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Technical Report

An Easy Method to Apply Matrix for MS Imaging

Koretsugu Ogata[1], Yumi Matsumoto[1], Takahiro Harada[1]

Abstract:

MS imaging with the iMScope allows matrices to be applied after detailed sample images are obtained under a microscope, without disturbing the positional information. While there are several methods that can be used to apply matrix, such as spraying, vapor deposition, or inkjet drop application, the way it is applied can have a significant effect on imaging results. The manual spraying method of applying matrix described below can be performed using simple equipment, but the proficiency level of the user can make a major difference in determining its success. This report introduces two simple matrix spraying methods which can make a significant difference to the image result.

Keywords: MS imaging, iMScope, iMLayer, matrix application

1. Importance of Matrix Application During MS Image Acquisition

MS imaging with the iMScope, Imaging Mass Microscope, allows the user to obtain the sample via the optical microscope before applying the matrix. After capturing the microscope image (optical image), matrix is applied and an MS image is acquired at spatial intervals as small as 5 µm. The *m/z* image can then be overlaid precisely onto the optical image, which is a key feature of the iMScope system. There are several methods that can be used to apply matrix, such as spraying, vapor deposition, or inkjet drop application, but each of the methods has advantages and disadvantages in terms of reproducibility, resolution, and processing time. Moreover, the matrix application method is known to have a significant effect on ionization efficiency and resolution, which can affect imaging results. In addition, resolution can also be affected by matrix crystal size. Manual spraying using an airbrush (Fig. 1) requires only working under a fume hood with inexpensive equipment commonly available for hobby use. Therefore, it can be started with relatively inexpensive equipment. On the other hand, since it is difficult to quantitatively control the size and volume of sprayed particles, the proficiency level of the user can determine whether or not the method is successful. This is especially significant when using a matrix such as DHB, where the crystalline sate significantly affects the resulting mass spectra. To achieve the best possible mass spectra and fine crystallization, it is considered important to refine the skills of the operator.

Fig. 1 Airbrush and Sample Holder

2. Problems with Using DHB as a Matrix

When using the matrix-assisted laser desorption ionization (MALDI) method, choosing the matrix for ionization is very important. For MALDI-MS imaging, common matrices include sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB), alpha-cyano-4-hydroxycinnamic acid (CHCA), and 9-aminoacridine (9-AA). Particularly when using the iMScope, it can be beneficial to select a matrix, such as DHB, CHCA, or 9-AA, to enhance the detection of the target substance. For lipid analysis, DHB is often selected as the matrix. When preparing samples for the MALDI method, the matrix is dissolved in a volatile solvent and mixed with the sample before being crystallized by spraying. Using DHB as the matrix results in is the formation of large, heterogeneous crystal needles. Therefore, the imaging resolution can vary widely depending on the needle size. Based on our experience, it is more difficult to achieve uniform needle size with good reproducibility using DHB than using other matrix types. Fig. 2 shows an example of applying matrix in the same manner to the same sample, but on different days, which significantly changed the appearance. The image on the left indicates that the matrix formed longer crystal needles.

Furthermore, the matrix was applied relatively thickly, so the state of the tissue section is difficult to see. The iMScope has a special function whereas the detailed parameters of data acquisition can be specified based on the optical image obtained in advance.

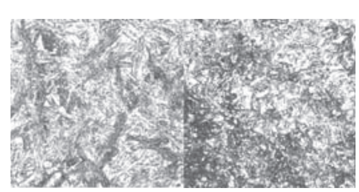

Fig. 2 Images Obtained After Applying DHB

1 Analytical and Measuring Instruments Division, Shimadzu Corporation

3. Confirming the Effects of the Quantity Applied During Spraying

The surface states of microscope slides were compared after spraying different amounts of solvent on them.

Method:

Sprayer: Procon Boy FWA Platinum 0.2 Double Action (Fig. 3)
Compressor: Mr. Linear Compressor, L5

(Specifications)

Compatible gas: Air
Rated pressure: 0.1 MPa
Max. pressure: 0.12 MPa
Air spray quantity: 5.27 L/min at 0.05 MPa
Matrix: DHB (Sigma-Aldrich)
DHB preparation: 30 mg/mL in 70 % methanol/0.1 % TFA
Total quantity of DHB solution: 1 mL added to attached cup.
Spray with the airbrush at a fixed 170 mm distance from the microscope slide.
Air pressure: 0.065 MPa
Air adjustment screw: Fully open
Semi-easy soft button: Press and then pull all the way.
In this example, the spray quantity was adjusted by turning the needle stopper (from 0.25 to 2 turns).

Fig. 3 Airbrush Components

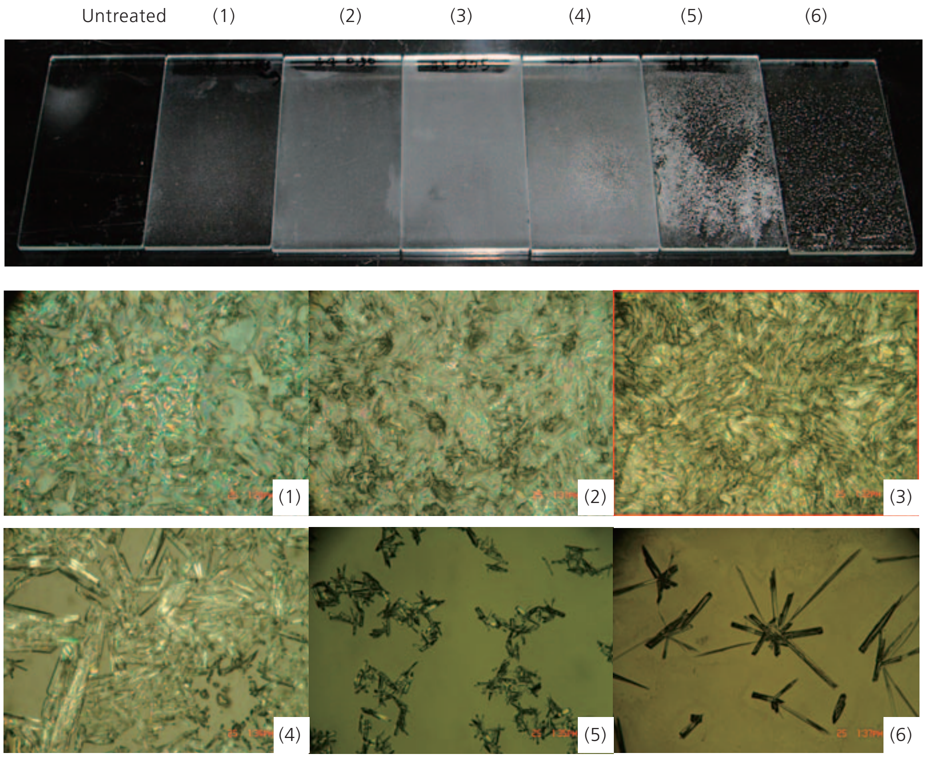

Fig. 4 Microscope Slide Surface Images — Upper: Overall Views; Lower: Magnified Images
Needle stopper rotations: (1) 0.25, (2) 0.5, (3) 0.75, (4) 1.00, (5) 1.50, and (6) 2.00

Results: A 1 mL matrix solution (DHB: 30 mg) was sprayed as the needle stopper was rotated to six levels—0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 turns—corresponding to images (1) to (6), respectively. Then the required spray time, surface wetness, and surface appearance (crystalline state) were compared. As the number of rotations was increased, the spray time was decreased from 1,200 seconds to 320, 100, 70, 25, and 20 seconds, respectively. With the stopper rotated more than one turn (images (4), (5), and (6)), the surface became wet during spraying and uniformity was lost. Since the glass surface was visible when the crystalline state was viewed in the magnified surface images obtained under the same conditions, the resulting image was expected to have poor resolution. At 0.25 and 0.5 rotations (images (1) and (2)), the glass surface appears completely coated, but looking at the entire glass surface indicates that coverage is not uniform. The images compared here reveal that rotating the stopper 0.75 turns and spraying for 100 seconds, as shown in image (3), gives the most appropriate matrix state for data acquisition.

From our experience, we believe that the spray quantity (adjustable by the stopper) applied when about 0.7 to 1.2 mL of the matrix solution is sprayed for about 100 to 150 seconds with the airbrush about 150 to 180 mm away from the glass slide provides relatively good data acquisition results.

The optimal amount of matrix to apply can vary widely depending on the sample state and target substances being detected. Therefore, you should try to determine the appropriate spray volume for yourself.

4. Ensuring Reliable Matrix Application

The applied quantity can also vary due to compressor performance, nozzle state, or other factors. Therefore, until you are comfortable with the process, we recommend using Kimwipes or other means to check how much the matrix solution spreads out or practicing spraying regular inexpensive slides, instead of spraying actual samples.

Depending on the operator's experienced of spraying the matrix, they may devise their own preferred method, such fixing or moving the airbrush, orienting the slide vertically, or laying it flat.

If moving the airbrush in relatively large movements is preferred, it may occasionally miss some areas of the surface, even though it consumes more matrix solution. Since it is difficult to keep the amount of airbrush movement consistent, the surface state needs to be as uniform as possible after matrix application. To make the process more reliable, ensure reproducibility by photographing matrix surfaces and recording weights before and after matrix application. Doing so will give you a more accurate understanding of the quantities applied and crystalline state, enabling more quantitative discussions.

Also, any residual undissolved matrix can be completely dissolved by processing it for about 10 minutes in an ultrasonic cleaner after the matrix is dissolved by a Vortex mixer or other means.

Nozzle blockage and other problems can be prevented by centrifuging (for 2 minutes at least 8000 rpm) and using only the supernatant.

5. Applying Multiple Layers of Matrix

The MALDI method can be thought of as mixing the sample with matrix and solvent, evaporating the solvent, and then ionizing the sample obtained when the matrix crystallizes.

Meanwhile, crystallization occurs by initially forming a tiny crystal nucleus that grows by attaching components identical to the nucleus to its periphery.

Matrix crystallization that forms on sample surfaces in MS imaging occurs in the same way. Therefore, it should be possible to reproduce consistent matrix crystallization by covering the entire surface with uniform crystal nuclei and growing them.

These methods described below were intended as ways to achieve good crystallization even with relatively little practice. In practice, modify the method to suit individual circumstances.

5-1. Multilayer Low-Concentration Matrix Application by Airbrush

Matrix: DHB (Sigma-Aldrich)
DHB preparation:
Solution (1) 20 mg/mL in 70 % methanol/0.1 % TFA, 350 µL
Solution (2) 50 mg/mL in 70 % methanol/0.1 % TFA, 700 µL
Distance from airbrush tip to glass slide:
At least 20 cm for the first and second times, and 15 to 20 cm for the third.
Spray and other conditions: Same as in section 3.

Apply low-concentration matrix solution (1): With the needle stopper rotated 0.25 to 0.3 turns, spray the entire sample plate by moving the airbrush in large movements (Fig. 5). After spraying about 350 µL, the glass surface should appear slightly cloudy.

Apply high-concentration matrix solution (2): Add 350 µL of solution (2) to the attached airbrush cup. With the needle stopper turned to the same position and the airbrush at the same distance as for solution (1), spray the entire amount with large airbrush movements.

Apply high-concentration matrix solution (2) again: Add the remaining 300 µL of solution (2) to the attached airbrush cup. Unscrew the needle stopper slightly to increase the spray quantity. At this point, move the airbrush slightly closer and use smaller airbrush movements. Watch the surface state, and stop as soon as sufficient solution is applied.

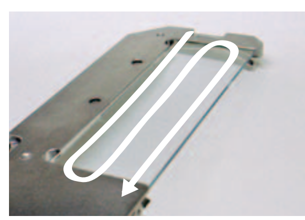

Fig. 5 Example of How to Move the Spray Nozzle

The objective of this technique is to ensure that crystal nuclei are formed by first applying a low-concentration solution. Then, once formed, they only need to be grown—a step that is unlikely to fail even if performed somewhat approximately.

Keep in mind, however, that the amount of low-concentration solution initially applied should not be too little. Until you get used to it, apply the low-concentration solution twice. Because the low-concentration solution should be applied beyond the edges of the glass slide, it is not unusual for this step to take over 10 minutes per sample.

However, the method is so fool-proof that some operators were able to acquire data successfully after practicing only twice. Therefore, we suggest trying the above until you are comfortable with the technique.

With the iMScope system, the sample plate is mounted in the provided sample holder and viewed with the optical microscope before spraying. The sample holder includes positioning markers, which allow you to attach a provided spray mask to prevent contamination by the matrix.

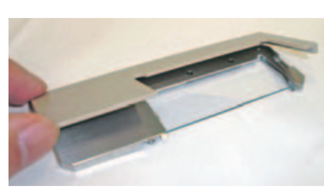

Fig. 6 Spray Mask

5-2. Multilayer Spraying with Vapor Deposition

This method uses an iMLayer vapor deposition system, which is available as an option for iMScope, Imaging Mass Microscope systems, to form the crystal nuclei. Vapor deposition of matrices has been reported to have some problems with imaging due to poor detection of certain molecules, and in particular water soluble molecules.

Here we introduce a multilayered spray method using the iMLayer system that ensures reliable matrix application.

In this method, a thin-film sample is viewed under the microscope and then a layer of matrix is formed by vapor deposition. Matrix is then applied using an airbrush.

Vapor deposition system: iMLayer (E-250MA)
Matrix: DHB (Sigma-Aldrich)
DHB preparation: 50 mg/mL in 70 % methanol/0.1 % TFA, 100 μL
Distance from airbrush tip to microscope slide: 15 to 20 cm
Spray and other conditions: Same as in section 3.
Vapor deposition: 0.4 to 0.6 μm thick (normally 2 μm)
Airbrush: 100 μL of DHB at 50 mg/mL (normally 300 μL)

The vapor-deposited layer serves as the base of crystal nuclei for the matrix subsequently sprayed on with the airbrush and helps form a uniform matrix crystal layer. Fig. 7 compares surfaces formed by only spraying and by combining vapor deposition with spraying. The spray-only method allows an extremely fine surface structure to be created using DHB, which forms needle crystals.

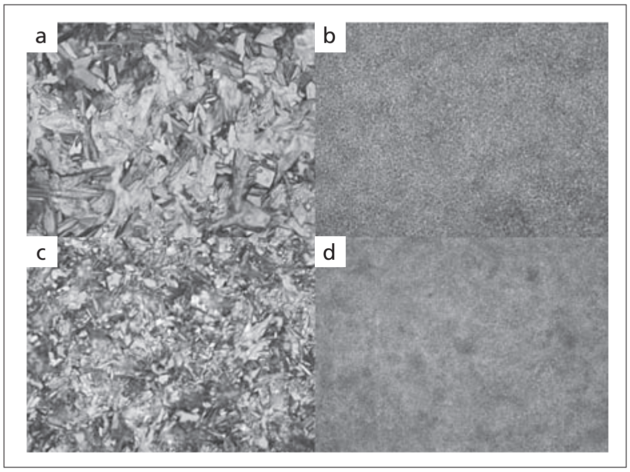

Fig. 7 Magnified Views of Sample Surfaces

Upper (a and b): On a sample section
Lower (c and d): On a microscope slide only
Left (a and c): Sprayed with DHB only (300 μL)
Right (b and d): Vapor deposition plus spray (100 μL)

Acquired spectral patterns revealed many peaks that could not be detected without vapor deposition (unreleased data). Due to the fine crystal structure, it is possible to obtain high-resolution imaging data. Just as with the multilayer low-concentration matrix solution method described above, this makes it possible to apply matrix properly without special operator skills.

Fig. 8 shows a diagram of the entire procedure. The solid lines indicate the time during which the operator is involved, whereas the dashed lines indicate processes that do not require operator involvement. Using both vapor deposition and airbrushing together may seem to be time consuming, but in fact it actually shortens the overall operation time.

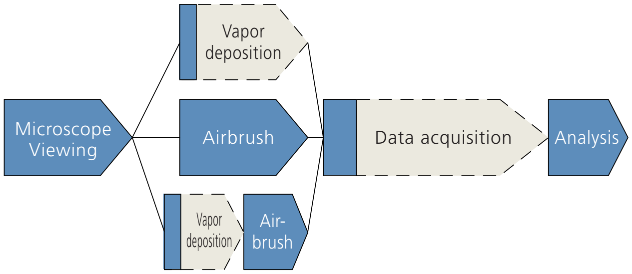

Fig. 8 Entire Procedure

Colored: Operator time required
Dashed lines: System operating time (for illustrative purposes)

6. Concluding Remarks

While applying matrices may be relatively easy for users with expert skills, the two methods presented in this report can be used even by operators that have not applied matrices very often.

The iMScope, Imaging Mass Microscope system is capable of acquiring imaging data of molecules, ionized with a laser beam diameter as small as 5 μm and measured with a mass spectrometer, and then overlay them accurately onto high-resolution morphological images viewed under the optical microscope.

Overlaying MS imaging data onto morphological microscope images increases the value of high-resolution data.

Using an airbrush in combination with the iMLayer vapor deposition system makes sample preparation, including matrix application, more reliable, which helps obtain even more information rich data.

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