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Application News

Imaging Mass Microscope iMScope™

High spatial-resolution MS imaging of longitudinal- and transverse- cross sections of drug-incorporated hair

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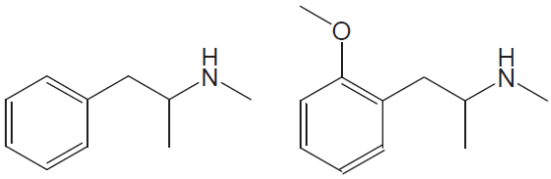
User Benefits

- The use of iMScope and iMLayer enables high spatial resolution MS imaging of a minimum of approximately 5 µm.
- High spatial-resolution MS imaging makes it possible to visualize drug distribution even in fine regions such as hair interior.
- The analysis of the longitudinal section of the hair allows us to observe the history of drug intake, and the analysis of the transverse section of the hair allows us to observe the degree of drug penetration.

■ Introduction

It is known that hair accumulates incorporated drugs over a period of months to more than a year, and therefore, the analysis of hair provides scientific evidence that includes time periods of drug exposure. For this reason, hair analysis using LC and LC-MS is often used in the investigations of drug crimes. However, these techniques are cumbersome, and it is difficult to obtain information about the localization of drugs in the hair or on the surface. In order to solve this problem, MS imaging technology has been receiving attention in recent years. To demonstrate the effectiveness of MS imaging, we have used methoxyphenamine (MOP) (Figure 1), which is structurally similar to methamphetamine, a type of antihypnotic, as a model drug to visualize the drug distribution in hair by MS imaging. In order to verify its usefulness, high spatial resolution MS imaging of both 'hair after MOP administration' ("user's hair") and 'hair immersed in MOP solution' ("soaked hair") has been performed for each longitudinal- and transverse- cross sections. As a result, the significance of visualization using MS imaging was confirmed and thus it is introduced in this article.



Methamphetamine
Formula : $C_{10}H_{15}N$
MW : 149.233

Methoxyphenamine (MOP)
Formula : $C_{11}H_{17}NO$
MW : 179.263

Figure 1 Structural Formula of Methamphetamine and Methoxyphenamine

■ Preparation of User's Hair and Soaked Hair

Male volunteers with black hair took an over-the-counter drug containing 50 mg of MOP hydrochloride 3 times daily for 5 consecutive days, followed by a 19 day rest period, then another 5 days, followed by a 13 day rest period. Hair was collected from the roots after the course of drug intake and rest periods were completed (Table 1). Soaked hair was prepared by taking hair from the roots of male volunteers before drug intake and immersing them in MOP hydrochloride solution (The amount of drug in the hair was determined to be 20 to 83 ng/mg by LC.; Please refer to Application News No. B75).

■ Structure of Hair and Preparation of Longitudinal- and Transverse- Cross Sections

The hair is about 50 to 150 µm in diameter. From the surface, hair is composed of three layers: the cuticle, the cortex, and the medulla. A cross section parallel to the hair axis is called the longitudinal section and a cross section perpendicular to it is called the transverse section (Figure 2). The longitudinal section

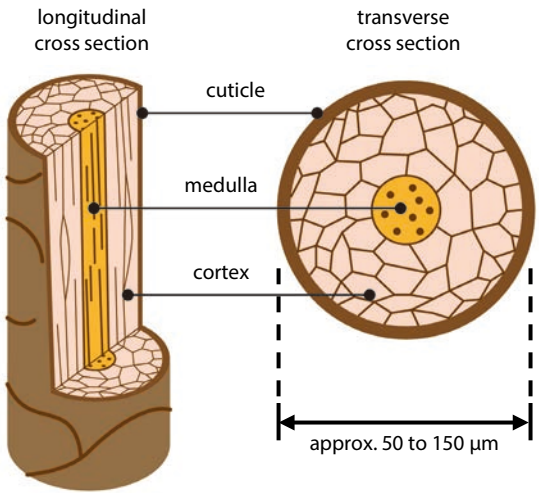


Figure 2 Enlarged View of Cross Sections of Hair

Table 1 Administration History of Drug Containing MOP Hydrochloride

(1) First dose *	Day 1 to 5
(2) Suspended	Day 6 to 24
(3) Second dose *	Day 25 to 29
(4) Suspended	Day 30 to 42
(5) Sampling	Day 43

* 50 mg each in the morning, noon and evening

Figure 3 iMLayer™

was made with a microtome and fixed to an ITO slide glass via conductive double-sided tape. Transverse sections were embedded in carboxymethylcellulose (CMC), sectioned using a cryostat, and fixed on ITO slides.

■ MS Imaging Analysis Conditions

CHCA was used for ionization assistance as matrix. In order to realize high spatial resolution imaging, it is necessary not only to apply the matrix uniformly but also to minimize the size of matrix crystals. Therefore, iMLayer (Figure 3) was used for the matrix coating. A mass spectrometer equipped with a microscope is suitable for observing fine parts such as cross sections of hair. For this reason, we used an imaging mass microscope, iMScope (Fig. 4), which can perform operations seamlessly from microscopic observation to mass spectrometry. The analysis conditions for MS imaging are shown in Table 2.

■ MS Imaging of Longitudinal Cross Section of Hair

First, we performed MS imaging of longitudinal section of hair with a low spatial resolution of 50 µm. As mentioned above, hair has a chronological history of taking the drug. Figure 5 shows the prediction diagram for drug distribution based on the dosing history shown in Table 1. Two drug-positive areas

Figure 4 iMScope™ QT
Successor to iMScope *TRIO*™. Adoption of Q-TOF type mass spectrometer (LCMS -9030) realizes dramatic improvements in mass resolution, mass accuracy, detection sensitivity, analysis speed and so on.

Table 2 Analysis Conditions for MS Imaging

Matrix Coating		
Instrument Name	:	iMLayer
Matrix Used	:	CHCA
Coating Method	:	Deposition with 0.7 µm Thickness
Mass Spectrometry		
Instrument Name	:	iMScope *TRIO*
Spatial Resolution (Pitch)	:	5 /10 / 50 µm
Polarity	:	Positive
Mass Range	:	*m/z* 100 - 185
MS Stages	:	2 (MS/MS)
Precursor Ion	:	*m/z* 180.1
Laser Irradiation Number	:	50 or 100 [shots]
Laser Repetition Frequency	:	1000 [Hz]
Laser Diameter Setting	:	0 (Approx. 5 µm) / 1 (10 µm) / 4 (50 µm)
Laser Intensity	:	0 / 21.7-30.0 / 56.4-63.0

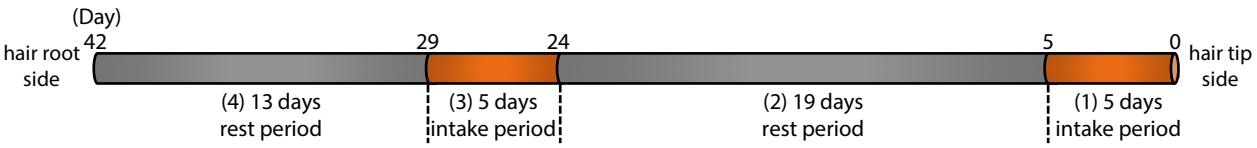


Figure 5 Prediction Diagram for Drug Distribution Based on the Dosing History

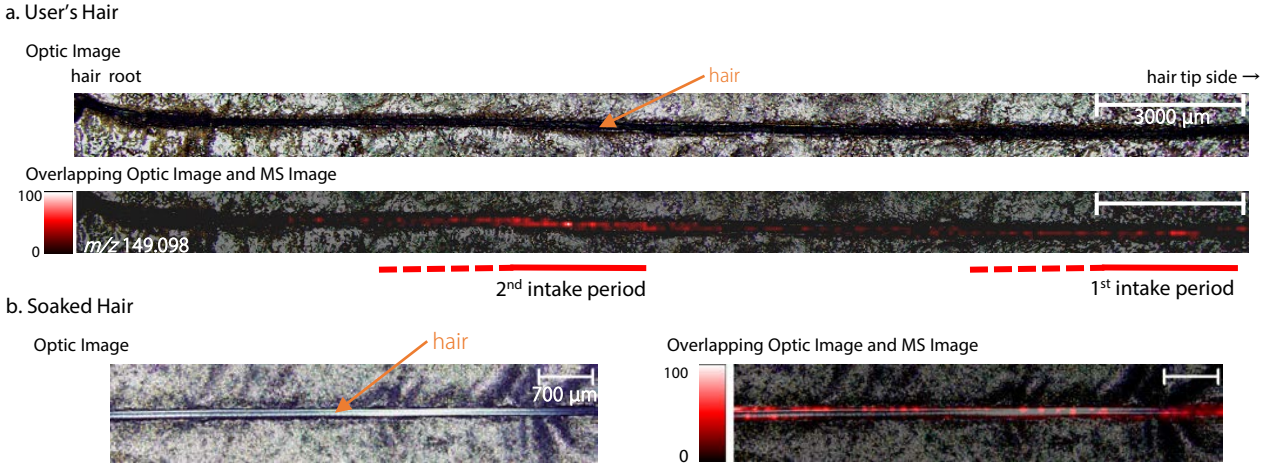


Figure 6 MS Images of Longitudinal Section of Hair at Low Spatial Resolution of 50 µm
(a) User's Hair, (b) Soaked Hair

corresponding to a 5-day dosing period and drug-negative areas corresponding to a suspended period between them were visualized in the user's hair (Figure 6a). This visualized MS image correlates with the prediction diagram for drug distribution, suggesting that hair MS imaging is an effective means to confirm drug dosing history. On the other hand, almost uniform drug distribution was confirmed in the length direction of the soaked hair sample (Fig. 6b). Next, we performed MS imaging of the longitudinal sections of hair samples with a high spatial resolution of 10 µm (Figure 7). The drug was clearly observed to be localized to the peripheral of the soaked hair (Figure 7b), but not in the user's hair (Figure 7d).

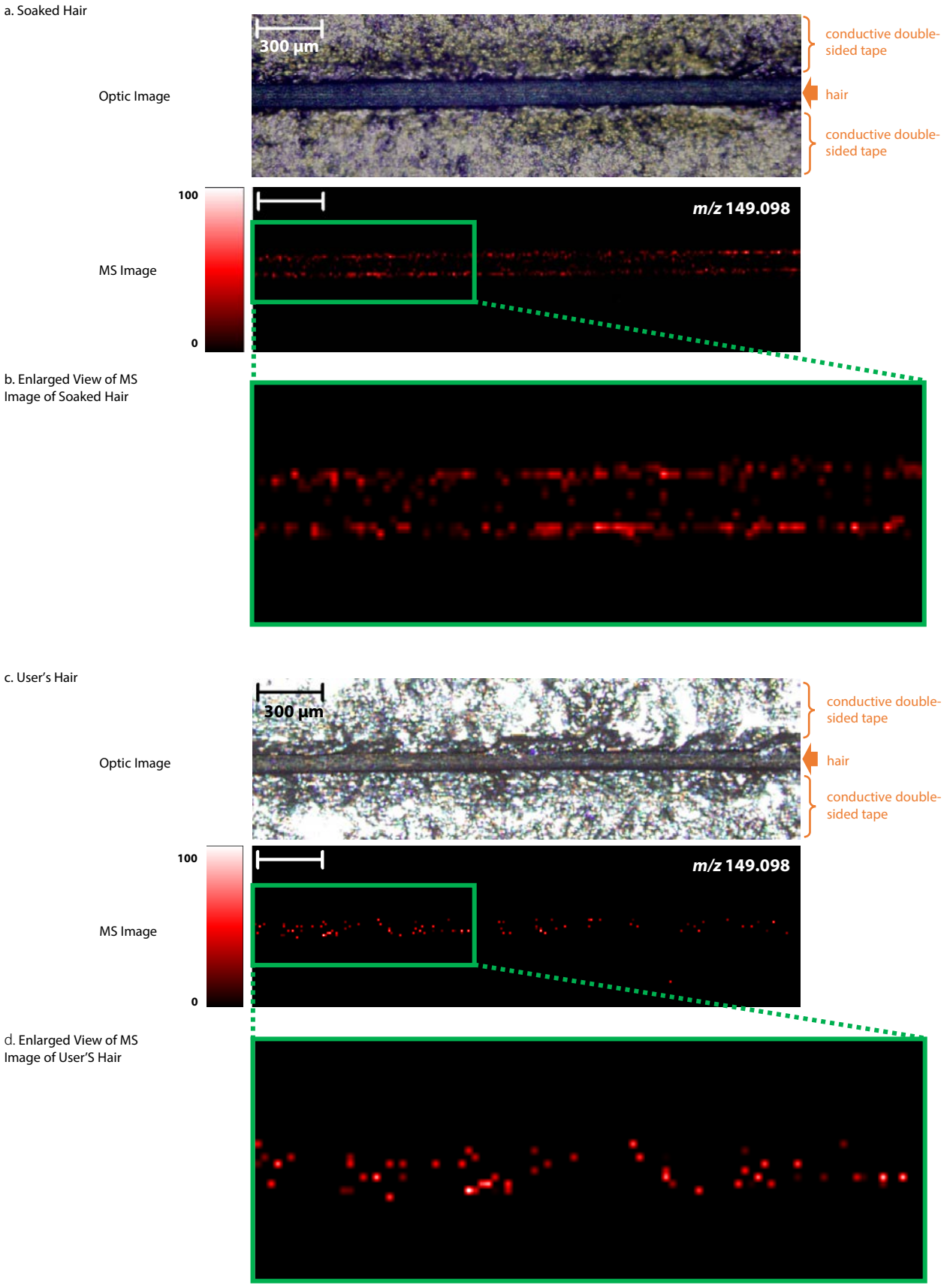


Figure 7 Detailed MS Images of Longitudinal Section of Hair at High Spatial Resolution of 10 µm
Soaked Hair (a, b), User's Hair (c, d)

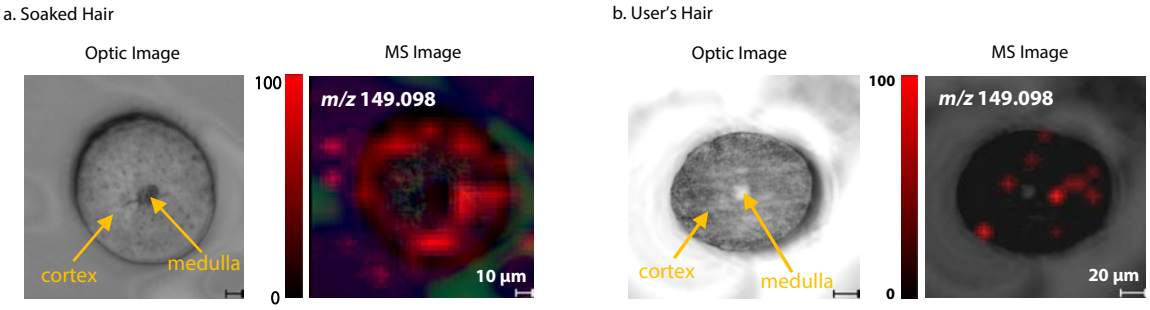


Figure 8 MS Images of Transverse Section of Hair at High Spatial Resolution of 10 µm
(a) Soaked Hair, (b) User's Hair

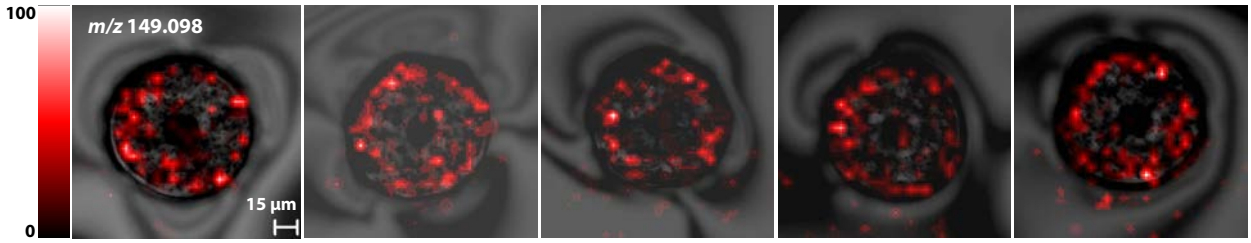


Figure 9 5 µm High Spatial Resolution MS Images of Transverse Serial Sections of Soaked Hair

■ MS Imaging of Transverse Cross Section of Hair

In order to observe drug distribution in hair more clearly, we performed MS imaging with a high spatial resolution of 10 µm using transverse cross section of a hair sample. The circular localization of the drug in the periphery was observed in the soaked hair (Figure 8a), but not in the user's hair (Figure 8b). These results are consistent with the MS imaging of the longitudinal section shown in Figure 7. Next, we performed MS imaging using serial sections of soaked hair with an even higher spatial resolution of 5 µm. A clearer MS image was obtained than the MS image obtained at a spatial resolution of 10 µm, and the drug localization in the hair periphery became clearer (Fig. 9). In addition, it was confirmed from each MS image of the serial sections that this method including pretreatment and mass spectrometry was performed with good reproducibility. It is important to distinguish between voluntary and passive (for example, by smoking) drug use, but conventional methods such as LC and LCMS cannot distinguish between them. High spatial resolution MS imaging of transverse section of hair is expected to be a new analytical method that makes this possible..

■ Conclusion

Hair can be likened to magnetic tape, which records the history of drug use, but the detailed mechanism of drug uptake is not revealed. Visualization of drugs in hair is an important and difficult subject in forensic medicine and forensic toxicology. In addition, in order to visualize small amounts of drugs buried in complex matrices on a microscopic scale, it is important to detect drugs with high spatial resolution and high sensitivity. As mentioned above, high spatial resolution MS imaging using iMLayer and iMScope enables easy and clear observation of drug localization in longitudinal and transverse sections of hair samples. This method can be applied not only to drug analysis of hair in forensic medicine and doping tests, but also to the development and evaluation of various hair care products for the purpose of maintaining and promoting hair beauty and health.

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