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.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate</td>
<td>200 µL/min</td>
</tr>
<tr>
<td>Spray Nozzle Velocity</td>
<td>1,200 mm/min</td>
</tr>
<tr>
<td>Spray Nozzle Temperature</td>
<td>75°C</td>
</tr>
<tr>
<td>Nitrogen gas pressure</td>
<td>10 psi</td>
</tr>
<tr>
<td>Track Spacing</td>
<td>3 mm</td>
</tr>
<tr>
<td>Number of Passes</td>
<td>8</td>
</tr>
<tr>
<td>Time per path</td>
<td>4 minutes, 30 seconds</td>
</tr>
<tr>
<td>Drying time between passes</td>
<td>30 seconds</td>
</tr>
<tr>
<td>Total approximate run time</td>
<td>40 minutes</td>
</tr>
</tbody>
</table>

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.

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).

**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).

**Experimental Summary**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue type</td>
<td>Rat whole body</td>
</tr>
<tr>
<td>Tissue cut</td>
<td>20 µm thickness</td>
</tr>
<tr>
<td>MALDI Plate</td>
<td>Bruker MTP Target Plate</td>
</tr>
<tr>
<td>Matrix deposition</td>
<td>DHB 40mg/ml, in 70:30 Methanol/H₂O</td>
</tr>
<tr>
<td>MALDI Laser</td>
<td>Smartbeam 1 kHz</td>
</tr>
<tr>
<td>Acquisition mode</td>
<td>Full Scan</td>
</tr>
</tbody>
</table>

**Instrumentation and Supplies**

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtome</td>
<td>LEICA CM3600</td>
</tr>
<tr>
<td>MALDI plate</td>
<td>BRUKER MTP Plate</td>
</tr>
<tr>
<td>Matrix</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Matrix Sprayer</td>
<td>HTX TM-Sprayer™</td>
</tr>
<tr>
<td>MALDI MS</td>
<td>BRUKER SolarIX™</td>
</tr>
<tr>
<td>Imaging software</td>
<td>BRUKER flexImaging</td>
</tr>
</tbody>
</table>
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.

HTX TM-Sprayer™ is a registered trademark of HTX Technologies, LLC. All other trademarks are the property of their respective owners.
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™ guar-
antees a very fine, uniform and consistent matrix coating
crucial for high-resolution imaging and relative quantifica-
tion 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 130°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: 24V Power Supply
Dimensions/Weight: 17 x 15 x 13in (43 x 38 x 33cm),
38lbs (17Kg)