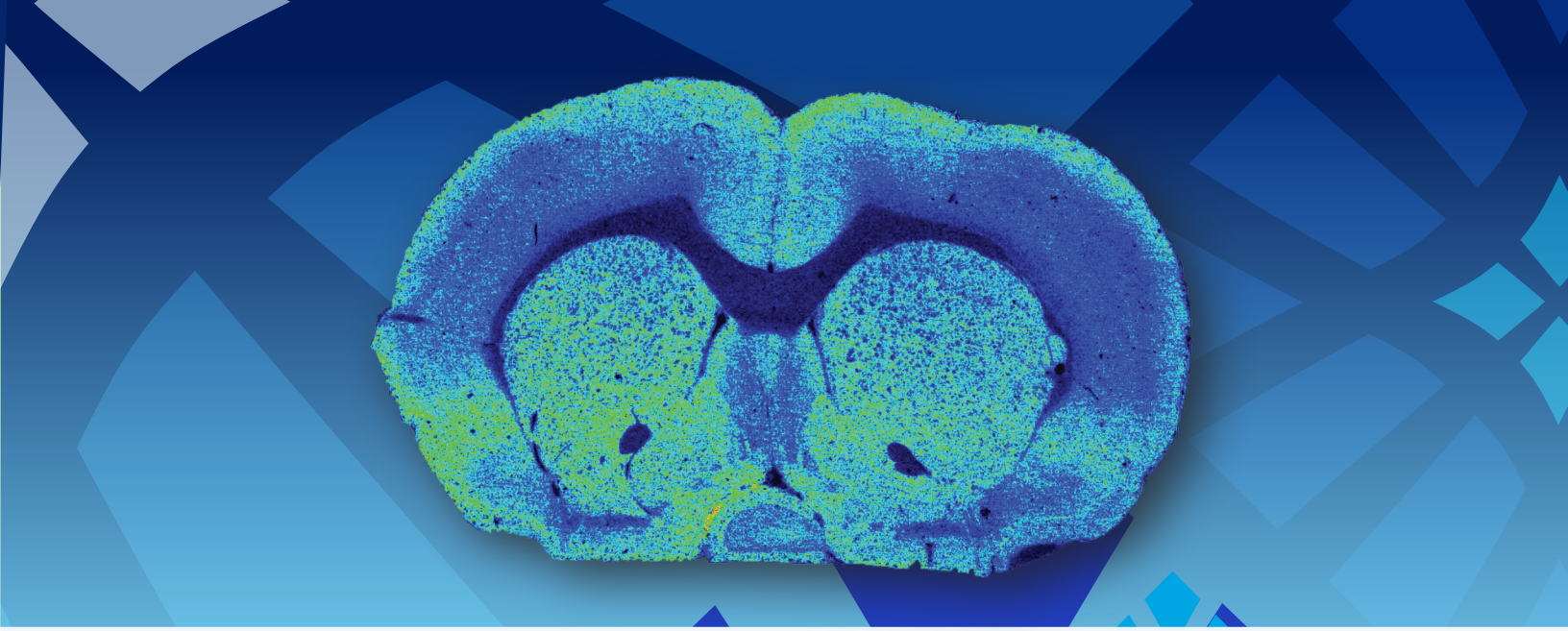
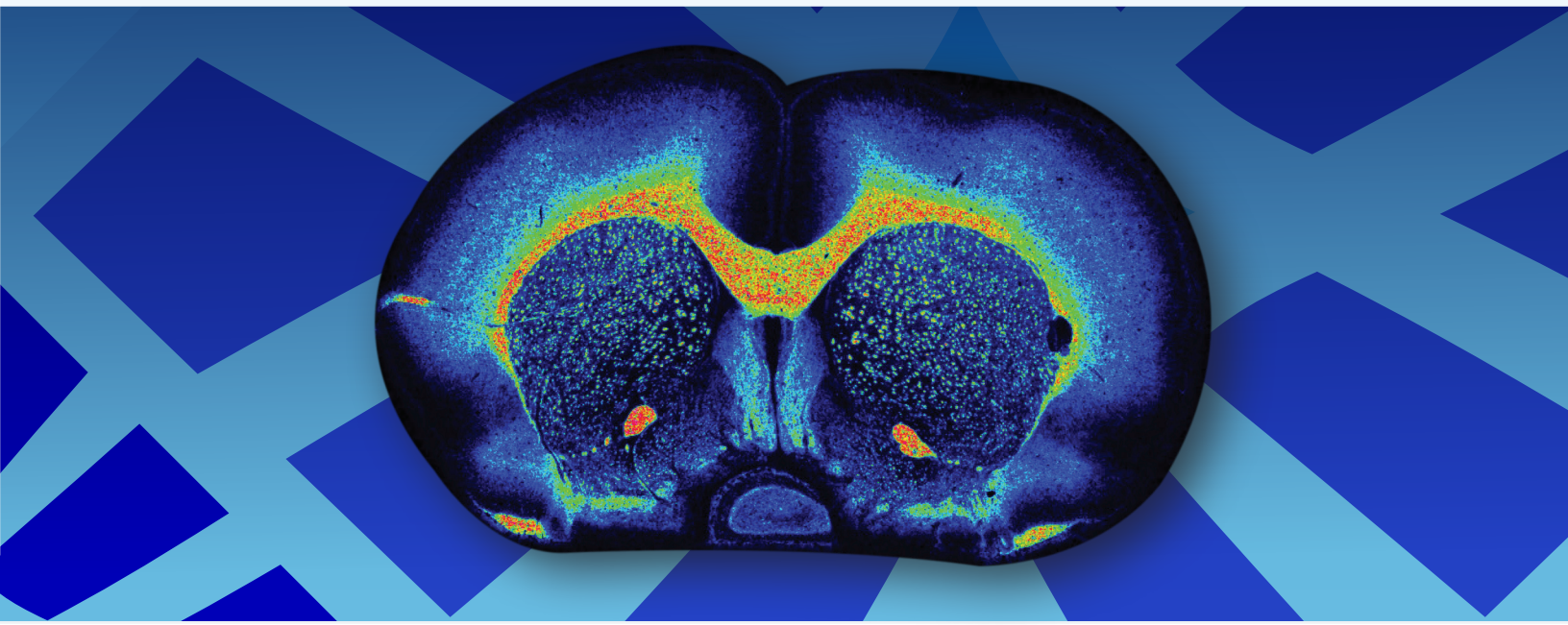


HTX M5 Sprayer™

Tissue MALDI Sample Preparation System

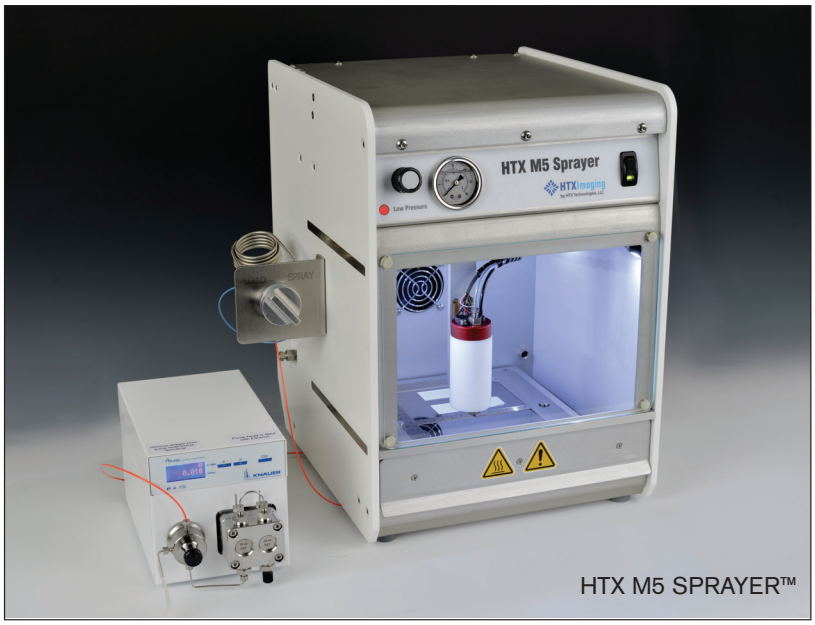


HTX Imaging

by HTX Technologies, LLC

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 Mass Spectrometry Imaging



The HTX M5 Sprayer™ is an easy-to-use, versatile spraying system that provides automated process for Sample Preparation in Mass Spectrometry Imaging.

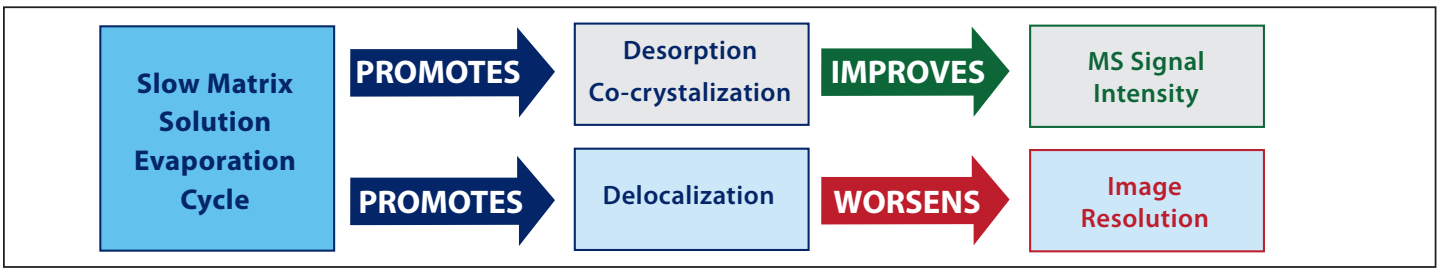
The proprietary spray technology of the HTX M5 Sprayer™ guarantees a very fine, uniform and consistent matrix coating crucial for high-resolution imaging and relative quantification of analytes.

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 valided 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



MATRIX DEPOSITION CHALLENGE

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 analytes, and the negative effects of analytes delocalization.

The HTX M5 Sprayer™ builds on the proven capabilities of its predecessor, the HTX TM-Sprayer™, to offer even greater performance. 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 (with optional humidity chamber)
- On-tray sample re-hydration (with optional humidity chamber)

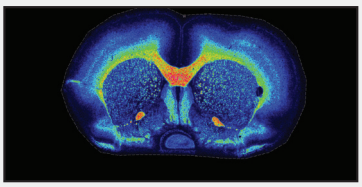
On the next page view a comparison of the two models.

SUCCESSFUL SPRAY DEPOSITION OF 2,4,6-TRIHYDROXYACETOPHENONE MALDI MATRIX

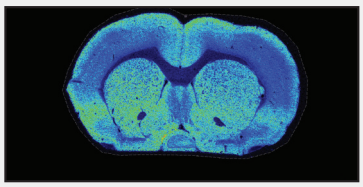
2,4, 6 Trihydroxyacetophenone or THAP offers great potential for MALDI analysis and Imaging of lipids, acidic glycans and glycol-peptides in negative ion mode, but its deposition by spray is challenging due to the tendency of THAP to form hydrates. When deposited by spray under normal conditions, the hydrated solution results in a liquid film layer that dries very slowly and forms a non-uniform coating. In the example below we devised a two steps process to deposit THAP successfully and create high resolution MS images.

Taking advantage of the high velocity of the XY stage of the HTX M5 Sprayer, the first four layers are deposited in super dry mode to create a seeding layer, then the next four layers, better optimized for extraction, are deposited on top resulting in a very good balance of analytes extraction, high signal intensity and limited spatial delocalization. This protocol did not require rehydration to produce a high intensity spectrum.

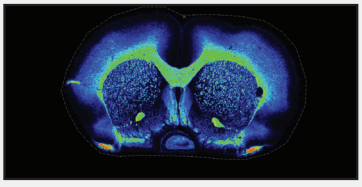
Data and images courtesy of Dr. Junhai Yang, Vanderbilt University.



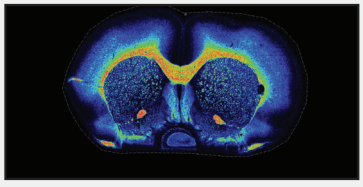
m/z 806.3 +/- 0.5Da



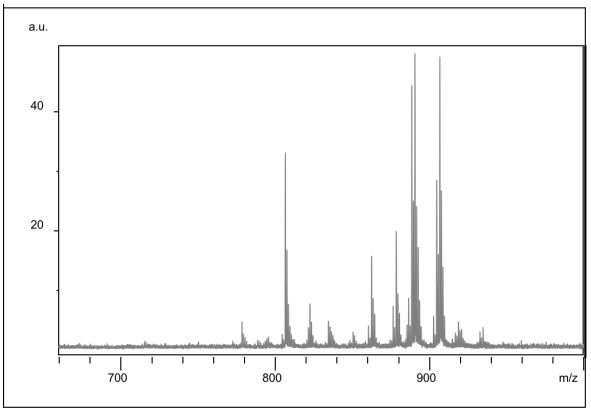
m/z 885.3 +/- 0.5Da



m/z 888.4 +/- 0.5Da



m/z 906 Da +/- 0.5 Da



PROTOCOL

First 4 passes:

Matrix:	2,4,6-THAP at 1mg/ml in 100% Acetone
Flow Rate:	0.050 ml/min
Velocity:	2,000 mm/min
Temperature:	30°C
Track Spacing:	1.5 mm

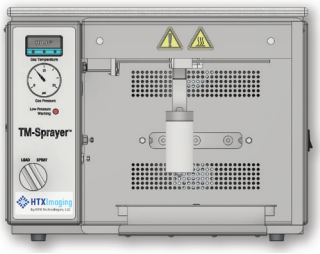
Next 4 passes:

Matrix:	2,4,6-THAP at 11.1mg/ml in 66% Chloroform, 34% Methanol
Flow Rate:	0.100 ml/min
Velocity:	3,600mm/min
Temperature:	30°C
Track spacing:	1.5 mm

INSTRUMENTS

Sample Prep:	Prototype HTX M5 Sprayer
Analysis:	Bruker rapifleX TissueTyper
Acquisition raster:	15µm

HTX MALDI Sample Preparation Systems: M3 vs. M5

Product Name	**HTX TM-Sprayer**	**HTX M5 Sprayer**
Front view		
Chassis	M3 chassis	M5 chassis
Instrument footprint	16.5”W x 13”H x 13”D	11.5”W x 16.3”H x 14”D
Weight	40 lbs. (18Kg)	35 lbs. (16Kg)
Available spray area	180 x 140mm	180 x 140mm
Drawer loading	No	Yes
Heated tray	No	Yes
Waste/Drain	Cup	Trough gutter EZ in/out
XY stage		
Maximum stage velocity	1,350 mm/min	5,600 mm/min
Nozzle height	Fixed (40mm)	Adjustable (25, 40, 52mm)
Heated Spray Nozzle	24V 40W RED Nozzle	Same
Electronics/Software		
Location	Left panel	Top panel
COM channels	2 COM Ports XY, Temperature	4 COM Ports XY, Temp1, Temp2, Pump
Software version	TMSP CS 4.1	TMSP CS 5.1
Protocols	Methods.xml file offering 30+ validated protocols	Expended but fully compatible with all previous methods.

HTX TECHNOLOGIES PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTICS PROCEDURES.

HTX M5 Sprayer™ is available worldwide exclusively from HTX Technologies, LLC and authorized distributors. To request further information contact:

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Andrew.Reeder@verulamscientific.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.

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SUPPLY & SERVICE OF ANALYTICAL INSTRUMENTS

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