Supporting information

Rapid MALDI TOF Mass Spectrometry Imaging with Scanning Desorption Laser Beam

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This supporting information includes:
- Description of the laboratory-built mass spectrometer and settings.
- Commercial mass spectrometer settings.
- Figure S1. Schematic diagram of the high-voltage pulsing circuit.
Description of the laboratory-built mass spectrometer. The instrument features a 20-cm cubic 6-way cross chamber with three large UV-transparent windows (R. M. Jordan Co., Grass Valley, CA) used for laser beam introduction and sample observation. The chamber contains the ion source. A 2.6-m long flight tube is connected to the source through a gate valve, which allows a high vacuum to be maintained in the detection system during sample changes. A dual-stage ion mirror with dimensions and details is shown in Fig. 1A. The MALDI sample plate was mounted on a holder (repeller). The extraction electrode had a 10-mm central hole covered with a fine mesh. The distance between sample plate and the mesh was 4.5 mm. Small apertures were made on both sides of the extraction electrode to focus the tight laser beam on the sample plate from the side bypassing the mesh (Fig. 1B). The small diameters and symmetrical arrangement of these holes provided minimal distortion of the electrical field near the irradiated spot on the sample plate. The distance between the extraction and the last electrodes (the 25-mm hole in the latter was also covered with fine mesh) was 11.0 mm. UV aluminum mirrors allowed the laser beam and CCD camera to be focused on the target (Fig. 1B).

The system was evacuated by two turbomolecular pumps, Turbovac TW700 and Turbovac TW300 (Leybold Vacuum, Cologne, Germany) located directly under the ion source chamber and the flight tube, respectively.

The laser optics featured a beam expander, an iris shutter and a variable neutral density filter to adjust the laser pulse power, a set of mirrors and a quartz lens with a focal length 254 mm focusing the laser beam on the sample plate. The laser spot size was 30 x 120 µm (~3600 µm²).

A laboratory-developed programmable hardware unit controlled the output of high-voltage power supplies, six electro-pneumatic valves monitored the vacuum conditions inside the ion
source and the flight tube. It was also able to automatically pump/vent the system. The voltage in the linear mode was set to 14.20 kV on both the repeller and the extraction electrode. To accomplish a pulse-delay focusing, a 0.8-kV HV pulse with 70-ns rise time was applied across the repeller 730 ns after the laser pulse. The resolving power for the ACTH fragment 19-38 (2464.2 Da is a monoisotopic mass) in a linear mode was 4,000. To operate in the reflector mode at frequencies up to 1 kHz, the voltage on the repeller and the extraction electrode was set to 11.00 kV; a 4.00 kV pulse delayed by 190-ns was applied to perform the pulse-delay focusing. The ion mirror voltage was set to 10.98 kV across the first mirror stage and 15.05 kV across the second stage. To operate at a 4-kHz repetition rate, the voltage across the first grid was reduced to 10.45 kV in order to compensate for the higher current load and preserve resolving power. Details concerning the power supplies and the delayed extraction circuitry are shown in Fig. S1. The resolving power for ACTH ion signal \((m/z=2464.2)\) reached 15,000 for frequencies up to 4 kHz.

**Commercial mass spectrometer settings.** Measurements were made using the manufacturer’s supplied method \(RP-900-4500Da\): voltages on the ion source 1, ion source 2, lens reflector 1 and reflector 2 were set to 19.00, 16.70, 8.30, 21.00 and 9.65 kV, respectively. The diameter of the laser spot was 90 µm (~6400 µm²). The AutoExecute method \(Imaging_FC33_NoRaster\) for the imaging experiment used a constant laser power, 100 shots per a single-position MS acquisition, and no rastering at a measured pixel.
Figure S1. Schematic diagram of the high-voltage pulsing circuit. HV1: 0-30 kV power supply (model 2591P, CPS – Computer Power Supply, Tigard, OR, USA); HV2: 0-15 kV power supply (model 2591P, CPS); HV MOSFET Switch: 15 kV/30A (model HTS 151, Behlke, Kronberg, Germany); C1 = 9 nF; C2 = 8.5 nF; C3 = 1.4 µF; R1 = 2MΩ; R2 = 250 Ω; R3 = 436 kΩ. The TTL synch pulse from the delay generator is sent to operate the high voltage switch. During the 1-kHz measurements, HV1 was used to maintain a stable electric potential on both the repeller and extraction electrode. During the 4-kHz measurements, HV1 was used to maintain a stable electric potential on the repeller while power supply HV3 (model 15A24-P30 Ultravolt, Inc., Ronkonkoma, NY, USA) was used to maintain a stable potential on the extracting electrode.