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Investigating MALDI MSI parameters (Part 1) – A systematic survey of the effects of repetition rates up to 20 kHz in continuous raster mode

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ABSTRACT

Recent developments in laser performance, combined with the desire for increases in detected ion intensity and throughput, have led to the adoption of high repetition-rate diode-pumped solid-state (DPSS) lasers in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI). Previous studies have demonstrated a more complex relationship between detected ion intensity, stage raster speed and laser pulse repetition rate than the simple linear relationship between number of pulses and detected ion intensity that might be expected. Here we report, for the first time, the interrelated influence of varying laser energy, repetition rate and stage raster speed on detected ion intensity. Thin films of PC 34:1 lipid standard and murine brain tissue with CHCA are analysed by continuous stage raster MALDI MSI. Contrary to previous reports, the optimum laser repetition rate is found to be dependent on both laser energy and stage raster speed and is found to be as high as 20 kHz under some conditions. The effects of different repetition rates and raster speeds are also found to vary for different ion species within MALDI MSI of tissue and so may be significant when either targeting specific molecules or seeking to minimize bias. A clear dependence on time between laser pulses is also observed indicating the underlying mechanisms may be related to on-plate hysteresis-exhibiting processes such as matrix chemical modification.

1. Introduction

The N_2 laser operating at around 20 Hz was, until relatively recently, standard within UV matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) applications. More recently the desire for improvements in pulse-to-pulse stability, laser lifetime and repetition rate has led to a widespread uptake in the standard employment by manufacturers of diode pumped solid state (DPSS) lasers. UV DPSS lasers used within MALDI MS are typically variations of neodymium doped lasers including: Nd:YAG [1–3], Nd:YLF [4,5] and Nd:YVO_4 [6] with a variety of repetition rate, temporal pulse width and energy characteristics.

The desire for increased laser pulse repetition rate is driven primarily by the need in MALDI mass spectrometry imaging (MSI) for increased throughput when imaging over larger tissue areas or with smaller pixel sizes and is considered to be of great importance for the next-generation instrumentation. Within MALDI MS it is generally understood that there will be a trade-off between the time spent interrogating a given region and the detected ion intensity achieved for a given analyte of interest; notwithstanding the complete removal of available matrix-analyte crystal in that region. Therefore, the ability to increase the rate of sampling, typically necessitating an increase in repetition rate, is needed.

The benefit of increased repetition rate for faster data acquisition has been demonstrated for spot data acquisition [3,7] and has also been shown in conjunction with improvements in detected ion intensity over a standard N_2 laser at atmospheric pressure [8]. More recently Bruker Daltonics have developed a new high-throughput MALDI-TOF imaging platform with a 10 kHz Nd:YAG laser enabling the acquisition of 50 pixels per second. This platform was recently employed to demonstrate the use of low vapour-pressure matrices within high-vacuum systems for MALDI MSI [9].

In order to access the fastest image acquisition times it may also be considered necessary to apply these high-repetition rate lasers to MALDI MSI utilising continuous raster mode. This provides throughput benefits over spot mode image acquisition due to the

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continuous movement of the laser over the sample. The performance of high-repetition rate lasers in MALDI MSI in raster mode was the topic of three recent publications [6,10,11]. These studies show that high-repetition rate lasers can provide high-quality tissue imaging data in short timeframes but also suggest that there is an optimum repetition rate for use in raster imaging below or above which a decrease in detected ion intensity may be observed. An early adoption of a DPSS high-repetition rate laser, similar to the one used within this study, was reported by Trim et al. where the most effective repetition rates were found to be at around 5 kHz, coinciding with the highest energy-per-pulse emitted by the laser in question [6]. Mass spectra from tissue were also shown within this study using 15 kHz repetition rate which was the highest reported repetition rate used for tissue imaging within MALDI MSI. Interestingly, Spraggins et al., when investigating peptides in α-cyano-4-hydroxycinnamic acid (CHCA) thin films, reported that a reduction in detected ion intensity was observed at higher repetition rates or at lower stage raster speeds, stating that this was due to excessive laser pulse-to-pulse overlap and that a limit of 50 laser pulses per unit area should be observed for optimum performance [10].

Porta et al. [12] investigated the effect of altering a number of variables including laser repetition rate between (50–1000 Hz) and stage raster speed (1–4 mm/s) on quantification in MALDI MSI by single reaction monitoring (SRM). Within their work it was observed that an increased ion intensity was observed where faster raster speeds or higher repetition rates were employed.

The interrelated influence of stage raster speed, laser repetition rate and varying laser energy has never previously been studied, particularly in a tissue imaging context. With the continuing progression of MSI systems toward high-throughput performance, this knowledge is increasingly important to best understand and optimise experiments within MALDI MSI. Within this study, the influence of laser pulsed repetition rate, energy and stage raster speed in continuous stage raster MALDI MSI are studied using both thin-film CHCA preparations of PC 34:1 lipid standard and CHCA-coated murine brain tissue sections. For thin-film studies the repetition rate is varied between 100 Hz and 20 kHz for stage raster speeds between 0.2 and 2.8 mm/s at four different single pulse energy values between 1.7 and 6.4 μJ. For tissue imaging studies repetition rates of 2 and 20 kHz, raster speeds of 0.5 and 2.8 mm/s and energies of 1 and 3 μJ are assessed.

2. Methods

2.1. Materials

Methanol (HPLC grade) used in preparation of all matrix solutions was purchased from Fisher Scientific (Leicestershire, UK). The water used was purified by an ELGA Purelab Option system (Marlow, UK). Trifluoroacetic acid (TFA, 99.9% purity) and CHCA (99% purity) were purchased from Sigma Aldrich (Dorset, UK). MALDI MS stainless steel imaging plates from Sciex (Ontario, Canada) were used for all experiments. The lipid standard PC 34:1 (18:1/16:0) with mass of 759.578 Da was purchased from Avanti Polar Lipids Incorporated (Delf Zyl, Netherlands).

2.2. Tissue preparation and sectioning

Mice were sacrificed humanly at the School of Cancer Sciences, University of Birmingham, in accordance with the Home Office Animals (Scientific Procedures) Act 1986 [13]. Mouse brain was flash frozen in liquid nitrogen immediately after excision. Four serial sections (10 μm thick) were collected and thaw-mounted onto a single MALDI imaging plate. Sectioning was performed on a Leica CM 1850 Cryostat (Milton Keynes, UK).

2.3. Matrix application

For the thin-film experiments using the lipid standard, a PC 34:1 solution (0.04 mg/mL in 80% CH_3OH) was mixed 1:1 (v:v) with a CHCA solution (10 mg/mL in 80% CH_3OH, 0.2% TFA) and for tissue imaging a CHCA solution at 5 mg/mL in 80% CH_3OH, 0.1% TFA was used. A MALDI imaging plate was used in the case of the thin–film measurements of the lipid standard. The solution, in either case, was then applied to the imaging plate using a TM Sprayer (HTX Technologies, Carrboro, NC) with a nebulizer temperature of 90 °C, a solvent flow rate of 0.115 mL/min, a gas pressure of 10 psi, and a spray head speed of 1333 mm/min. Eight sequential passes across the whole plate were used, each with a spacing of 3 mm between lines, even passes were performed horizontally, and odd passes vertically, and an offset of 1.5 mm was used on passes 3, 4, 7, and 8. This gave a density of matrix and lipid on the plate of 0.115 mg/cm^2 and 0.0005 mg/cm^2, respectively, resulting in an even distribution of matrix/analyte across the whole plate.

2.4. Mass spectrometry

The laser energy per pulse was measured using a pyroelectric sensor (PD10-C, Ophir Photonics). Further details and a schematic of the laser delivery setup are shown in the supplementary information (SI) Fig. S1 and associated text.

MALDI-TOF MS analysis was carried out on a QSTAR XL QqTOF instrument using Analyst QS 1.1 with oMALDI server 5.1 (Sciex). A Nd:YVO_4 (SPOT-10-100-355; Elforlight, Daventry, UK) DPSS laser with a wavelength of 355 nm, a repetition rate of <40 kHz and a pulse length of <1.5 ns was used in this study. The Nd:YVO_4 laser was continuously triggered by a function generator (TTi – TG2000 20 MHz DDS) during all analyses whilst the path of the laser was blocked by a shutter system triggered by the QSTAR oMALDI software to 'turn the laser on and off', rather than triggering the laser directly, ensuring a greater degree of pulse-to-pulse laser stability. The laser was coupled to the MALDI source via a 100 μm core diameter fiber optic patch cord of 4 m (Fiberguide Industries via AMS Technologies, Leicestershire, UK; NA = 0.22) for the tissue imaging experiments and a 105 μm core diameter fiber optic patch cord (Thorlabs Ltd; NA = 0.22) for the thin-film experiments. The raster speeds employed were dictated by the preset software speed values available within the oMALDI (Sciex) software named slowest, slower, slow, medium and fast and correspond to 0.2, 0.3, 0.5, 1.0 or 2.8 mm/s respectively. An m/z range of 50–1000 was used for all experiments. All data were acquired in positive ion mode.

For the thin-film lipid standard analysis the Nd:YVO_4 laser was operated at repetition rates of 100, 500, 1000, 2500, 5000, 7500, 10,000, 12,500, 15,000 and 20,000 Hz, at raster speeds of either 0.2, 0.3, 0.5, 1.0 or 2.8 mm/s and at single-pulse energies of either 1.7, 2.8, 4.2 or 6.4 μJ. A table of parameter combinations is shown in Table S1. The irradiated area on the sample as measured by the fluorometric method [14] was 2.8×10^{-8} m^2 giving fluence values of 225.7, 148.1, 98.8 and 60.0 J/m^2. One raster line of 56 pixels (360 μm diameter per pixel) in length was acquired for each variable combination. The energy of the laser was measured before each of these acquisitions. The average of these values are discussed in the text and used in the fluence calculation.

Tissue MSI data were acquired at stage speeds of 0.3 and 2.8 mm/s ('slower' and 'fast' as labeled within the oMALDI software) at repetition rates of 2 and 20 kHz, and energies of 3 and 1 μJ per pulse. The irradiated area on the sample as measured by the fluorometric method [14] was 2.05×10^{-8} m^2 giving fluence