APPLICATION NOTE

#35 MALDI Mass Spectrometry Imaging of lipids in positive and negative ion mode

The tissue images and MS data presented in this document were provided courtesy of Dr. Alain Brunelle of the French National Centre for Scientific Research (CNRS), Paris, France.

Application & Backtround

Mass spectrometry imaging (MSI) is the only analytical method that allows researchers to locate and identify various chemical compounds without selection *a priori* a chemical class or compound.[1,2] Since this method does not require targeting compounds prior to the analysis, it makes it possible to draw anatomical images of any ion detected in the mass spectra in one single experiment.

It is known that lipids are important cell signaling molecules, acting as neurotransmitters and precursors in the regulation of various cellular functions.[3] Here, we demonstrate lipid imaging in rat brain sections using two different matrices. α-cyano-4-hydroxycinamic acid (CHCA) is an excellent matrix for *in situ* analysis of lipids on tissue sections in the positive ion mode,[4] while in negative ion mode 9-aminoacridine (9-AA) leads to very good signal-to-noise ratio and contrast of the ion images, as well as enhanced capabilities of direct MS/MS measurements.[5]

Experimental

Sample Preparation

For imaging experiments, rat brains were immediately frozen on dry ice upon necropsy. The samples were then stored at -80°C until sectioning at 16 µm. Samples were thaw-mounted onto indium-tin oxide (ITO) microscope slides.

Matrix Application

Both CHCA and 9-AA were applied to tissue sections using the HTX TM-Sprayer. The unique capability of the HTX TM-Sprayer to spray matrices at different temperatures allowed for optimized protocols for each individual matrix to be used to obtain the best results. Specifically, as the use of 9-AA as an imaging matrix intrinsically leads to a better signal-to-noise ratio, a higher spray temperature, which results in a drier spray, could be used. Additionally, only one pass was needed for the coating of 9-AA to obtain sufficient signal, while two was needed for CHCA. The ability to easily change these parameters between spraying protocols not only helps optimize MSI results, but also saves researchers time in the lab. The spraying parameters used for each matrix are displayed in **Table 1**.

	Positive Mode	Negative Mode
Matrix	CHCHA	9-AA
Solvent	70% ACN, 30% H_2O, 0.1% TFA	70% EtOH; 30% H_2O
Matrix Concentration (mg/mL)	10	
Flow Rate (mL/min, FR)	0.24	
Spray Nozzle Velocity (mm/min, V)	1200	
Spray Nozzle Temperature (°C)	75°C	100°C
Track Spacing (mm, TS)	3	
Number of Passes (NP)	2	1
Nitrogen Pressure (psi)	10	
Spray Pattern	CC	
Drying Time (sec)	30	
Nozzle Height (mm)	40	

Table 1. Spraying parameters for MALDI MSI in both positive and negative mode.

MALDI MSI

Spectra were collected across the entire tissue area using the UltrafleXtreme (Bruker Daltoniks, Bremen, Germany) with a tripled-frequency Nd/YAG pulsed laser (355 nm) operating at 2 kHz in reflector mode. The emitted ions were accelerated with a voltage of 20 kV and a delayed extraction time of 120 ns. Analog offset was 1.8 mV. Ten µm laser spot size was set for cerebellum imaging while 50 µm laser spot size was used for the whole sagittal brain section, with 500 laser shots per pixel for both positive and negative ion modes. Mass spectra were acquired over a mass range of *m/z* 140 to 1200 with a resolution of about 20,000 at *m/z* 885.6.

Data Processing

Data acquisition and preprocessing were performed using FlexControl 3.4 and FlexImaging 4.0 (Bruker Daltoniks, Bremen, Germany), respectively. All the spectra were normalized by total ion counts.

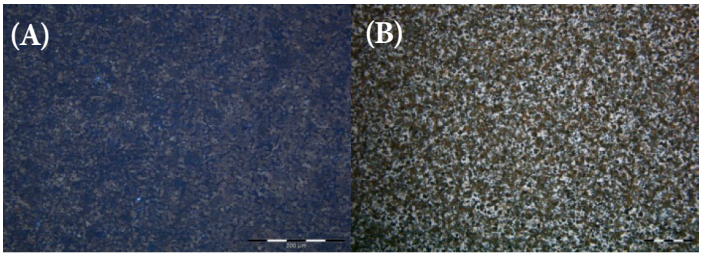


Figure 1. Crystals of (A) CHCA and (B) 9-AA on-tissue.

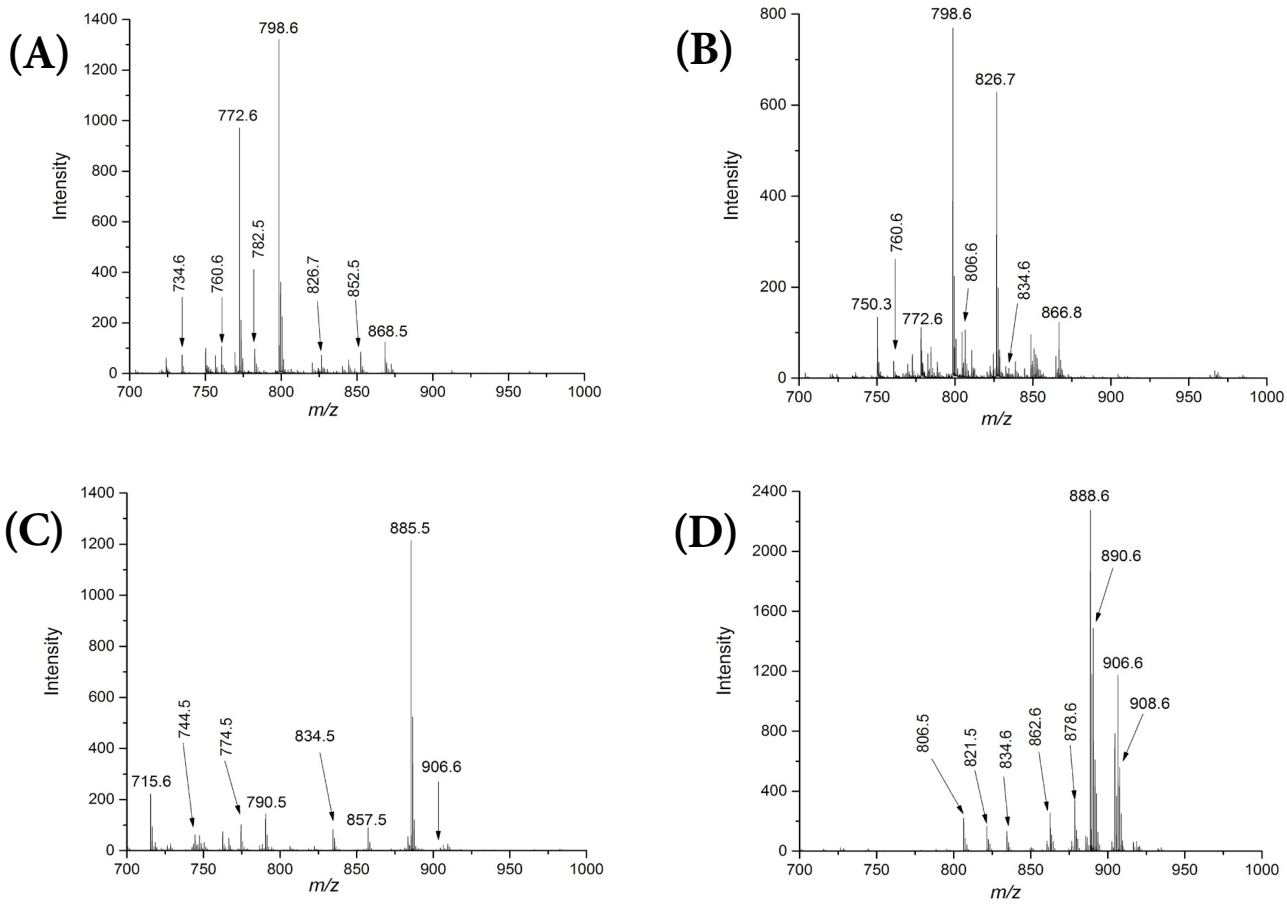


Figure 2. Representative mass spectra recorded on rat brain sections in (A) positive ion mode, CHCA matrix, cerebral cortex, (B) positive ion mode, CHCA matrix, corpus callosum (white matter), (C) negative ion mode, 9-AA matrix, corpus callosum (white matter), and (D) negative ion mode, 9-AA matrix, cortex. Many lipid peaks are visible in spectra.

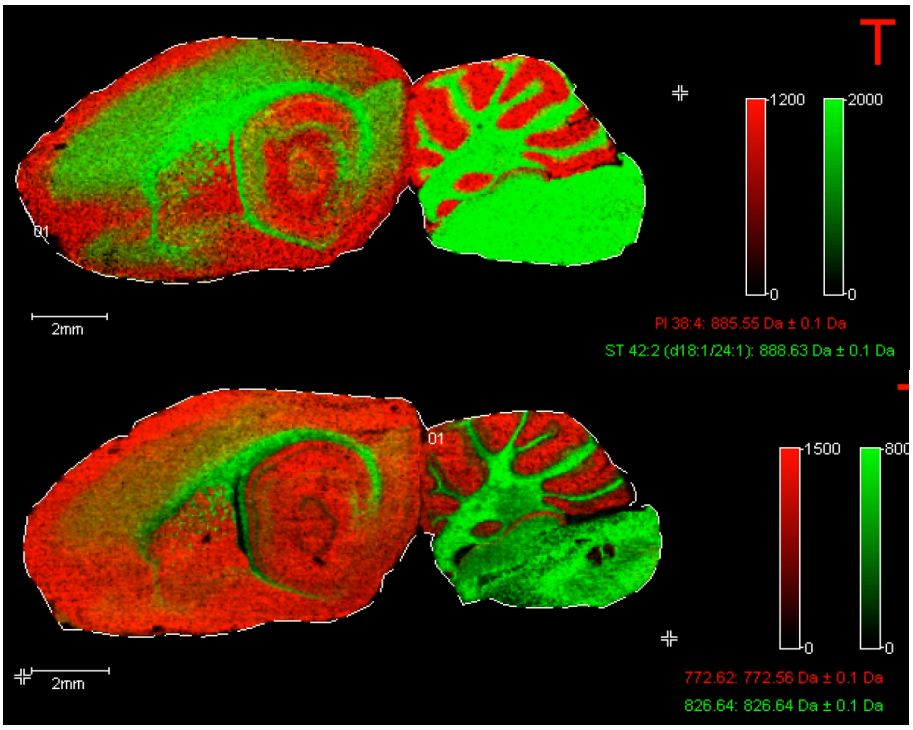


Figure 3. MALDI MSI image of rat brain recorded in negative ion mode (9-AA matrix). Two color overlay, Red *m/z* 885.55 ($[PI38:4-H]^-$) Green *m/z* 888.63 ($[ST42:2-H]^-$). Pixel size: 50 µM, acquisition time ~15 h. Scale bar represents 2 mm.

Figure 4. MALDI MSI image of rat brain recorded in positive ion mode (CHCA matrix). Two color overlay, Red *m/z* 772.7 ($[PC32:0+K]^+$); Green *m/z* 826.8 ($[PE40:0+Na]^+$). Pixel size: 50 µM, acquisition time ~15 h. Scale bar represents 2 mm.

Results & Conclusions

The HTX TM-Sprayer allows for easy preparation of serial sections with various matrices for imaging in positive and negative mode. The number of parameters that are able to be altered in the HTX TM-Sprayer protocols allows for simple optimization of spray methods for different matrices. The reproducibility of these protocols from lab to lab enables wide-spread sharing of sample preparation protocols for MALDI MSI, which is of critical importance as the science of MSI continues to grow.

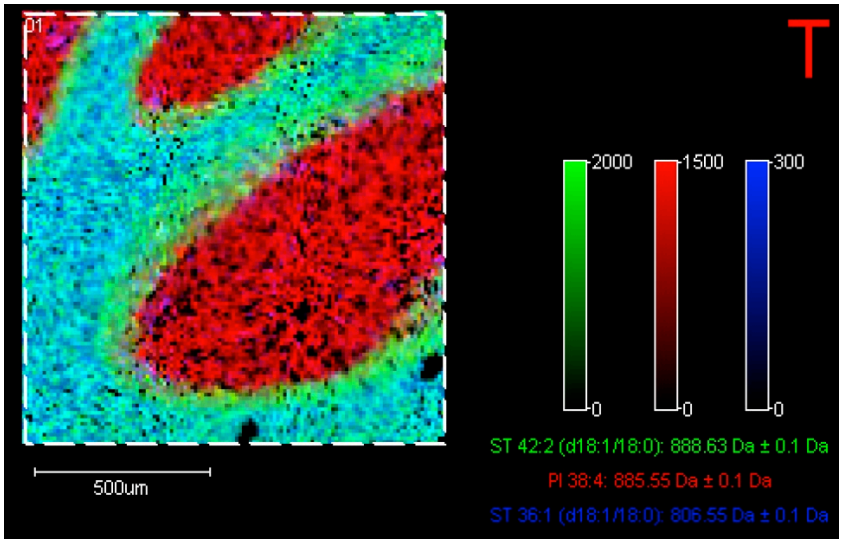


Figure 5. MALDI MSI image of rat cerebellum recorded in negative ion mode (9-AA matrix), Three color overlay, Red *m/z* 885.55 ([PI38:4-H]-); Green *m/z* 888.63)[ST42:2-H]-); Blue *m/z* 806.55 ([ST36:1-H]$^-$). Pixel size 10 µm, acquisition time ~5 h. Scale bar represents 500 µm.

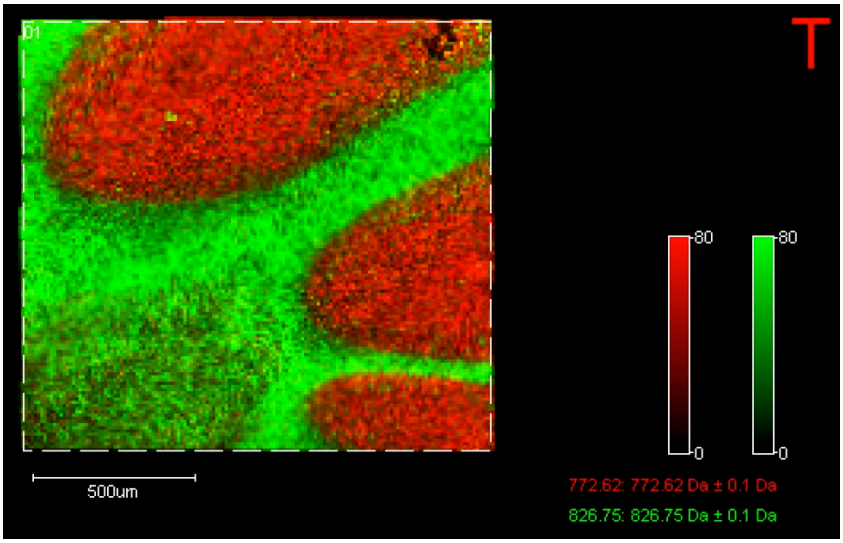


Figure 6. MALDI MSI image of rat cerebellum recorded in positive ion mode (CHCA matrix). Two color overlay, Red *m/z* 772.7 ([PC32:0+K]$^+$); Green *m/z* 826.8 ([PE40:0+Na]$^+$). Pixel size 10 µm, acquisition time ~5.5 h. Scale bar represents 500 µm.

Experimental Summary

Tissue type	Rat brain
Preservation	-80°C
Tissue cut	16 µm thickness
MALDI Plate	ITO-coated glass slides CHCA
Matrix deposition	(10 mg/mL in 70%acetonitrile (ACN, 30% H_2O, 0.1% trifluoroacetic acid (TFA) 9-AA 10 mg/mL in 70% ethanol (EtOH), 30% H_2O
MALDI Laser	355 nm
Acquisition mode	Reflector mode

Instrumentation and Supplies

Microtome	LEICA 3050- S
MALDI plate	ITO coated glass slides
Matrix	Sigma-Aldrich
Matrix	HTX TM-Sprayer™
Sprayer	Bruker Daltonics
MALDI MS	UltrafleXtreme
Imaging software	FlexImaging 4.0

References

[1]Pól J, Strohalm M, Havlíček V, Volny M: Molecular mass spectrometry imaging in biomedical and life science research. Histochem Cell Biol 2010, 134:423-443.

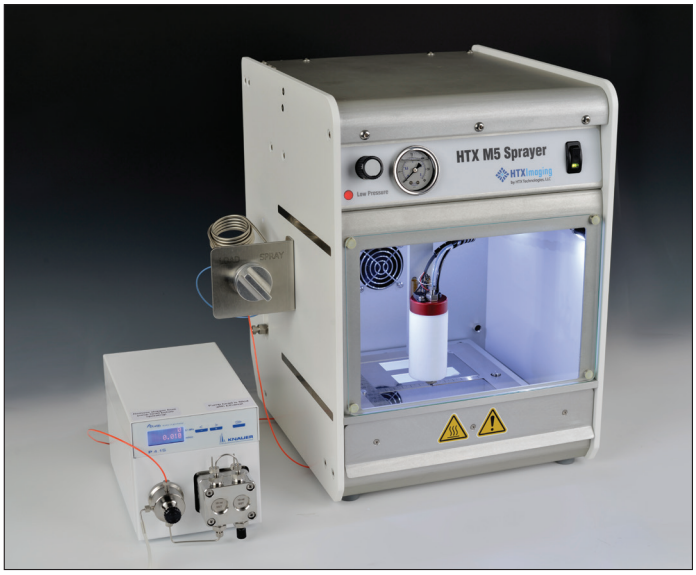
[2]McDonnell LA, Heeren RMA: Imaging mass spectrometry. Mass Spectrom Rev 2007, 26:606-643.

[3]Fernandez JA, Ochoa B, Fresnedo O, Giralt MT, Rodríguez-Puertas R: Matrix-assisted laser desorption ionization imaging mass spectrometry in lipidomics. Anal Bioanal Chem 2011, 401:29-51.

[4]Cerruti CD, Touboul D, Guérineau V, Petit VW, Laprévote O, Brunelle A: MALDI imaging mass spectrometry of lipids by adding lithium salts to the matrix solution. Anal Bioanal Chem 2011, 401:75-87

[5]Cerruti CD, Benabdellah F, Laprévote O, Touboul D, Brunelle A: MALDI imaging and structural analysis of rat brain lipid negative ions with 9-aminoacridine matrix. Anal Chem 2012, 84: 2164-2171.

HTX M5 Sprayer™ Tissue MALDI Sample Preparation System



HTX M5 Sprayer™ System is an Automated MALDI Matrix Deposition System Offering **High Reproducibility** and **Superior Data Quality** for Imaging Mass Spectrometry

The HTX M5 Sprayer™ is an easy-to-use, versatile spraying system that provides automated processes for sample preparation in imaging mass spectrometry.

The proprietary spray technology of the HTX M5 Sprayer™ guarantees a very fine, uniform and consistent matrix coating crucial for high-resolution imaging and relative quantification of analytes.

The unique ability to control liquid and propulsion gas temperature creates a fine solution mist that can be deposited in a precise and adjustable pattern over all or part of any MALDI plate.

Spray characteristics (wet or dry) are easily adjustable via the intuitive operator interface. Users can create and save methods for reproducible operation.

Key Characteristics

- Proprietary technology providing very small matrix droplets (<5 microns)
- High flow rate and fast sample prep (2 to 18 minutes per slide)
- Highly consistent matrix deposition across entire sample area (+/- 3% by weight)
- Unique use of temperature and nitrogen flow to control evaporation rate and matrix crystal formation
- More than 30 validated protocols covering trypsin and most matrices (e.g.: SA, CHCA, DHB, DAN, 9-AA, DHA, CMBT, THAP)
- Validated protocols for Trypsin digestion of FFPE
- Continuous matrix coverage as needed for high-resolution imaging
- Rugged operation and easy clean-up

Addressing the Matrix Deposition Challenge

The main challenge when preparing samples for MALDI Mass Spectrometry Imaging is to balance the positive effects of the matrix solution penetrating the tissue and co-crystallizing with the analyte, and the negative effects of analytes delocalization.

The all-new M5 chassis, high velocity stage and heated sample holder drawer contribute to a greater user experience and expanded process capabilities including:

- Faster and drier deposition capability
- On-tray trypsin digestion capability
- On-tray sample re-hydration

HTX M5 Sprayer™ is available worldwide exclusively from HTX Technologies, LLC.

To request further information contact:

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HTX Technologies offers innovative sample preparation systems for advanced analytical platforms. Our integrated workflow solutions include user training, instruments, software, consumables and method development services.

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