TECHNICAL NOTE

#30 Demonstration of MALDI matrix deposition for whole animal tissue imaging. Description of experimental conditions. Localization of parent and metabolites in multiple tissues.

Application

The need for consistent, homogeneous MALDI matrix deposition is crucial for imaging studies that span multiple analyses. This may be true for imaging studies with large numbers of subjects, timepoints, or oversized samples that do not fit onto a single target plate (and will require multiple MS analyses with subsequent stitching of data; e.g., whole-rat sections). Uncontrolled variations in the matrix coating step of an imaging experiment can adversely affect the extraction efficiency of your analytes of interest, as well as, affect the imaging parameters of the experiment such as laser power and number of laser shots to accumulate for each acquisition. These variations can make comparisons of imaging data produced from successive experiments challenging, and in the case of oversized samples, unnecessarily difficult to define normalization factors in order to make the images comparable and amenable to stitching.

Here we describe a robust and reproducible MALDI matrix coating protocol using the TM-Sprayer for the preparation of oversized whole-rat tissue sections.

Intended Use Of This Technical Note

The goal of this document is to illustrate possible uses of the TM-Sprayer for Research Purpose Only. HTX, the manufacturers referenced in this note and the users that have accepted to share their data do not make any guarantees as to the performance of the illustrated workflow, and each lab should insure that replicating these experiments respects applicable health and safety regulations.

Imaging Workflow

A single 10 mg/kg PO dose of Olanzapine was administered to a male Sprague-Dawley rat, euthanized at 6 hours post-dose and flash frozen.

The whole-animal carcass was sectioned (20 µm thickness) and sagittal whole-body sections were transferred to MALDI target plates using double-sided tape.

Tissue sections were then sprayed with 2,5-Dihydroxbenzoic (DHB) matrix (40mg/ml, 70/30 Methanol/H2O spiked with 2 uM IS) using the HTX TM-Sprayer and the following conditions:

Flow Rate	200 µL/min
Spray Nozzle Velocity	1,200 mm/min
Spray Nozzle Temperature	75°C
Nitrogen gas pressure	10 psi
Track Spacing	3 mm
Number of Passes	8
Time per path	4 minutes, 30 seconds
Drying time between passes	30 seconds
Total aproox	

Optical image of whole-rat section after completion of the MALDI spray coating using the optimized HTX TM-Sprayer protocol described herein.

Images were collected across the entire tissue area at 500 µm pixel resolution using a 7.0T SolariX FTMS system (Bruker Daltonics) equipped with a dual ESI-MALDI source employing smartbeam-II™ technology. The laser was operated at 1 kHz and a total of 500 laser shots were accumulated from each pixel position. Data were collected in full scan mode over a mass range of m/z 100 to 1500.

Full scan data were processed and drug and metabolite images were extracted and displayed using FlexImaging software 3.0 (Bruker).

Experimental Summary

Tissue type	Rat whole body
Tissue cut	20 µm thickness
MALDI Plate	Bruker MTP Target Plate
Matrix deposition	DHB 40mg/ml, in 70:30 Methanol/H_20
MALDI Laser	Smartbeam 1 kHz
Acquisition mode	Full Scan

Instrumentation and Supplies

Microtome	LEICA CM3600
MALDI plate	BRUKER MTP Plate
Matrix	Sigma-Aldrich
Matrix Sprayer	HTX TM-Sprayer™
MALDI MS	BRUKER SolariX™
Imaging software	BRUKER flexImaging

Results and MALDI MS Images

High-resolution FTMS data were imported into FlexImaging for processing and ion image extraction. To assess overall matrix coverage and image performance, the ion image representing the matrix-spiked internal standard was extracted (Figure 1). It can be seen that the non-tissue regions (along the outer edge of the tissue section) provided the highest signal intensities. There also appears to be regions or specific organs of the whole-rat tissue where the IS signal has been suppressed. Representative pixels from the top, middle, and bottom of the non-tissue regions were selected and spectra were compared for peak intensity, resolution, and signal to noise (Figure 2). Since the IS response proved consistent throughout the run, subsequent olanzapine and metabolite ion images were extracted and stitched, allowing for the visualization of analyte distributions across a whole-rat section in the proper orientation (Figures 3-5).

Figure 1. MALDI MS Image of Internal Standard

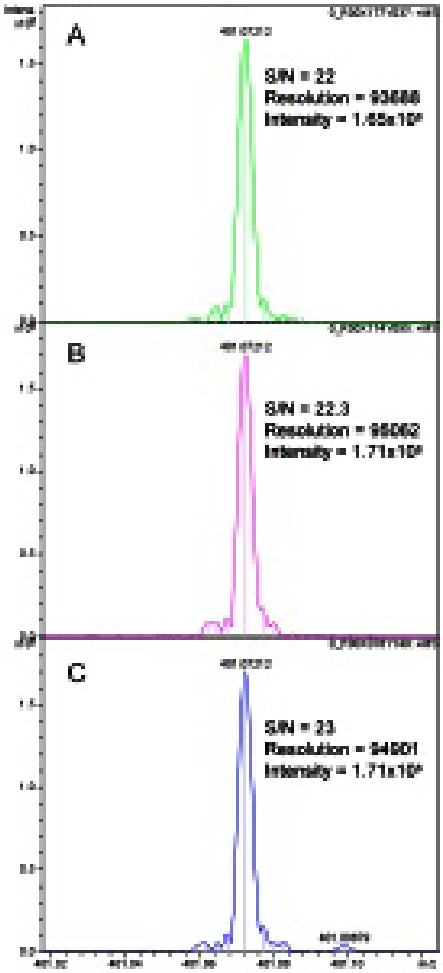


Figure 2.Representative Spectra of Internal Standard Response Throughout Image Run. Non-tissue pixels selected from A) top, B) middle, C) bottom of image

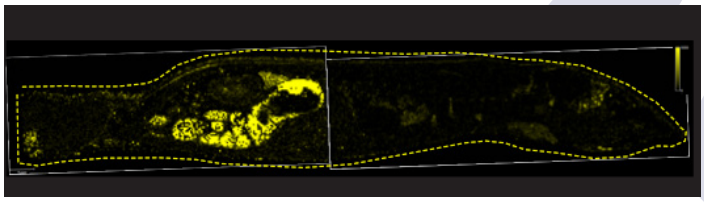


Figure 3. MALDI MS Image of Olanzapine (stitched) (313.1517 m/z)

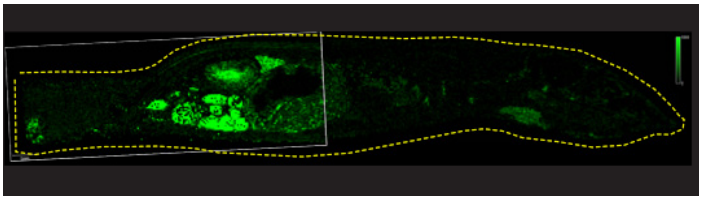


Figure 4. MALDI MS Image of Oxidative Metabolite (stitched) (329.1484 m/z)

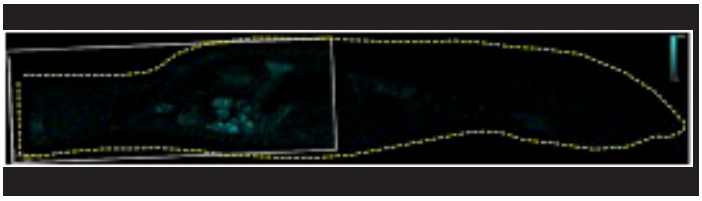


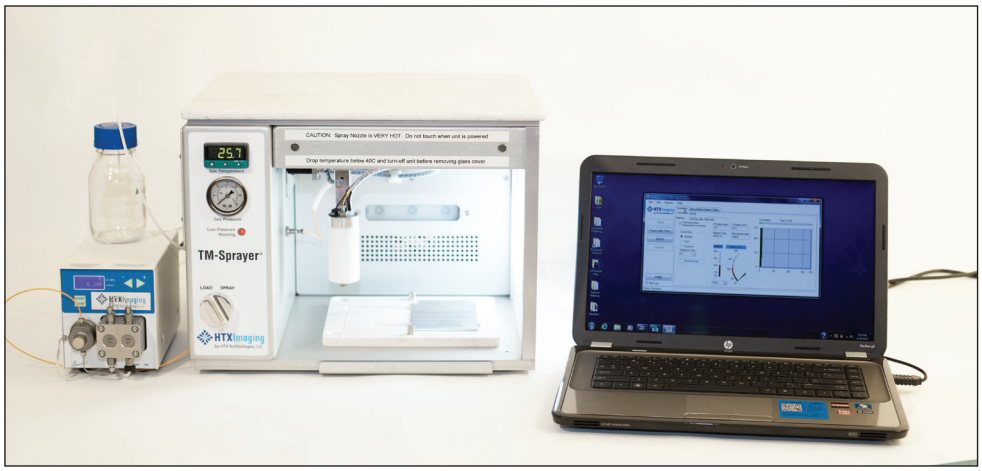
Figure 5. MALDI MS Image of N-desmethyl Metabolite (stitched) (299.1356 m/z)

Acknowledgements

The tissue images and MS data presented in this note were provided by Dr. Sheerin Shahidi-Latham and Cristine Quiason, Genentech Inc. South San Francisco, CA, USA.

TM-Sprayer™ Tissue MALDI Sample Preparation System

The HTX TM-Sprayer™ System is an automated MALDI matrix deposition system offering high reproducibility and superior data quality for Mass Spectrometry Imaging



The HTX TM-Sprayer™ is an easy-to-use, versatile spraying system that provides an automated process for Sample Preparation in Mass Spectrometry Imaging.

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

The new HTX Technologies' spray nozzle, featured in the next generation TM-Sprayer, creates a fine solvent 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

- Patented technology providing very small matrix droplets (<10 microns)
- High flow rate and fast sample prep (10 to 20 minutes per plate)
- 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
- Validated protocols for most matrices (e.g.: SA, CHCA, DHB)
- Validated protocols for Trypsin digestion
- Continuous matrix coverage as needed for high-resolution imaging
- Rugged operation and easy clean-up

TM-Sprayer™ Specifications

Deposition: Spray deposition in linear or serpentine modes with variables offsets

Spray Nozzle Flow: 50 to 1000µl/min

Sheath Gas: Ambient to 150°C (+/- 2°C), software selected

Gas Supply: Sheath gas flow 5-15.5 liter/min

Spray Nozzle Position: Spray nozzle mounted on Cartesian stage

Electrical: 36V Power Supply

Dimensions/Weight: 17 x 15 x 13in (43 x 38 x 33cm), 38lbs (17Kg)

TM-Sprayer™ is available worldwide exclusively from HTX Technologies, LLC. To request further information contact:

Alain Creissen
Imaging Product Manager, HTX Technologies
acreissen@htximaging.com

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.

HTXImaging
by HTX Technologies, LLC

PO Box 1036, Carrboro, NC 27510, USA
Tel +1-919-928-5688 ◆ Fax +1-919-928-5153
info@htximaging.com ◆ www.htximaging.com