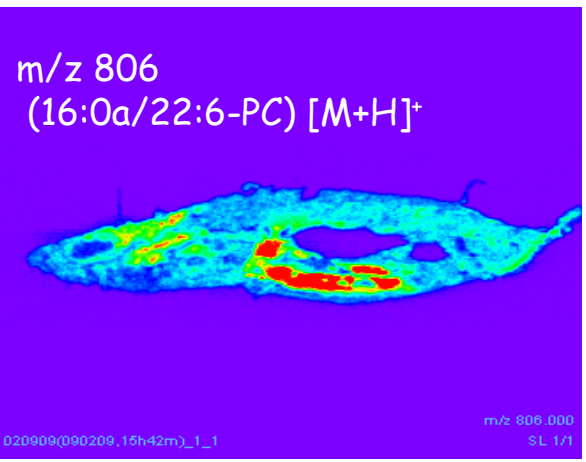
# LIPID MAPS Lipidomics Workshop

April 19, 2009

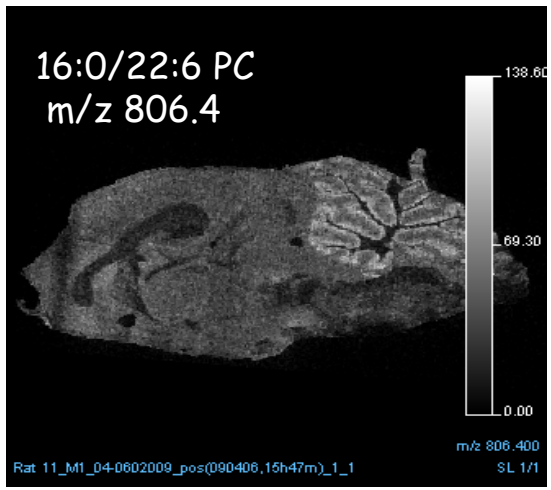
www.lipidmaps.org




## Future Directions: Tissue and Cell Imaging

Robert C. Murphy

Department of Pharmacology
University of Colorado Denver




Other LIPID MAPS Bridge C (Imaging Group):

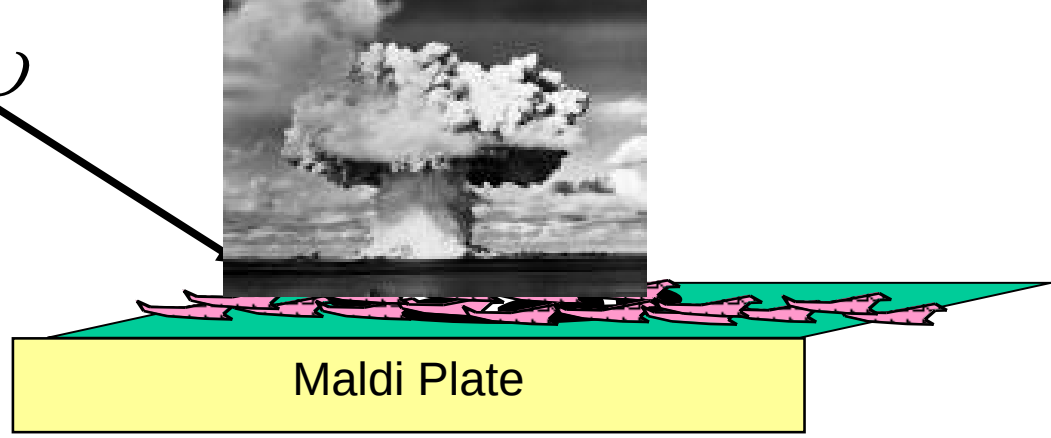
| University of Colorado Denver | Penn State |
|---|---|
| Robert Barkley | Nicolas Winograd |
| Joseph Hankin | Melissa Passarelli |

## Outline:

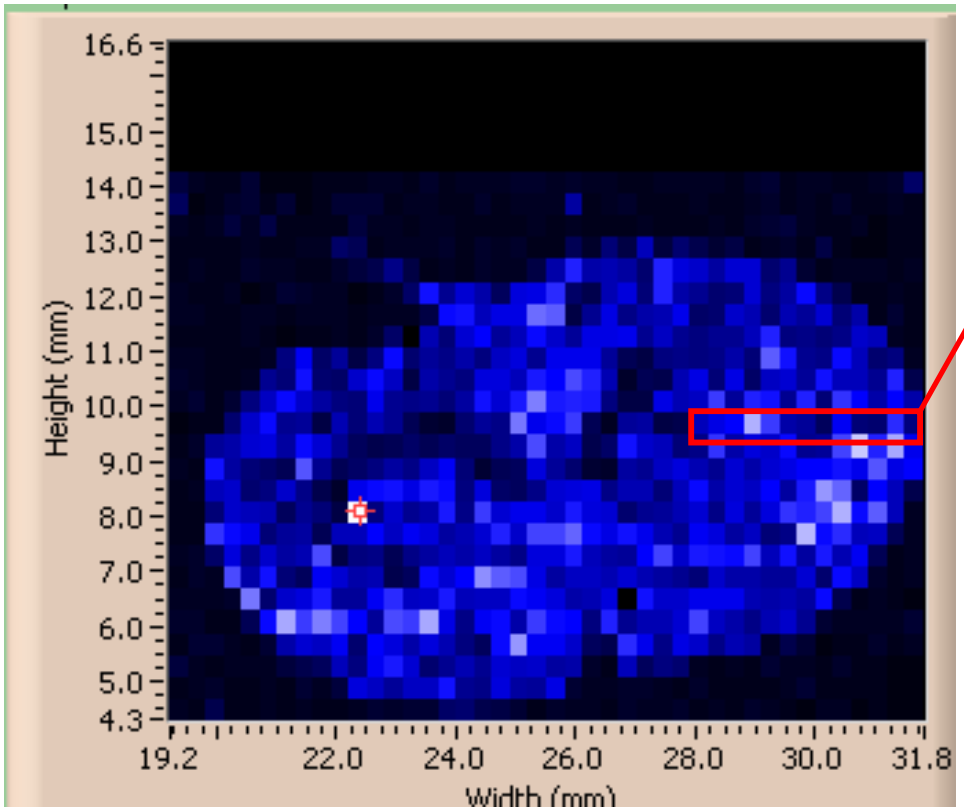
A. Brief introduction of tissue molecular imaging by mass spectrometry
B. Sample preparation issues: tissue preparation, matrix application
C. Compound identification: Molecular weight (high resolution) and MS/MS
D. Brain images-Abundance of Ions
    i. Abundance of ions/abundance of phospholipids
    ii. Microdissection and LC/MS/MS quantitation
E. Buckyball ($C_{60}^{+}$) images
F. Future
G. References

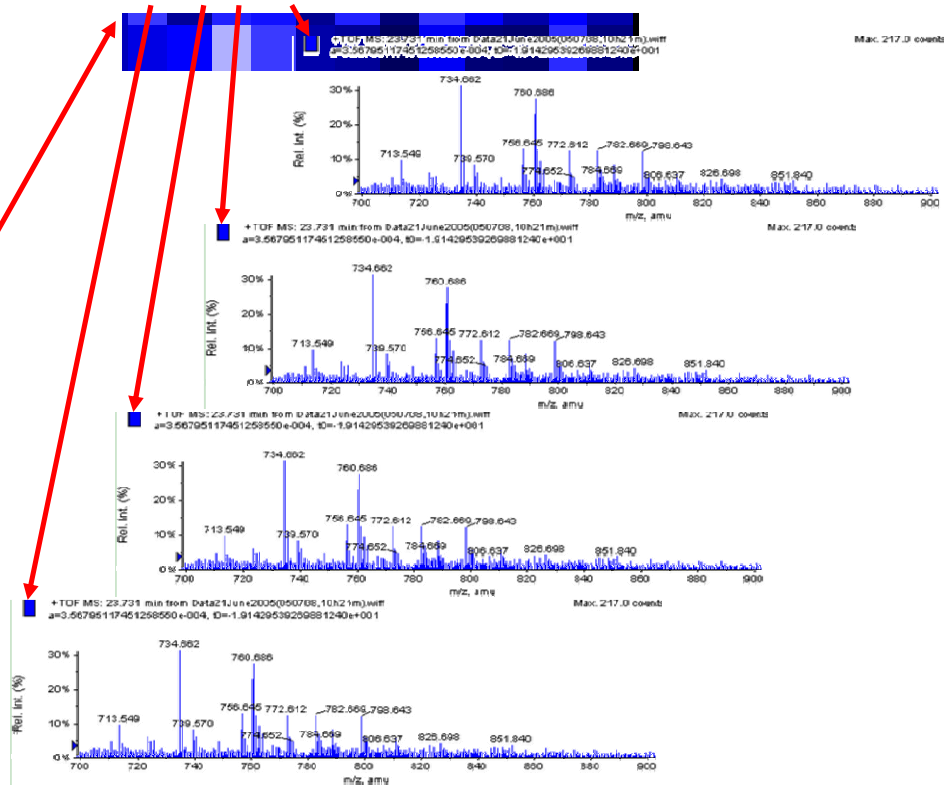
$h\upsilon$

**$N_2$-laser**
**337 nm**

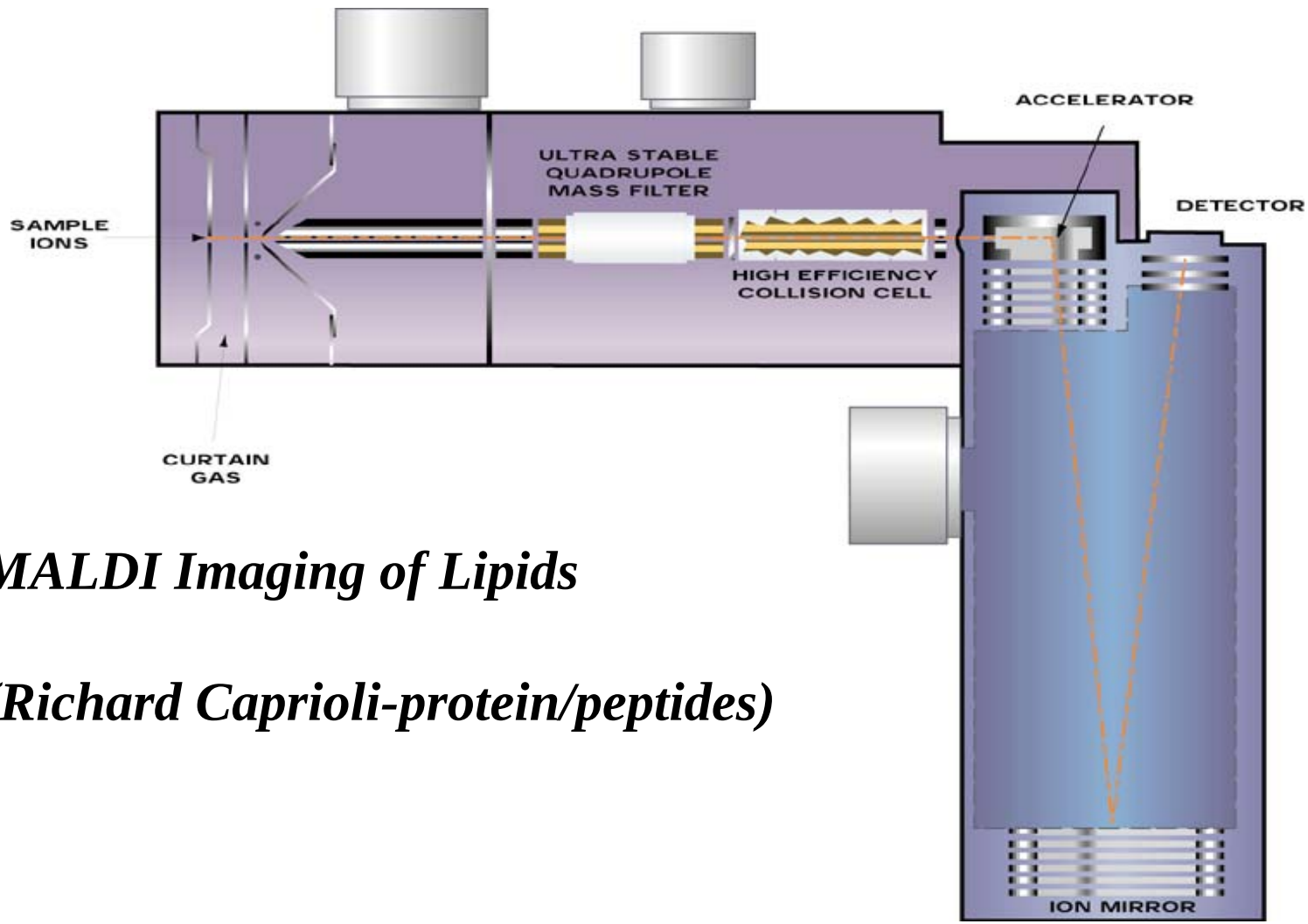



Matrix: ***2,5-dihydroxybenzoic acid***



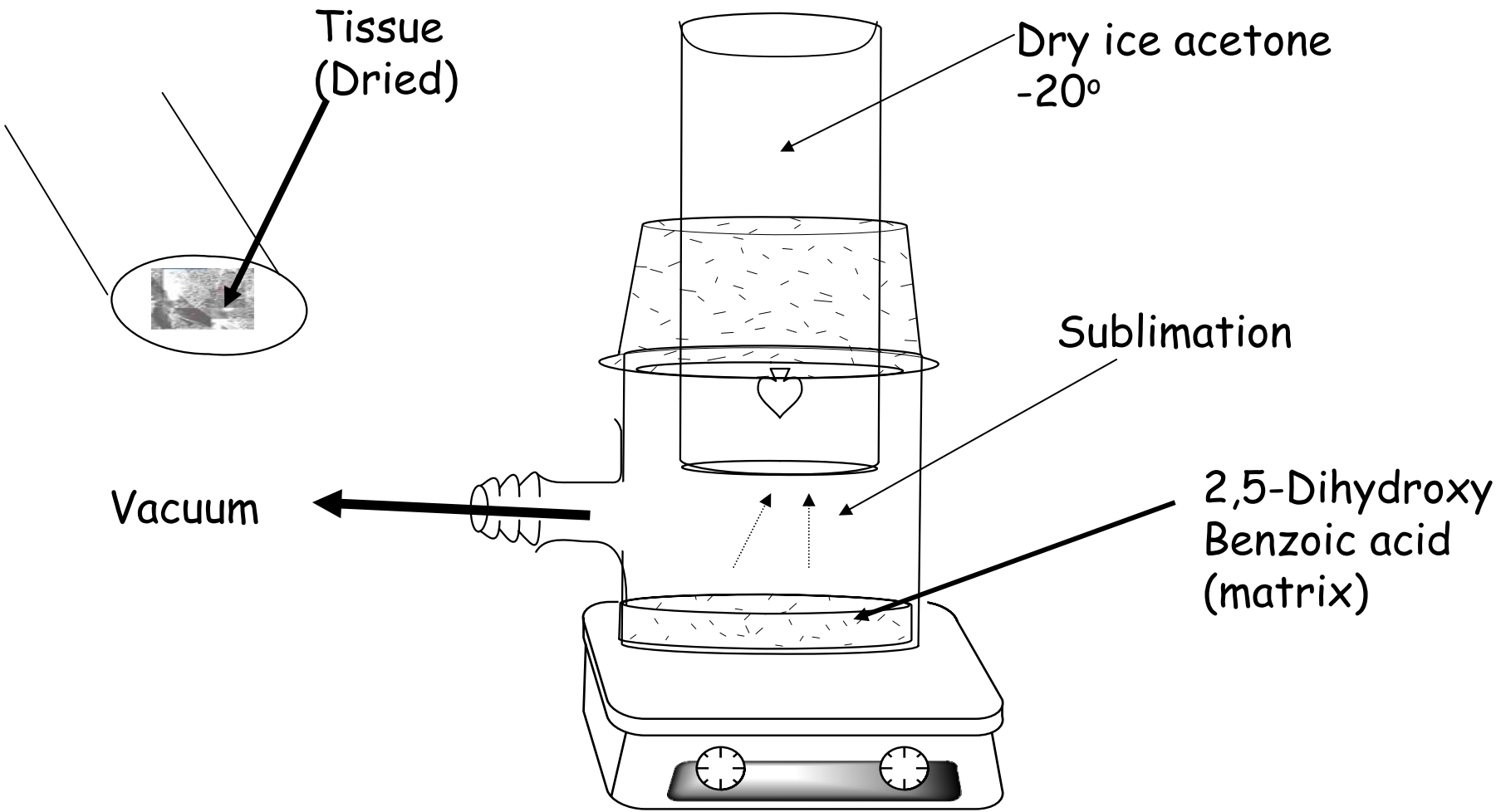
# MALDI- QStar (Q-TOF)




***MALDI Imaging of Lipids***

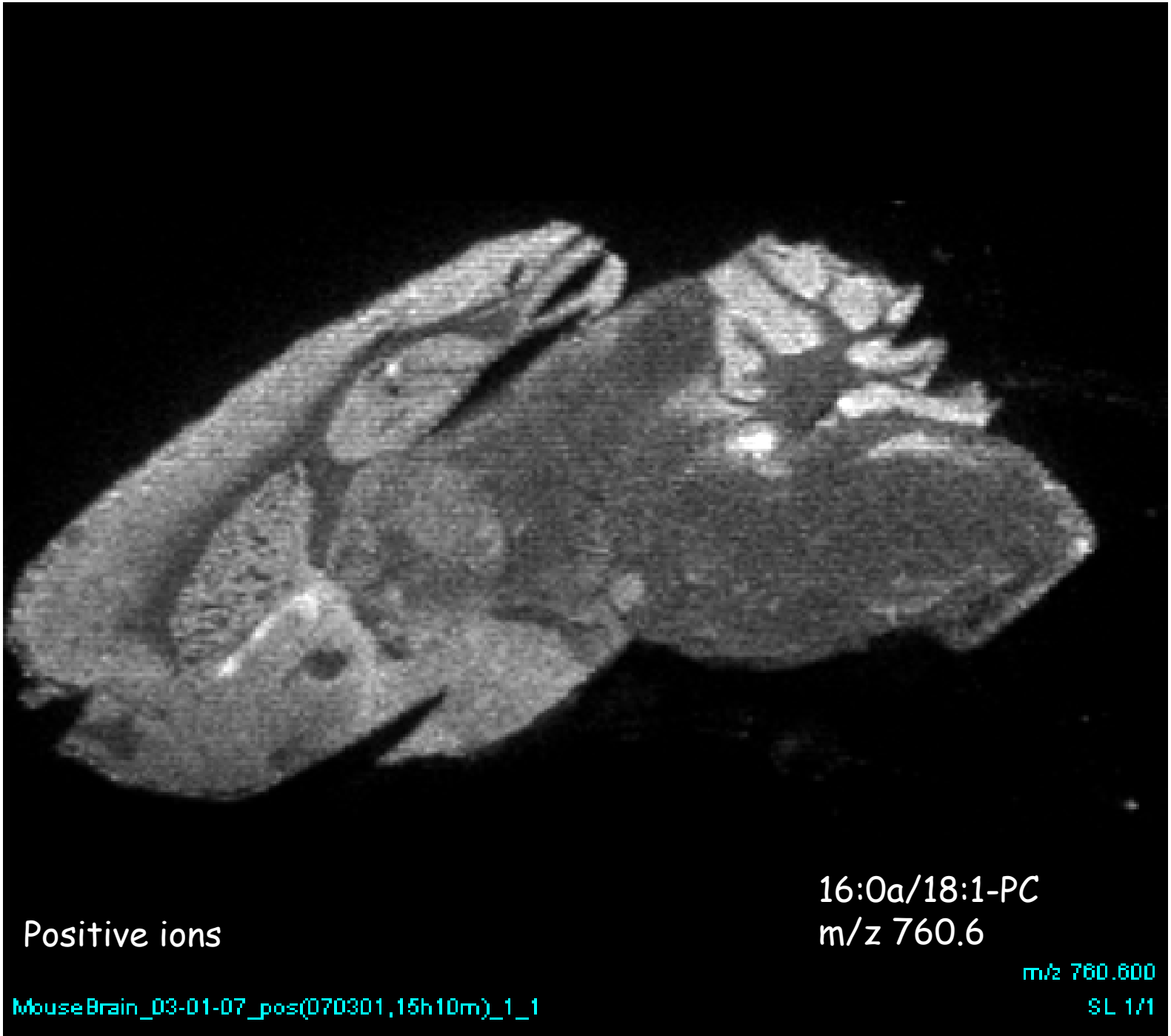
***(Richard Caprioli-protein/peptides)***

# Sublimation

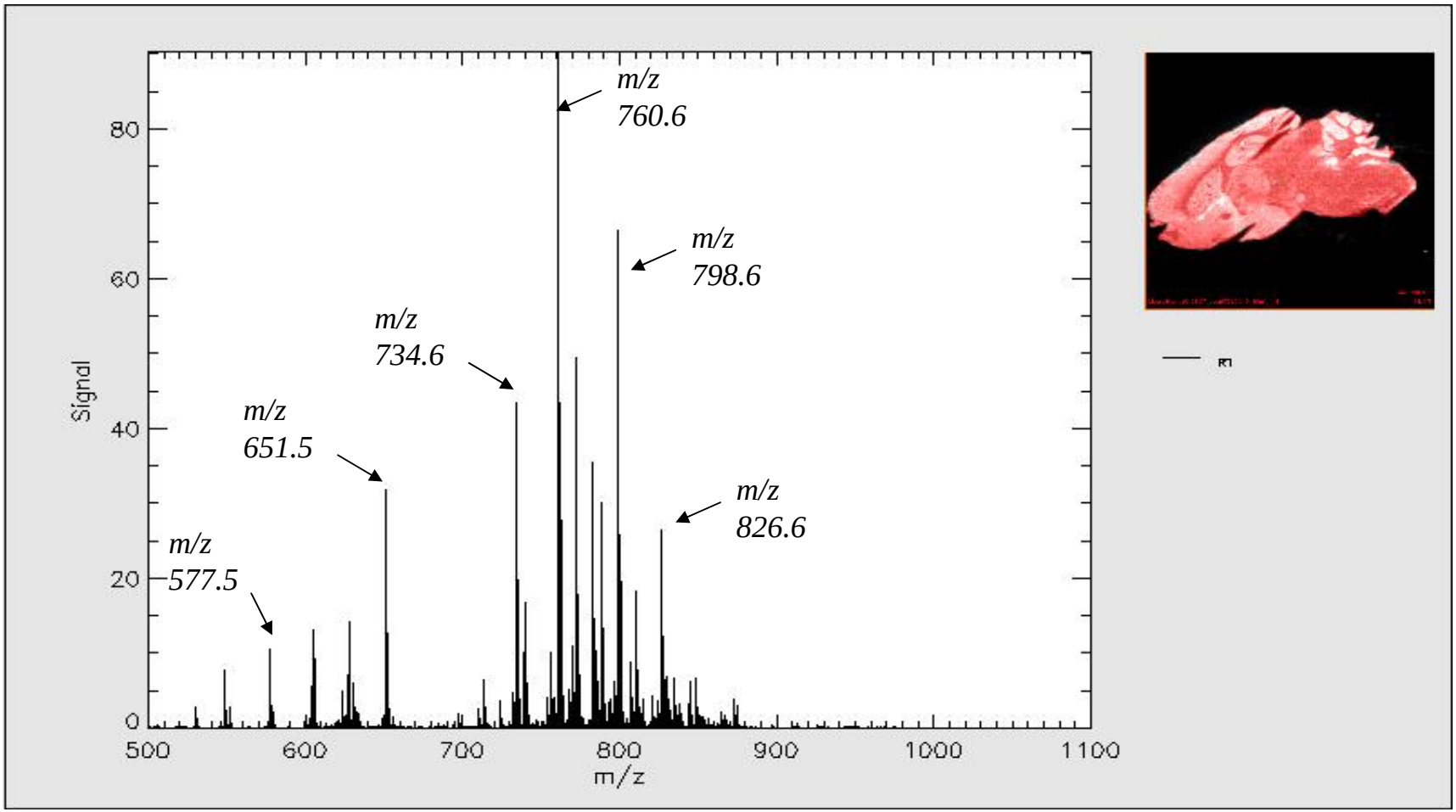


# Sample preparation

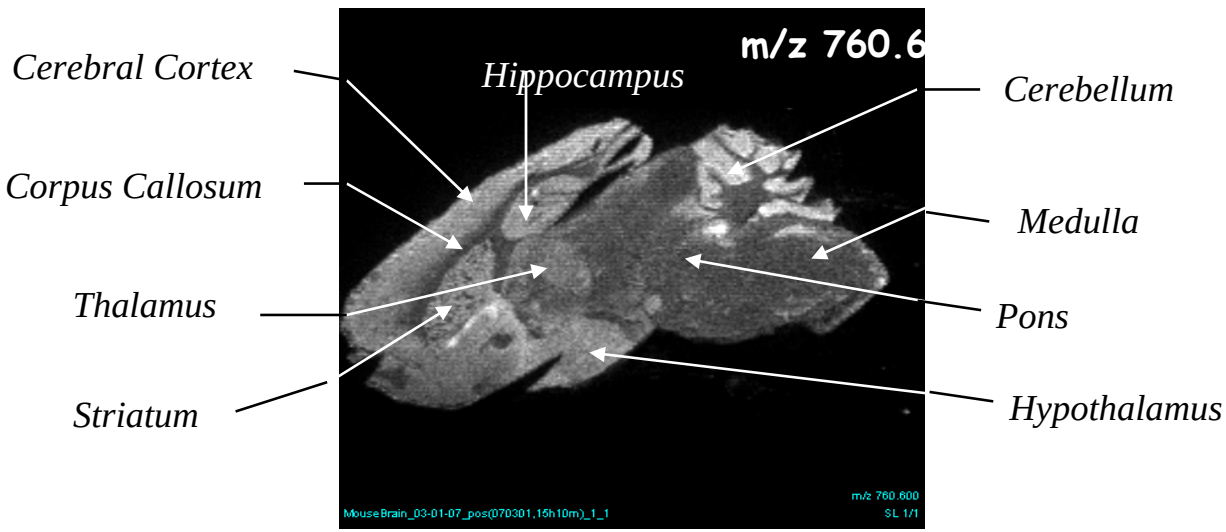
- Mouse brain flash frozen-70$^{o}$C
- Warmed to -15$^{o}$C, mounted with Optimal cutting temperature compound
- Sliced (cyrostat) 10 um thickness
- Placed directly on glass cover slips or MALDI steel plate
- Stored -20$^{o}$C

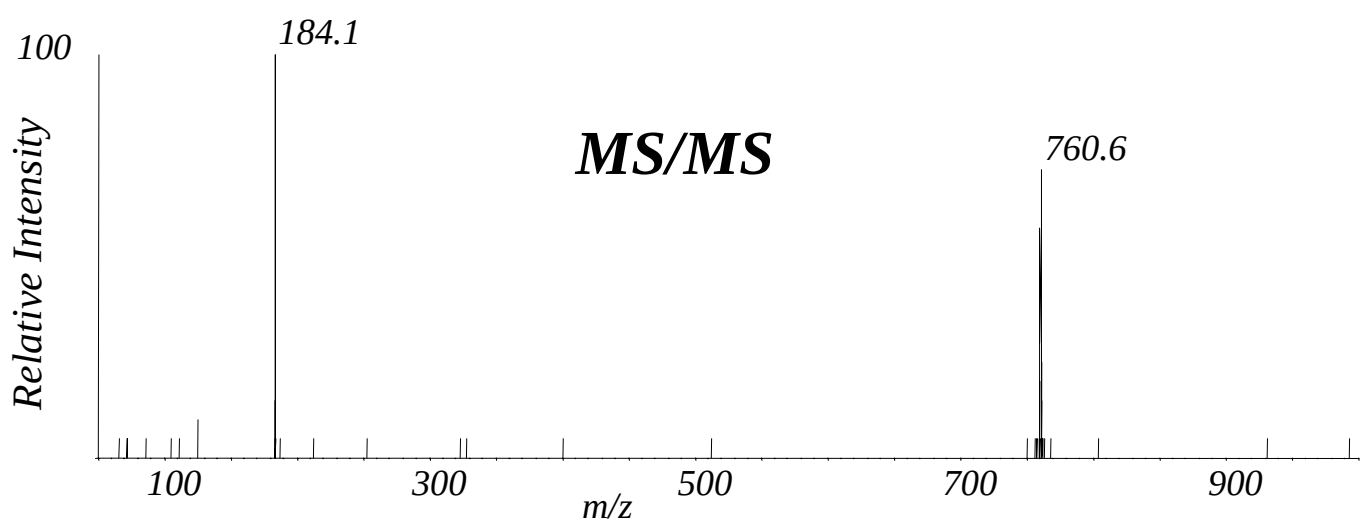

Positive ions
16:0a/18:1-PC
m/z 760.6
m/z 760.600
MouseBrain_03-01-07_pos(070301,15h10m)_1_1
SL 1/1

# Full Scan Spectrum of image

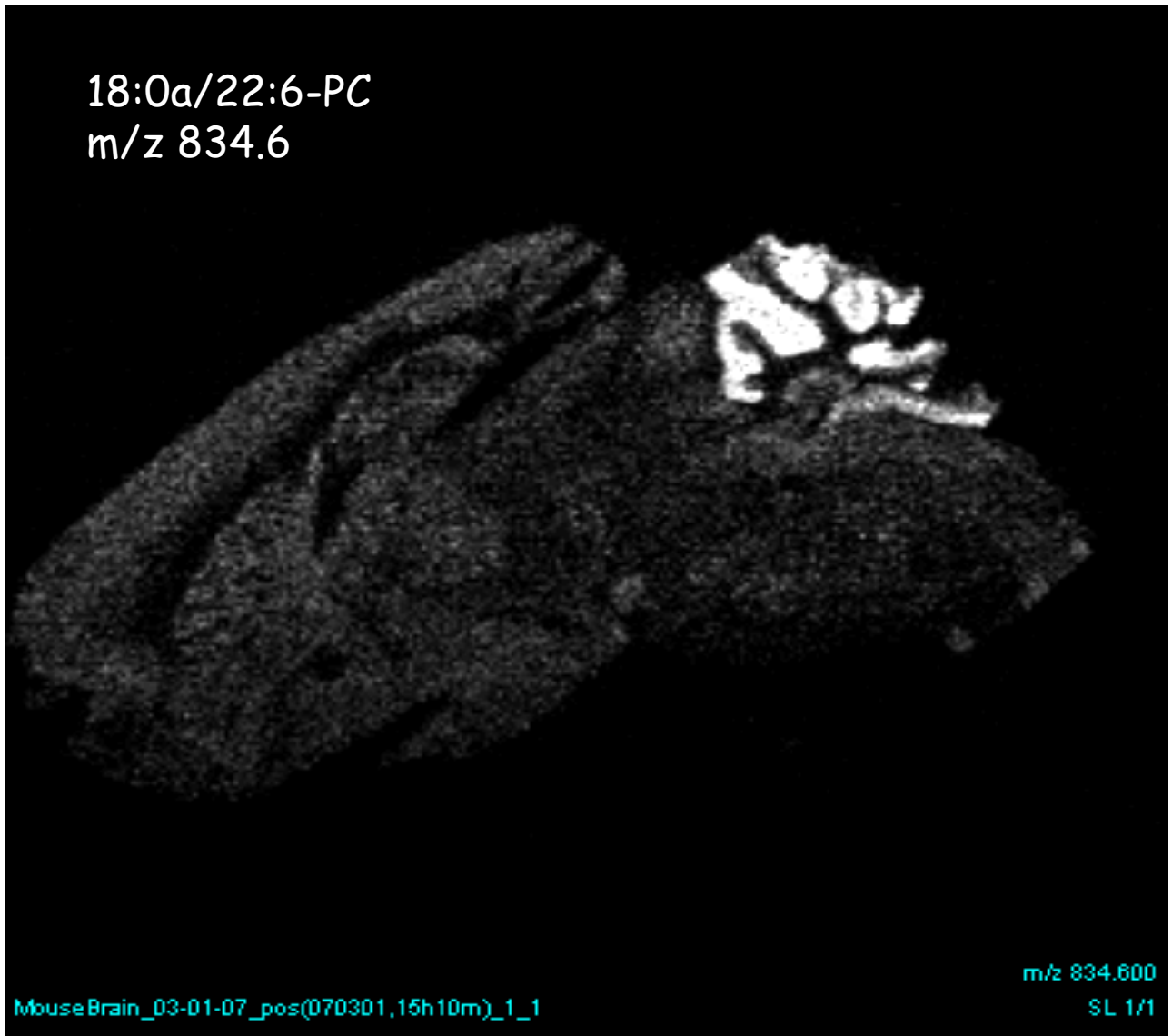


# Identification of Lipids (MS/MS)

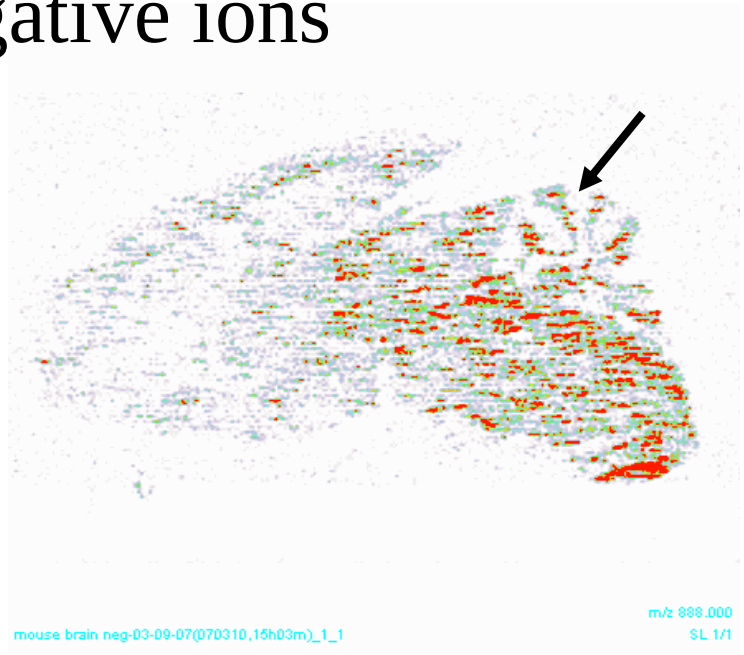


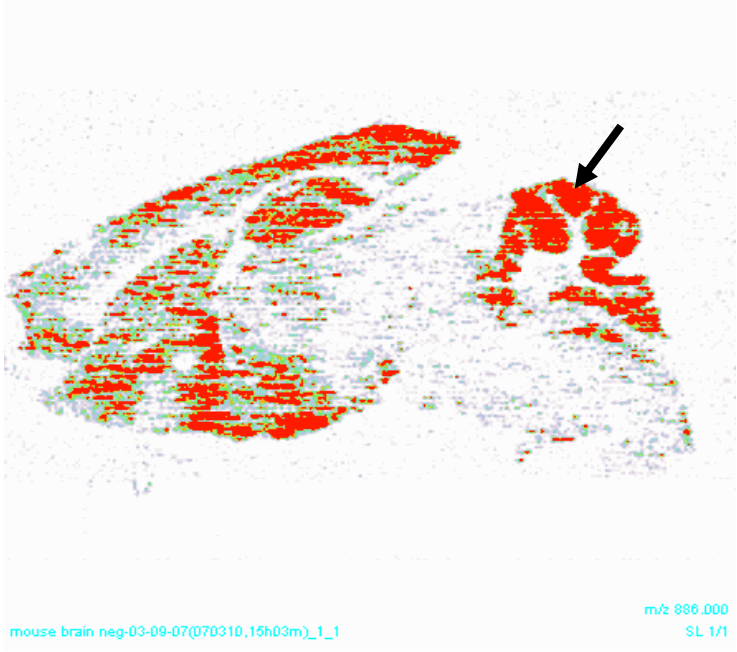
# Docosahexaenoic Acid Containing Phosphatidylcholine



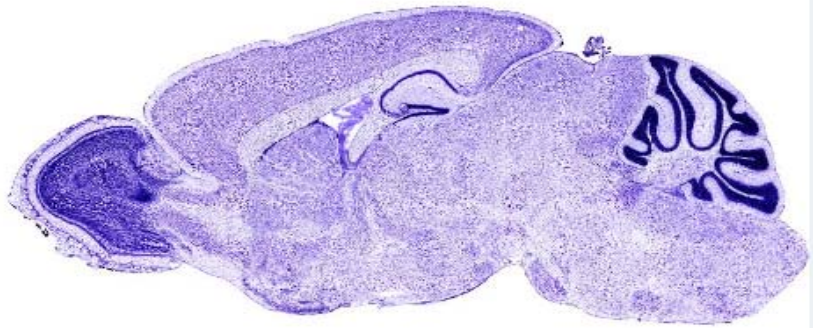
# Negative ions




d18:0/24:1 – ST
m/z 888.7

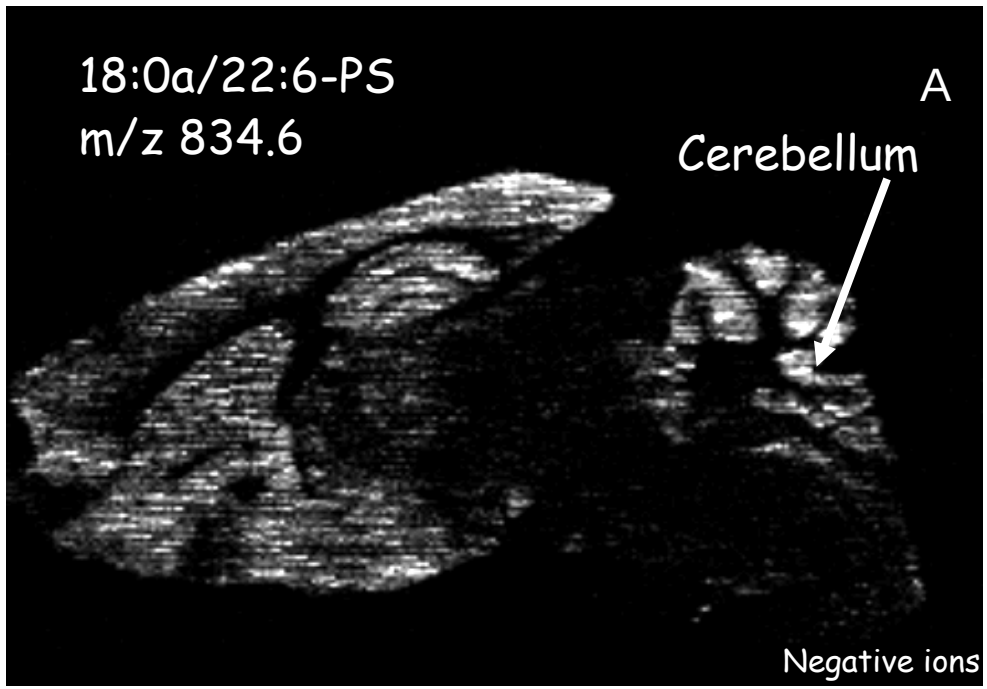


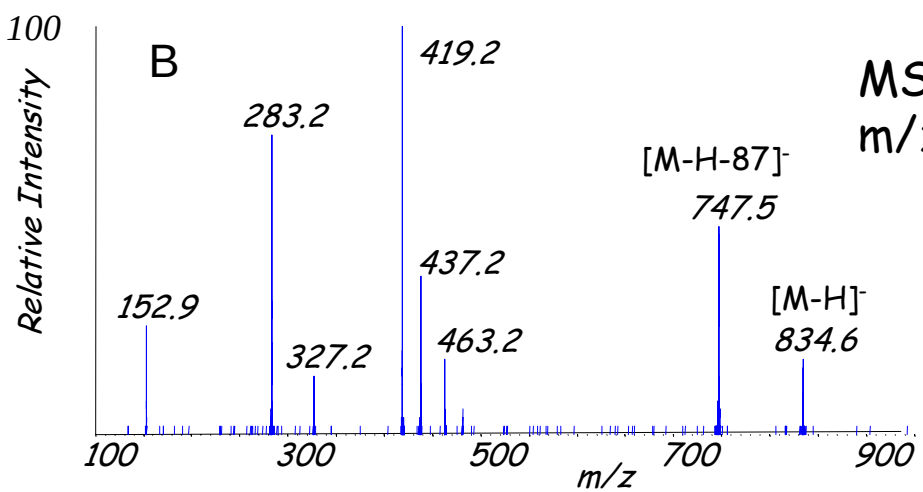

18:0a/20:4-PI
m/z 885.6



Stain image
Allen Brain Atlas
www.brainatlas.org

# Negative Ions



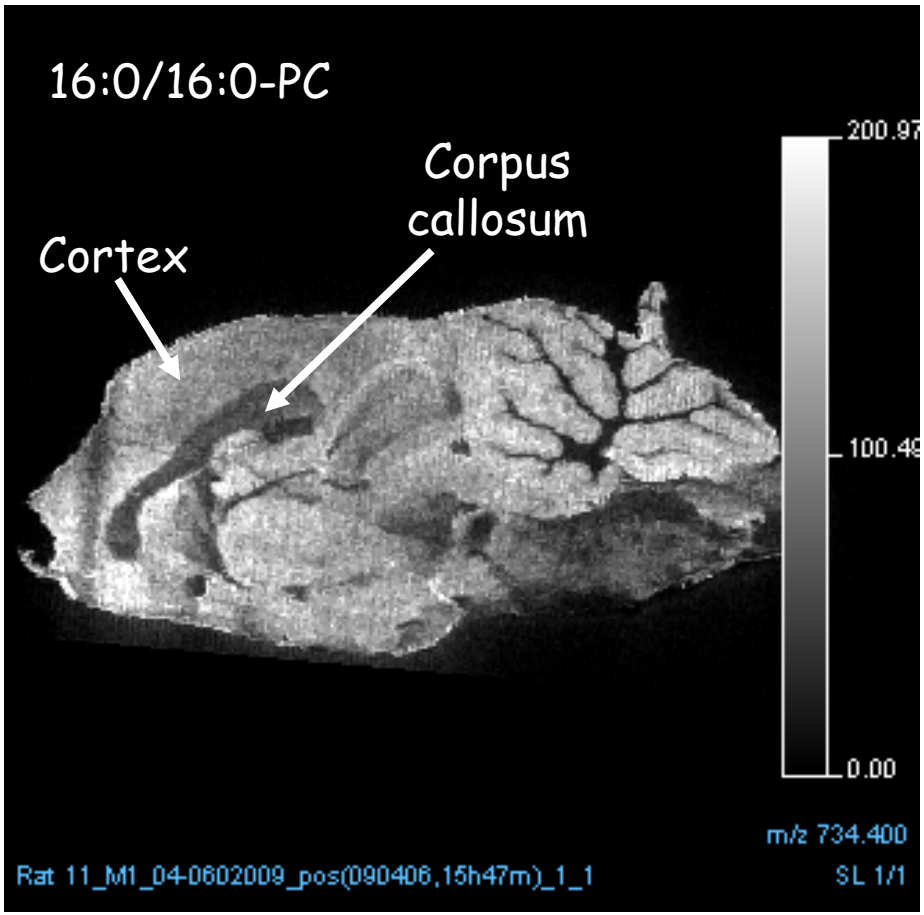



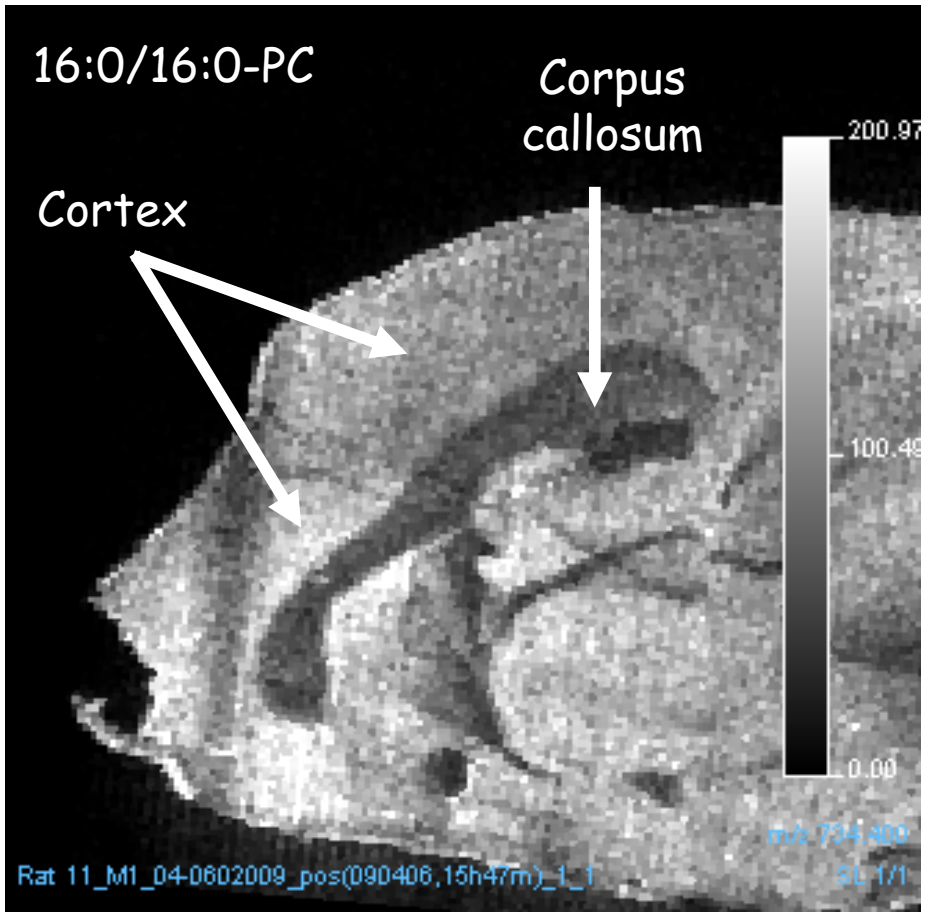

MS/MS(CID)
m/z 834.6

# What do the ion abundances mean?

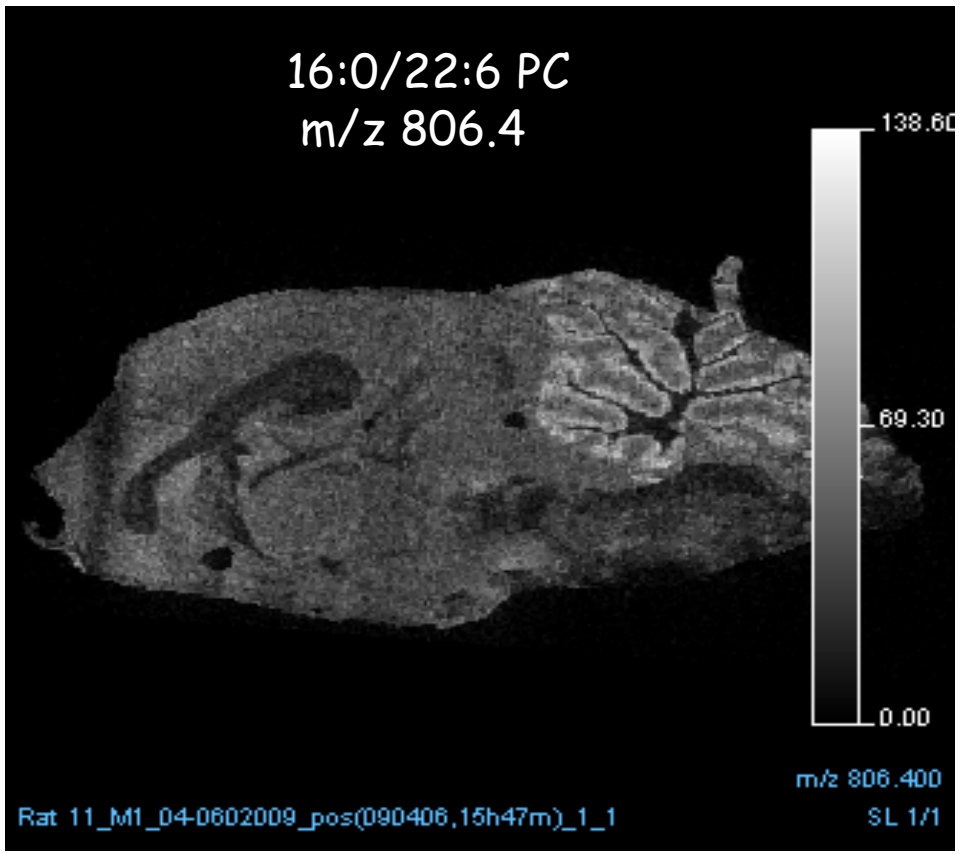
- Phospholipid species is present?
- Concentration is higher than other regions?

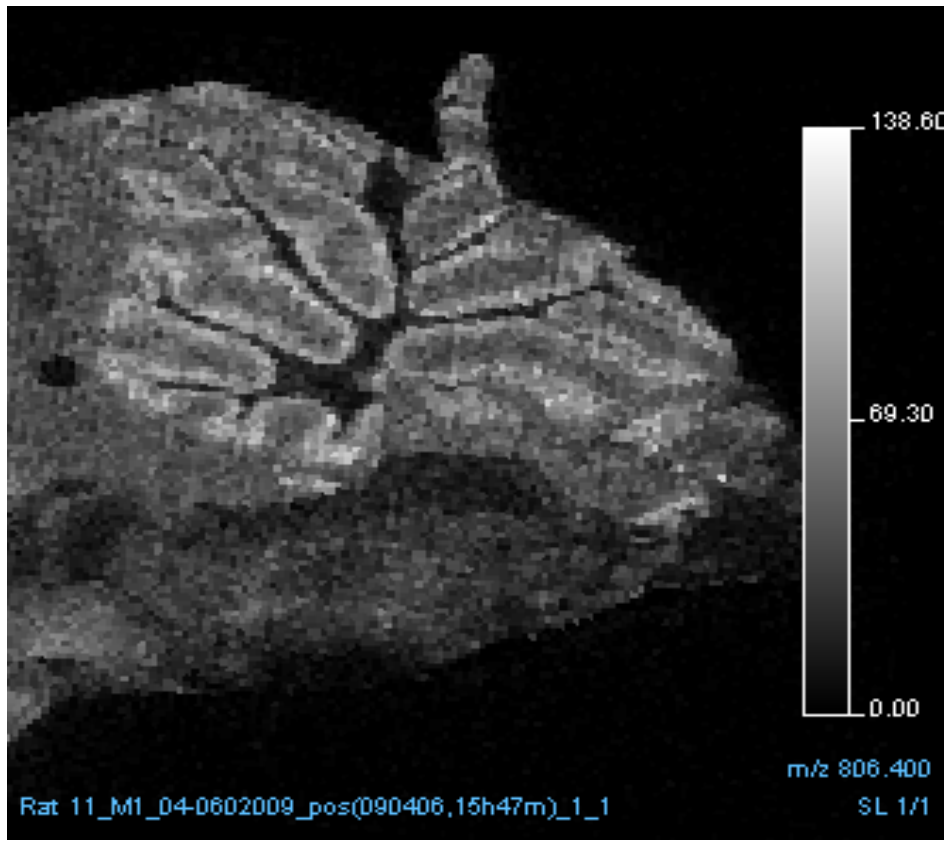
# Is there more 16:0/16:0-PC ( m/z 734.4) in cortex than in corpus callosum?



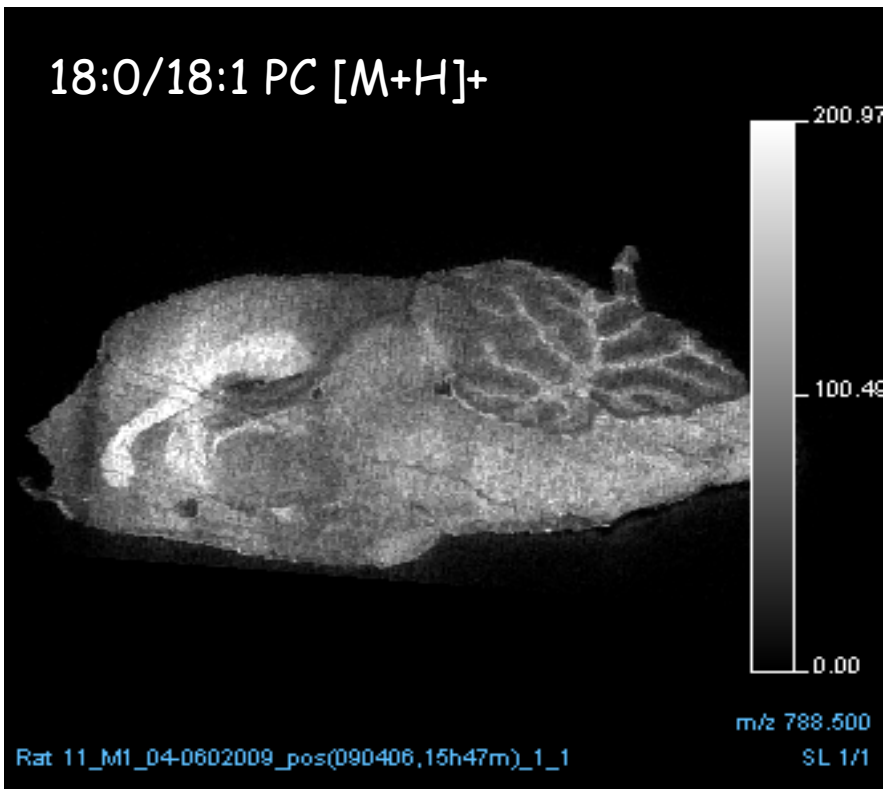



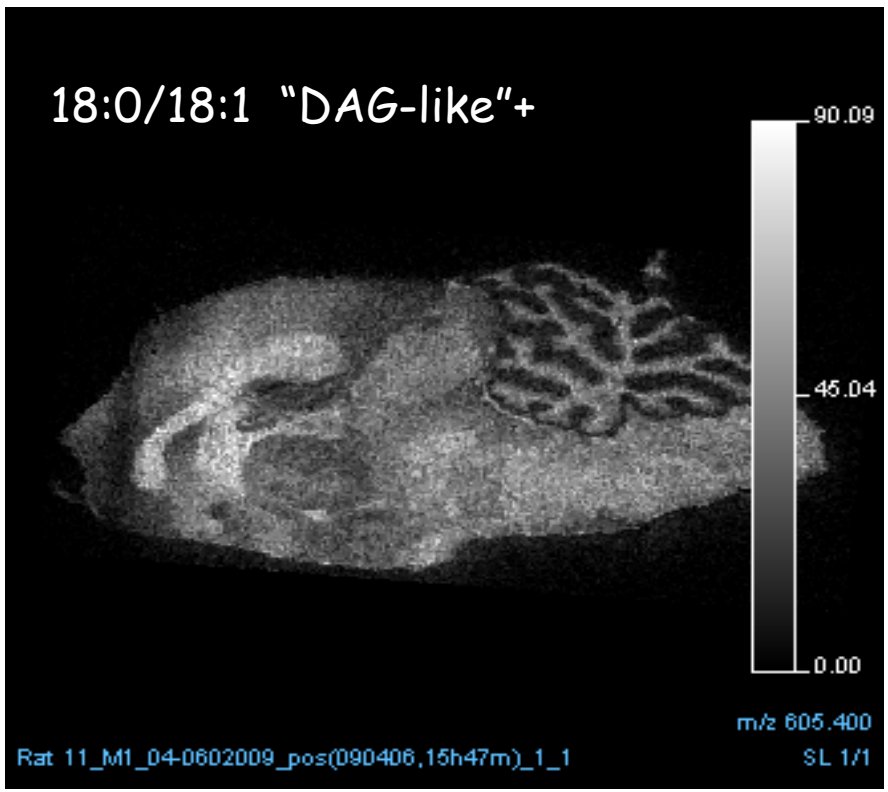
# Is there really more esterified 22:6 in the rat cerebellar grey matter?



# Is there really more esterified 18:0/18:1 in the rat white matter?



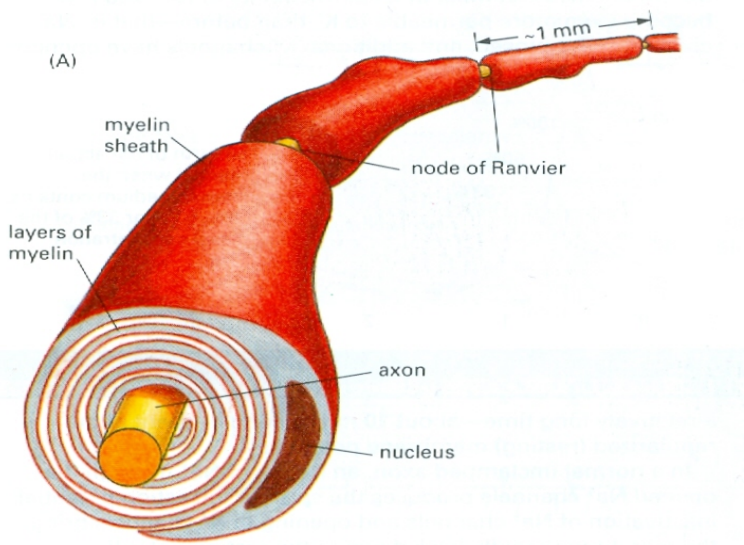
# Do observed ions reflect actual concentrations?

- Image of major phospholipids from a slice
- Isolate mouse brain regions (micro dissection) on adjacent slice
- Extraction (added 100ng deuterated-PC internal standard)
  - Normal phase LC/MS (electrospray ionization)
    - Positive ions

# Abundance of ions in images: False negatives/no false positives

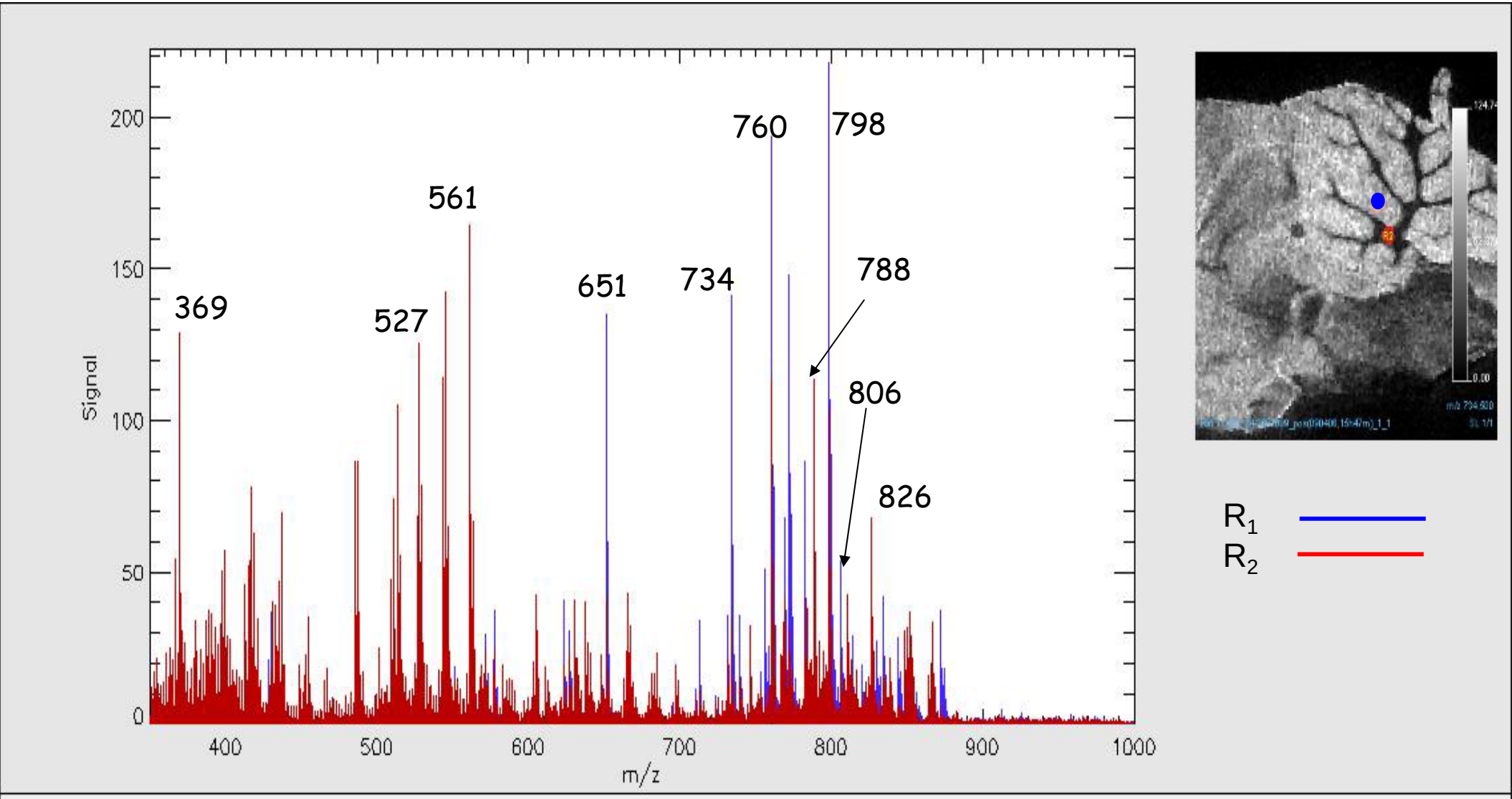
- Imaging of Phospholipids in tissue slices
  - Striking distribution of specific molecular species in regions (50 micron resolution)
  - Observance of m/z means PL is present at site
    - False negative information likely
  - Mechanism of lipid secondary ion release?
  - Abundance of ions
    - $I_{m/z}$ = f([Lipid] x [ionization cross section] x [local environment] x …)

# Why is local environment so different about white matter?




Myelin sheath

# Total MALDI ions in celebellar grey(blue) and celebellar white (red)



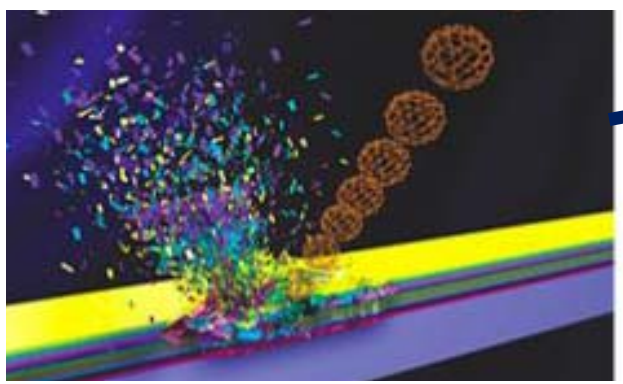
# Collaboration : Nick Winograd Penn State

- SIMS (secondary ion mass spectrometry) based imaging
  - Buckyball ion beam ($C_{60}^+$)
  - Better lateral resolution
    - Lipid bilayers with SIMS ($Ga^+$) Science, 2004
    - 200 nm ion beam
- Compare sublimation MALDI with SIMS
  - Prepare rat cerebellum on In oxide glass slides
  - Serial sections analyzed UCHSC/Penn State

# Buckyballs $[C_{60}]$+
# Nick Winograd-Penn State

Melissa Passarelli

Primary ion beam focused to a submicron spot



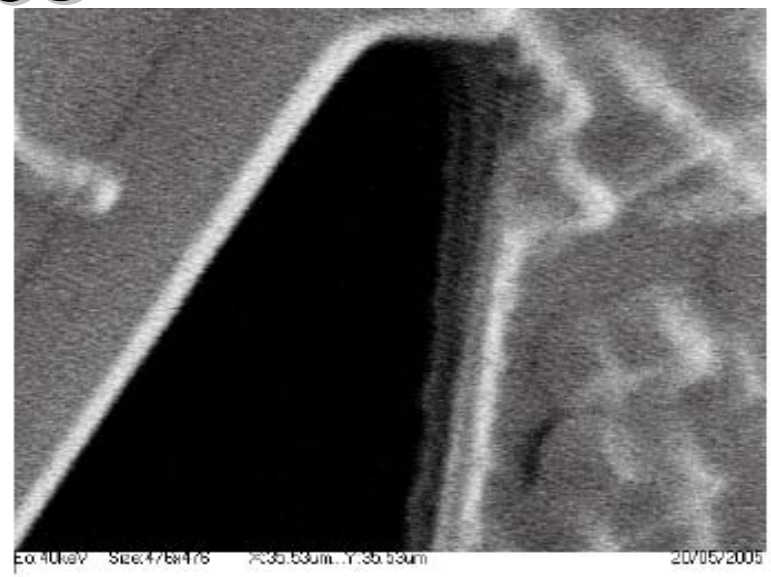
Each carbon atom carries 1/60$^{th}$ of total incident kinetic energy

Other ion beams- $Au_3$, $Bi_3$, $SF_9$, $Au_{400}$

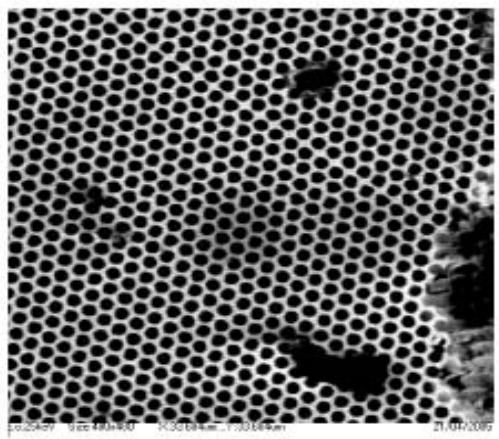
*Weibel, Wong, Lockyer, Blenkinsopp, Hill and Vickerman, Anal. Chem., 2005.*

# $C_{60}$ Ion Source

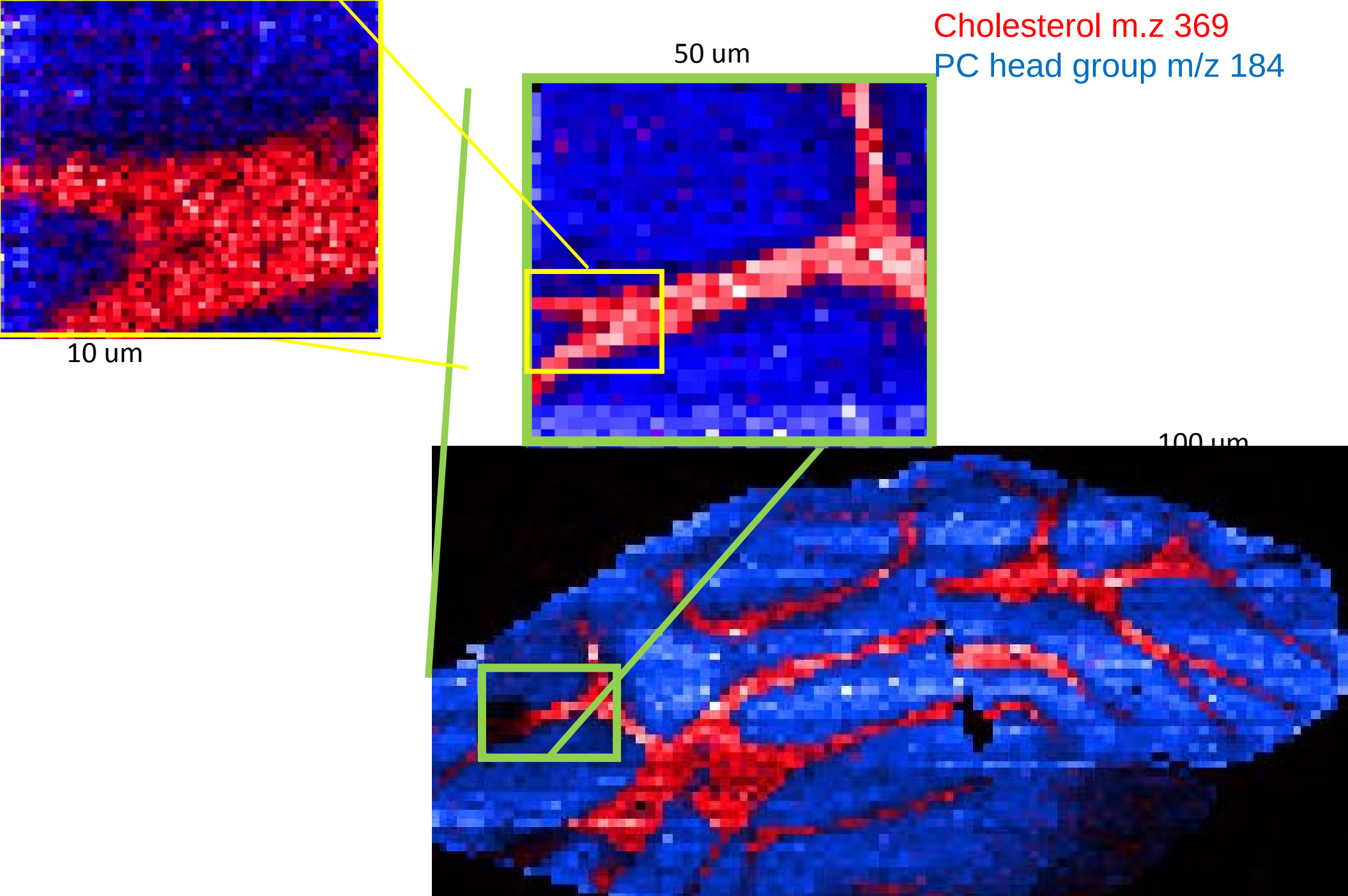
Ionoptika



40keV C60+, 1pA, 400nm
Image of 10um MCP



Spatial Resolution < 300 nm
Energy range 10 keV – 40 keV
Source lifetime > 600 hours


Cholesterol m.z 369
PC head group m/z 184
50 um
10 um
100 um

# Conclusions

- Imaging of Lipids in the brain
  - Striking distribution of specific PL-molecular species and even cationized species
  - Abundance information relevant within tissues of similar cellular structure
  - Rich biochemical information
  - Can we improve lateral resolution?

# G. Examples of imaging lipids in tissues

**Sublimation as a method of matrix application for mass spectrometric imaging. (2007).** Hankin JA, Barkley RM, Murphy RC. *J Am Soc Mass Spectrom* 18:1646–1652

**Imaging MALDI mass spectrometry using an oscillating capillary nebulizer matrix coating system and its application to analysis of lipids in brain from a mouse model of Tay-Sachs/Sandhoff disease** (2008) Chen Y, Allegood J, Liu Y, Wang E, Cachón-Gonzalez B, Cox TM, Merrill AH Jr, Sullards MC. Anal Chem. 80(8):2780-8.

**Molecular imaging of proteins in tissues by mass spectrometry** (2008). Seeley EH, Caprioli RM Proc Natl Acad Sci;105:18126-31.

**Nanoparticle-assisted laser desorption/ionization based mass imaging with cellular resolution.**( 2008 )Taira S, Sugiura Y, Moritake S, Shimma S, Ichiyanagi Y, Setou M. Anal Chem. 80:4761-6.

**Solvent-free matrix dry-coating for MALDI imaging of phospholipids**.( 2008 ) Puolitaival SM, Burnum KE, Cornett DS, Caprioli RM. J Am Soc Mass Spectrom. 19:882-6.

# Acknowledgements

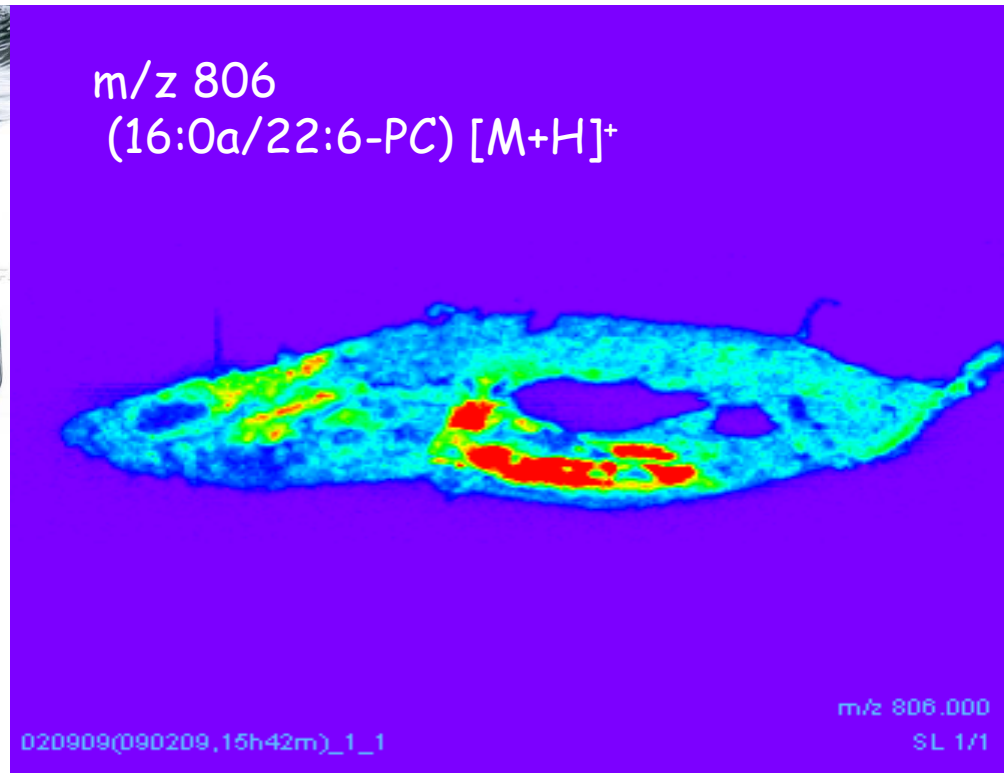
MS-Imaging


UCDenver

- Joseph Hankin
- Robert Barkley
- Santiago Farias

Penn State

- Nick Winograd
- Melissa Passarelli



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