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Two-Step Matrix Deposition for Improved MS Imaging Resolution

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1. Introduction

Mass Spectrometry Imaging (MSI) is a technique of visualizing components within a sample, usually a biological tissue by their m/z. In MALDI, the matrix chosen for analysis plays a key role in analyte detection. There are two main ways of depositing matrix needed for MALDI based MSI analysis: spraying and sublimating. Matrix sublimation provides small and uniform matrix crystals, while spraying comes with more variability in crystal sizes but comes with an increase in sensitivity through analyte extraction. For imaging experiments at 10 µm and below, having small matrix crystals is critical for high quality images. As spatial resolution increases, signals tend to decrease through ionization of a smaller area making sublimation alone inefficient for high quality data. Combining these matrix application techniques can provide both the small crystal size and signal intensity needed for successful experiments. This work demonstrates the benefits and limitations for using two-step matrix deposition for MSI analysis.

2. Methods

Matrix spraying was performed using the Shimadzu iMLayer Aero, matrix sublimation was performed using the Shimadzu iMLayer. Data acquisition was performed using the iMScope QT, an atmospheric pressure MALDI Imaging system, specific details are available in **Table 1.** Data was analyzed using IMAGEREVEAL MS.

Four matrix application conditions were compared: spraying, sublimation, sublimation with recrystallization and sublimation with matrix spraying (Two-Step). The resulting DHB crystals for each application type are shown in **Figure 1**. Two samples were tested, strawberry slices[1] and mouse brain.

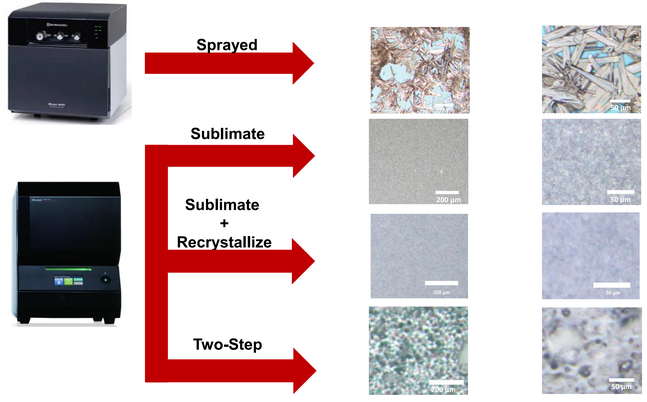

Fig. 1 Microscope images obtained on the iMScope QT using the 10x and 40x objectives,showing size differences between the different matrix application methods.

3. Results

Matrix deposition is a critical step for MALDI based MSI affecting both spatial resolution and analyte sensitivity. Sublimation is typically the choice for high resolution imaging due to the small crystals that are produced. One limitation from sublimation is typically lower signal intensity compared to spraying, a common solution to this is using recrystallization to wet the sample and increase analyte extraction. Shimadzu has introduced a “Two-Step” method that combines sublimation and spraying to increase analyte extraction while using sublimation to maintain small crystal sizes necessary for high resolution imaging. As a proof of concept, we compared four application methods on strawberries[1] at 25 µm before moving to mouse brains for 25 µm and 5 µm imaging experiments.

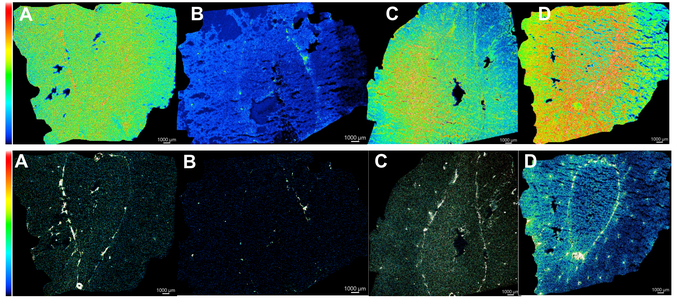

Fig. 2 MSI Images of strawberries imaged at 25 µm **(A)** sprayed, **(B)** Sublimated, **(C)** Sublimated + Recrystallized, **(D)** Two-Step.

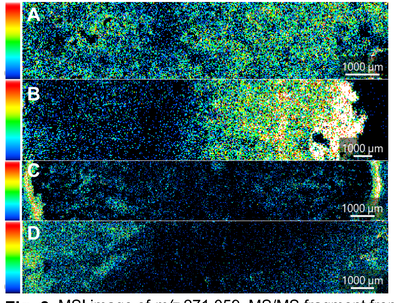

Fig. 3 MSI image of *m/z* 271.059, MS/MS fragment from Pelargonidin-3-O-glucoside at 25 µm from **(A)** Sprayed, **(B)** Sublimated, **(C)** Sublimated + Recrystallized, **(D)** Two-Step.

Table. 1 Analytical settings of MSI

Mass Spectrometer	
System	: iMScope QT+LCMS-9050
Polarity	: Positive
DL temp	: 250 ℃
Heat block temp	: 450 ℃
MS Range	: *m/z* 100-600 / 600-950
Spatial Resolution (Pitch)	: 5 / 25 µm
Laser Diameter Setting	: 0 / 2
Laser Intensity	: 27 / 65
Laser Repetition Frequency	: 2 kHz
Matrix Coating	
System	: iMLayer
Matrix Used	: DHB
Coating Method	: Sublimation
Matrix Coating	
System	: iMLayer AERO
Matrix Used	: 30 mg/mL DHB 70% MeOH, 0.1% TFA
Coating Method	: Sprayed with 10 layers at 75 mm/s

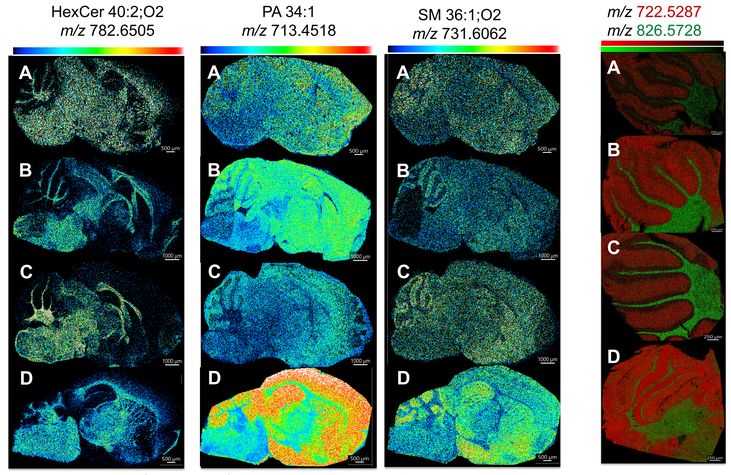

Fig. 4 MSI Images of mouse brain imaged at 25 µm and mouse brain cerebellum imaged at 5 µm **(A)** sprayed, **(B)** Sublimated, **(C)** Sublimated + Recrystallized, **(D)** Two-Step. Tentative lipid ID from Lipid Maps search.

The use of Two-Step matrix application increased analyte extraction for the strawberry metabolites shown in **Figure 2**. When looking at MS/MS of Pelargonidin-3-O-glucoside **(Figure 3)**, the signal obtained from the sublimated data has the highest intensity compared to the other techniques. **Figure 4** shows different lipids commonly found in mouse brain with varying intensity based on the preparation technique. When moving to high resolution imaging, brain cerebellum imaged at 5 µm showed higher definition of cell layer details with the Two-Step method compared to the other methods.

4. Conclusion

The two-step method of matrix application increased detection levels of various plant metabolites and lipids. The increase in detection was seen with most, but not all analytes within the tested samples. The two-step method can be a useful tool when looking to highlight specific analytes within a sample to increase high resolution detection.

References
1) Food Chemistry (2021) Vol 345, 128838, Wang *et. al.*

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