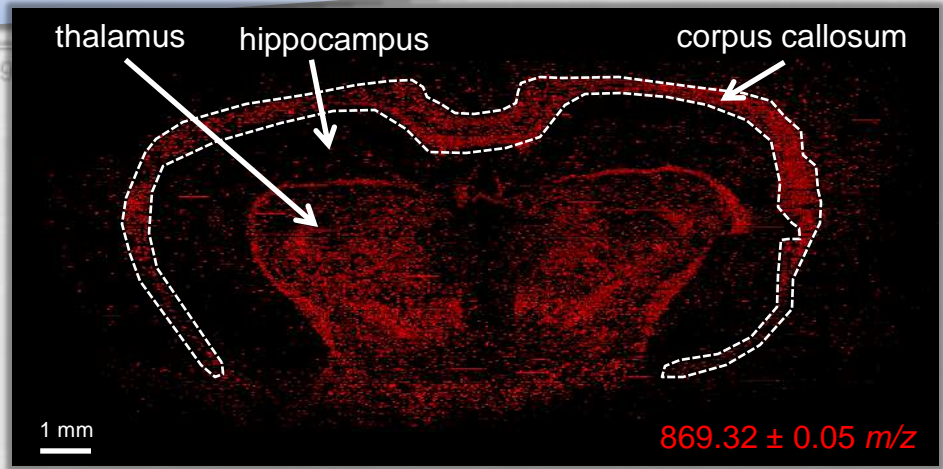
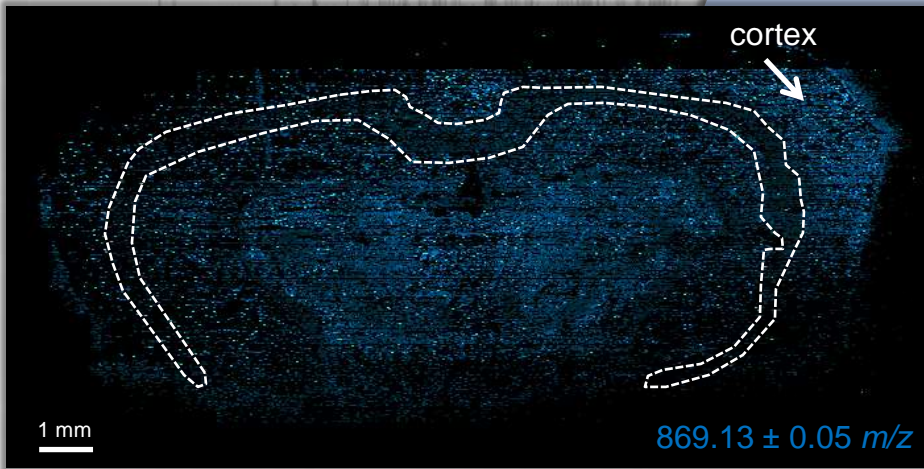
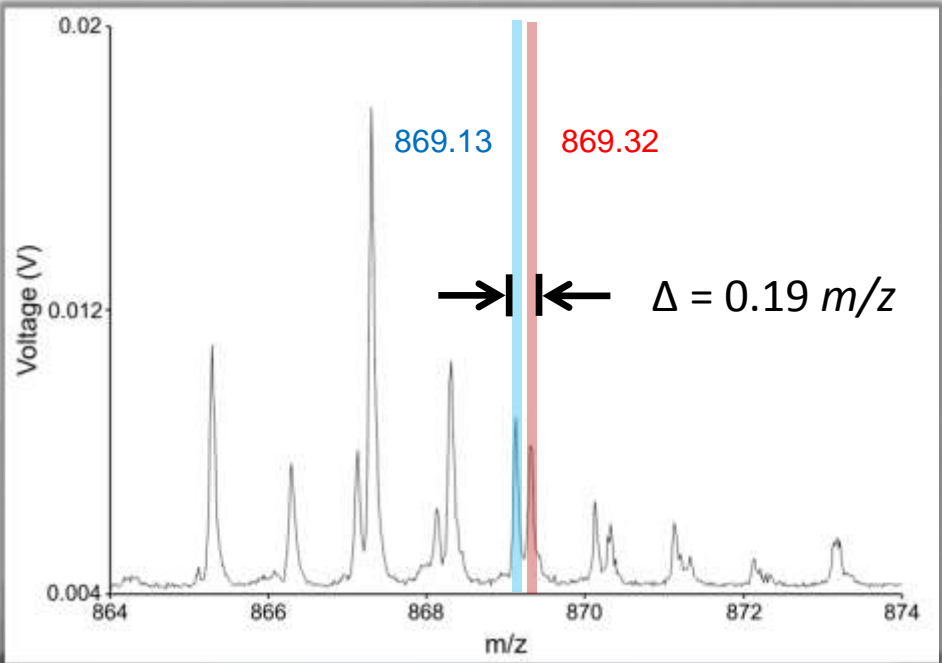
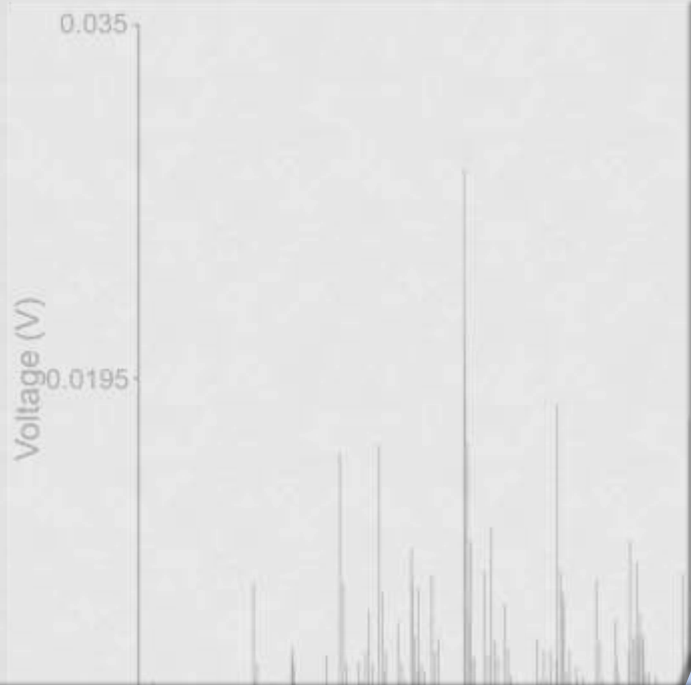


**Sample:** +Rat Brain (lipids)  
**Lateral Spatial Resolution:** 25  $\mu\text{m}$   
**Vertical Step:** 25  $\mu\text{m}$   
**Matrix:** CHCA

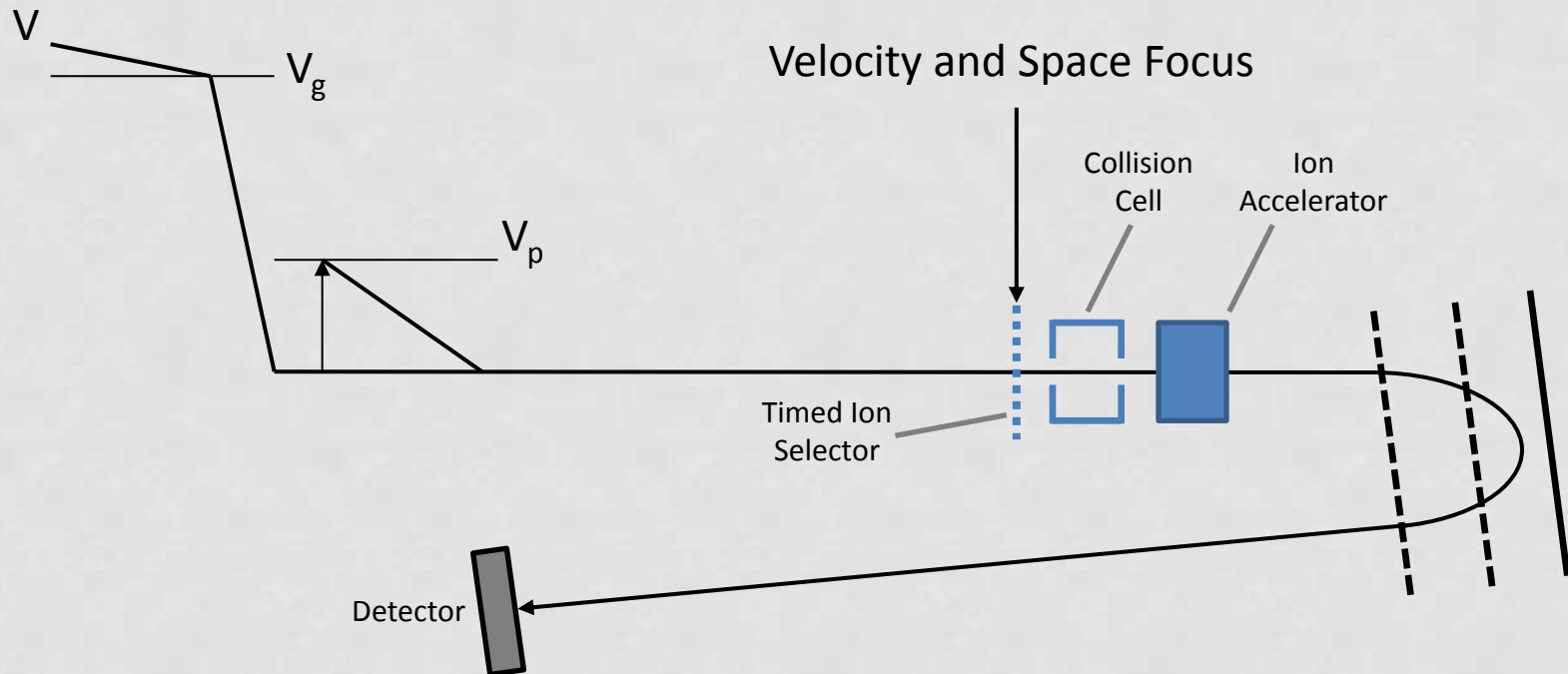
**Laser Rep. Rate:** 5000 Hz  
**Acquisition Rate:** 100 pixels/second  
**Pixels:** 178,154 pixels  
**Analysis Time:** ~90 minutes

*~12,000 FWHM resolution at 869 m/z*

# The Need for High Mass Resolution



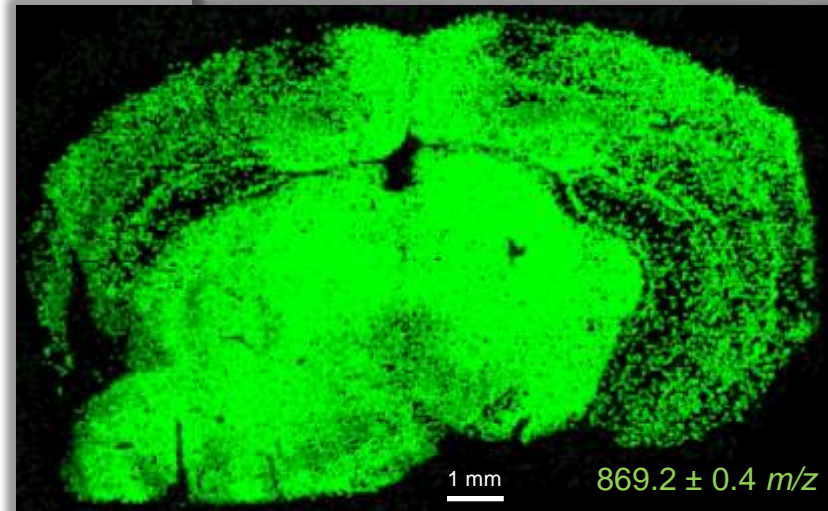
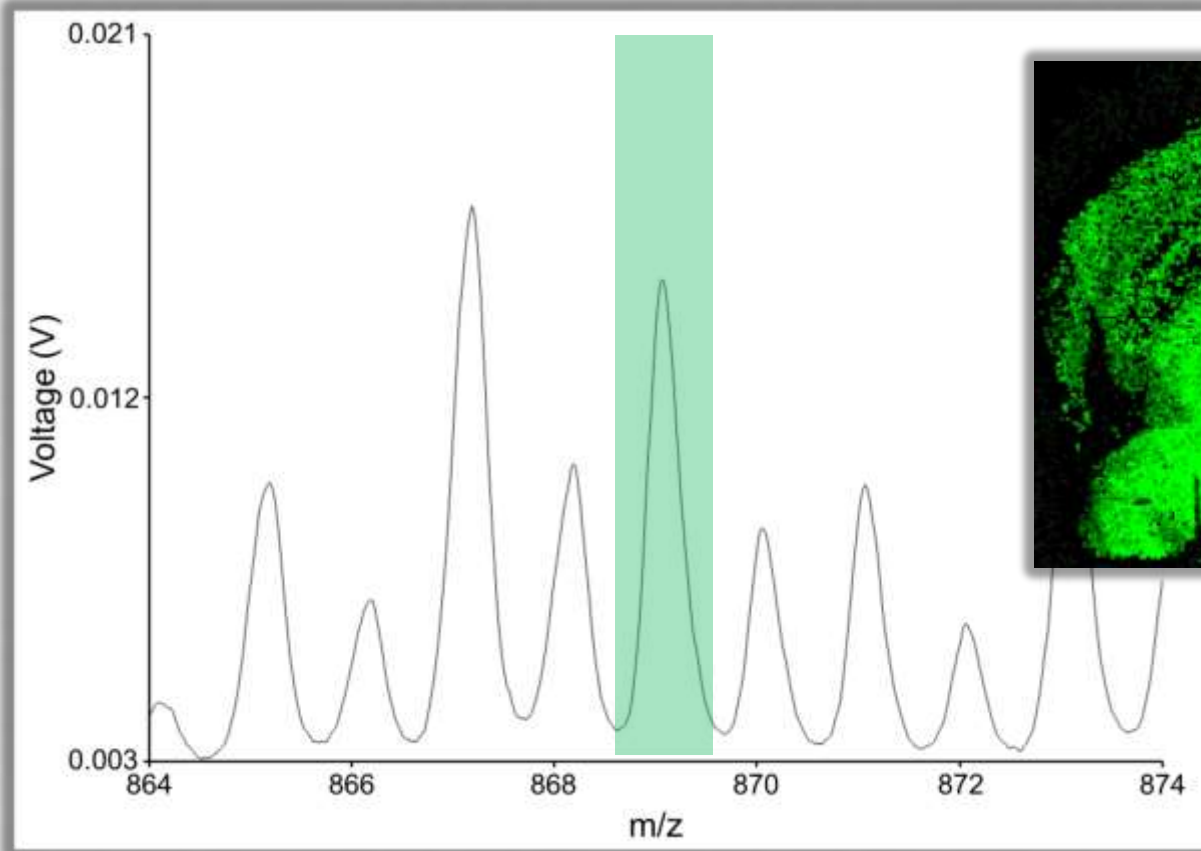
# MALDI TOF/TOF



*MS/MS can provide a way to distinguish isobaric species and give sequence information on an analyte of interest.*

- High resolution timed ion selector (<5 Da)
- Collision cell for efficient fragmentation
- High repetition rate laser (5 kHz Nd:YLF)
- High digitizer acquisition rate (50-100 pixels/sec)
- Continuous laser raster sampling

# MS Lipid Imaging

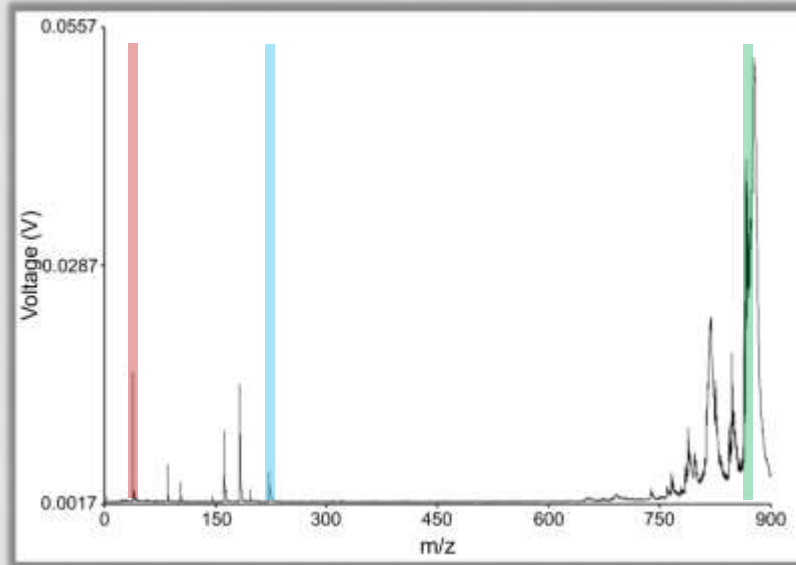


**Sample:** +Rat Brain (lipids)  
**Lateral Spatial Resolution:** 50  $\mu\text{m}$   
**Vertical Step:** 50  $\mu\text{m}$   
**Matrix:** DAN

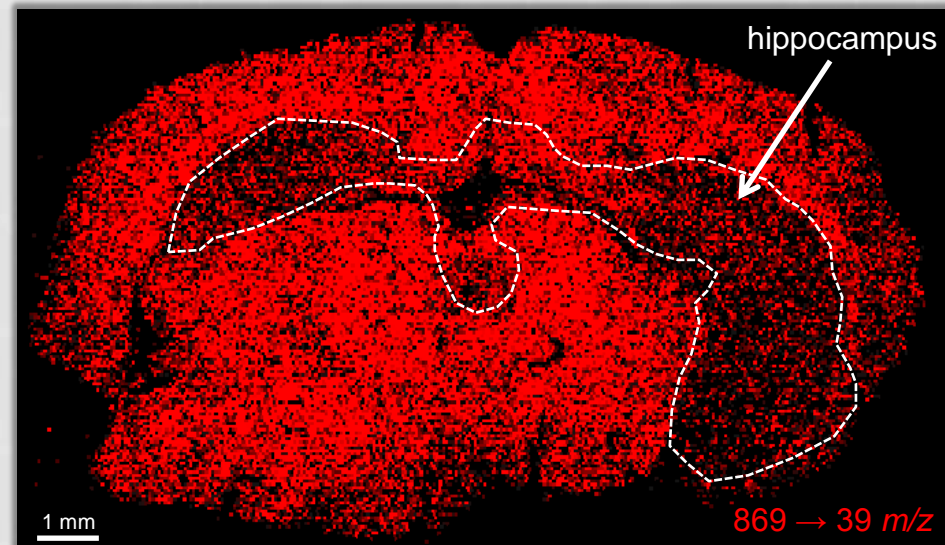
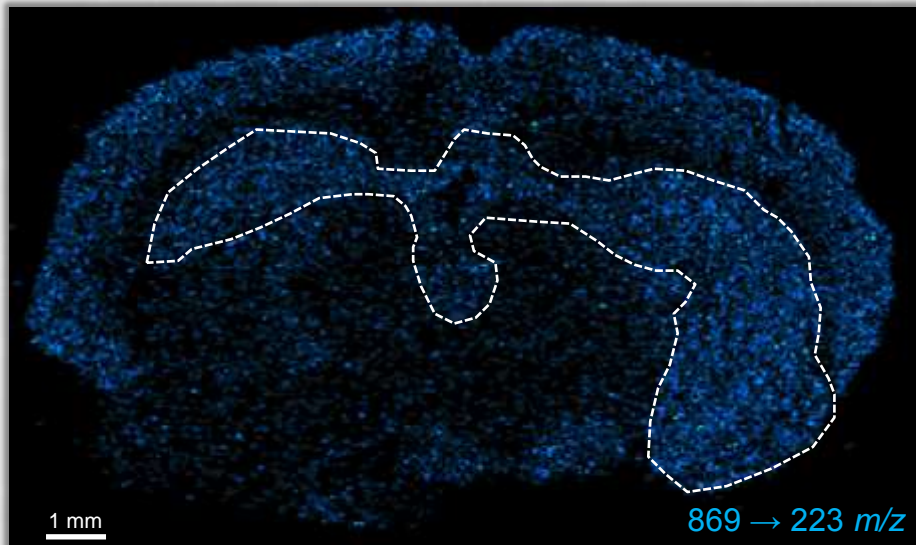
**Laser Rep. Rate:** 1000 Hz  
**Acquisition Rate:** 20 pixels/second  
**Pixels:** 51,120 pixels  
**Analysis Time:** ~45 minutes



# MS/MS Lipid Imaging

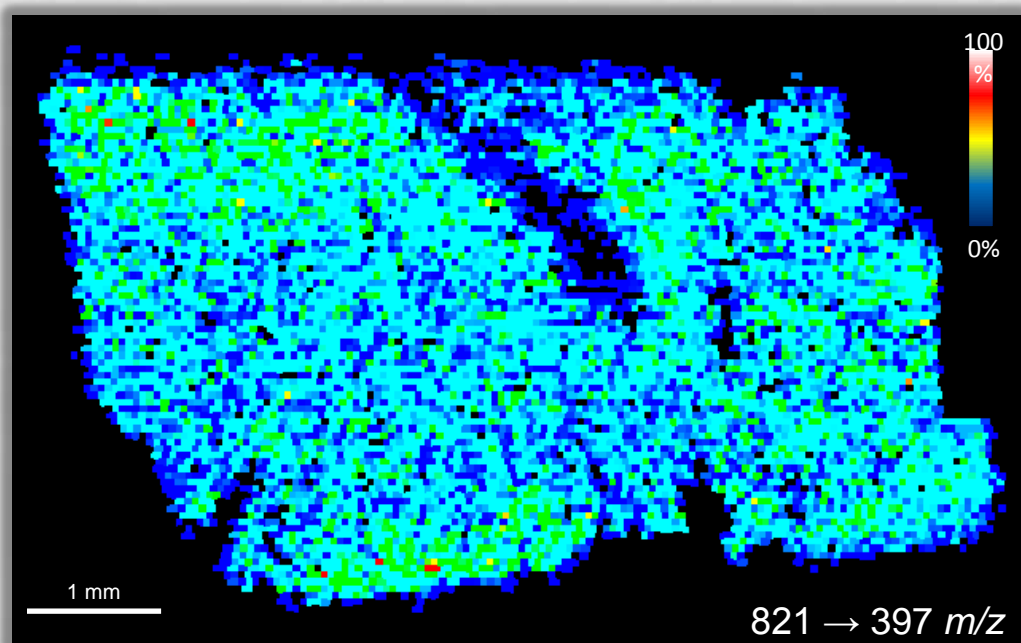
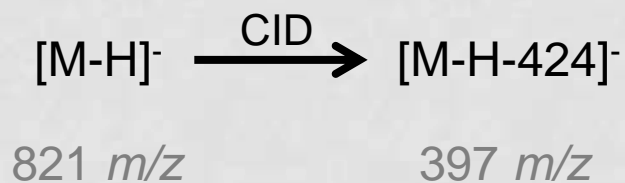
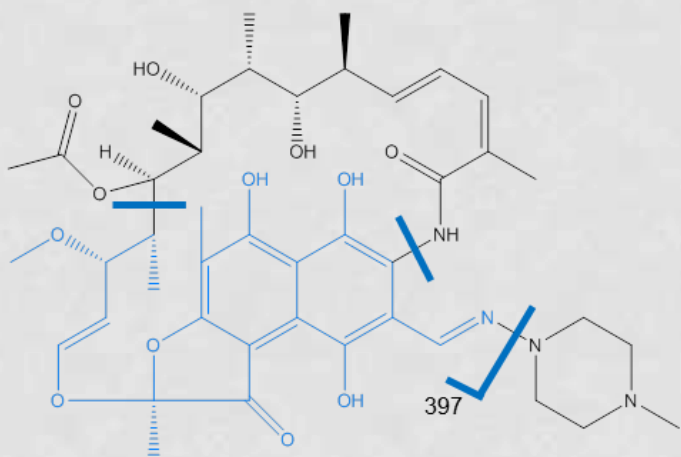
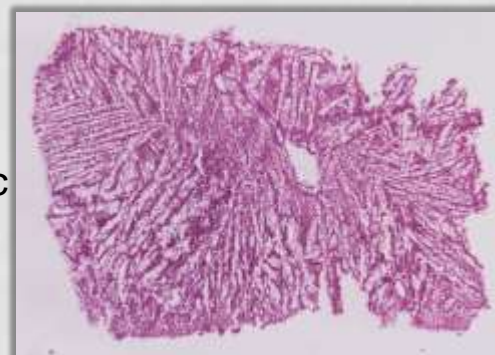


**MS/MS Precursor:** 869.2  
**Sample:** +Rat Brain (lipids)  
**Lateral Spatial Resolution:** 50  $\mu\text{m}$   
**Vertical Step:** 50  $\mu\text{m}$   
**Matrix:** DAN  
**Laser Rep. Rate:** 1000 Hz  
**Acquisition Rate:** 20 pixels/second  
**Pixels:** 48,441 pixels  
**Analysis Time:** ~45 minutes

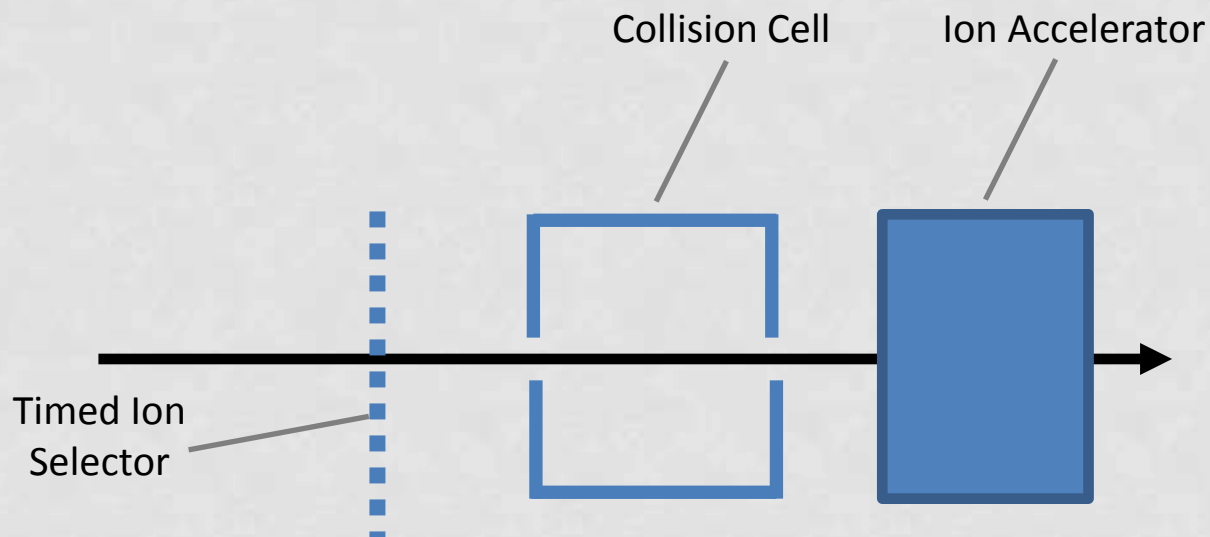


# MS/MS Drug Imaging

**Sample:** +Rat Liver (synthetically does with Rifampicin, 400 mg liver immersed in 200  $\mu$ M RIF solution for 100 hrs)  
**Matrix:** THAP  
**Laser Rep. Rate:** 1000 Hz  
**Acquisition Rate:** 20 pixels/sec  
**Pixels:** 14,192 pixels  
**Analysis Time:** ~15 minutes



# “Multiplexed” MS/MS Analysis



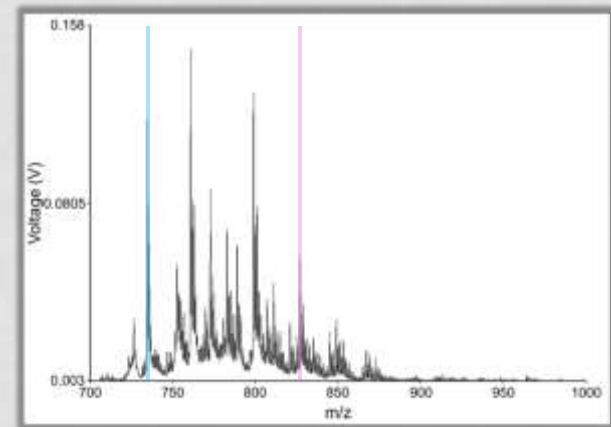
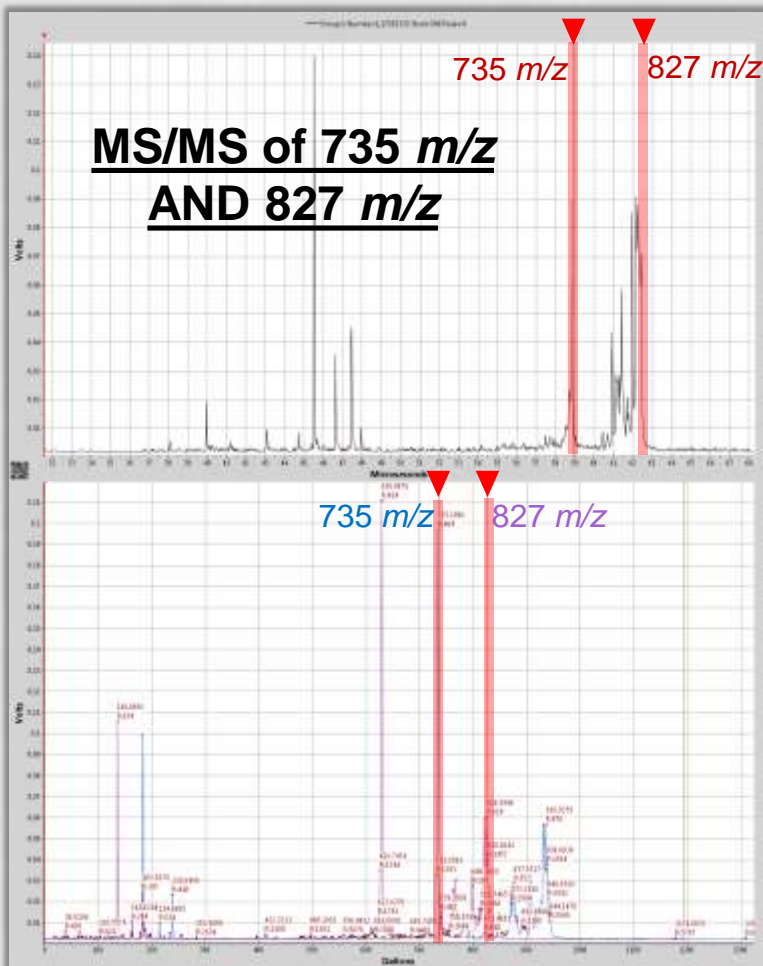
*Monitoring MS/MS transitions is useful, but throughput is limited when only examining a single precursor ion.*

- High resolution timed ion selector (<5 Da)
- Selection of multiple precursor ions allows for multiple MS/MS transitions to be performed in a single laser shot.

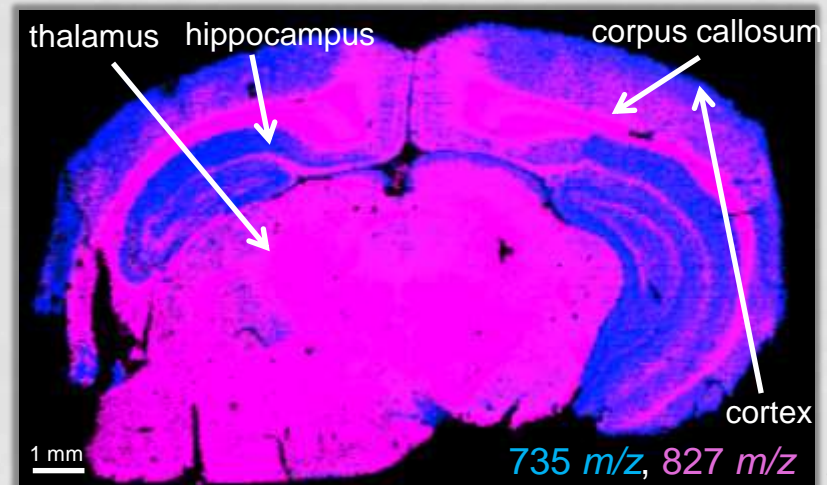
# “Multiplexing”

**Sample:** +Rat Brain (lipids)  
**Lateral Spatial Resolution:** 50  $\mu\text{m}$   
**Vertical Step:** 50  $\mu\text{m}$   
**Matrix:** DAN

**Laser Rep. Rate:** 1000 Hz  
**Acquisition Rate:** 20 pixels/second  
**Pixels:** 47,204 pixels  
**Analysis Time:** ~40 minutes

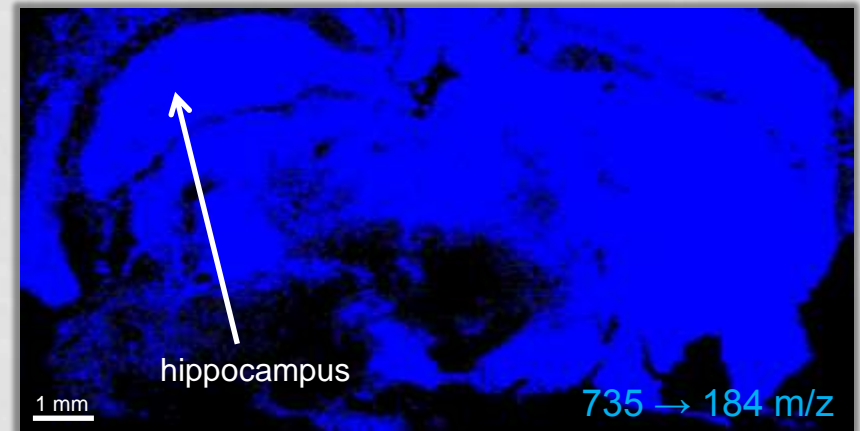
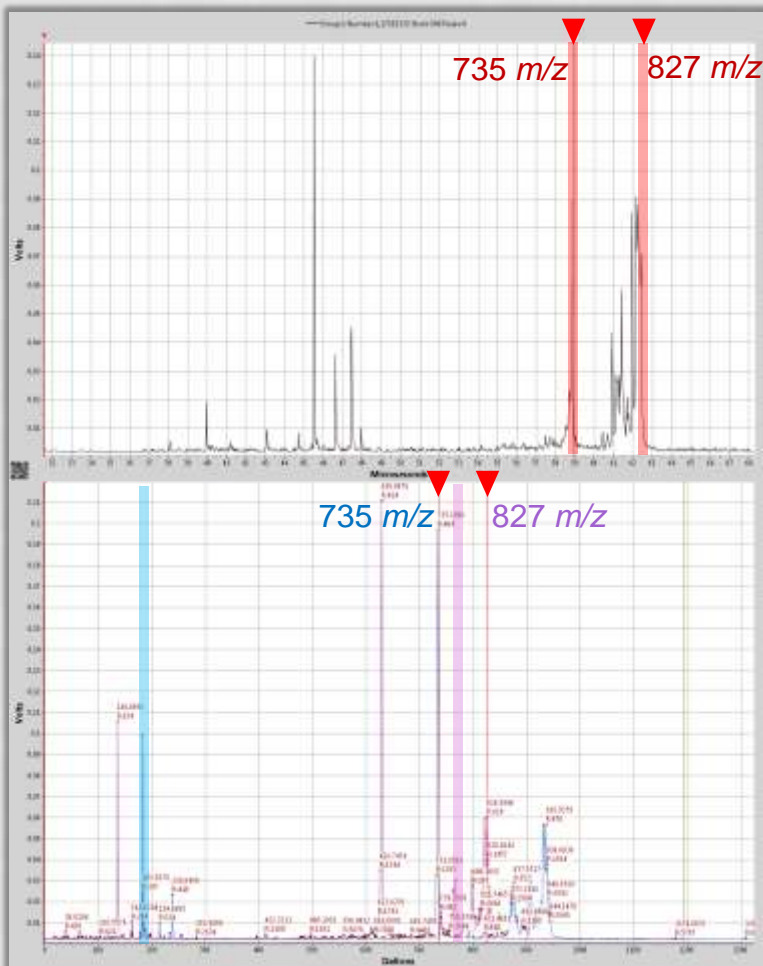


**MS Image**

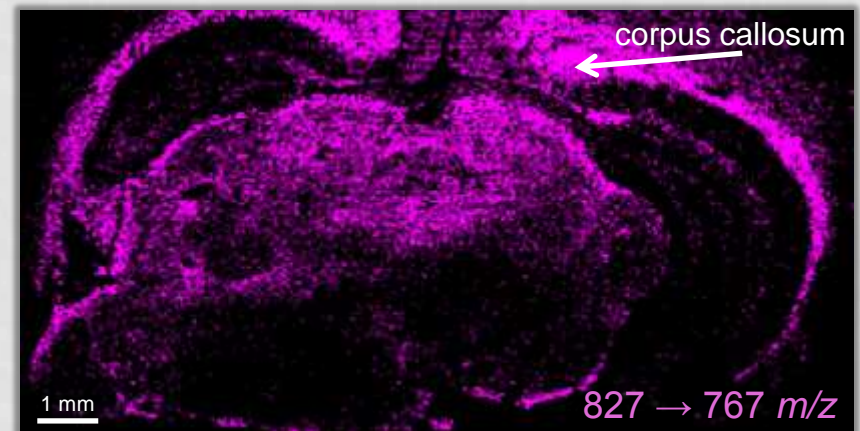


# Multiplexed Imaging

## MS/MS of 735 m/z AND 827 m/z



## MS/MS Images



# Conclusions

- **Going from hours to minutes:** High speed IMS produces high quality molecular images.
- **Making the unseen, seen:** Isobaric or near isobaric species require special consideration.
  - High resolution MS
  - MS/MS
- **Informing biology:** Coupled with the multiplexed nature of MS-based acquisition, the high throughput methodologies described herein offer viable means for studying complex biological systems *in situ*.

# Acknowledgements

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VANDERBILT UNIVERSITY



School of Medicine

Advanced Imaging Mass Spectrometry

# AIMS.2014

## Laboratory Course

**April 22 - 25, 2014**

Vanderbilt University, Nashville TN



### CURRICULUM

- Over 18 hours of hands-on workshops
- Mixed lecture/workshop format
- 3.5 day course with gala dinner
- Instrument vendor on-site demos

### COST & DEADLINE

- Jan 15 - Mar 15, early registration - \$750
- Mar 16- Mar 31, late registration - \$950
- Registration closes March 31
- Limit 40 participants



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General Medical Sciences

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