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Development of a high sensitivity atmospheric MALDI-2 source for imaging mass spectrometer

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1. Introduction

◆ MALDI imaging enables label-free and wide-range analysis of the spatial molecular distribution. Cutting-edge applications of MALDI imaging require high sensitivity to get deeper insights. Unfortunately, the inherent ionization efficiency of MALDI is low. Therefore, it is important to improve MALDI sensitivity through post ionization. Laser based post ionization (MALDI-2) was introduced by Soltwisch et al[1]. Although MALDI-2 is mostly performed in low-vacuum condition, Niehaus et al.[2] and Frache et al.[3] have applied MALDI-2 on transmission MALDI and normal MALDI sources operated in atmosphere, respectively. Herein, we completed MALDI-2 upgrade on a commercial instrument with atmospheric reflection-geometry MALDI source, and the superior features of AP MALDI-2 were investigated through experiments and demo applications.

2. Methods

◆ **Instrument upgrade:** We developed AP MALDI-2 on Shimadzu Imaging Mass Microscope (iMScope^{TM} QT) (**Fig. 1**). A second nanosecond laser (laser-2) with 266-nm wavelength was installed, and the beam of laser-2 was parallel to the sample plate while at 0.5 mm above the plate. The position of the laser-2 beam could be precisely adjusted to aim at the particle plume ablated by laser-1. Microscope function of iMScope was maintained in our upgraded instrument.

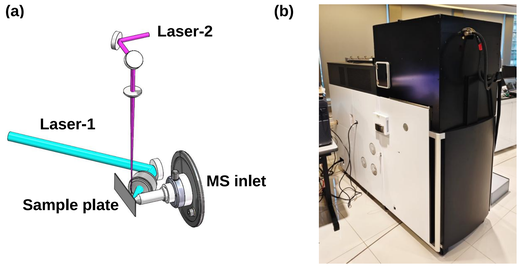

Fig. 1 **(a)** Illustration of the second laser for post ionization. **(b)** Photo of the upgraded iMScope QT with MALDI-2.

◆ **Sample preparation:** 8-μm-thick tissue sections were produced by a cryotome and were mounted onto ITO glass slides. DHB matrix was vapor-deposited on the sample surface using iMLayer^{TM} (Shimadzu).

◆ **Data acquisition:** Following parameters were used to acquire data with the upgraded iMScope QT. Polarity: positive. Laser repetition rate: 1000 Hz. Delay time between laser-2 and laser-1 pulses: 105 μs. Laser-2 pulse energy: 600 μJ. Pixel size: 20 μm. Laser shots: 30. DL temperature: 250 ℃. Heat block temperature: 450 ℃.

◆ **Data analysis:** MSI data were analyzed using IMAGEREVEAL^{TM} software (Shimadzu).

3. Results

◆ During acquisition of standard samples, the position of laser-2 beam was scanned across MALDI particle plume using movable reflectors as illustrated in **Fig. 1(a)**, and changes in signal intensity were recorded according to relative positions of laser-2 beam (**Fig. 2**). It was found that the positional tolerance of laser-2 beam for optimal sensitivity was very critical, that is, within 0.1 mm at the transverse direction of MALDI plume. As a result, real-time positional adjustment of laser-2 beam was performed before imaging experiments. Besides, the delay time between laser-2 and laser-1 pulses was found to be less critical than vacuum MALDI-2, and the optimal delay time was longer.

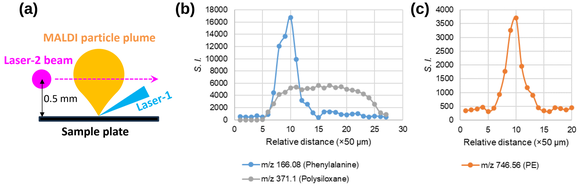

Fig. 2 **(a)** Illustration of the scanning of the position of laser-2 beam. **(b)** Phenylalanine sample. Polysiloxane ($(C2H6SiO)_5$, m/z = 371.10) from ambient air was also ionized by laser-2. **(c)** PE (18:0-18:1) sample.

◆ Using rat liver homogenate (**Fig. 3(a)(b)**), it was found that AP MALDI-2 could significantly increase the signal intensity of cellular lipids around m/z 600, where protonated diacylglycerols (DG) were detected with a loss of water. For lipids around m/z 700~900, although the signal intensity improvement for the predominant phosphatidylcholines (PC) was not significant, the number of detected m/z features was increased, including those lipids not well-detected in original MALDI due to ion suppression effects.

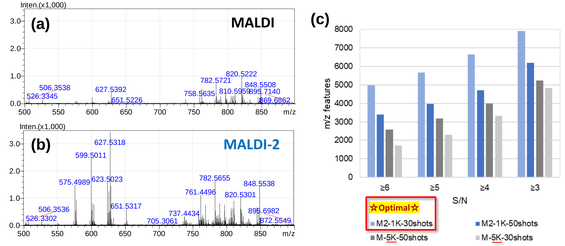

Fig. 3 Averaged MS spectra of rat liver homogenate obtained with **(a)** MALDI and **(b)** MALDI-2. Laser repetition rate was 1 kHz. **(c)** Number of detected m/z features from averaged MS spectra obtained with 1-kHz MALDI-2 and 5-kHz MALDI. Mass range: 500~900 m/z. Optimal parameters: M2 (MALDI-2) – 1K (laser repetition rate) – 30 shots (per pixel).

◆ Although the highest repetition rate of laser-2 was only 1 kHz, we found that the number of detected m/z features in MALDI-2 was 50% more than that in the original instrument operated with 5 kHz laser (**Fig. 3(c)**, S/N ≥ 3).

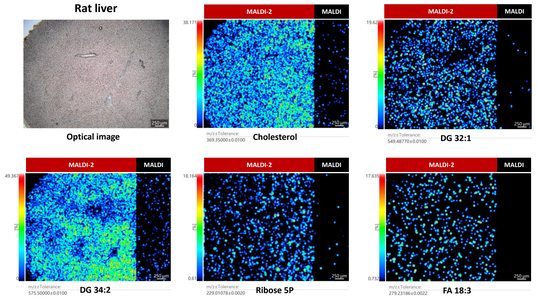

Fig. 4 Optical image and MS images acquired from rat liver using iMScope with MALDI-2. Left half of each MS image was acquired with laser-2 ON, while right half was acquired with laser-2 OFF. Displayed metabolites: [cholesterol - H_2O + H]$^+$, [DG 32:1 – H_2O + H]$^+$, [DG 34:2 – H_2O + H]$^+$, [ribose 5P + H]$^+$, [FA 18:3 + H]$^+$.

◆ In the imaging of rat liver (**Fig. 4**) and rat heart (**Fig. 5**), we turned on/off laser-2 to compare MALDI and MALDI-2. The visualized results revealed that the sensitivity of cellular lipids and small-molecule metabolites was improved by MALDI-2, and many previously unseen metabolites was now detected. These results indicated that AP MALDI-2 upgrade would promote MSI differentiation based on a more extensive chemical signature.

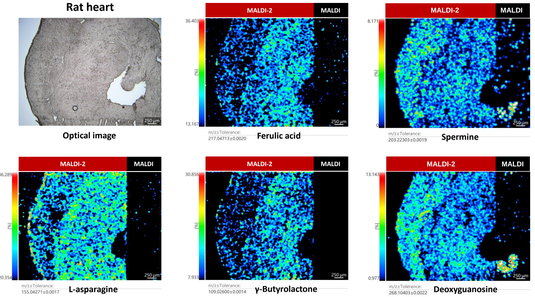

Fig. 5 Optical image and MS images acquired from rat heart using iMScope with MALDI-2. Left half of each MS image was acquired with laser-2 ON, while right half was acquired with laser-2 OFF. Displayed metabolites: [ferulic acid + Na]$^+$, [spermine + H]$^+$, [L-asparagine + Na]$^+$, [γ-butyrolactone + Na]$^+$, [deoxyguanosine + H]$^+$.

4. Conclusion

◆ AP MALDI-2 was implemented on iMScope QT.

◆ Alignment of laser-2 beam was critical in AP MALDI-2.

◆ AP MALDI-2 upgrade could improve the sensitivity of cellular lipids and small-molecule metabolites and reveal more m/z features in MS imaging.

Reference

1) J. Soltwisch et al., *Science* **348**, 211-215 (2015).
2) M. Niehaus et al., *J Am Soc Mass Spectrom* **31**, 2287-2295 (2020).
3) G. Frache, **ASMS 2024**.

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