# Imaging of Gangliosides using 2,6-Dihydroxyacetophenone with an AP-MALDI Source

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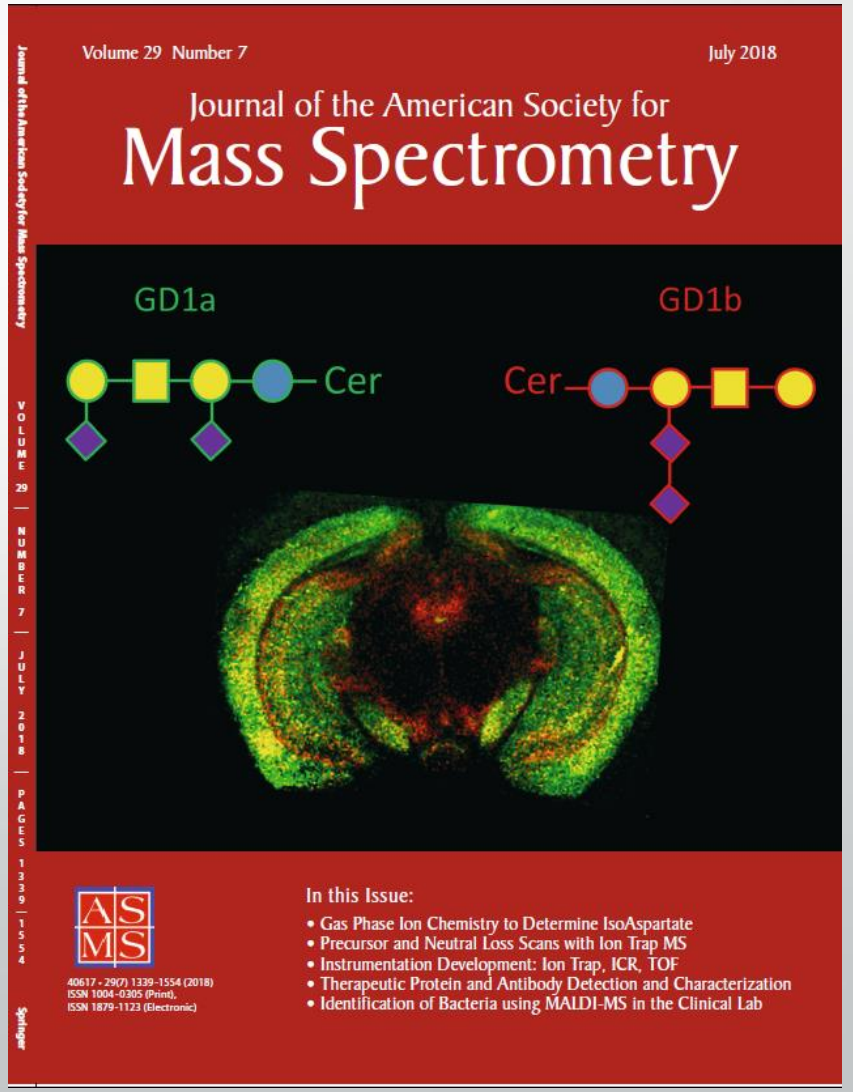
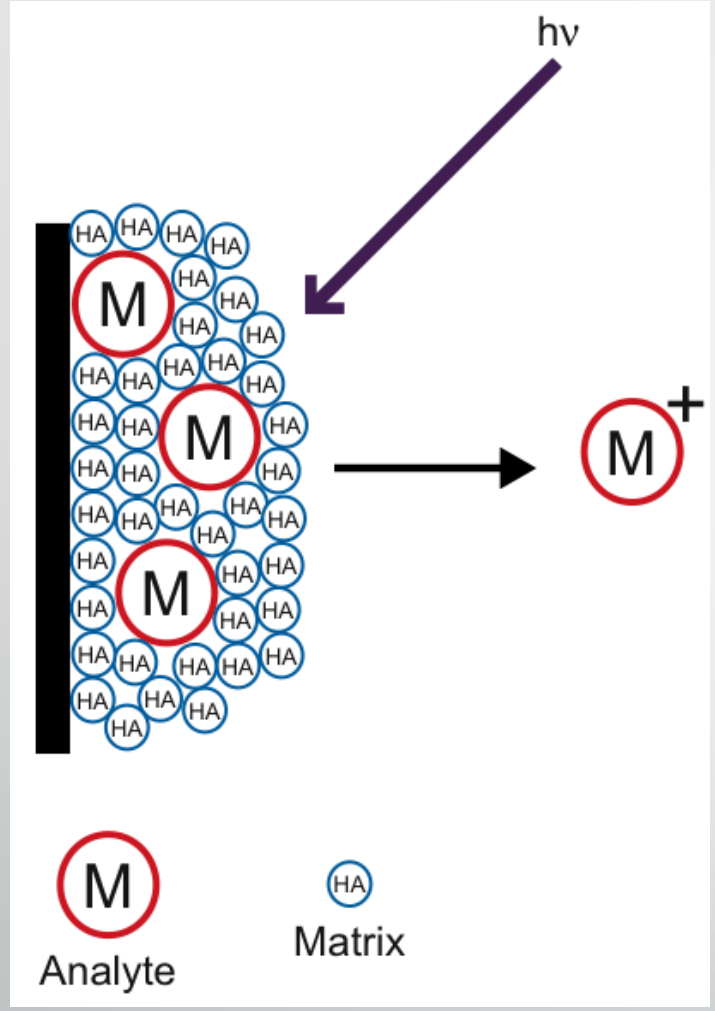
Structural Biology Core

NIDA-IRP, NIH

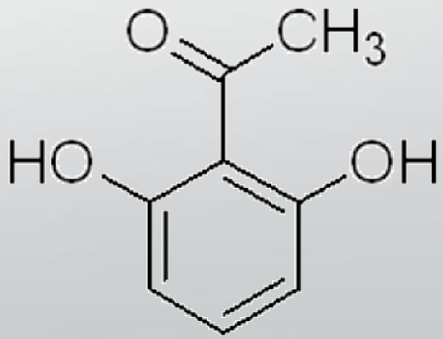



National Institute on Drug Abuse

# Matrix-Assisted Laser Desorption/Ionization (MALDI)




- Matrix
  - Weak organic acid
  - Absorbs laser wavelength
- Low detection limit
- High tolerance for impurities
- Surface analysis (tissue)

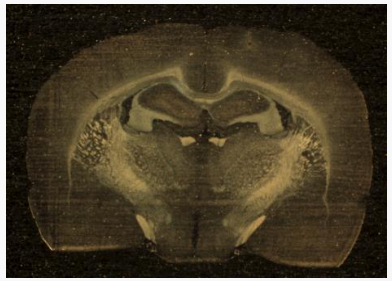



2,6-Dihydroxyacetophenone
(DHA or DHAP)

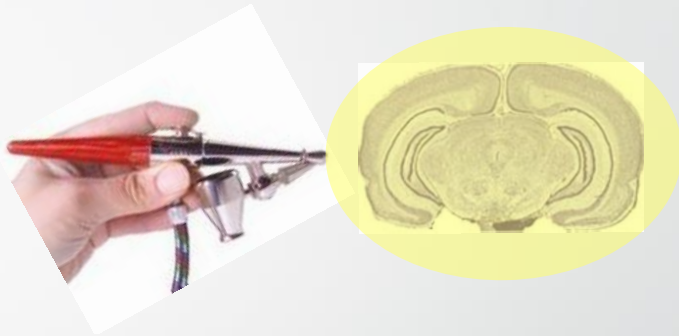
# Work Flow for MALDI MSI

1. Harvest Organ/Storage

2. Tissue Sectioning

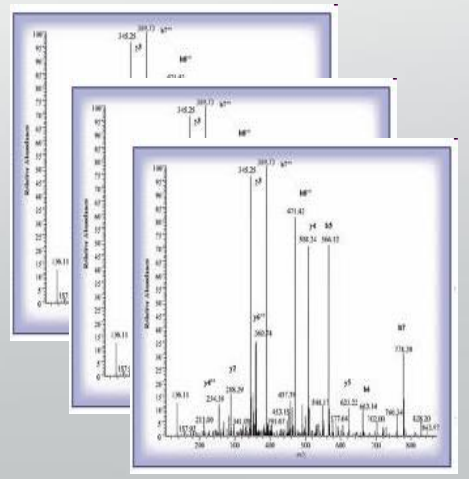



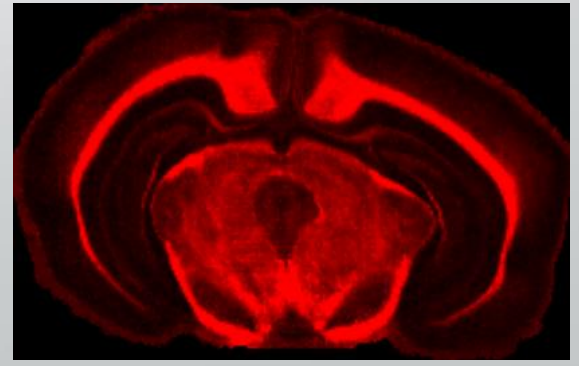
3. Matrix Deposition



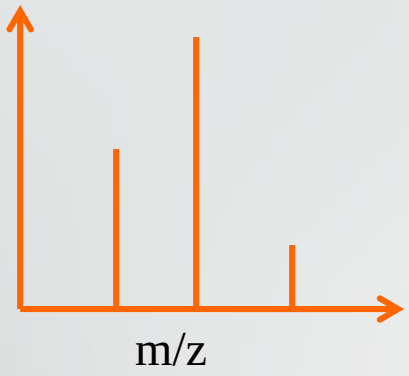
4. Mass Analysis

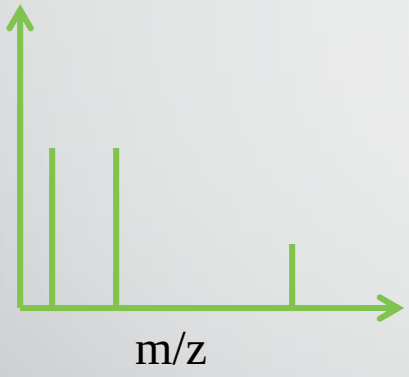

5. Data Processing/Image Construction

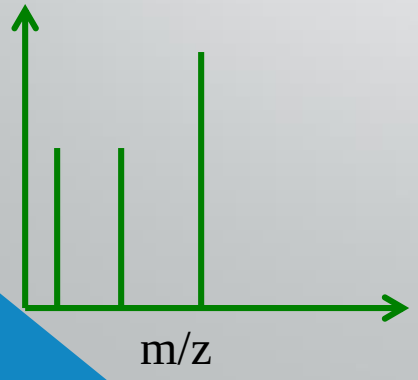


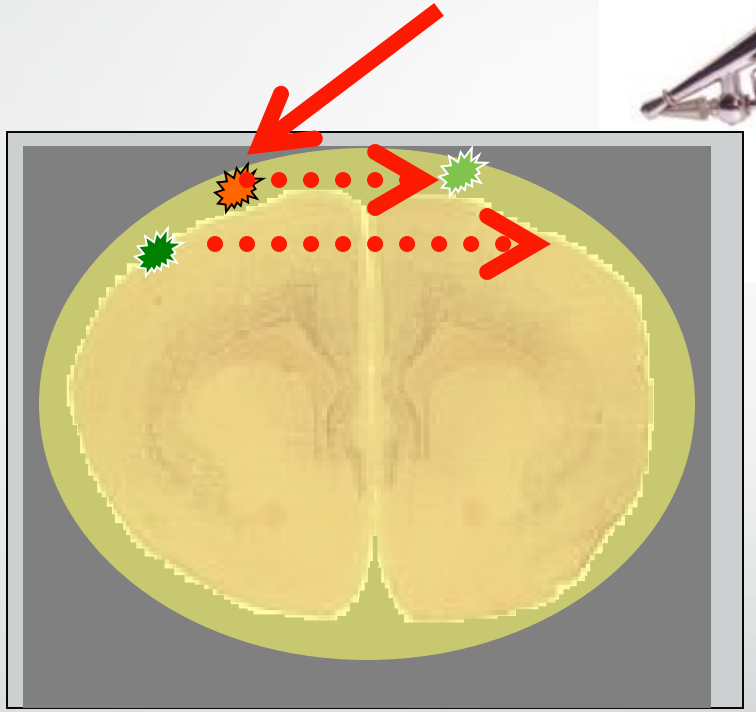


# Matrix deposition with an airbrush
## Data acquisition

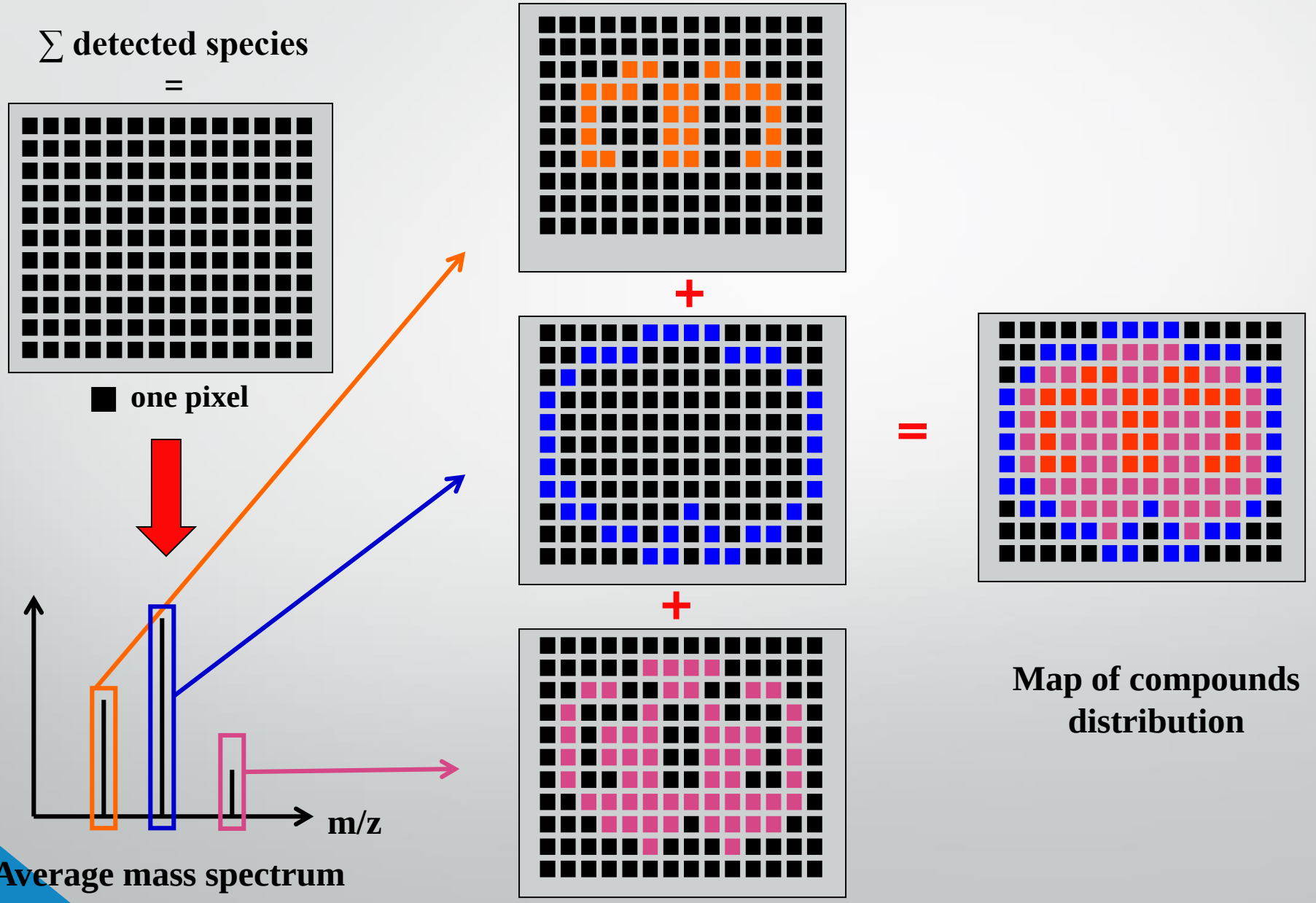


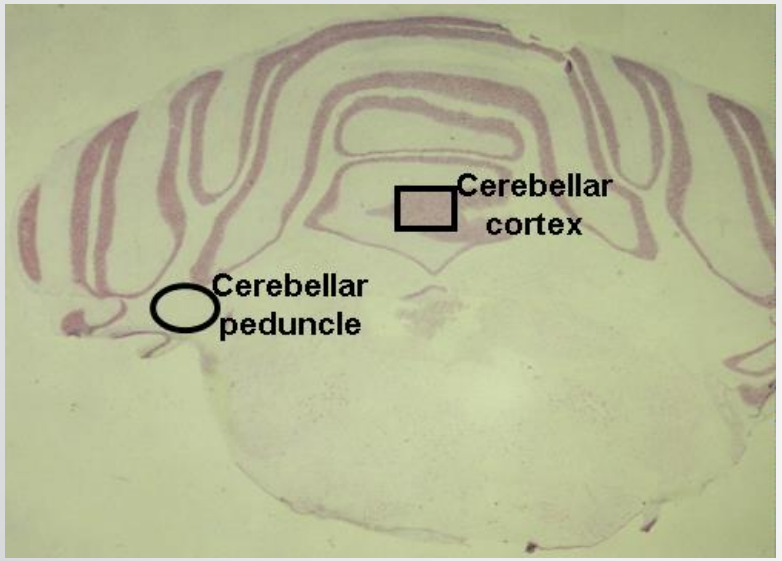




# Mass Spectrometry Imaging Data processing

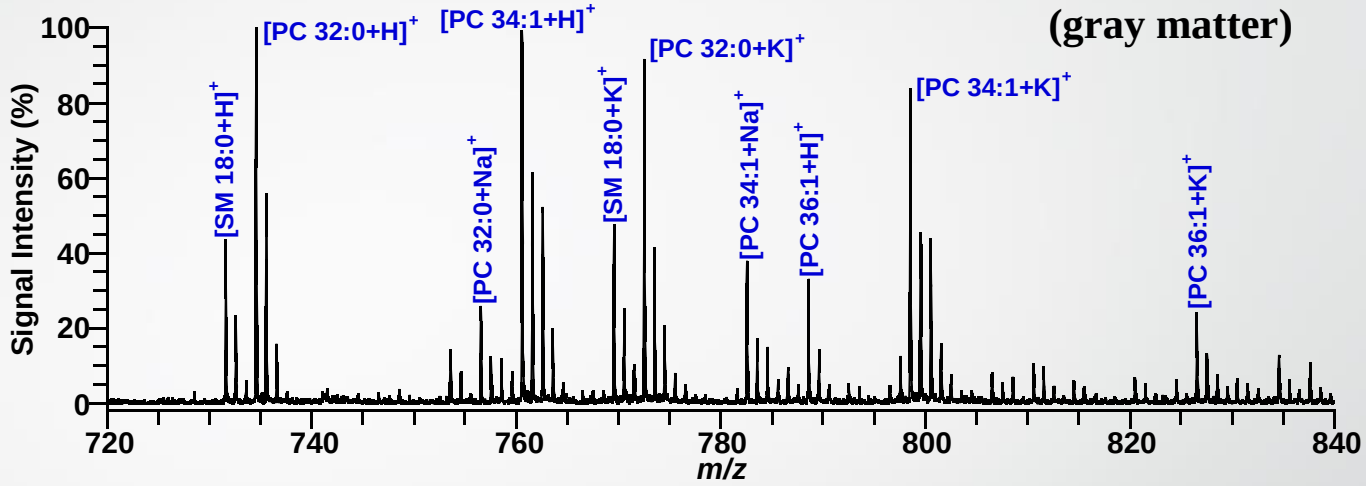


# Direct Profiling of Lipids in Brain Tissue by MALDI-MS

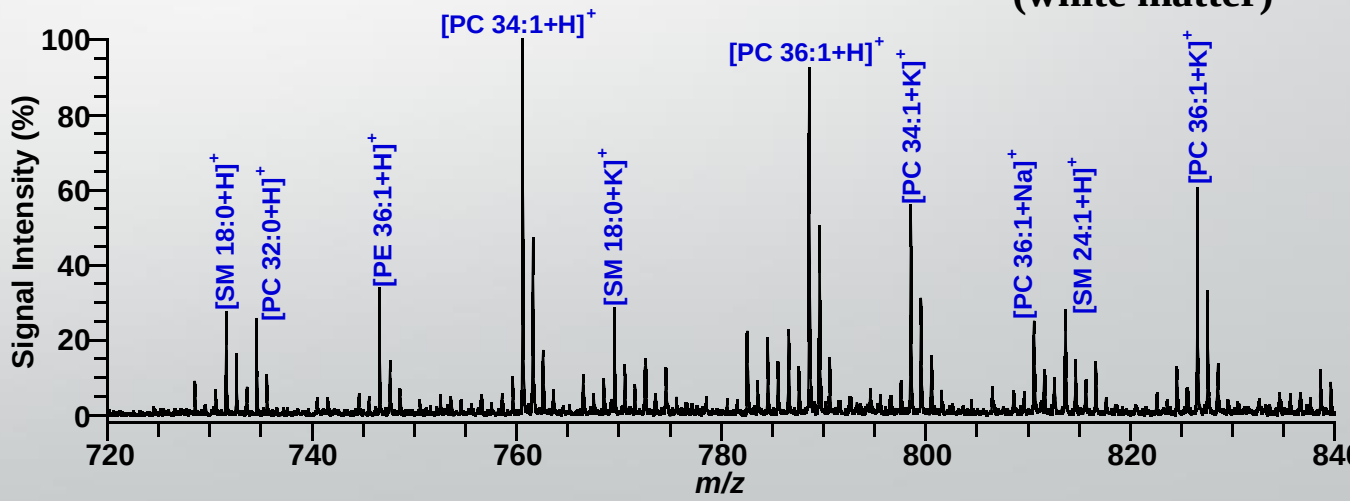



- Rat cerebellum tissue sections (14µm)
- Voyager DE-Pro in Positive Ion Mode
- DHA matrix excellent matrix for all major lipid classes in positive/negative ion mode
- DHA matrix sublimes under vacuum

**Cerebellar cortex (gray matter)**



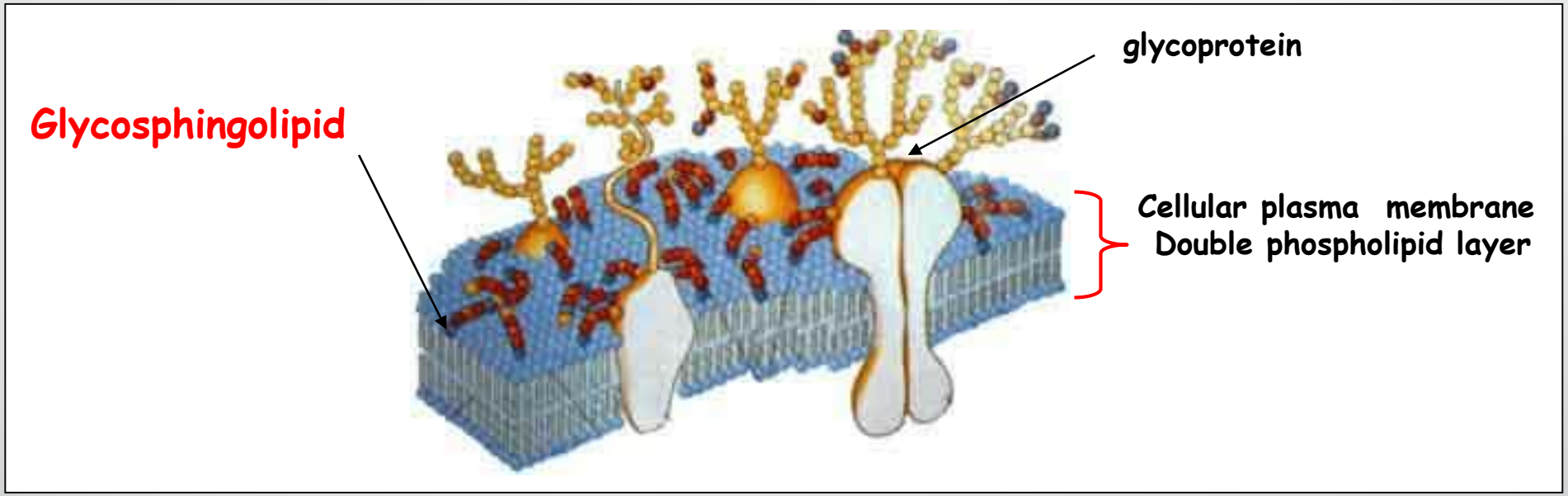

**Cerebellar peduncle (white matter)**




Jackson et al. *Anal. Chem* **77**, 4523-4527 (2005).

# Gangliosides: general aspects

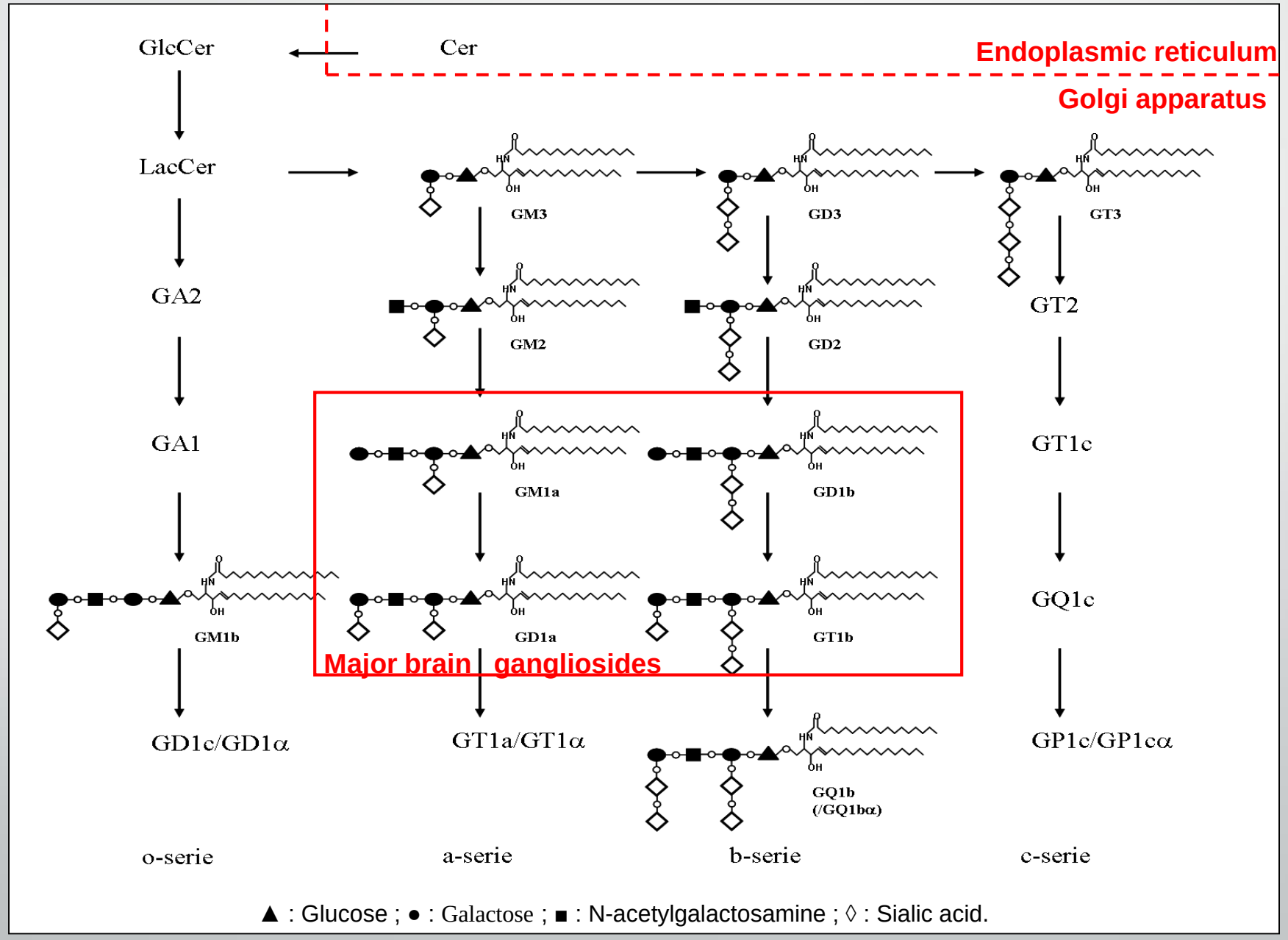
• Glycosphingolipids are natural substances which are found in cell membranes of all living organisms.




• They are particularly abundant in the central nervous system.

• They are present on membranes of neurons and glial cells (oligodendrocytes, myelin, astrocytes and microglia).

# Ganglioside species in mammalian brain : biosynthesis



# Ganglioside species and related diseases

**Neurological diseases:**

- **GM1 Gangliosidosis: (betagalactosidase)**
- **GM2 Gangliosidosis: (betahexosaminidase)**
- **Sialidosis : GM3 (sialidase)**

**catabolic enzyme defect results in accumulation of gangliosides in lysosomes.**
(Kolter and Sandhoff 2006)

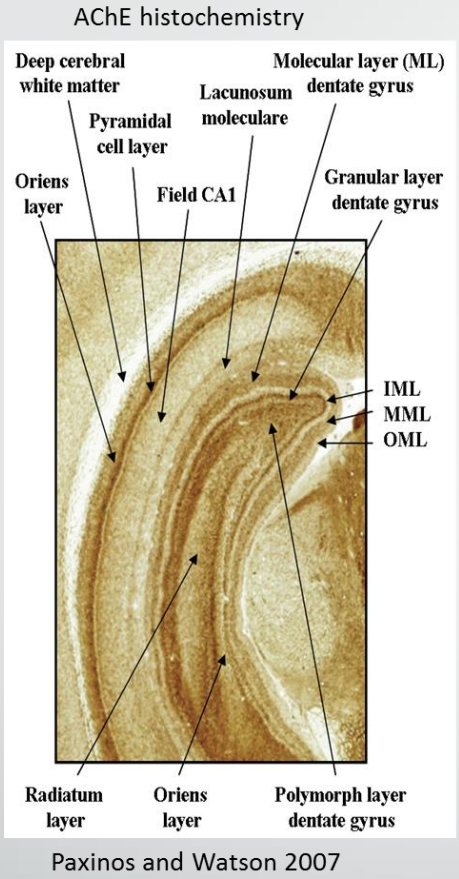
- **Alzheimer disease : Ganglioside binding with β-amyloid peptides. (Ariga et al. 2008)**
- **Guillain-Barre syndrome : autoimmune neurological disease involving gangliosides (Miller-Fisher syndrome). (Kaida et al. 2009)**

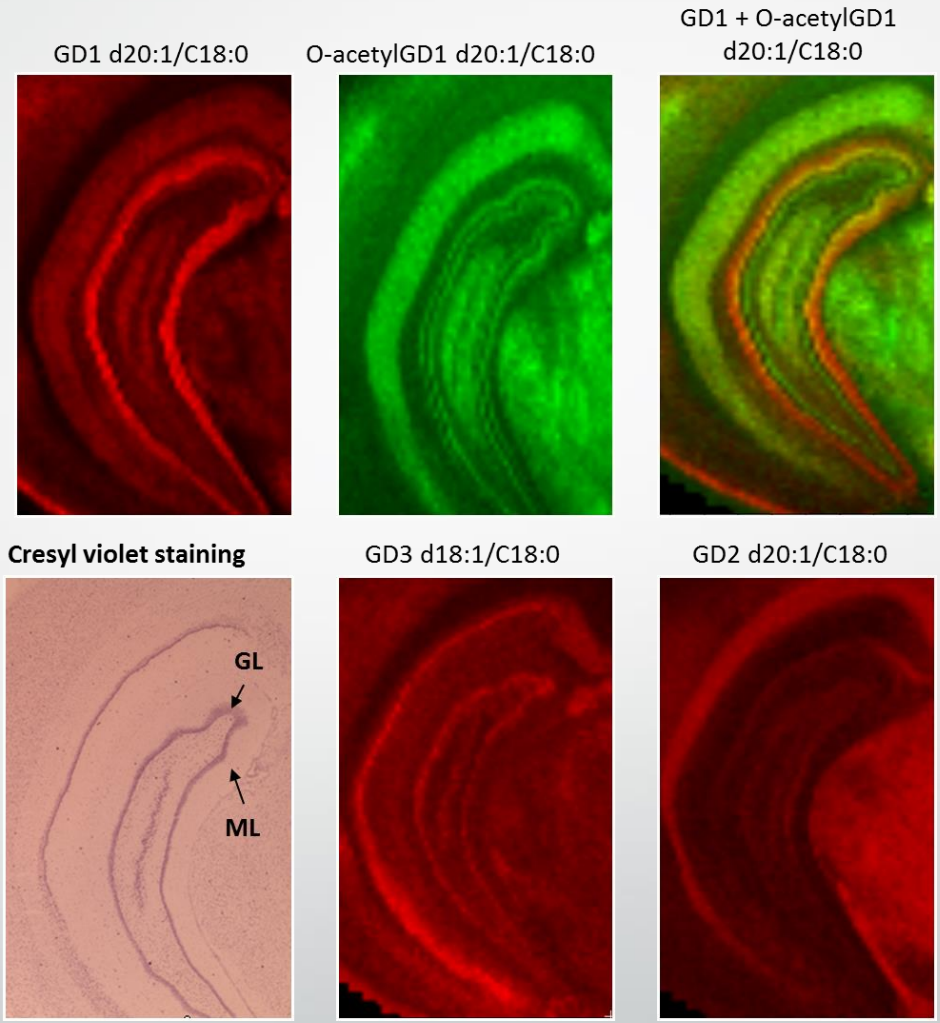
**Cancer: aberrant glycosylation in cancer cells (Hakomori 1996)**

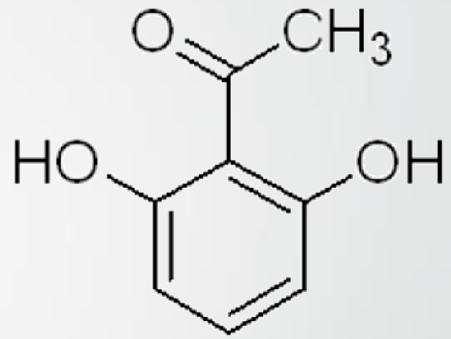
- **GD3, GM2 and GD2 are highly expressed in human melanomas.**
- **GD2 abundant on neuroblastoma cells.**

# DHA 2,6-Dihydroxyacetophenone

(mass resolution 1000, step 80 um)



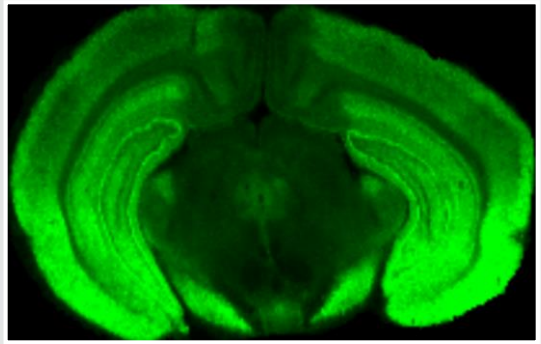



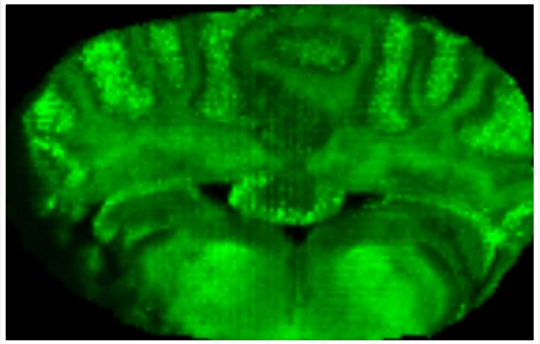

**Colsch B. *et al* . Molecular Microscopy of Brain Gangliosides: Illustrating their Distribution in Hippocampal Cell Layers. ACS Chem. Neurosci., 2 (4), pp 213–222(2011)**

# In-source fragmentation of Gangliosides and image processing

**GM1 d18:1/C18:0 (*m/z* 1544.6) + GM1 Fragment from GD1**

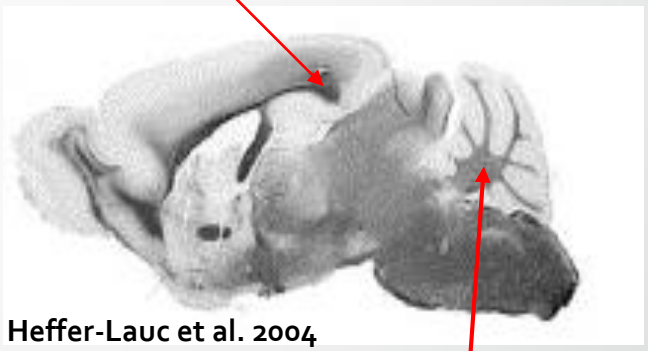


**GM1 d18:1/C18:0 (*m/z* 1544.6) + GM1 Fragment from GD1**



**GM1 antibody results**

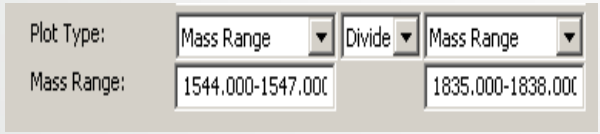
Corpus Callosum



Heffer-Lauc et al. 2004

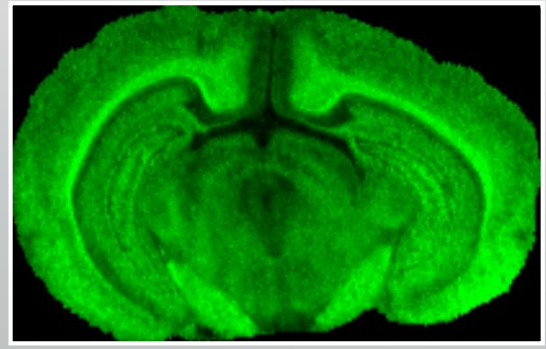
Cerebellar White matter

**GM1/GD1 : signal normalization**

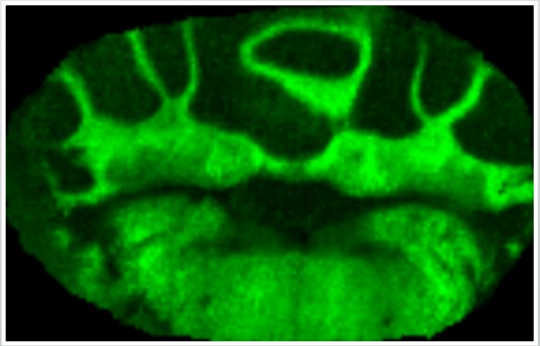


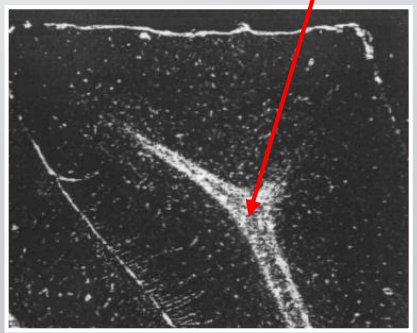

GM1 GD1

**Native GM1 d18:1/C18:0 (*m/z* 1544.6)**



**Native GM1 d18:1/C18:0 (*m/z* 1544.6)**



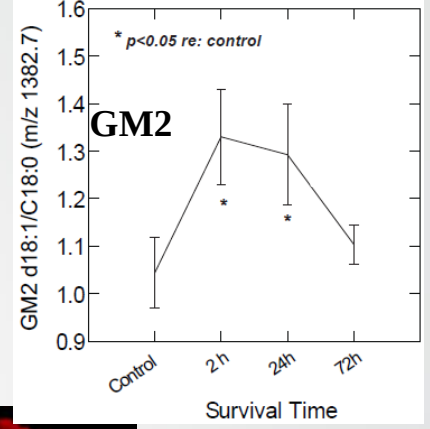


Kotani et al. 1993

**In-source fragmentation of sialic acids can be minimized by signal normalization**

# Blast Induced TBI

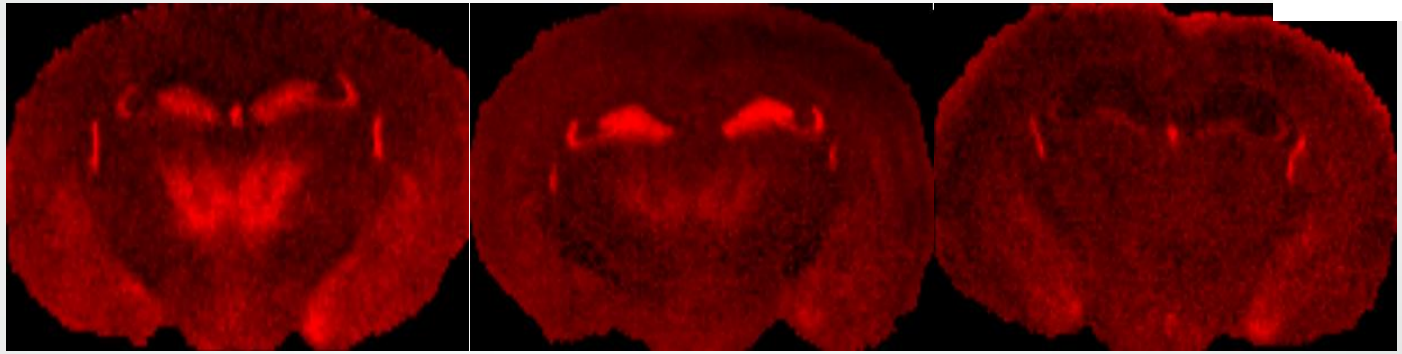
(mass resolution 1000, step 80 um)



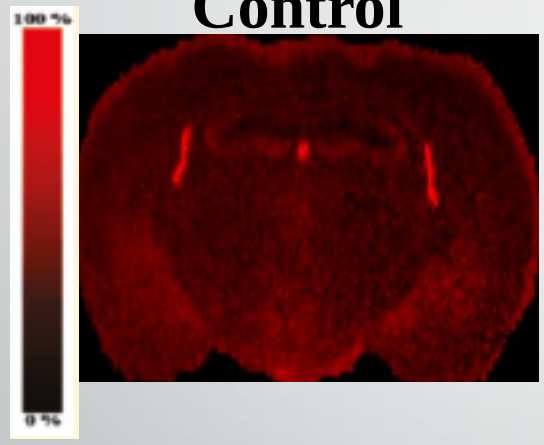

**GM2 ganglioside**

**5.5 PSI and**
**4 M Blast Radius**

**Post Blast** **2Hr** **24 Hr** **72 Hr**

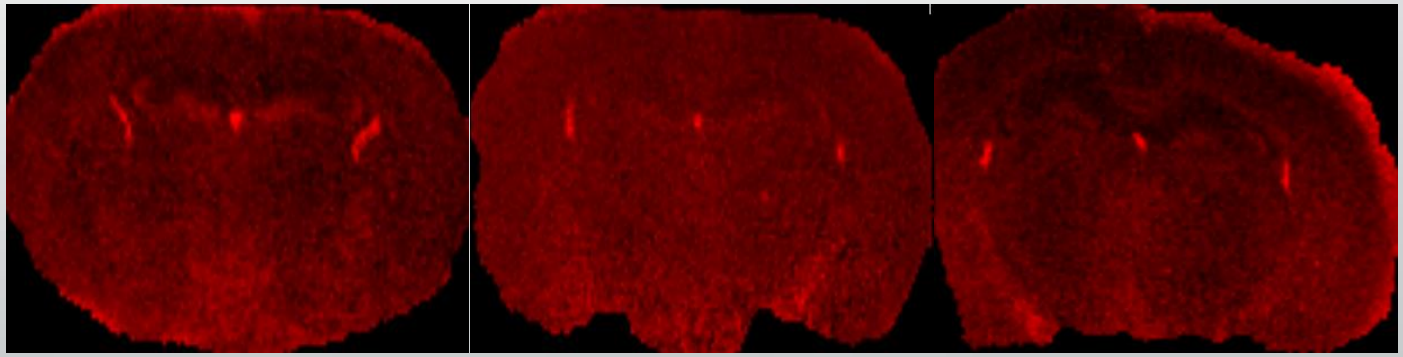


**Control**



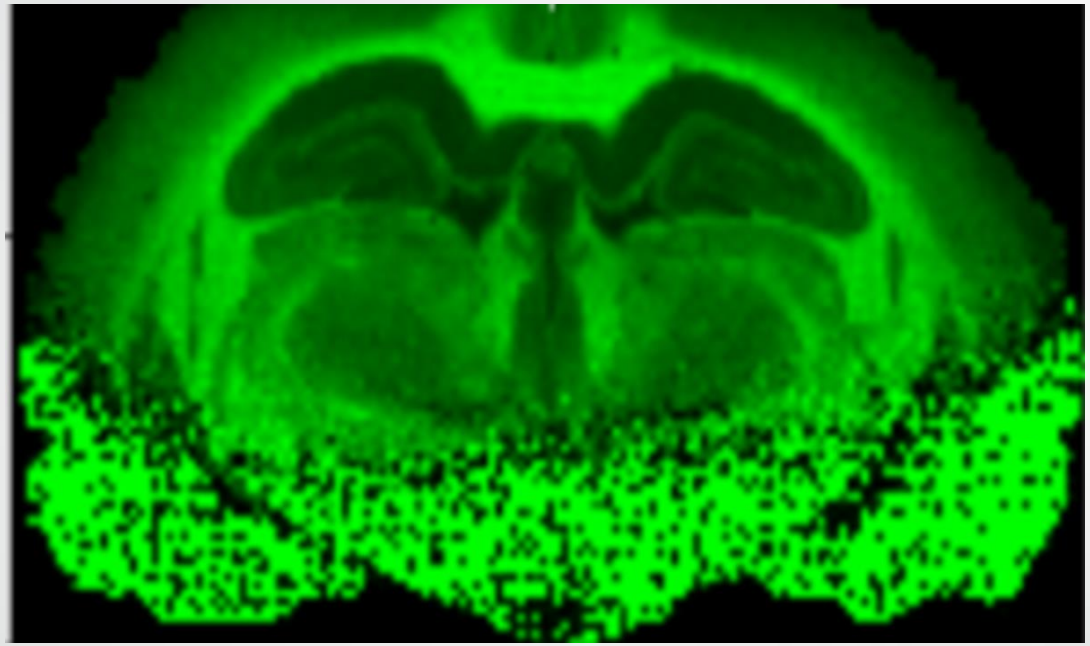

**2.5PSI and**
**7 M Blast Radius**

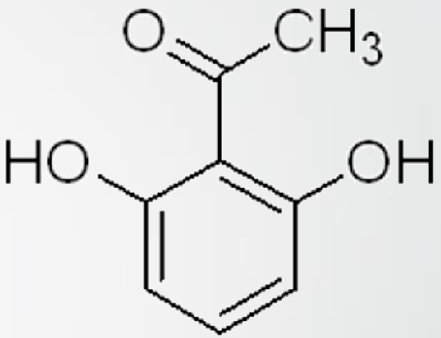
**Post Blast** **2 Hr** **24 Hr** **72 Hr**



**Woods AS *et al.* Gangliosides and Ceramides Change in a Mouse Model of Blast Induced Traumatic Brain Injury. *ACS Neuroscience* 4, 594-600 (2013)".**

# High Mass Resolution with DHA

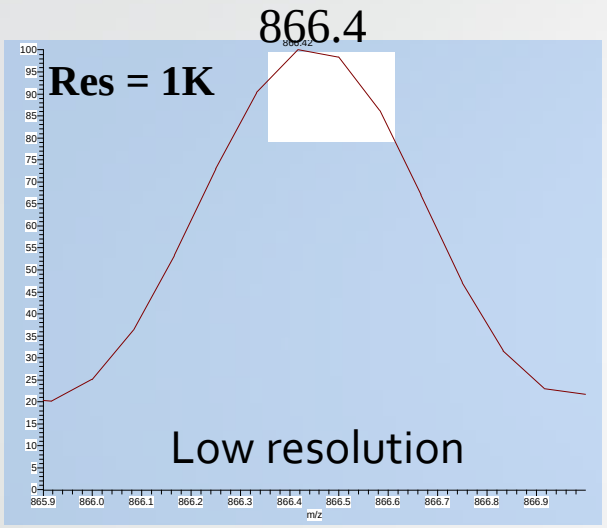


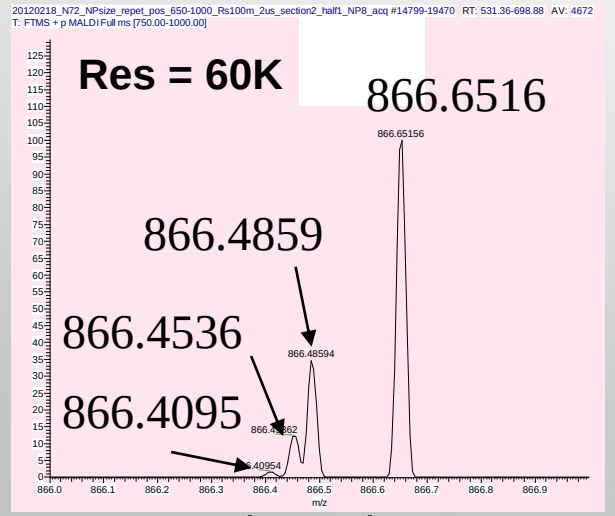



First try for a longer analysis in vacuum

Major drawback stability under vacuum only for few hours

# High Mass Resolution Improves Assignment in Imaging




High resolution

| | |
|---|---|
| PS36:4-2H+2Na+K | 866.43208 |
| PS38:7-H+Na+K | 866.43449 |
| PE40a:7-H+2K | 866.44989 |
| PS36:1-H+2K | 866.47103 |
| ST C18:1or18:0/d18:1or18:2-H+Na+K | 866.48249 |
| ST C20:4/d18:1+K | 866.48489 |
| ST C18:1(OH)or18:0(OH)/d18:1or18:2-H+2Na | 866.50347 |
| ST C20:4(OH)/d18:1+Na | 866.50587 |
| ST C21:0(OH)/d18:1+H | 866.60218 |
| GalCer C24:0(OH)/d18:1+K | 866.64819 |
| SM 20:0-2H+AgKNa-N(CH3)3 | 866.39903 |
| GalCer C16:0/d18:1-2H+AgKNa | 866.40728 |
| GalCer C16:0(OH)/d18:1-2H+Ag+2Na | 866.42826 |
| PC 34a:1+Ag | 866.48235 |

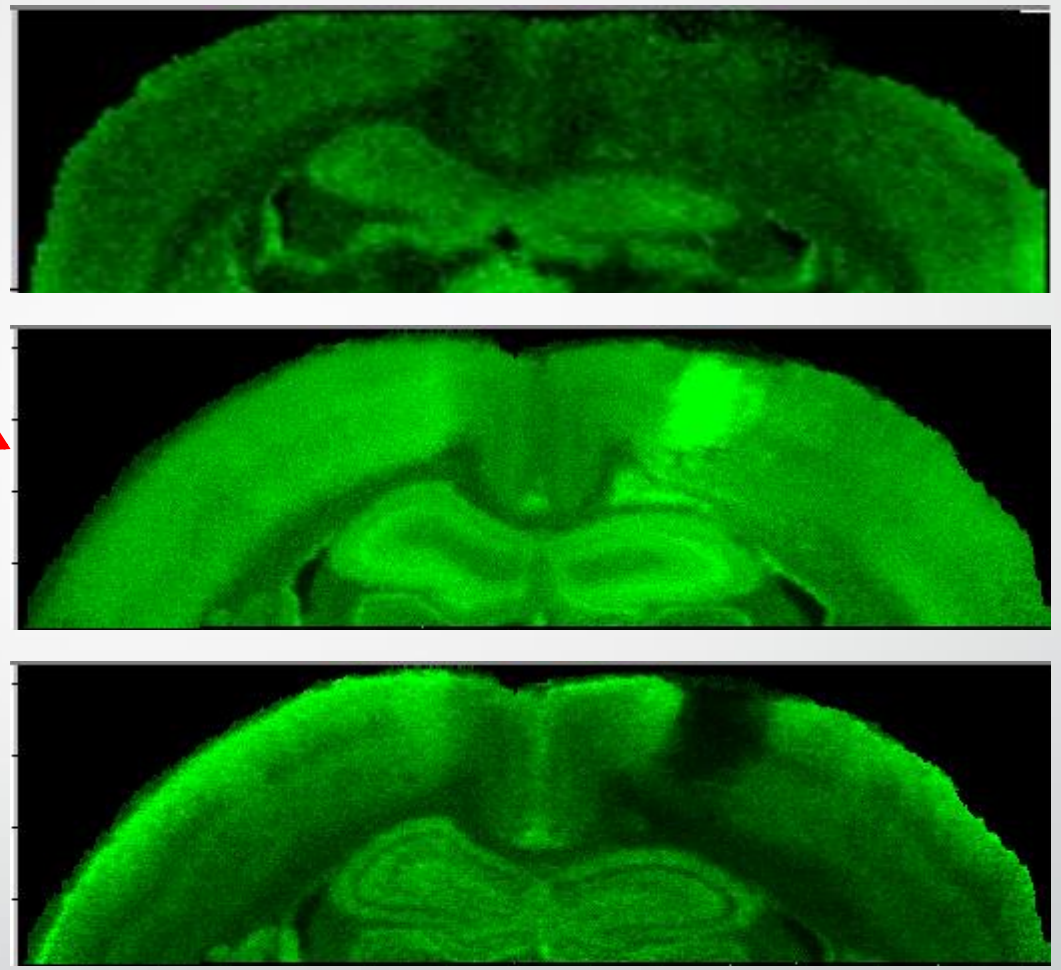
| | |
|---|---|
| GalCer C16:0/d18:1-2H+AgKNa | 866.40728 |
| PE40a:7-H+2K | 866.44989 |
| PC 34a:1+Ag | 866.48235 |
| GalCer C24:0(OH)/d18:1+K | 866.64819 |

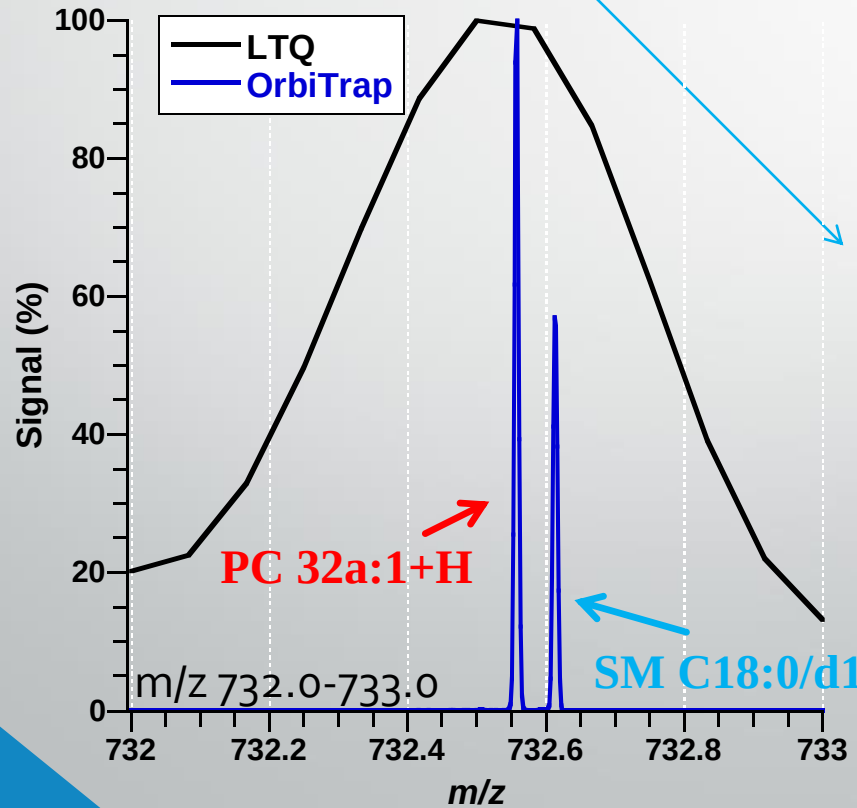
# High Mass Resolution Provides a Clearer View in Imaging

**LTQ**

**PC 32a:1+H**

**SM C18:0/d18:1 (C13) +H**



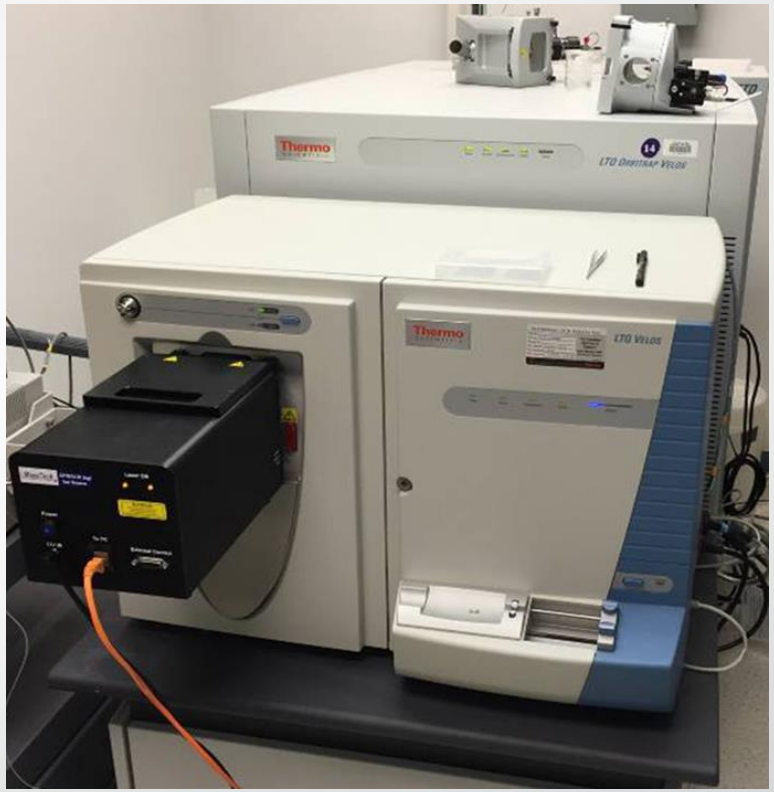


# MALDI vs AP-MALDI

Under vacuum ($10^{-2}$ torr)

- Reproducible
- More sensitive
- Soft ionization

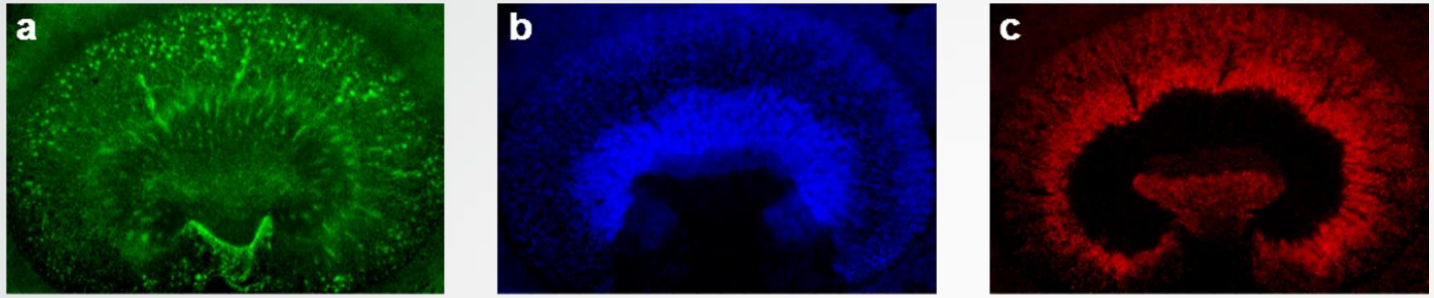
Atmospheric pressure (760 torr)




- Modular source
- Easy to use
- Softer ionization
- But need to realign laser

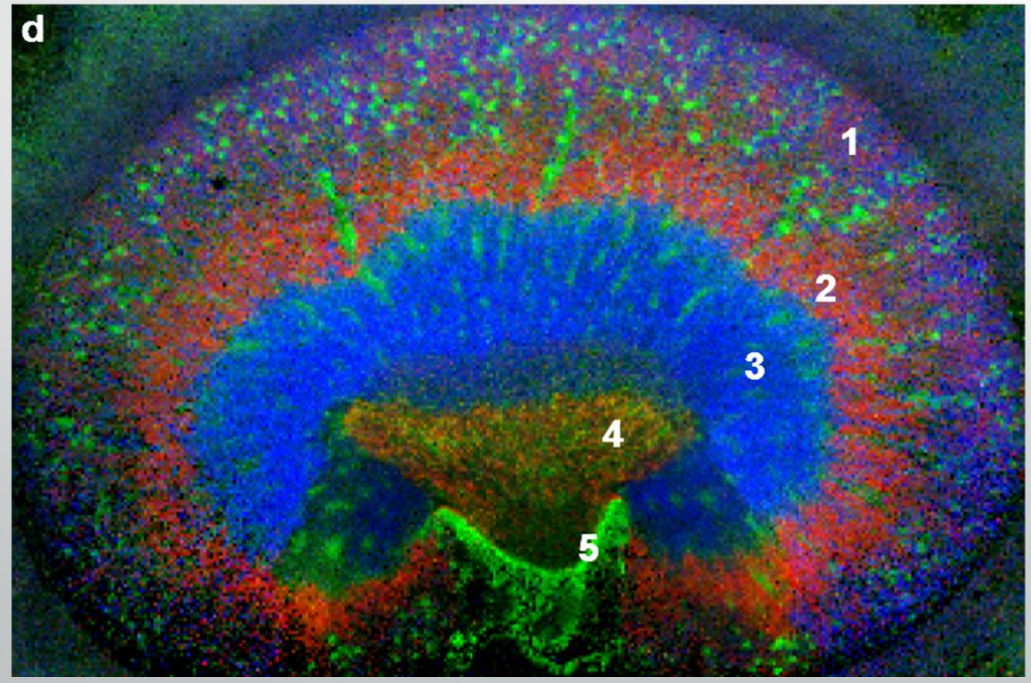
# High Mass Resolution with DHA

**(mass resolution 60 000 , step 60 um), AP-MALDI source on Orbitrap Velos**




SM d18:1/18:0+H, 731.606 | PE 38a:5+H, 766.538 | SM d18:1/26:0, 843.731

**Rat Kidney**




1. Cortex
2. Outer Medulla
3. Inner Medulla
4. Pelvis
5. Hilum

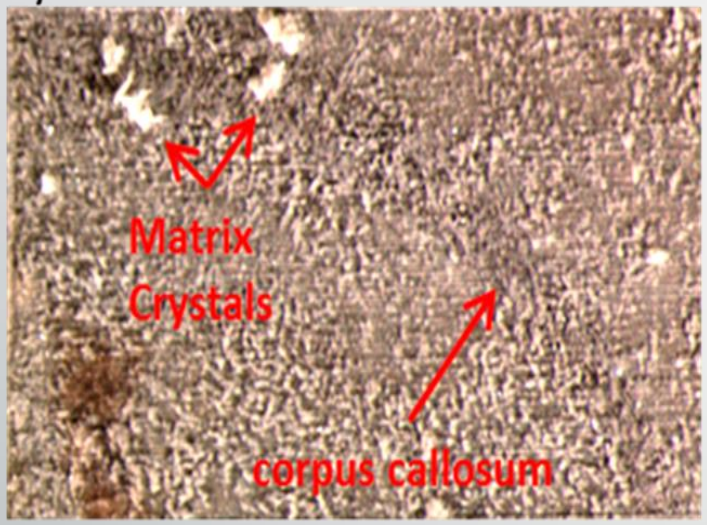
**The total run time for this analysis was approximately 40 hours**

# High Spatial Resolution with DHA

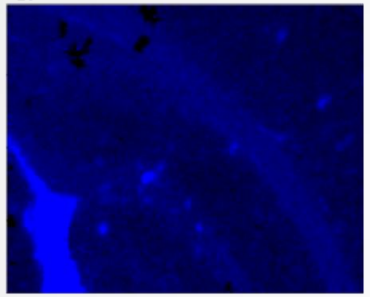
(mass resolution 60 000 , step 10 um)

**Rat coronal brain section**

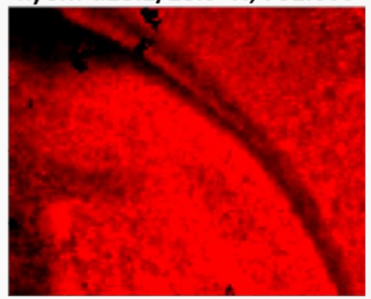
a)



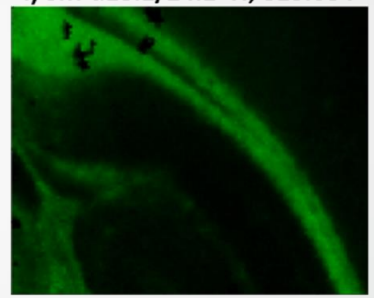

g) SM d18:1/16:0+H, 703.575



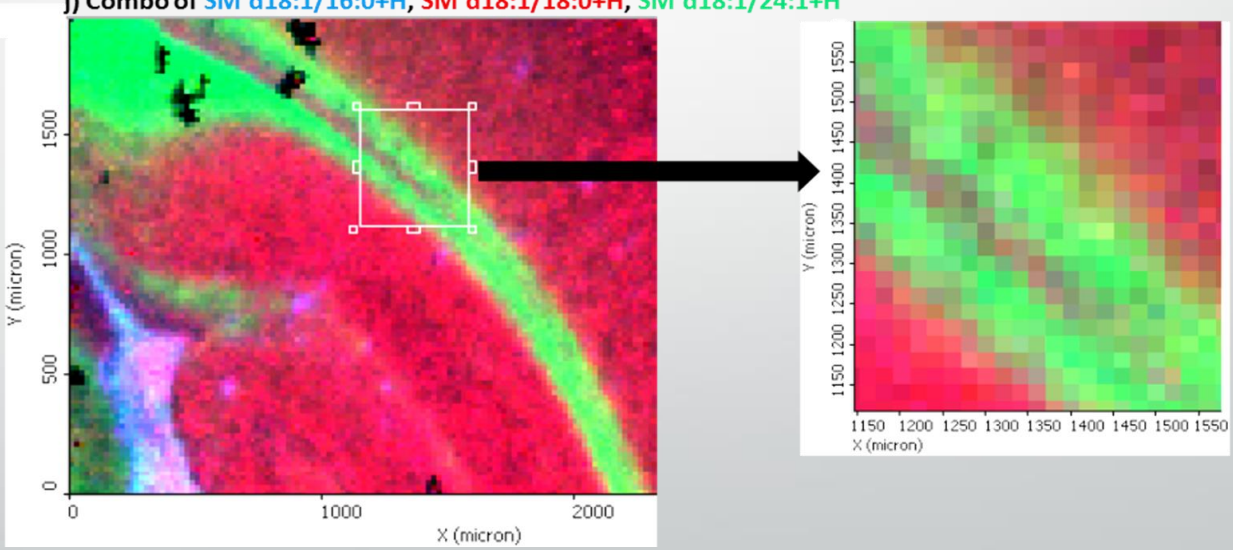
h) SM d18:1/18:0+H, 731.606



i) SM d18:1/24:1+H, 813.684

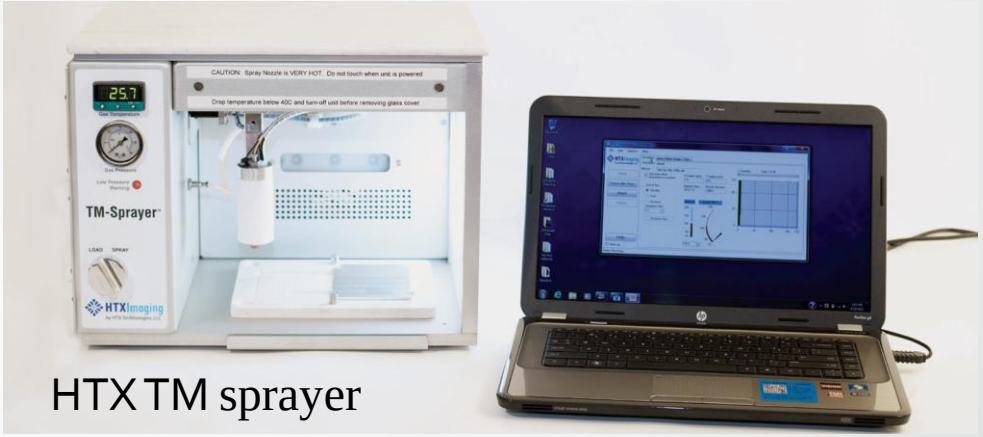


j) Combo of SM d18:1/16:0+H, SM d18:1/18:0+H, SM d18:1/24:1+H



# Automatic Matrix Sprayer

- Better sample to sample reproducibility
- Control flow rate, spray nozzle rate and temperature
- Applicable to wide range of biomolecules.

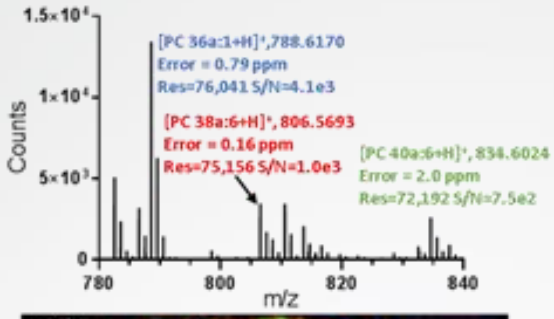


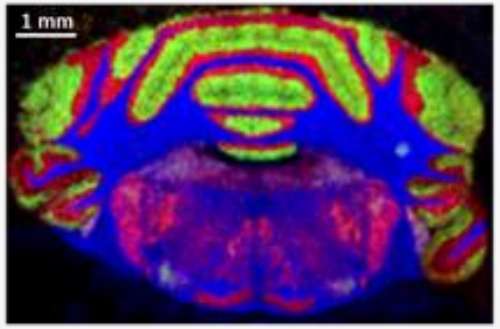
HTX TM sprayer

To control the amount of matrix coated onto the tissue sections we used an automatic sprayer as a better way to compare AP (760 torr) with IP ($10^{-2}$ Torr)

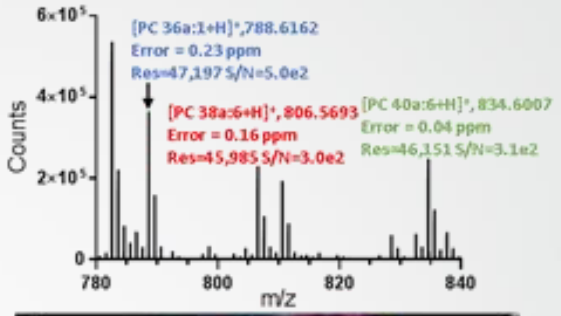
| Matrix Layers | Matrix Density ($mg/mm^2$) |
|---|---|
| 4 | 0.0013 |
| 20 | 0.0067 |
| 50 | 0.0167 |
| 100 | 0.0333 |

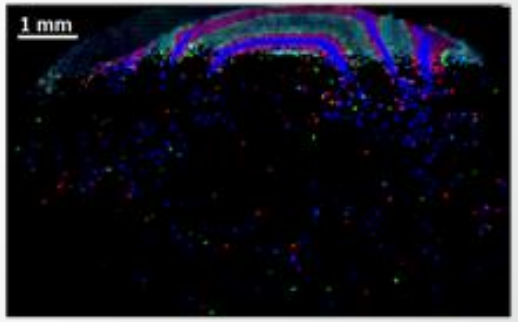
AP-MALDI. 4 matrix layers



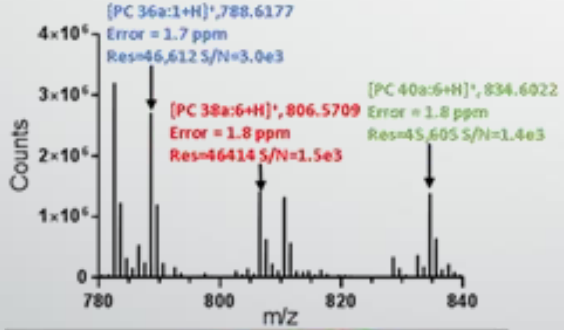



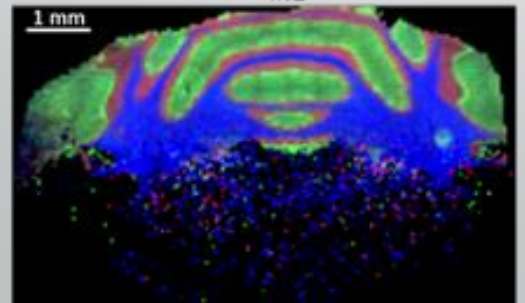

IP-MALDI. 4 matrix layers



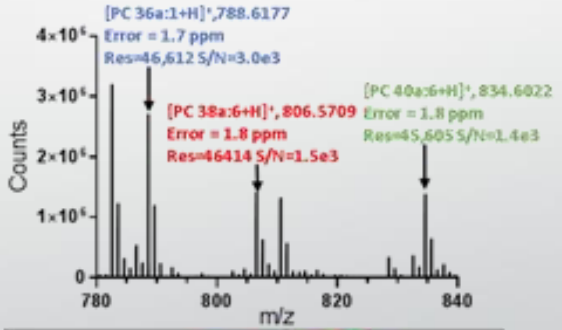



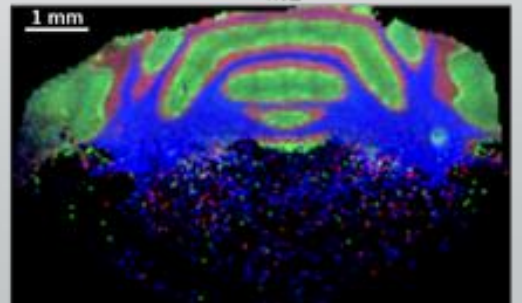

IP-MALDI. 20 matrix layers



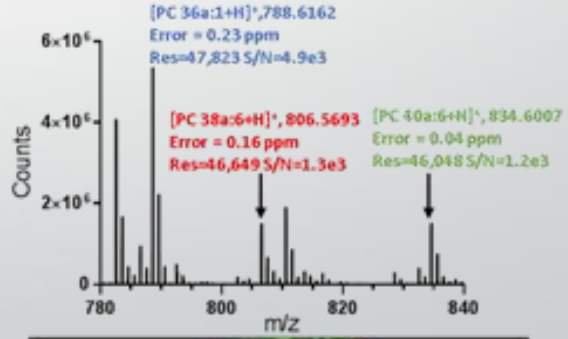



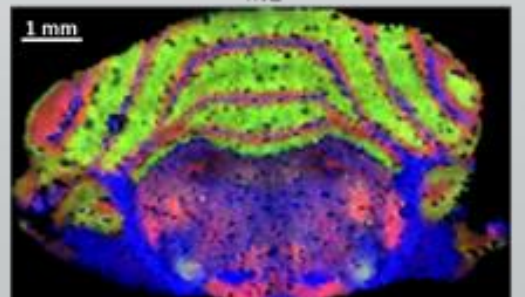

IP-MALDI. 50 matrix layers




IP-MALDI. 100 matrix layers



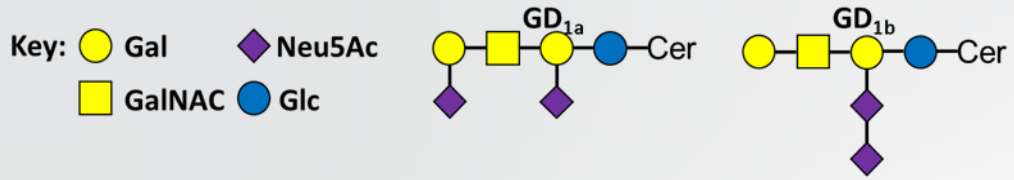



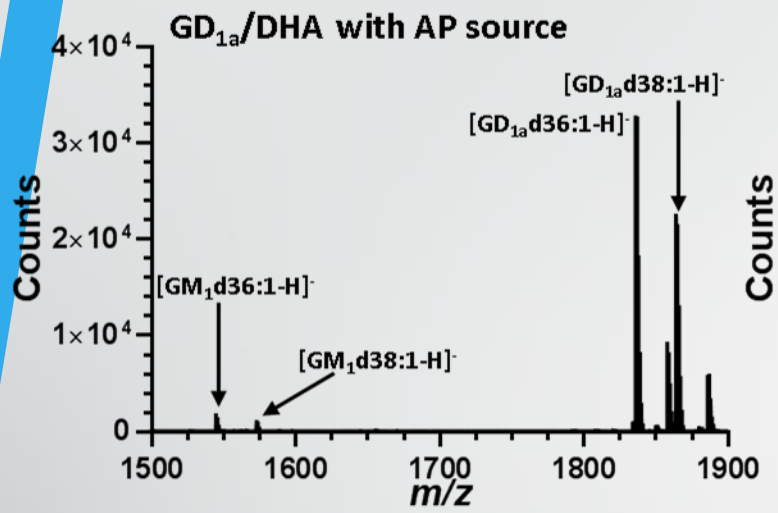
# Gangliosides and MALDI mass spectrometry

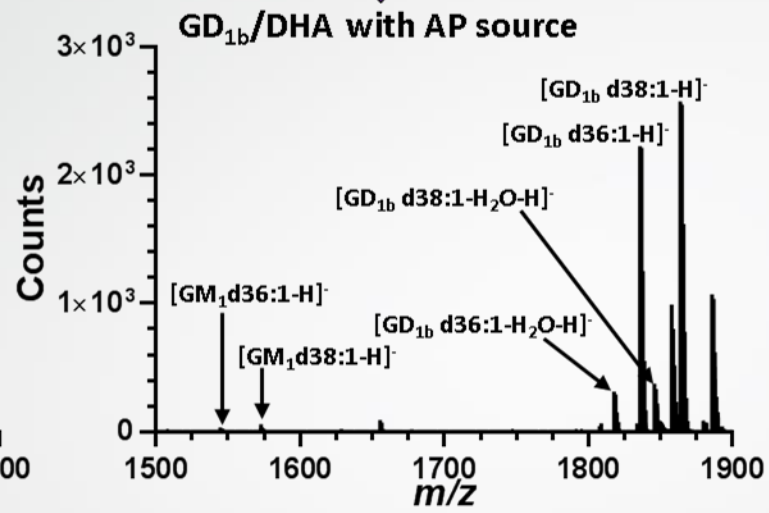
**MALDI matrices:**
**DHB, CHCA (Sugiyama *et al* 1997)**
**Others matrices were tested (Harvey 1999, Ivleva *et al.* 2004)**
**DHA (Jackson *et al.* 2005)**

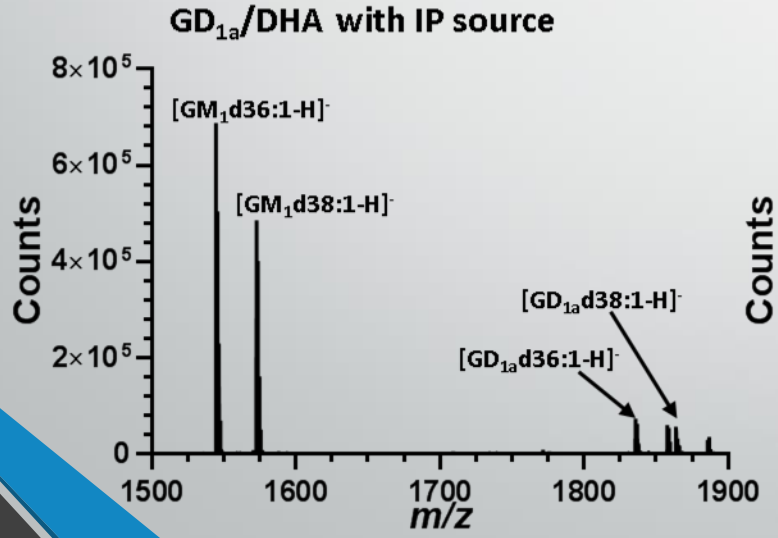
**MALDI Imaging:**
**DHB/TFA (Sugiura et al. 2008)**
**Ionic Liquid Matrix ImCHCA (Chan et al. 2009)**
**DHA sat/ammonium sulfate/HFBA (Colsch and Woods 2010)**
**9-AA (Post *et al*. 2017)**
**DAN (Whitehead *et al*. 2017)**

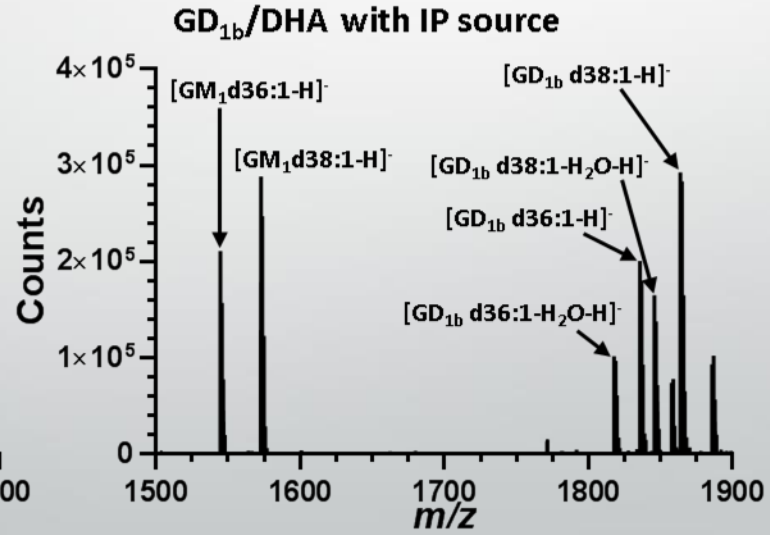
***In-source* Fragmentation of sialic acid moiety (Costello *et al*. 1994, Harvey 1999) is reduced using higher pressure ion source (O'Connor and Costello 2001,Ivleva *et al*. 2004,2005)**



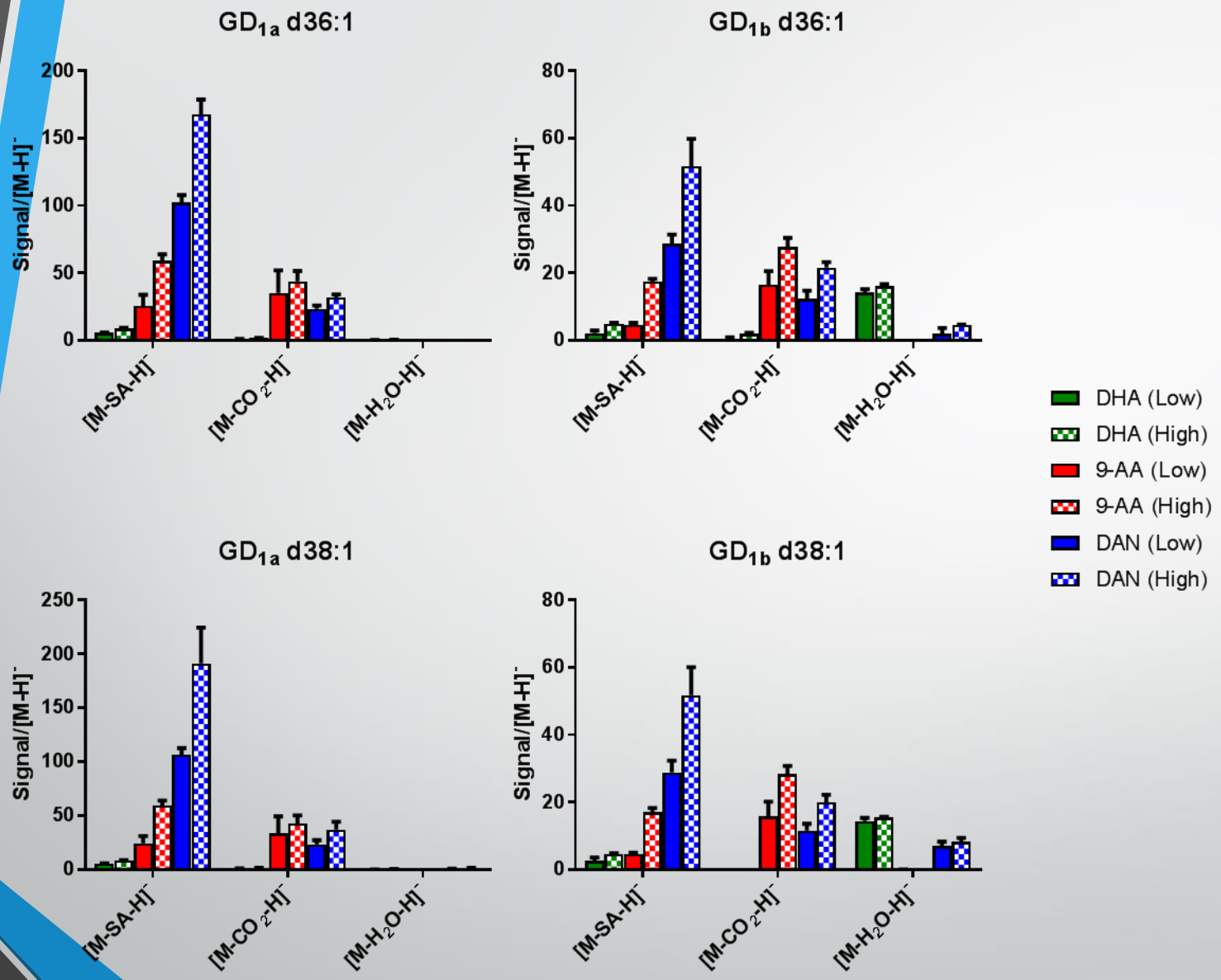



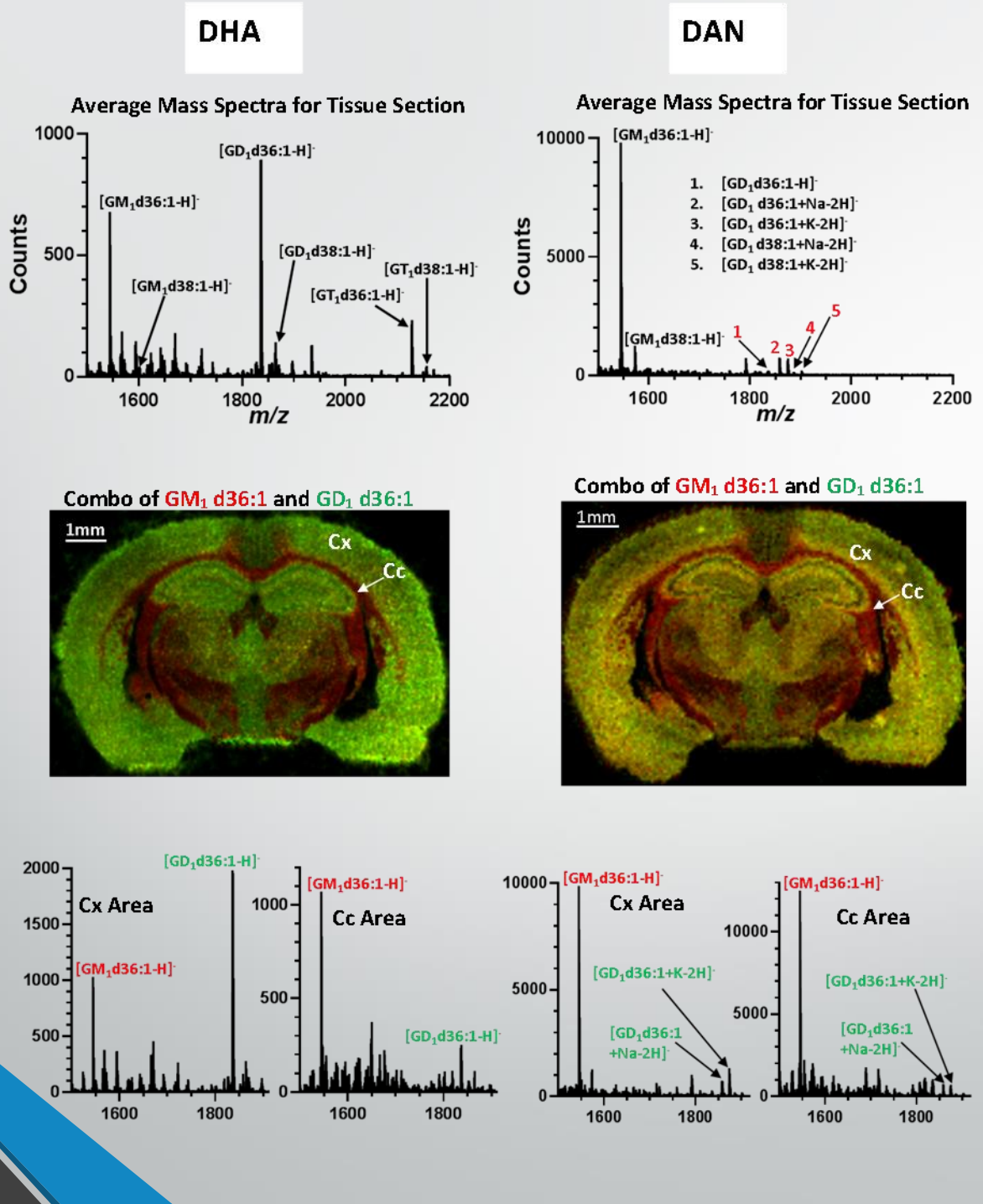




- **GD1a and GD1b stds with DHA**
- **Negative Ion Mode**
- **Laser fluence just above threshold**
- **AP has very little *in source* fragmentation compared to IP**
- **GD1b has a fragment peak due to the loss of water that is very weak/not detected in GD1a**

# DHA Matrix versus DAN & 9-AA Matrix for Ganglioside Analysis




- GD1a and GD1b stds
- AP MALDI Source on Q-Exactive
- Negative Ion Mode
- High (twofold) and Low (~15%) Laser fluence above threshold
- DHA produces less *in source* fragmentation compared to DAN and 9-AA




- **MSI of serial mouse coronal sections with DHA and DAN**
- **AP MALDI Source on Q-Exactive**
- **Negative Ion Mode**
- **DHA shows correct distribution of GM1 and GD1in white and gray matter**
- **DAN shows incorrect distribution with GM1 being the dominant specie in both white and grey matter**
- **Using AP-MALDI source with DHA matrix, no need to use software to normalize for *in source* fragmentation.**

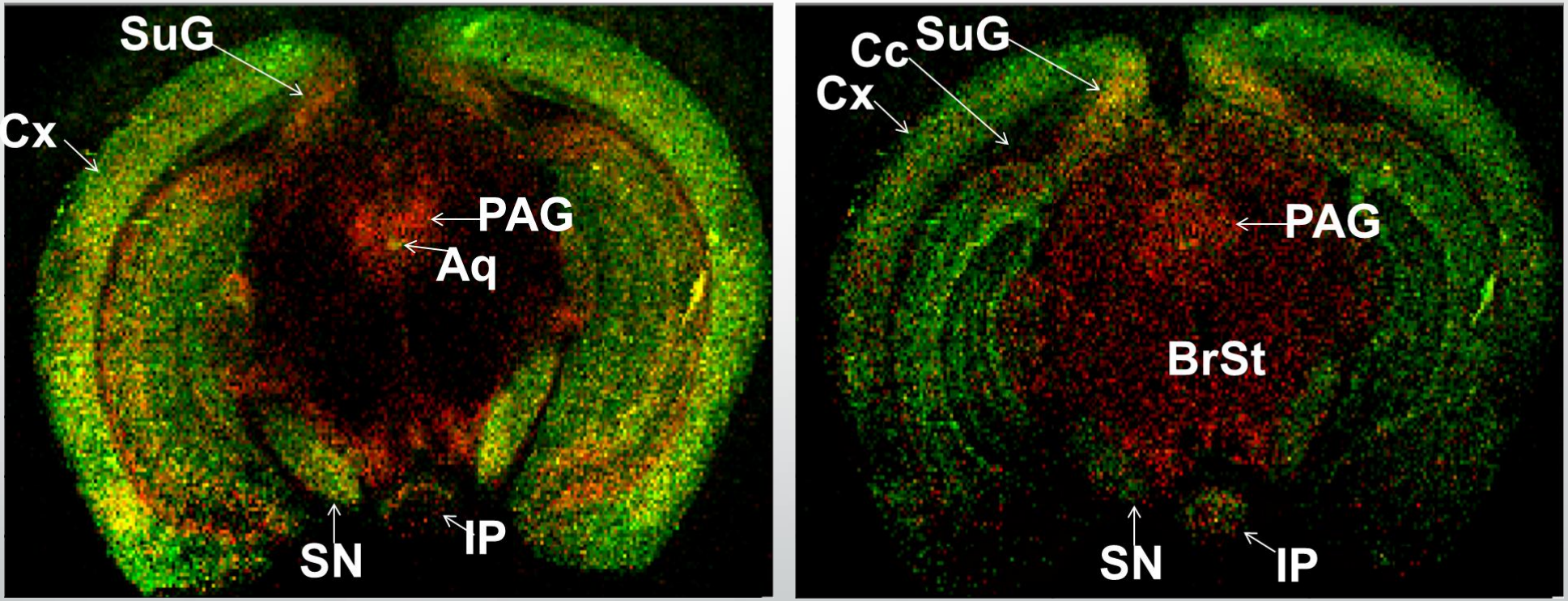
# Metastable fragments allow for the mapping of GD1a and GD1b isomers

**GD1 d18:1/18:0-$H_2O$-H and GD1 d18:1/18:0-H**

**GD1 d20:1/18:0-$H_2O$-H and GD1 d20:1/18:0-H**

$GD_1b$

$GD_1a$




Aq = Aqueduct; BrSt = Brain Stem; Cc = Corpus Callosum; Cx = Cortex; IP = InterPedencular nucleus; PAG = PeriAqueductal Grey; SN = Subtentia Nigra; SuG = Superficial Grey layer of the superior colliculus.

## Conclusion

**Coupling of an AP-MALDI source with an Orbitrap mass spectrometer**

1. **Eliminated the problem of DHA matrix sublimation that prevented completing long MSI runs in vacuum, intermediate, and low-pressure MALDI sources.**

2. **Significantly reduced the amount of fragmentation observed for gangliosides when compared to other matrices used with any MALDI source and to DHA used with an intermediate pressure source.**

3. **It was also demonstrated that by increasing the metastable fragments, it was possible to distinguish GD1a and GD1b isomers. The mapping of the metastable fragment of the loss of water from mostly GD1b ganglioside permitted the comparison of its localization in the brain compared to the $[M–H]^-$ GD1 mass peak that consists mostly of GD1a isomers.**

# Acknowledgments



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# Thank you for your attention