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iMLayer™ Matrix Vapor Deposition System (Imaging Mass Microscope iMScope™ QT)

Application News

Optimal matrix application methods for MS imaging

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User Benefits

- The iMLayer enables users of any experience level to apply matrices easily and with high repeatability.
- With the vapor deposition method, the matrix can be applied evenly and with very small crystal size.
- The 2-step method involving spraying after vapor deposition allows MS imaging with high sensitivity and high spatial resolution.

■ Introduction

The technique of MS imaging, initially common only in drug discovery and metabolomics research, has become more widespread in a variety of fields over recent years. In the case of MS imaging using MALDI as the ionization method, it is particularly important not only to select the most appropriate matrix for detection of the target compound, but to consider the optimal matrix application method.

Spraying of the matrix provides effective extraction from the biological tissue. However, this method has shortcomings in that the compounds may spread outside the tissue sample and the matrix crystals are large and non-uniform. On the other hand, the vapor deposition method of matrix application results in very small and uniform crystals, but does not provide effective extraction. In light of this, we have developed a 2-step method (Patent No: 6153139) which combines spraying and vapor deposition, as well as a recrystallization technique that can be used on the vapor-deposited matrix. In this document, we report the differences observed in matrix crystal size and shape depending on the type of matrix and the application method used, and introduce examples showing the benefits of the 2-step method for MS imaging.

■ The iMLayer matrix vapor deposition system

The increasing spatial resolution power of MS imaging has put constraints on the matrix crystal size and uniformity. To this end, the vapor deposition technique of matrix application was developed.

Amongst many types of matrix available, the most commonly used in MS imaging are 2,5-dihydroxybenzoic acid (DHB), 9-aminoacridine (9-AA) and α-cyano-4-hydroxycinnamic acid (CHCA). The iMLayer™ vapor deposition system (Fig. 1) is pre-programmed with vapor deposition methods optimized according to the boiling points etc. of these three compounds. The system is operated via a simple touch panel and allows MS imaging novices to apply matrices consistently and with confidence. In addition, an automatic layer thickness control unit allows the matrix layer thickness to be set in units of 0.1 µm, ensuring repeatability of the application process and allowing quantitative comparison of MS imaging data between different sample slides.

■ Choosing an application method

It is important to select an appropriate matrix according to the target compounds to be detected, but in the case of MS imaging, the matrix application method is also extremely important. When carrying out MS imaging with high spatial resolution, it is necessary not only for the matrix to be applied evenly but also for the crystals to be very small. Limited spread of compounds and effective extraction are also factors to be considered.

Fig. 1 The iMLayer ™

The three main methods of matrix application are spraying, vapor deposition, and the 2-step method. In the spraying method, the liquid matrix solution is sprayed directly onto the sample plate. In vapor deposition, the solid powdered matrix is heated under a vacuum and sublimated to coat the sample. In the 2-step method, the matrix is first applied through vapor deposition before a second layer is added via spraying. Here we consider the optimal application method taking into account both target compounds and four factors that impact analysis results: crystal size, uniformity of application, compound spreading, and extraction efficiency.

Fig. 2 The OLS4100 laser microscope

■ Surface observations of matrix crystal size and uniformity of application

To check the crystal size and uniformity of the applied matrix, we applied three common matrices (DHB, 9-AA, CHCA) to ITO glass slides with each of the three application methods, then observed the resulting matrix surfaces with the OLS4100 laser microscope (Fig. 2) and the SPM-9700HT™ scanning probe microscope (Fig. 3).

Fig. 3 The SPM-9700HT™ scanning probe microscope

Sprayed matrices were observed with the OLS4100. For DHB, we observed uniform needle-shaped crystals with a length of a few tens of µm (Fig. 4A). 9-AA formed irregular tree-shaped crystals with a length of a few tens of µm (Fig. 4B). CHCA formed an irregular granular surface with grains of a few µm in diameter (Fig. 4C). From these observations, it can be seen that the spraying method is not ideal for high-resolution MS imaging where the laser beam diameter may be as small as 5 µm, since spraying results in relatively large and irregular crystals.

Next, we used the SPM-9700HT scanning probe microscope to observe slides with matrices applied via vapor desorption. Taking into account the resolution of MS imaging, the observation field was set as a square with 5 µm sides. In this case, DHB formed uniform crystals of around 1 µm in length (Fig. 4D). 9-AA formed a uniform surface where individual crystals could not be distinguished but were estimated to be a few tens of nm across (Fig. 4E). For CHCA, we observed regular grains of around 0.1 µm in diameter (Fig. 4F).

Finally, we observed matrices applied via the 2-step method, again using the SPM-9700HT. DHB formed uniform crystals of a few µm in diameter (Fig. 4G). 9-AA formed uniform needle-shaped crystals of around 1 µm in length (Fig. 4H). Lastly, CHCA formed regular grains of around 1 µm in diameter (Fig. 4I).

These observations show that the vapor deposition and 2-step method provide appropriate crystal size and uniformity for MS imaging with a minimum laser beam diameter of 5 µm.

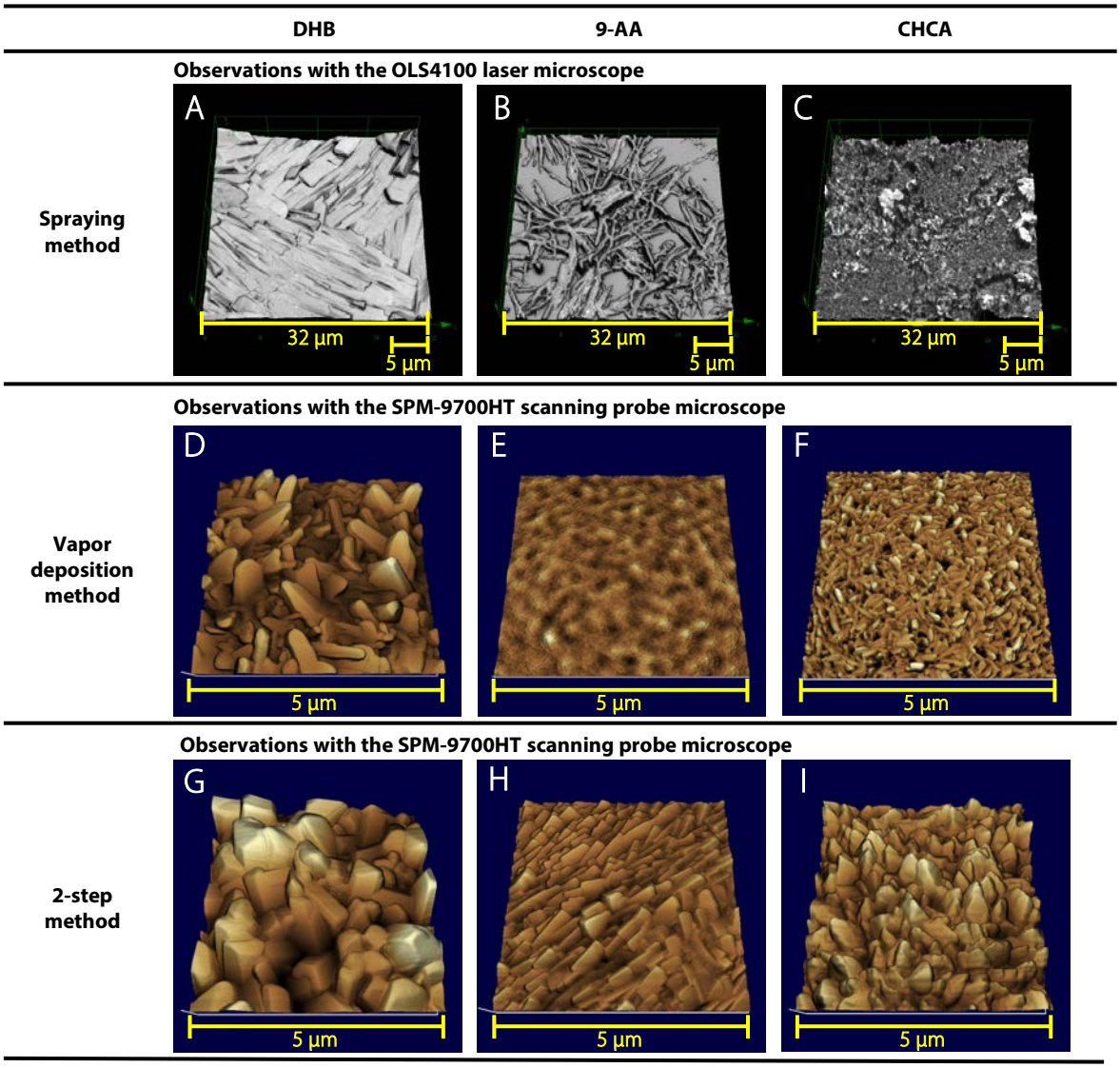

Fig. 4 Crystallized surfaces for matrices applied with three different application methods

Table 1 Application conditions for compound spreading comparison

Application method	Detailed method
Spraying method	20 mg/mL 9-AA 100% methanol sprayed on using the iMLayer AERO
2-step method	9-AA applied via vapor deposition with thickness of 1.0 µm using the iMLayer, then 10 mg/mL 9-AA 80% methanol sprayed on using the iMLayer AERO

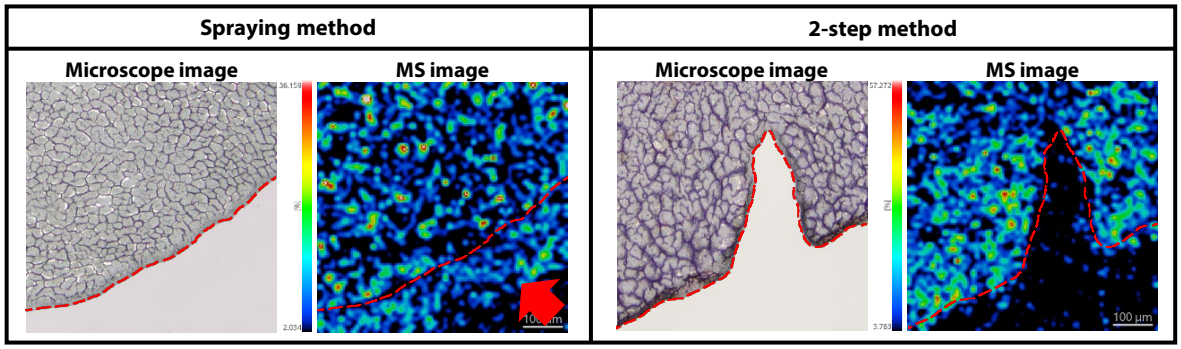

Fig. 5 Comparison of compound spreading in mouse liver samples using different matrix application methods

■ Comparison of compound spreading

The unwanted spreading of compounds across the sample plate can be an issue when applying matrices with the spraying method. To check the extent of compound spreading with the spraying method, and in turn how much this problem is alleviated by employing the 2-step method, we applied 9-AA to mouse liver samples using these two methods and carried out MS imaging. The conditions for applying the matrix are listed in Table 1. For both methods, spraying was carried out using the iMLayer AERO automatic matrix sprayer (Fig. 6). The iMScope QT mass microscope was used so that mass spectrometry could be carried out at the same positions as the optical observations.

With the spraying method, we observed that compounds spread around 200 µm outside the mouse liver sample (Fig. 5 left, red arrow), and that with the 2-step method, this spreading was limited to only around 5-20 µm (Fig. 5 right).

■ Comparison of extraction effects

For MS imaging of hair samples, both high sensitivity and high resolution are needed. Depending on the compounds involved, this may require high extraction efficiency. We expect the 2-step method to increase extraction efficiency while maintaining high resolution.

Fig. 6 The iMLayer™ AERO automatic matrix sprayer

Here we have carried out MS imaging on hair samples using the vapor deposition and 2-step methods to demonstrate the high extraction efficiency of the latter. These matrix application methods were carried out on cross-sections of hair that had been soaked in drug solutions for 24 hours. We then compared the sensitivity. It was found that the 2-step method resulted in a signal intensity about 4 times higher than for vapor deposition alone (Fig. 7b, d). This is believed to be due to higher efficiency of extraction from the sample.
(For a detailed analysis example of drug distribution in hair, refer to Application News No. B75.)

On the other hand, cholesterol is a compound which can only be detected using the vapor deposition method, not with the spraying or 2-step methods. That is to say, these methods do not always result in higher sensitivity than vapor deposition.

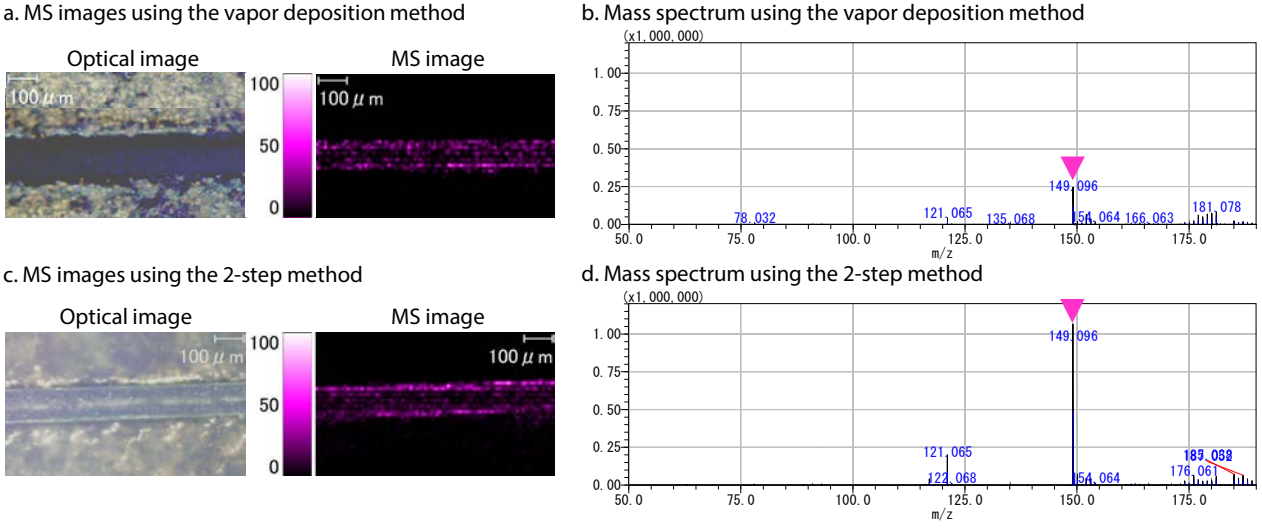

Fig. 7 Differences in sensitivity due to matrix application method in high-resolution (10 µm) MS imaging of hair samples

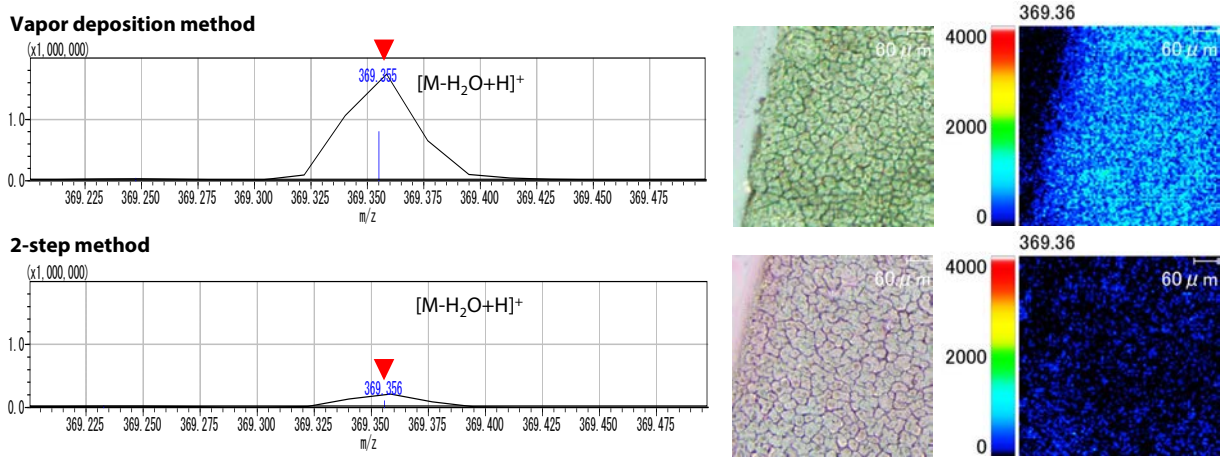

Fig. 8 MS imaging of cholesterol in mouse liver samples using the vapor deposition method and 2-step method

Fig. 8 shows an analysis of cholesterol in mouse liver samples, with the matrix CHCA applied via vapor deposition and the 2-step method. The extra spraying involved in the 2-step method results in a sensitivity 10 times lower. It can be seen that in some cases vapor deposition is the preferable application method, and the target compound must be taken into account when choosing the optimal method.

■ Conclusions

The results of our comparisons are summarized in Table 2.

We observed the matrices with a laser microscope and scanning probe microscope to determine crystal size (Table 2, ①) and uniformity (Table 2, ②). We found that the spraying method results in relatively large and irregular crystals, whereas the vapor deposition and 2-step methods allow the matrix to be applied more uniformly and with smaller crystals.

With regards to compound spreading (Table 2, ③), we compared the spraying and 2-step methods and found that spraying alone resulted in a spread of around 200 µm outside the tissue, whereas carrying out vapor deposition before spraying (the 2-step method) limited the spread greatly.

We then compared the vapor deposition and 2-step methods in terms of extraction efficiency (Table 2, ④). In the case of hair treated with a drug, the 2-step method provided superior extraction, resulting in a detection efficiency around 4 times higher. This indicates that the 2-step method is preferable when high extraction efficiency is required.

However, in the case of low-molecular-weight compounds, such as cholesterol, phospholipids and tertiary amines, which do not require high extraction efficiency, vapor deposition alone may be preferable if it is shown to provide high enough sensitivity. This is because the vapor deposition method is generally superior in terms of spatial resolution and limiting the spread of compounds.

The choice between vapor deposition and the 2-step method must be taken on a case-by-case basis. Therefore, when there are no previous examples to refer to, analysis must be carried out to determine which method is preferable. In this case, first apply the matrix via vapor deposition and analyze part of the sample. If the target compounds can be detected, carry on with MS imaging. If they cannot, apply an extra matrix layer via spraying (Fig. 9). In either case, the vapor deposition step is key for high-resolution MS imaging.

Table 2 Advantages and disadvantages of each matrix application method

	Spraying method		Vapor deposition method		2-step mehod	
① Crystal size	Large	(△)	V. small	(◎)	Small	(○)
② Uniformity	Low	(△)	High	(◎)	Medium	(○)
③ Compound spreading	High	(△)	None	(◎)	Low	(○)
④ Extraction efficiency	High	(◎)	Low	(△)	High	(◎)
If high extraction efficiency is not needed			Optimal			
If high extraction efficiency is needed					Optimal	

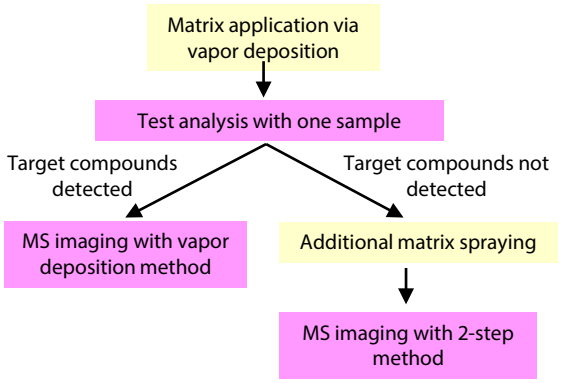

Fig. 9 Flowchart for selecting a matrix application method for MS imaging

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