APPLICATION NOTE

#41 Effects of Tray Temperature on Matrix Deposition and Mass Spectrometry Imaging

Application & Background

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) imaging is a powerful analytical technique. It allows researchers to map the distribution of thousands of analytes in one run without labeling. High spatial resolution imaging analysis is especially useful in many applications, such as checking drug distribution in and across tissues, looking for biomarkers specific to tumor cells, or resolving biomolecules at the cellular level. To achieve high spatial resolution images, it is essential to evenly deposit matrix on tissue sections, without letting the matrix crystals grow too large. While some matrices are known to generate fine crystals, 2,5-dihydroxybenzoic acid (DHB), one of the most commonly used matrices, is known to form large needle-shaped crystals. The HTX M5-Sprayer has many parameters that can be modified to optimize matrix deposition protocol, including flow rate, nozzle speed, number of passes, and nozzle temperature. In addition to these features, the HTX M5-Sprayer also features a heated tray that can precisely control the temperature of the sample slide while spraying MALDI matrix.

Intended Use Of This Application Note

In this application note, we aimed to compare the effect of tray temperature on DHB crystal size and the resulting quality of the MALDI MS images.

Experimental

Sample Preparation

Rat brain embedded in 10% gelatin solution was fresh frozen and cryosectioned sagittally at a width of 12 microns using a Leica CM 3050 cryomicrotome. Tissues were thaw-mounted onto ITO coated glass slides and kept frozen at -80°C until analysis. Prior to matrix deposition, slides were kept in a desiccator at room temperature for 30 minutes. No pre-treatment or washing step was used.

Matrix Application

DHB was applied to the slides at a concentration of 30 mg/mL (in 50:50 methanol:water) using the HTX M5-Sprayer (Figure 1a). The parameters of the HTX M5-Sprayer were as displayed below:

Flow Rate	0.1 mL/min
Spray Nozzle Velocity	1000, 1500 or 2000 mm/min
Spray Nozzle Temperature	75°C
Tray Temperature*	25, 35, 45 or 55°C
Track Spacing	3 mm
Number of Passes	8, HH pattern

**To minimize the time that the sample was exposed to an elevated temperature, all slides were kept off the tray until the target temperature was reached. The slides were then placed on the tray for 1 minute prior to spraying to allow the temperature to equilibrate. After spraying, the slide was taken off the tray and immediately placed in a desiccator.*

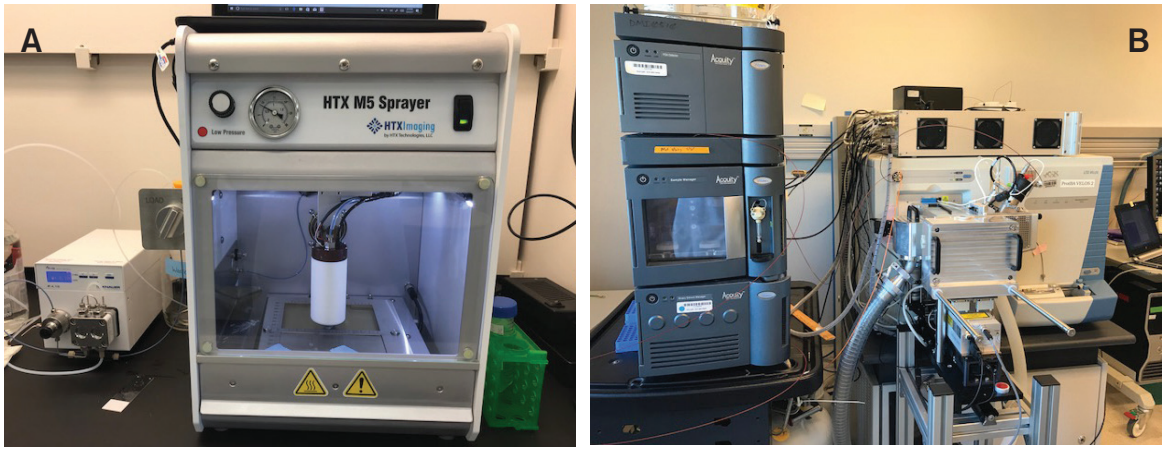


Figure 1. Instruments used in this study. (a) HTX M5 Sprayer; (b) MALDI/ESI injector coupled to Orbitrap Velos mass spectrometer.

Experimental Conditions:

Condition	1	2	3	4	5	6
Tray Temperature (°C)	55	45	35	25	25	25
Nozzle Velocity (mm/min)	1000	1000	1000	1000	1500	2000
Resulting Matrix Density (µg/mm²)*	8	8	8	8	5.3	4

**Matrix density (W, µg/mm²) was calculated by the equation:*

$$W = \frac{NP \times C \times FR}{V \times TS} \times 1000$$

NP: number of passes, C: concentration of matrix (mg/mL)
FR: flow rate (mL/min), V: nozzle velocity (mm/min)
TS: track spacing (mm)

The matrix coated slides were examined under a light microscope (Nikon Eclipse Ni-U Microscope) at 4x and 10x, and images were captured both on-tissue and off-tissue.

MS Analysis

MS imaging experiments were performed on a MALDI-Orbitrap system, where a MALDI/ESI injector (Spectroglyph) was coupled to an Orbitrap Velos mass spectrometer (Thermo Scientific) for high resolution MS analysis (Figure 1b). The MALDI source was equipped with an Explorer One Nd:YLF (349 nm) laser firing at 500 Hz and 2.2 Amp. Mass spectra were acquired for m/z 150-2000, with a resolving power of 7500 (at m/z 400) and a maximum injection time of 50 ms (automatic gain control target = 1 x 10^6). Rat cerebellum and brain stem areas were selected for imaging at a pixel size of 25 µm. Conditions 5 and 6 were not imaged due to poor matrix coverage.

Results

Slides sprayed with DHB at the various experimental conditions were examined under the microscope for comparison (Figure 2). It was found that a matrix density of 8 µg/mm², which corresponded to a nozzle velocity of 1000 mm/min (Conditions 1-4), gave good matrix coverage both on- and off-tissue. Matrix densities of 5.3 µg/mm² (Condition 5) and 4 µg/mm² (Condition 6) did not provide good coverage. Due to the long imaging time at high spatial resolution, Conditions 5 and 6 were not selected for MS imaging analysis. When comparing slides with the same matrix density (Conditions 1-4), slides sprayed when the sample tray was at higher temperatures clearly displayed finer DHB crystals compared to ones sprayed when the sample tray was at lower temperatures. For example, 10x on-tissue microscopic pictures (Figure 2b) revealed fine crystal structures in Conditions 1 and 2 (55°C and 45°C, respectively), but visible needle-shaped crystal structures in Conditions 3 and 4 (35°C and 25°C, respectively). The same trend was observed in the off-tissue microscopic pictures. Therefore, we concluded that increasing the sample tray temperature during matrix spraying helped to reduce matrix crystal size, which increases the quality of high spatial resolution MS images.

Rat cerebellum and brain stem areas were selected for high resolution MS imaging analysis at 25 µm (Conditions 1-4).

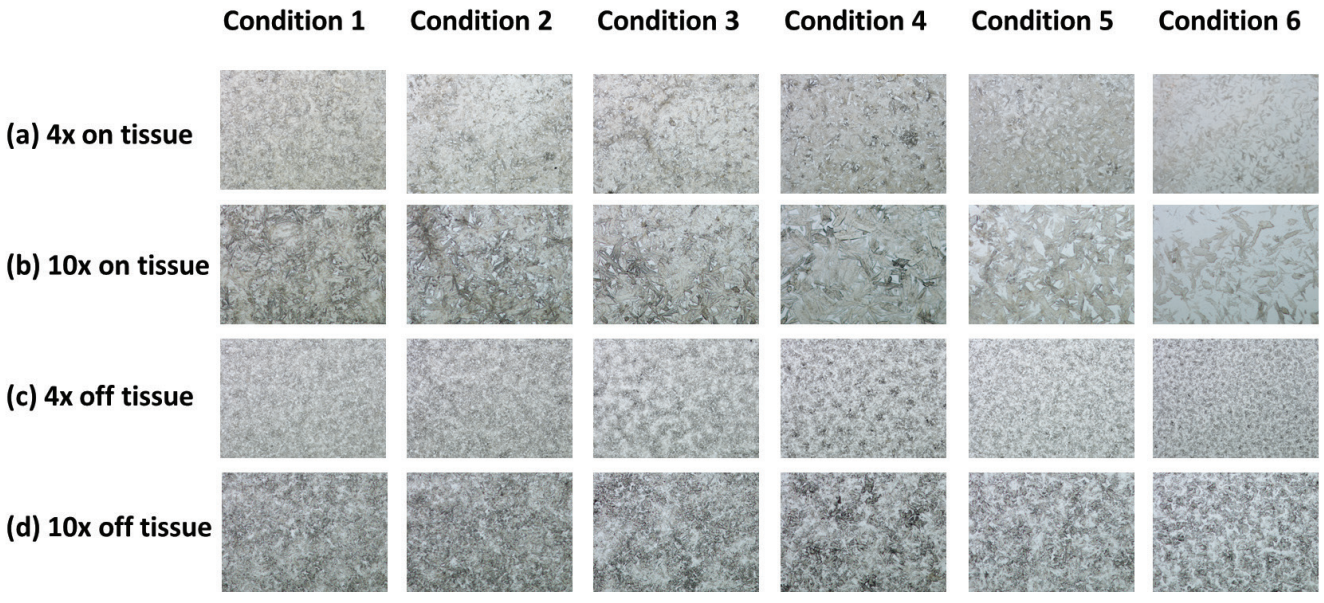


Figure 2. Microscopic pictures of matrix coated slides. (a) 4x magnified on-tissue images; (b) 10x magnified on-tissue images; (c) 4x magnified off-tissue images; (d) 10x magnified off-tissue images.

A common lipid list of 350 species was used to map distribution, and selected ion images are shown in Figure 3. It was observed that the slides sprayed at higher temperature generated sharper, more intense, and more evenly distributed signals. On the other hand, similar number of peaks was detected across all 4 conditions, so there is no obvious evidence suggesting that elevated temperature causes degradation of lipid species. Further research is needed on the effect of elevated tray temperatures on MS imaging analyses when working with heat sensitive or labile analytes.

Conclusions

Generating fine matrix crystals is essential for high resolution MS imaging experiments. Applying DHB matrix at elevated tray temperatures helped to create fine matrix crystals, which resulted in sharper and more intense MS image signals. Future research seeks to explore the effect of an elevated tray temperature on the quality of MS images across other analytes and matrices.

The tissue images and MS data presented in this note were provided by Bingming Chen from the Department of Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, USA.

Experimental Summary

Tissue Type	Rat brain
Preservation	Fresh frozen
Tissue Cut	12 µm thickness
MALDI Plate	ITO coated glass slides
Matrix Deposition	DHB (30 mg/mL) in 50:50 Me/H_2O
MALDI Laser	Explorer One 349-60 Nd:YLF (349 nm)
Acquisition Mode	FTMS full scan

Instrumentation and Supplies

Microtome	Leica CM3050 cryostat
MALDI Plate	ITO coated glass slides
Matrix	DHB
Matrix Sprayer	HTX M5-Sprayer™
MALDI MS	MALDI/ESI injector (Spectroglyph) coupled to Orbitrap Velos (Thermo Scientific)
Imaging Software	ImageInsight (Spectroglyph) and MSiReader (NC State)

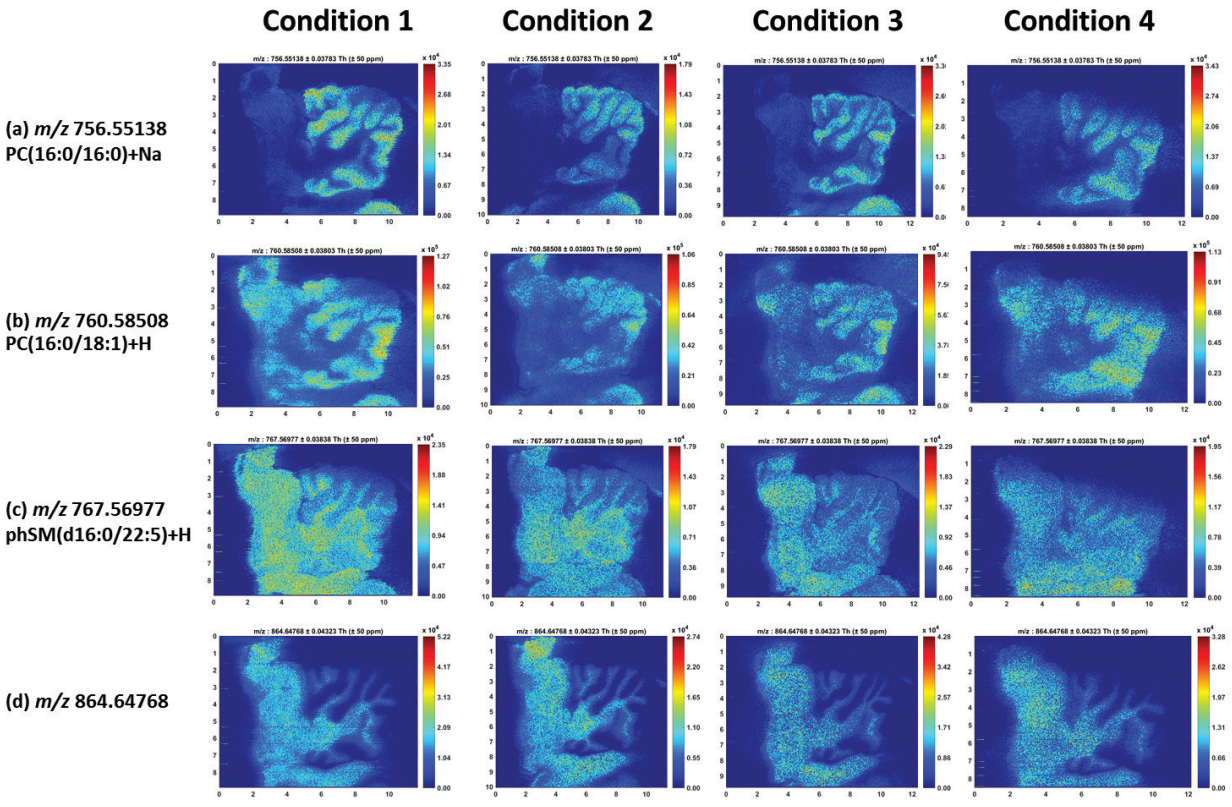
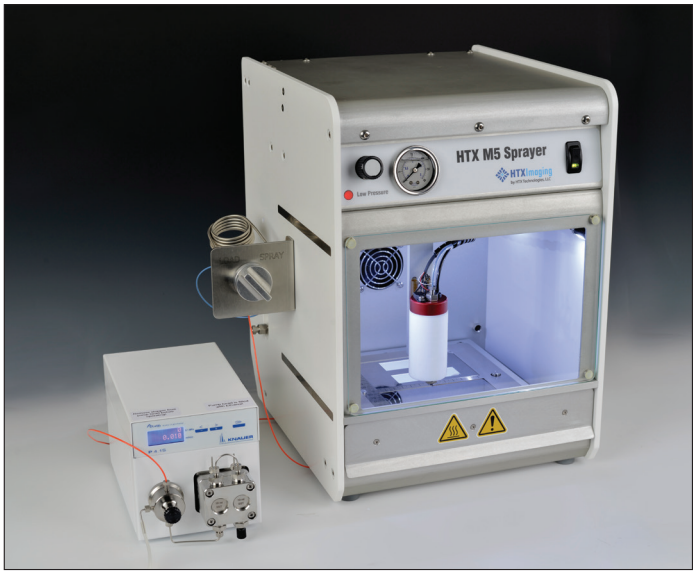


Figure 3. MS images of lipid species detected on rat brain sections at each condition. The m/z values, tentative lipid identification and MSI results are listed.

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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