TECHNICAL NOTE

#31 Demonstration of matrix deposition optimization. Improving protein detection and reduction of analyte delocalization

Application

When depositing MALDI matrix onto tissue samples for subsequent Imaging Mass Spectrometry (IMS), the selection of an appropriate sample preparation technique is key to obtaining maximum signal intensity and image resolution. The principle of MALDI IMS requires the co-localization of an ionization promoter (matrix) with the molecular species of interest[1]. The introduction of the matrix can be performed in a number of ways including spotting, spraying or sublimating onto the tissue surface[2-5].

Here we demonstrate how the adjustment of spraying conditions on the HTX TM-Sprayer enables the maximization of signal intensity for specific molecular species while limiting delocalization of these same species. The process optimization approach also provides a guideline for the MS imaging optimization of other molecular species.

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 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 illustrated workflow. Each lab should insure that replicating these experiments respects applicable health and safety regulations.

Background

In this example, the user was interested in imaging a wide molecular weight range of proteins (3-30 kDa) in a rat kidney tissue section. Previous studies have suggested that a wetter matrix deposition process (slower drying time) increases extraction of these species towards the surface of the tissue, promoting co-crystallization with the matrix and improving MS signal intensity. However, the laws of chemical diffusion also suggest that allowing the tissue surface to remain wet for a long duration will provide an opportunity for molecules to re-organize spatially to achieve a lower system entropy.

Chemical diffusion, which results in the delocalization of chemical species, works against our goal to image analytes in their original location in the tissue sample and we should seek to limit it during sample preparation.

Experimental Plan

The HTX TM-Sprayer provides the ability to control spray temperature (T) and nozzle lateral velocity (V) during the sample preparation. Because both parameters affect the matrix solution evaporation rate and can be adjusted independently from one another the user has the opportunity to optimize signal intensity while reducing delocalization.

In this experiment we varied temperatures (T) from 75 to 105°C (10°C increments) and velocity (V) from 700 to 1,300 mm/min (200 mm/min increments). For profiling experiments, average spectra were collected over 5,000 shots. For imaging experiments, complete ion images were produced for each of 16 samples.

Sample Preparation

Fresh frozen rat kidney was sectioned at 12 microns on a cryomicrotome and thaw-mounted on indium tin oxide (ITO) coated slides. Tissues were allowed to air dry for 30 minutes prior to washing. Slides were washed to remove salts and lipids in 70% EtOH (30 sec), 100% EtOH (30 sec), Carnoy's Solution - 6:3:1 EtOH:chloroform:acetic acid (2 min), 100% EtOH (30 sec), ddH_2O (30 sec), 100% EtOH (30 sec) as previously described[5] and then allowed to air dry.

Matrix Application

Sinapinic acid was applied to the slides at a concentration of 5 mg/mL using the TM-Sprayer. The slides were coated using the instrument parameters listed as follows:

Flow Rate	0.2 mL/min
Spray Nozzle Velocity	700 to 1,300 mm/min
Spray Nozzle Temperature	75 to 105°C
Track Spacing	2 mm
Number of Passes	8, criss-cross and offset
Resulting Matrix Density	*See chart*

Matrix Density

W = Matrix density (mg/mm^2) NP = Number of passes
C = Concentration of matrix (mg/mL) FR = Flow rate of matrix solution (mL/min)
V = Nozzle velocity (mm/min) TS = Track spacing (mm)

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

Velocity	NP	Conc.	Density
mm/min	*Count*	*mg/mL*	*mg/cm^2*
700	8	5	0.571
900	8	5	0.444
1100	8	5	0.363
1300	8	5	0.307

Rehydration/Recrystallization

Once the slides were coated with matrix, a rehydration step was performed as described previously[5]. Briefly, a 9% acetic acid solution was used as the rehydration solution. Slides were allowed to air dry for 5 minutes prior to further analysis.

MS Analysis

Spectra from m/z 3,000 to 30,000 were collected across the entire tissue area using an Autoflex Speed MALDI-TOF mass spectrometer (Bruker Daltonics) with a SmartBeam laser operating at 1 kHz in positive ion linear mode. For the imaging acquisition, a total of 200 laser shots were accumulated and averaged from each pixel, using a lateral spatial resolution of 250 µm. Image acquisition and analysis were carried out using FlexImaging 3.0, and spectral analysis was performed with FlexAnalysis 3.3.

Experimental Summary

Tissue type	Rat kidney
Preservation	Fresh frozen
Tissue cut	12 µm thickness
MALDI Plate	ITO Coated Glass Slides
Pre-treatment	70% EtOH (30 sec) 100% EtOH (30 sec) Carnoy's Solution (2 min) 100% EtOH (30 sec) ddH_2O (30 sec) 100% EtOH (30 sec)
Matrix Deposition	Sinapinic Acid (5 mg/mL) in 50:50 acetonitrile: H_2O with 0.2% TFA
Rehydration	9% acetic acid solution at 85°C for 3.5 min
MALDI Laser	Smartbeam 1 kHz
Acquisition mode	Positive ion linear mode

Instrumentation and Supplies

Microtome	Leica CM3050 cryostat
MALDI plate	ITO coated slides
Matrix	Sinapinic acid
Matrix Sprayer	HTX TM-Sprayer™
MALDI MS	Bruker Autoflex™ Speed
Imaging software	Bruker flexImaging

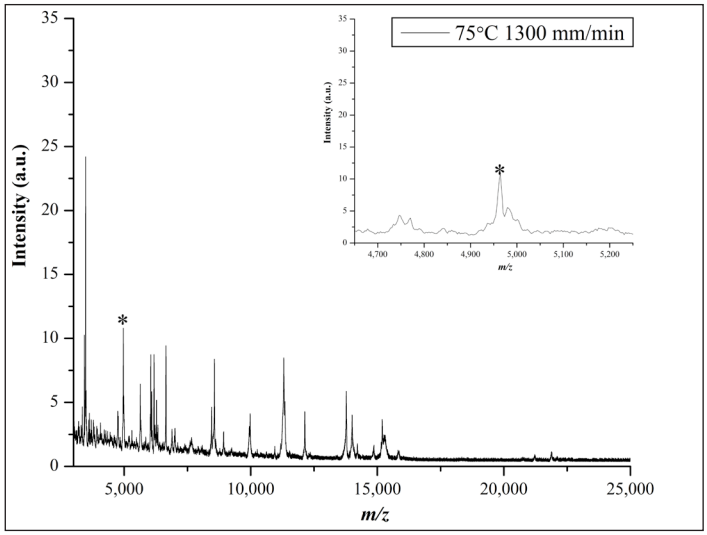
Conclusions

From initial experiments comparing spectra collected in the cortex region, it was found that lower temperatures (75-85°C) and medium velocities (900-1100 mm/min) gave rise to more intense protein signal. These conditions led to relatively wet coatings of the tissue, allowing for better analyte extraction and cocrystallization with the matrix. Alternatively, the higher temperatures and lower velocities gave a much thicker, drier coating with minimal analyte extraction. The imaging experiment took into account peak intensities as well as localization patterns within the tissue. The conditions previously found to give the most abundant signals had a great deal of delocalization. It was found that the middle temperatures (85-95°C) and the medium velocities (900-1100 mm/min) gave abundant, localized signal over

the majority of the mass range. This was due in large part to the optimization of the coating wetness to increase extraction while decreasing delocalization.

It should be noted that this is merely a starting point, and that methods must be optimized for different matrices, solvents, tissue types, and analytes of interest. The versatility of the HTX TM-Sprayer™ for matrix coating allows for more complete method optimization and enhanced imaging capabilities.

Figure 1. Ion intensity differences seen for lower molecular weight species (*m/z* 4,964) Representative spectra from rat kidney medulla showing signal intensity for the peak at m/z 4964, denoted with the asterisks, increasing from black spectrum (low - 10.8 a.u.) then red spectrum (high - 33.9 a.u.). Inset shows an expanded view of the peak of interest. Intensity axes were scaled to the same values.



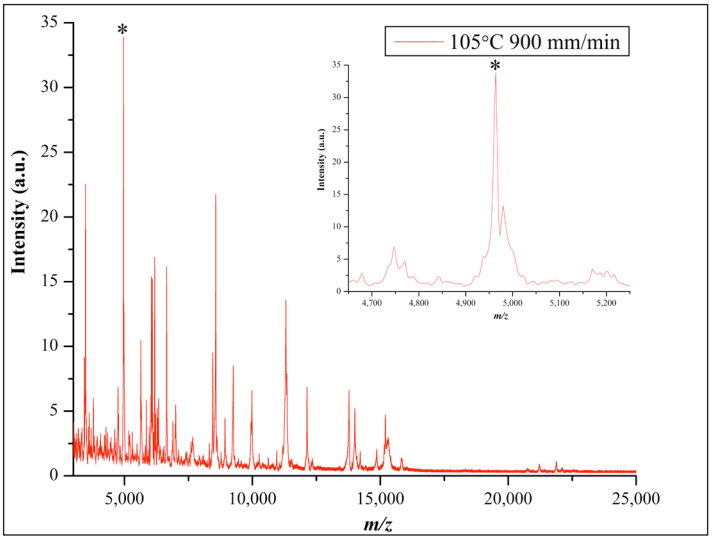
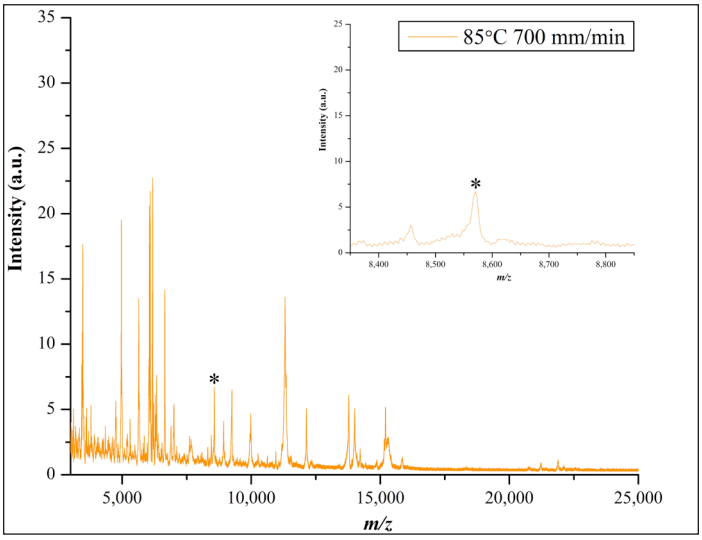


Figure 2. Ion intensity differences seen for medium molecular weight species (*m/z* 8,570) Representative spectra from rat kidney medulla showing signal intensity for the peak at m/z 8570, denoted with the asterisks, increasing from 8.35 x 102 a.u. orange spectrum (low - 12.3 a.u.) to blue spectrum (high - 22.5 a.u.). Inset shows an expanded view of the peak of interest. Intensity axes were scaled to the same values.



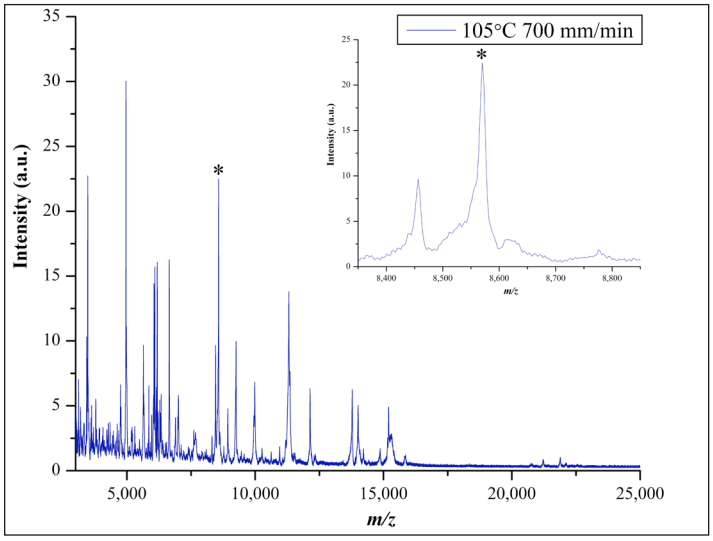


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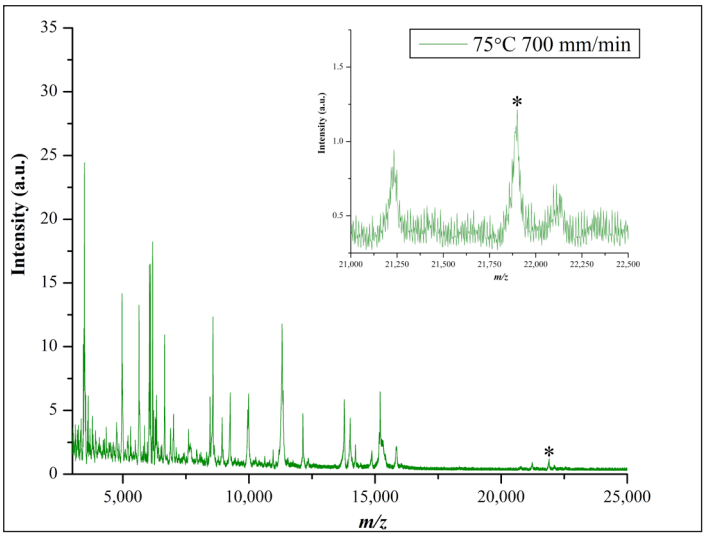
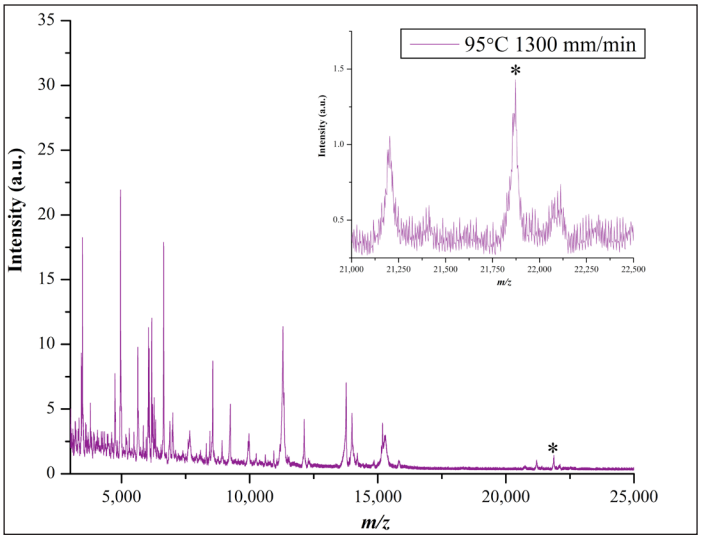
Figure 3. Ion intensity differences seen for higher molecular weight species (*m/z* 21,900) Representative spectra from rat kidney medulla showing signal intensity for the peak at m/z 21900, denoted with the asterisks, increasing from green spectrum (low - 1.2 a.u.) to purple spectrum (high - 1.4 a.u.). Inset shows an expanded view of the peak of interest. Intensity axes were scaled to the same values.





Acknowledgements

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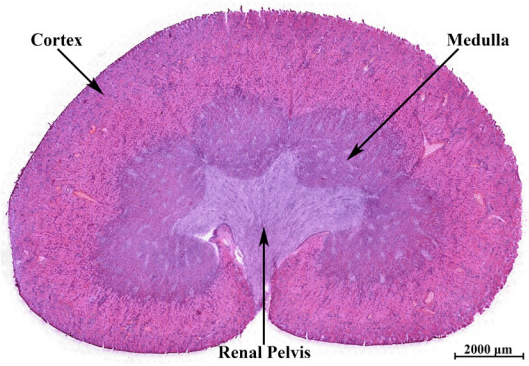
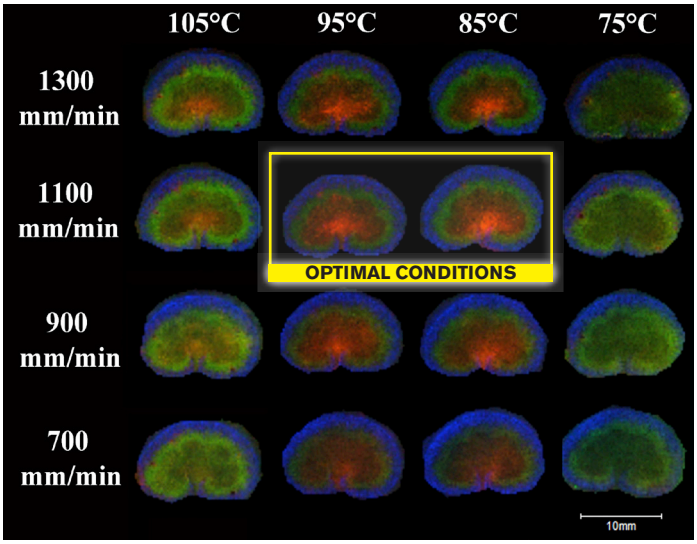
Figure 4: Imaging comparison using rat kidney. Eight passes of SA (5 mg/mL) were applied using the TM Sprayer at varying temperature and velocity settings. (a) H&E stained serial rat kidney section (12 µm-thick). Scale bar represents 2 mm. (b) Composite ion images collected from 12 µm-thick rat kidney sections at 200 µm lateral resolution. Colors correspond to multiple m/z values: 4,748 & 4,964 (red); 6,052 & 6,084 (blue); and 8,570 & 14,218 (green).





References

1. Caprioli, R. M.; Farmer, T. B.; Gile, J., *Analytical Chemistry* 1997, 69 (23), 4751-4760. | **2**. Schwartz, S. A.; Reyzer, M. L.; Caprioli, R. M., *Journal of Mass Spectrometry* 2003, 38 (7), 699-708. | **3**. Cerruti, C. D.; Touboul, D.; Guérineau, V.; Petit, V. W.; Laprévote, O.; Brunelle, A., *Anal Bioanal Chem* 2011, 401 (1), 75-87. **4**. Hankin, J. A.; Barkley, R. M.; Murphy, R. C., *Journal of the American Society for Mass Spectrometry* 2007, 18 (9), 1646-1652. | **5**. Yang, J.; Caprioli, R. M., *Analytical Chemistry* 2011, 83 (14), 5728-5734.

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

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