# **SHIMADZU**

# Multi-laser induced MALDI post ionization (MALDI-2+) on a MALDI/ESI dual source

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# I. Introduction

Laser induced MALDI post ionization (MALDI-2) has been reported, in which a second laser intersects and interacts with the MALDI plume ablated by the first laser<sup>1</sup>. MALDI-2 could produce a sizable increase in overall signal intensity, as well as the number of detectable peaks in the mass spectra. Herein we multiplexed the intersecting times between laser-2 and MALDI plume and proved that MALDI-2 sensitivity could be further improved, especially due to the increased spatial coverage of laser-2. We named this technology MALDI-2+. Our experiments were conducted on a novel MALDI/ESI dual source, coupled with Q-TOF. The combination of high sensitivity LC-MS and MALDI imaging could facilitate the study of spatial omics.

# 2. Methods

#### 2-1. MALDI/ESI dual source

We developed a MALDI/ESI dual source on a modified Q-TOF instrument (Shimadzu LCMS-9030<sup>®</sup>). A T-shaped segmented quadrupole ion guide (Fig. 1) was designed to transmit MALDI and ESI ions from different inlets. ESI ions goes straight forward, while MALDI ions enters the quadrupole orthogonally. This configuration could provide maximum ESI sensitivity, and allows the incident MALDI ablation laser to be set closer and perpendicular to the sample plate, increasing MSI resolution.



Figure 1 Design of the dedicated ion guide. Scheme (A), photo (B) and simulations (C) of T-guide.

#### 2-2. MALDI-2+

For MALDI-2+, with a concave reflector, the reflecting laser-2 intersected MALDI plume once more. Moreover, with two concave reflectors forming an optical cavity, laser-2 was reflected multiple times for multiple intersections. The reflectors were mounted on a three-dimensional piezoelectric moving stage in the vacuum chamber for real-time adjustment. (Fig. 2)



Figure 2 Top view (A) and side view (B) scheme of the ion source and principle of MALDI-2+

### 3. Results

#### 3-1. Evaluation of MALDI-2+

For standard samples of Caffeine, Chloroquine, Cholesterol, Reserpine and PE (18:0-18:1), MALDI-2+ could improve MALDI sensitivity by over an order of magnitude. Compared with MALDI-2, MALDI-2+ (SR, intersections x 2) showed ~2 fold sensitivity improvement (Fig. 3A). For Caffeine sample, MALDI-2+ (MR, intersections x 5) showed up to 3 fold improvement in Caffeine signal intensity, and >3 fold improvement in total ion signals, compared with MALDI-2. Some hardly detected signals in MALDI-2, such as DHB multimers, were increased by over an order of magnitude in MALDI-2+. (Fig. 3B)



Figure 4 Measuring the width of MALDI plume (A) and laser-2 spots (B).

#### **3-3. Detection of poorly soluble organic pigments**

MALDI is tolerant to the characteristics of samples, and thus it has the advantage in analyzing poorly soluble compounds which are difficult to detect with LC-MS. Some poorly soluble organic pigments, sometimes used in tattoo inks, are associated with tattoo side effects and health concerns<sup>2</sup>. Here we applied our system in detection of four organic pigments (P.R.254, P.B.15, P.V.19, P.R.170) and found that MALDI-2+ could significantly boost the sensitivity by  $1\sim2$  orders.

Then we diluted the pigment to test its LOD in tattoo ink (Table 1). P.R.254 was dopped in commercial tattoo ink to get a practical chemical background. We found that MALDI-2+ could reduce the detection limit of P.R.254 in tattoo inks by ~50 times. It is noteworthy that, for P.R.254 concentration of 5  $\mu$ g/mL, MALDI-2+ was able to detect this target analyte which was undetectable in conventional MALDI-2.

Concentration	Method	Signal Intensity	S/N	Result
Pigment / Tattoo ink = 1:1 P.R,254: 250 µg/mL	MALDI	260	1	Detected
	MALDI-2+	12900	/	Detected
Pigment / Tattoo ink = 1:10 P.R,254: 50 µg/mL	MALDI	×	/	Undetected
	MALDI-2+	4200	/	Detected
Pigment / Tattoo ink = 1:100 P.R,254: 5 µg/mL	MALDI	×	/	Undetected
	MALDI-2	132	2.3	Undetected
	MALDI-2+	327	4.1	Detected
Pigment / Tattoo ink = 1:1000 P.R,254: 0.5 μg/mL	MALDI	×	/	Undetected
	MALDI-2+	55	1.9	Undetected

Table 1 Detection of gradually diluted pigment doped in tattoo ink

#### 4. Conclusions and future directions

- An ESI/MALDI combined ion source is developed.
- Multi-laser induced MALDI post ionization (MALDI-2+) could improve MALDI-2 sensitivity.
  - Sensitivity improvement:
  - MALDI-2+(Single Reflection) / MALDI-2: 2 times
  - MALDI-2+(Multiple Reflections) / MALDI-2: up to 3 times
  - MALDI-2+ / MALDI: > 1 order of magnitude
- MALDI-2+ has been applied in detection of organic pigments from tattoo inks. • For the next step, we will apply MALDI-2+ in real-time microscope guided high-resolution MALDI imaging.

#### References

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