APPLICATION NOTE

#36 Homogenous Spray Application of DHB on Dried Agar for MALDI Imaging of Microbial Cultures

Background

MALDI imaging of microbial colonies on agar media is a growing application for mass spectrometry; however, matrix deposition on agar is often complicated. Typically, matrix is applied to wet agar using a fine sieve to dust matrix powder over the top of the slide, which often yields low to medium signal intensity and poor reproducibility. Here we present a more reproducible method of applying 2,5-dihydroxybenzoic acid (DHB) to dried agar slices for MALDI imaging of metabolites from bacterial colonies.

Intended Use Of This Application Note

The goal of this document is to illustrate possible uses of the TM-Sprayer for Research Purpose Only. HTX Technologies, its partners, 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

Part of the bacterial colony and the surrounding agar were sliced out of the petri dish using a razor blade spatula and carefully laid on a glass slide using a metal spatula, ensuring there were no bubbles underneath the agar. The slides were dried down in a desiccator at room temperature overnight. Note that drying time will vary depending on the size of the agar slice and the effectiveness of the desiccator.

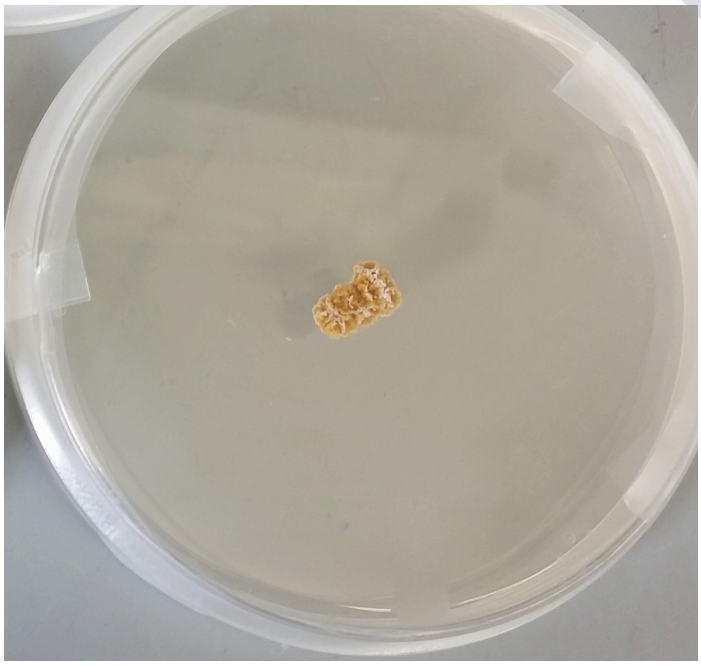


Figure 1. Bacterial colony grown on agar media

Tissue sections were then sprayed with DHB matrix (40 mg/ml, Methanol 50%, TFA 0.1%) using the HTX TM-Sprayer and the following conditions:

Flow Rate	100 µL/min
Spray Nozzle Velocity	950 mm/min
Spray Nozzle Temperature	80°C
Track Spacing	3 mm
Number of Passes	10, criss-cross and offset
Time per path	1.5–1.6 min
Nitrogen Pressure	10 psi

Spectra were collected across the entire agar/colony area using a MALDI- LTQ Orbitrap (Thermo Scientific, Waltham, MA, USA) analyzer equipped with a nitrogen laser in positive mode over a mass range of *m/z* 100 to 2000. The raster width was set to 100 µm for imaging.

APPLICATION NOTE

Experimental Summary

Tissue type	Agar/ bacterial colony
Preservation	Dried in desiccator
Tissue cut	Intact
MALDI Plate	Standard glass slides
Matrix deposition	DHB 40mg/ml, 0.1% FA in 50:50 MeOH/H_20
MALDI Laser	60 Hz Nitrogen Laser
Acquisition mode	Positive

Instrumentation and Supplies

MALDI plate	Standard glass slides
Matrix	Acros Organics
Matrix Sprayer	HTX TM-Sprayer™
MALDI MS	Thermo MALDI- LTQ Orbitrap™
Imaging software	Thermo ImageQuest™ MSiReader[1]

Results and MALDI MS Images

MALDI MS images of compounds detected in the agar and the bacterial colony when matrix was applied using the method above were compared to images acquired when the matrix was applied via dry coating with a sieve.

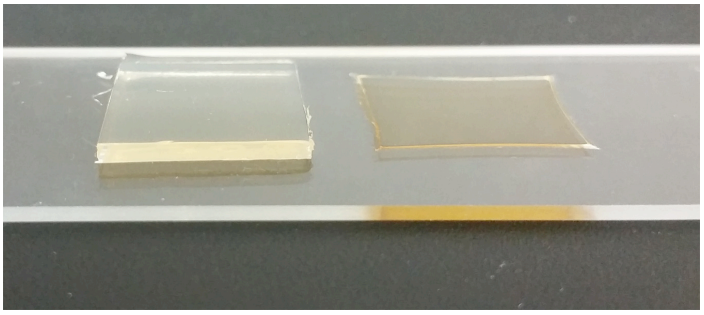


Figure 2. Agar before (left) and after (right) drying

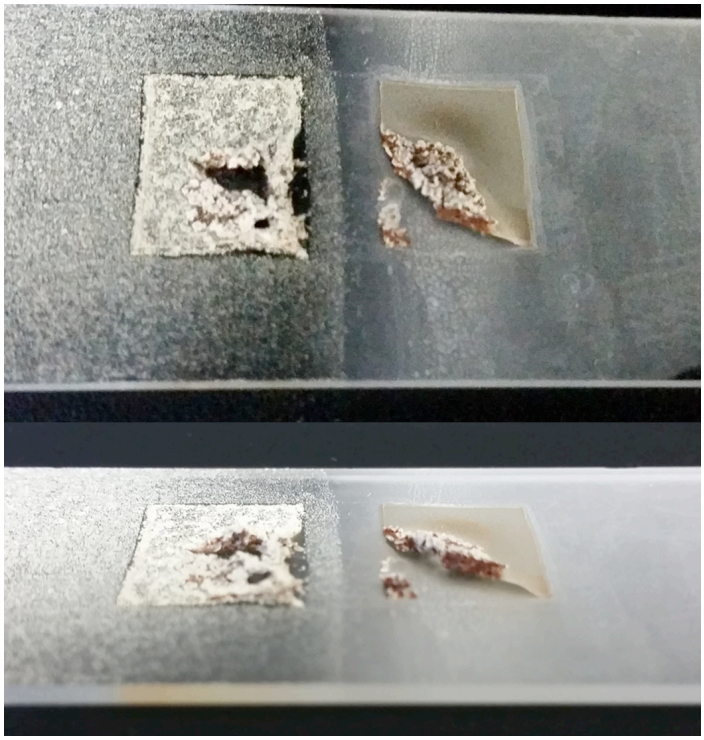


Figure 3. Agar that has been drying too long. It has begun to crack and flake away from the glass slide.

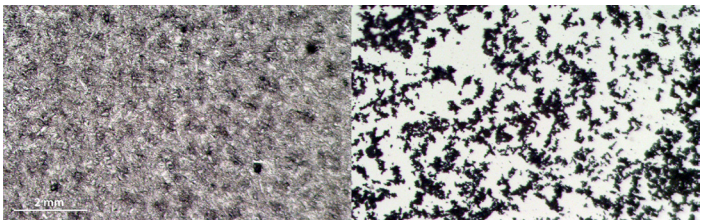


Figure 4. High resolution image of DHB matrix crystal size and coverage on a glass slide when applied with the TM-Sprayer compared to application via sieve.

Acknowledgements

The agar images and MS data presented in this note were provided by Erin Gemperline and Dr. Lingjun Li, Department of Chemistry and School of Pharmacy, University of Wisconsin- Madison, Madison, WI, USA

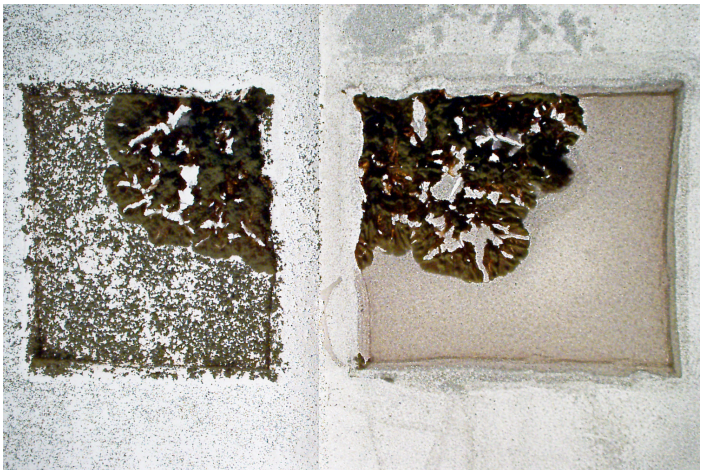


Figure 5. Agar and bacterial colony on glass slide with matrix applied via dry sieve (left) and TM-Sprayer (right).

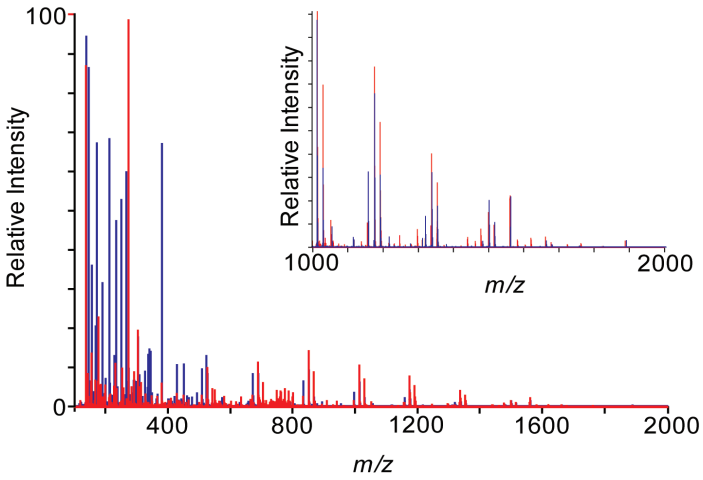


Figure 6. Mass spectra of bacterial colony samples when matrix is applied via TM-Sprayer or sieve. Metabolites with *m/z* 100-2000 were acquired. The inlay zooms in on the higher mass region, *m/z* 1000-2000, where dry sieving does not generate as many peaks as the TM Sprayer.

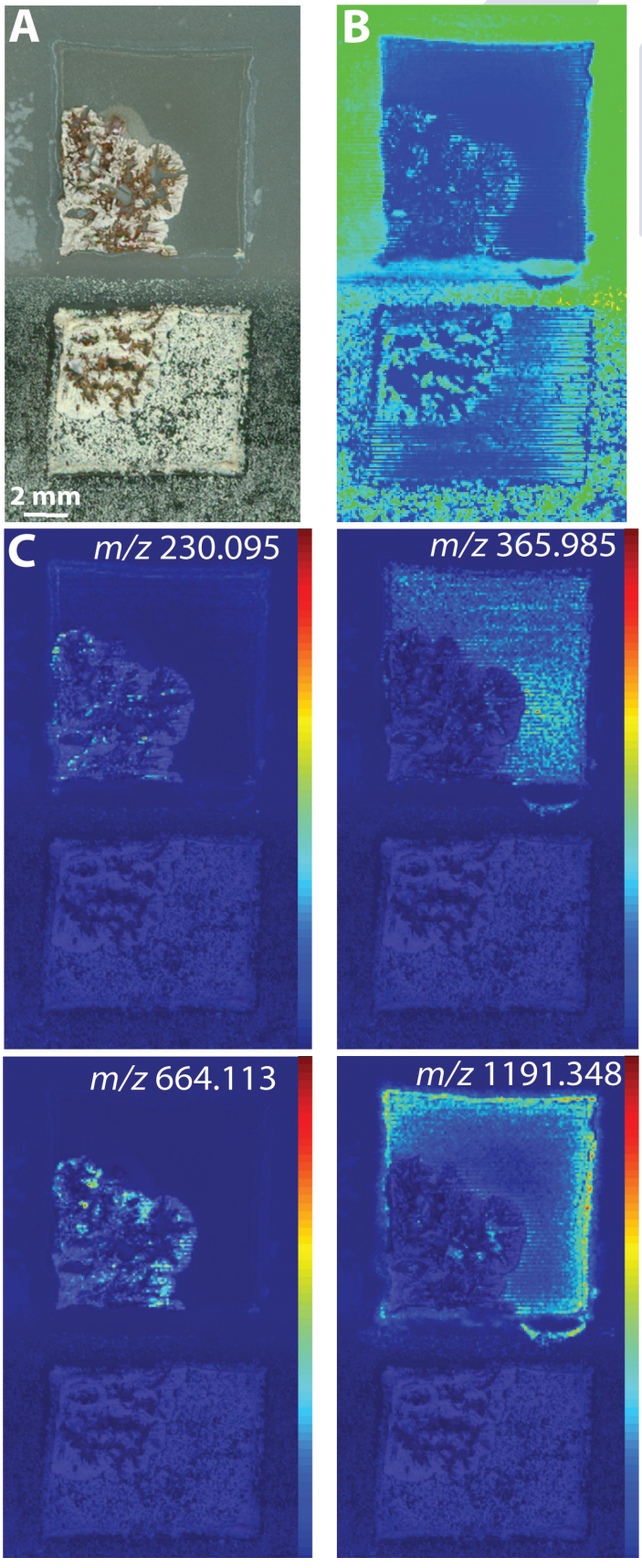
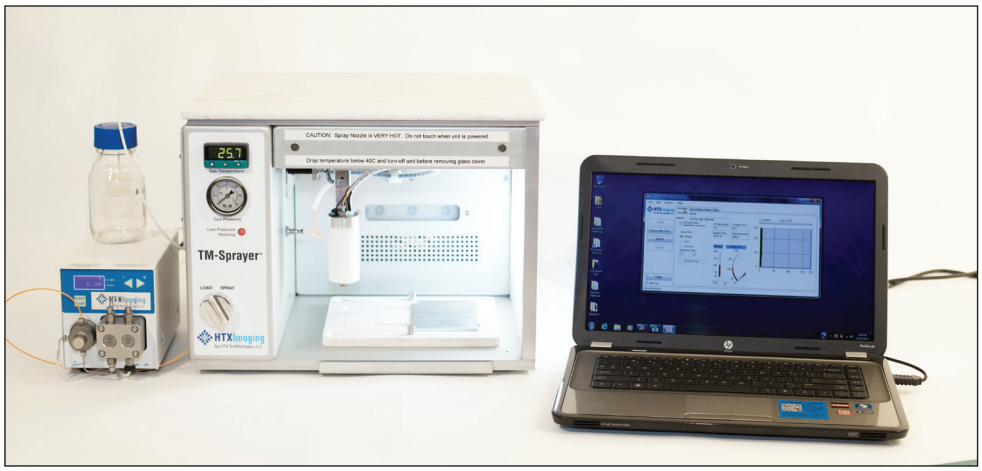


Figure 7. A) Optical image of the bacterial colony on agar with DHB applied via TM-Sprayer (top) or sieve (bottom). B) TIC image comparing the DHB coverage of the two application methods. C) Representative ion images of m/z 230.095, 365.985, 664.113, and 1191.348 comparing metabolite distribution when matrix is applied with the TM-Sprayer vs. sieve. Scale bar = 2 mm, Intensity scale = low abundance (blue) to high abundance (red).

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 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)

TM-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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