PO-CON1535E

Study of experimental conditions for MALDI Imaging by using highly controlled sublimation technique

ASMS 2015 WP 528

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Overview

- New sublimation technique controls thickness of matrices precisely.
- The sublimation technique is combined with a new MALDI-TOF/TOF MS.
- Analysis at 10 μm spatial resolution is conducted in both MS and MS/MS.
- Resolving power in MS/MS, up to 10,000, is utilized to visualize distinctive images.
- Classification of obtained images in similarity is performed by a software.

Introduction

Sublimation technique for coating matrices is known to generate highly uniform and fine crystals of matrices, which enables to visualize precise distribution of targeted molecule in MALDI imaging. A thickness of coated matrix is necessary to be controlled to achieve high reproducibility. However, controlling the thickness is frequently hard, because it is managed only by elapsed time of sublimation. Measuring increased weight of the coated subject is reliable, but it should be done after coating. Recently, we developed a new sublimation technique, in which the thickness of matrix is monitored in real time by variation of laser light transmission. We will report an experimental condition of the sublimation technique for MALDI imaging and apply it to a new vacuum MALDI-TOF/TOF.

Methods

Condition for Sublimation

Sample	: mouse cerebellum
Instrument	: iMLayer (Shimadzu Corp.)
Matrices	: 9-AA, DHB
Thickness of matrix	: 1 to 4um, monitored by variation of laser light transmission

Recrystallization[3] was conducted following the sublimation.

MALDI-tandem TOFMS

Instrument	: MALDI-7090 (Shimadzu/Kratos)
Measurement	: High-energy CID-MS/MS in positive ion mode.
Collision gas	: helium
Collision energy	: 20 keV (laboratory frame of reference).

Principle of Axial Spatial Distribution Focusing (ASDF)

MS/MS resolution is improved with correcting the spatial distribution of ions in ASDF cell. This is achieved by applying a pulsed electrostatic field at the point, at which the precursor and fragments are velocity focussed but are spatially resolved.

Statistical analysis

Imaging MS Solutions (Shimadzu Corp.) was applied to visualize all images and to classify them in similarity.

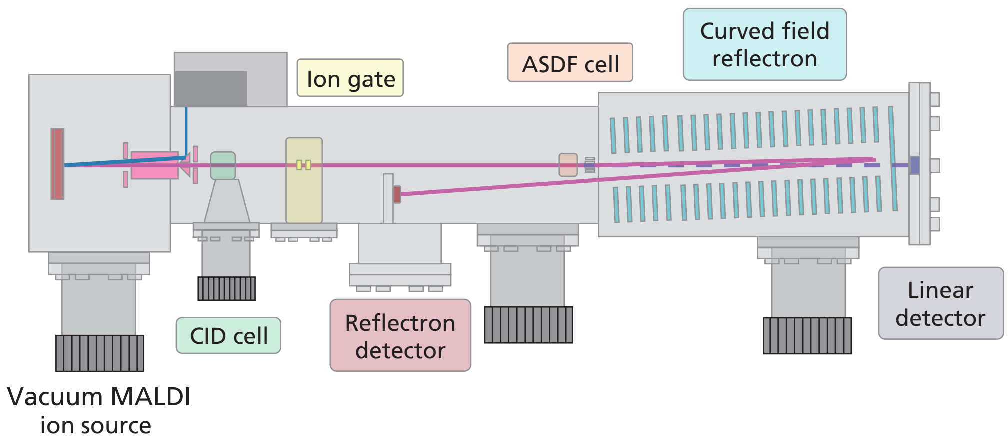


Fig.1 Inside view of MALDI-7090

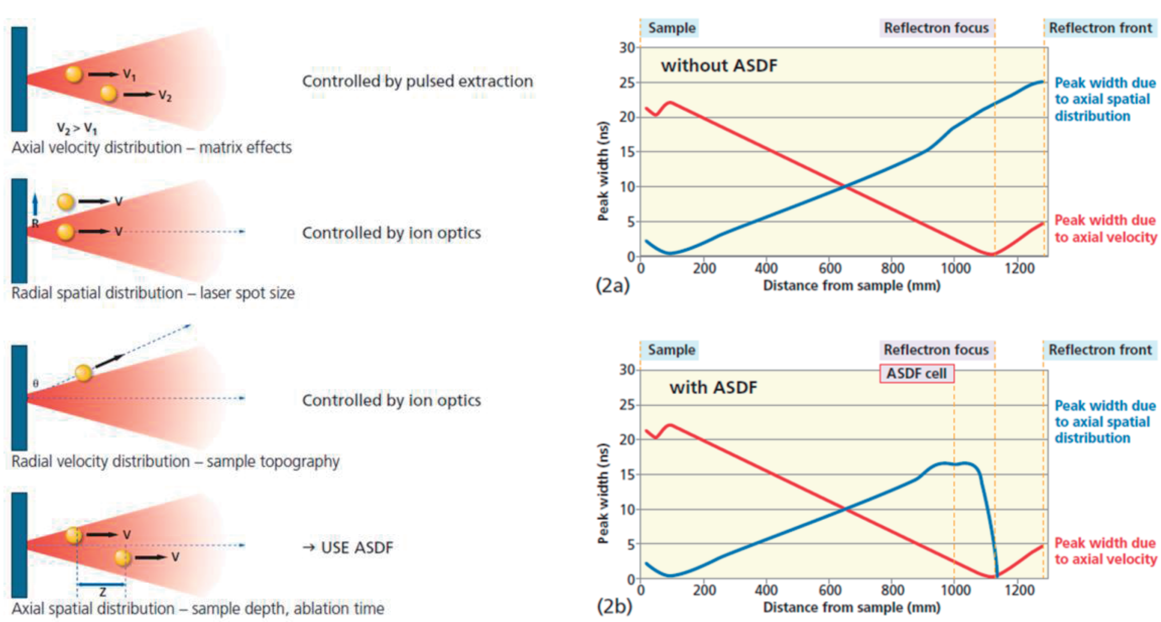
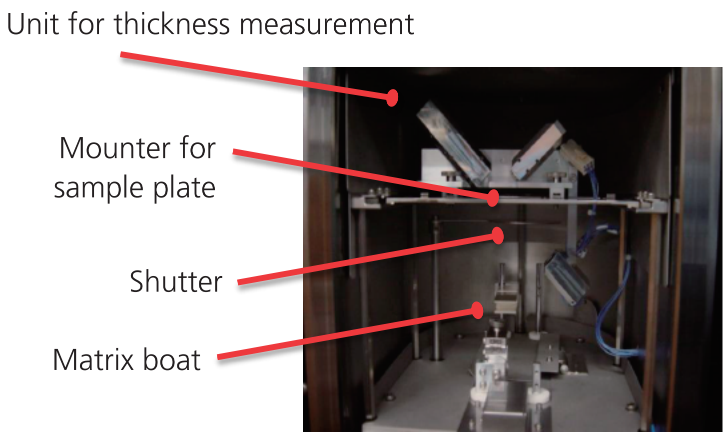


Fig.2 Axial Spatial Distribution Focusing

Results



Features

- Fine crystal size
- Control of matrix layer thickness
- Automated procedure
- Compact size, enough to be installed in a fume hood.

Fig.3 Principle of iMLayer

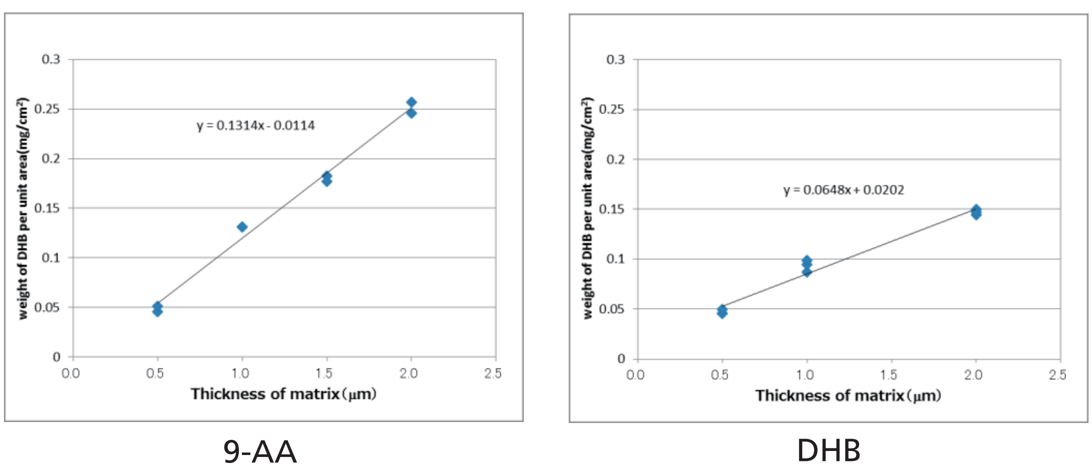


Fig.4 Thickness and amount of matrices

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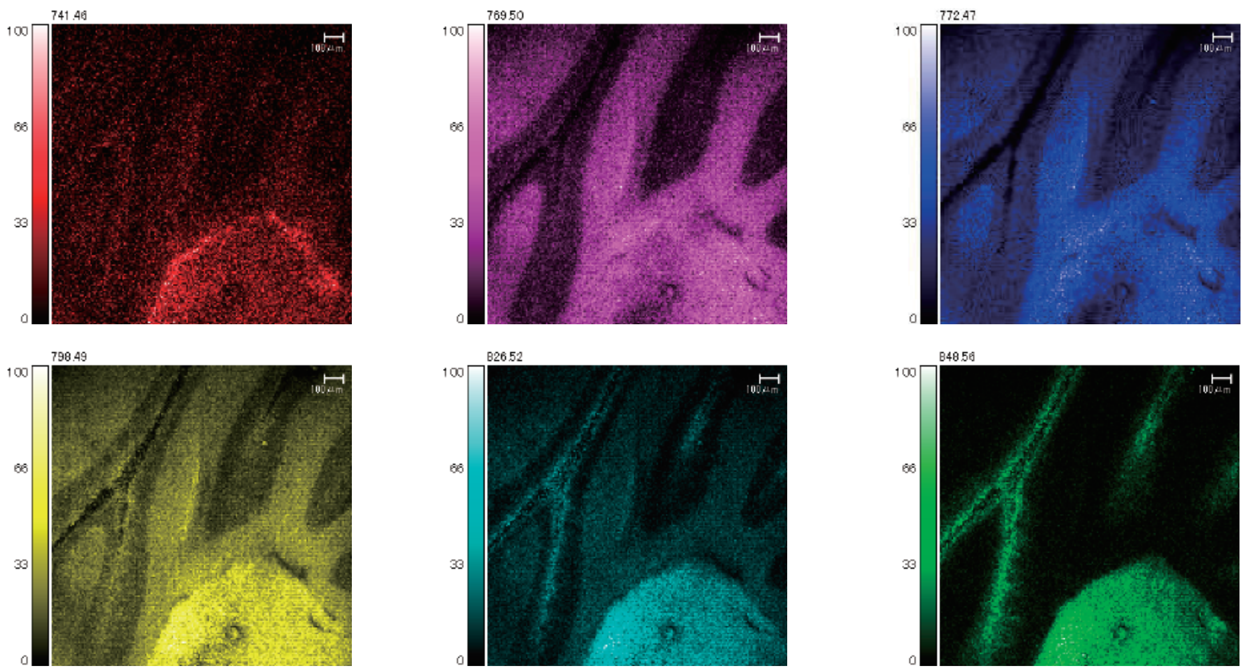
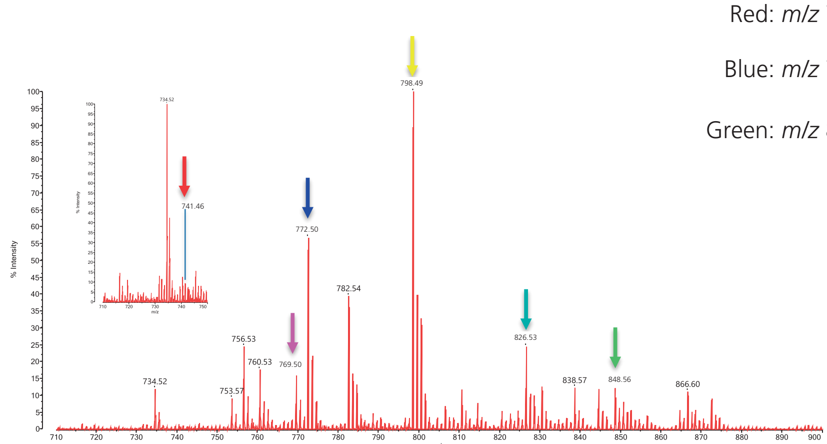
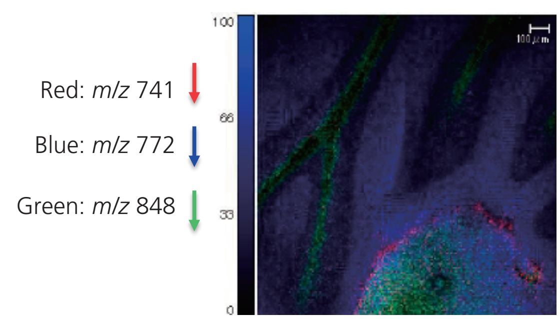


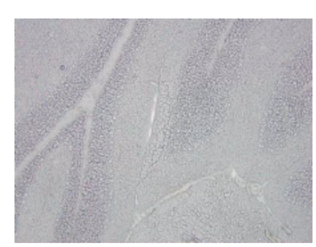
MS image of the *m/z* indicated with arrows in the spectrum below.



MS spectrum for a whole area of cerebellum



Merged view of MS imaging



Optical image of the tissue

Fig.5 MS imaging of cerebellum at 10μm spatial resolution

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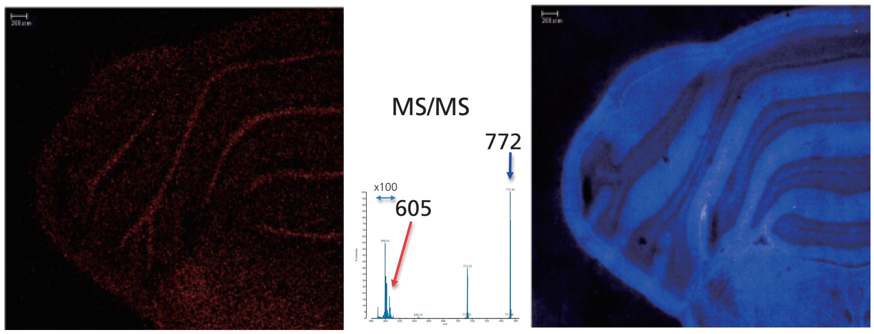
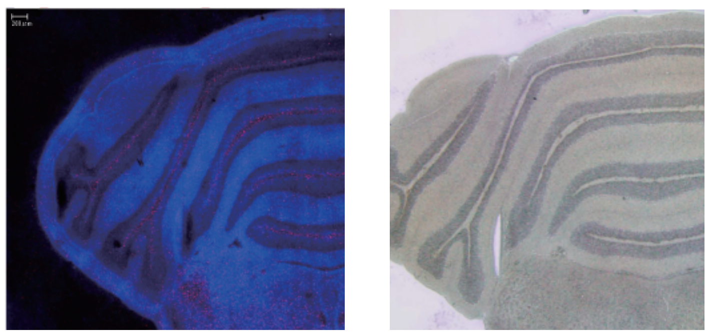
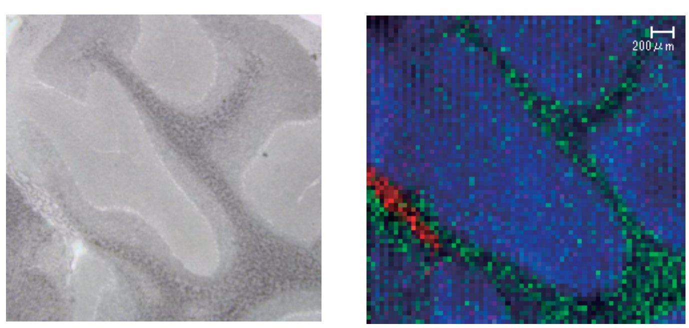


m/z 605; fragment ion   *m/z* 772 ; precursor



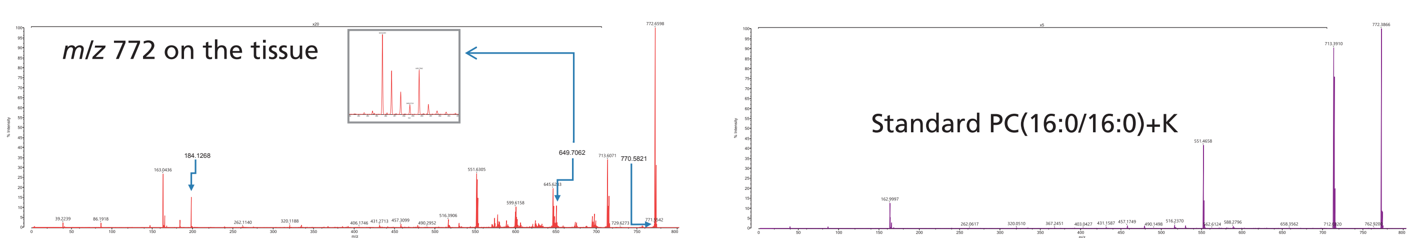
Merged view of *m/z* 772 & 605   Optical image of the tissue

Fig.6　MS/MS imaging at 10μm spatial resolution



Green: *m/z* 184
Red: *m/z* 649
Blue: *m/z* 772

Optical image of the tissue   Merged view of MS/MS imaging



Comparison of MS/MS spectrum between PC(16:0/16:0)+K and *m/z* 772 in the tissue.

Fig.7　MS/MS imaging at 30μm spatial resolution (Precursor: *m/z* 772, different specimen from “Fig.6”)

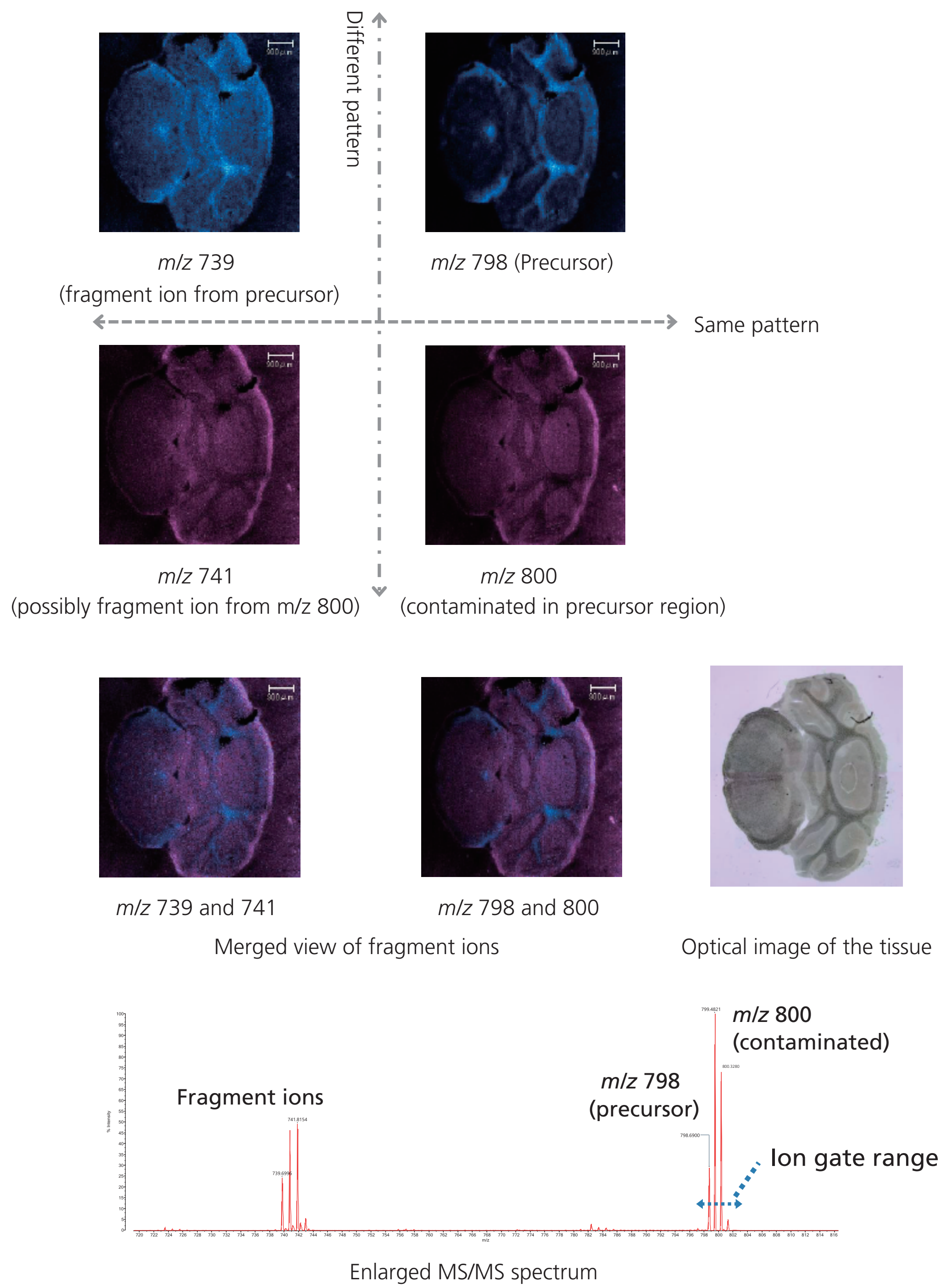


Fig.8 MS/MS imaging at 30μm spatial resolution (Precursor: *m/z* 798)

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Conclusions

- High reproducibility of sublimation was obtained by monitoring the thickness of matrices in real time.
- The sublimation technique and the new MALDI-TOF/TOF was optimum combination, especially, to have high quality MS/MS imaging in terms of reproducibility and high spatial resolution.
- Well-resolved signals at both precursor region and fragment ions by ASDF can be utilized to visualize distinctive images so clearly that they are classified in similarity using statistical analysis.

References

(1) Bouschen, W., et al; Rapid Commun. Mass Spectrom. 2010, 24 (3), 355–364.
(2) Hankin, J. A., et al; J. Am. Soc. Mass Spectrom. 2007, 18 (9), 1646–1652.
(3) Yang, J., et al; Anal. Chem. 2011, 83, 5728–5734

First Edition: May, 2015

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