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Influence of C-Trap Ion Accumulation Time on the Detectability of Analytes in IR-MALDESI MSI

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Abstract

Laser desorption followed by post electrospray ionization requires synchronized timing of the key events (sample desorption/ionization, mass spectrometry analysis, sample translation) necessary to conduct mass spectrometry imaging (MSI) with adequate analyte sensitivity. In infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) MSI analyses, two laser pulses are used for analysis at each volumetric element, or voxel, of a biological sample and ion accumulation in the C-trap exceeding 100 ms is necessary to capture all sample-associated ions using an infrared laser with a 20 Hz repetition rate. When coupled to an Orbitrap-based mass spectrometer like the Q Exactive Plus, this time window for ion accumulation exceeds dynamically controlled trapping of samples with comparable ion flux by automatic gain control (AGC), which cannot be used during MSI analysis. In this work, a next-generation IR-MALDESI source has been designed and constructed that incorporates a mid-infrared OPO laser capable of operating at 100Hz and allows requisite C-trap inject time during MSI to be reduced to 30 ms. Analyte detectability of the next-generation IR-MALDESI integrated source has been evaluated as a function of laser repetition rate (100 – 20 Hz) with corresponding C-trap ion accumulation times (30 – 110 ms) in both untargeted and targeted analysis of biological samples. Reducing the C-trap ion accumulation time resulted in increased ion abundance by up to three orders of magnitude for analytes ranging from xenobiotics to endogenous lipids, and facilitated the reduction of voxel-to-voxel variability by more than 3-fold.

Graphical Abstract

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INTRODUCTION

Mass spectrometry imaging (MSI) entails the systematic acquisition of mass spectral information from an array of discrete positions across a sample, allowing the distribution of analytes to be visualized. Initially conceived of by Castaing and Slodzian in the 1960's¹, MSI began as a relatively crude technique for biological ion imaging² that through technological advancements^{3–9} now combines the high specificity and sensitivity of mass spectrometric detection with high spatial resolution for unparalleled measurement of analyte distribution within biological samples. MSI has been shown to be sensitive for analysis of metabolomics^{10,11}, proteomics^{10,12–14}, lipidomics^{15–18}, as well as targeted analysis of pharmaceutical drugs^{19–23}.

For MSI analysis of small molecules and lipids within biological matrices, often including analytes that are labile and/or semi-volatile, ambient ionization approaches that preserve the native state of the sample for *in situ* analysis of intact tissue are desired. Ambient MSI techniques such as infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI)²⁴ have the added benefit of requiring minimal sample preparation, which for more preparation intensive MSI approaches like MALDI can dictate analyte sensitivity²⁵. The mechanism of MALDESI involves a two-step process wherein material is first desorbed from a sample surface by an impinging laser pulse, with the resulting plume of desorbed neutrals then gently ionized by entrainment in an orthogonal electrospray²⁶. By operating at mid-infrared wavelengths, IR-MALDESI laser stimulation of biological tissue samples leads to the resonant excitation of species natively present, such as endogenous water or an exogenous layer of ice²⁷, eliminating background interferences associated with organic matrices as used in MALDI. The thin layer of exogenous ice also accounts for variation in water content in different tissue compartments, and has been shown to greatly influence tissue desorption²⁷ and improve ion abundance²⁸.

MSI analysis of complex samples such as biological tissue specimens can lead to the simultaneous generation of hundreds to thousands of unique ions. These ions are sampled into a mass spectrometer in concert, demanding high instrument mass resolving power to distinguish ions that are nearly isobaric. The capability of assigning peak identities based on

exact mass provided by high mass resolving power and accuracy mass spectrometry reduces the need for additional analysis steps for identification like tandem mass spectrometry that can either reduce sample throughput or limit the breadth of multiplexed sample analysis. To this end, the IR-MALDESI source has recently been integrated with a hybrid quadrupole Orbitrap mass spectrometer, the Q Exactive, and the utility of this combination has been demonstrated for sensitive detection of lipids and metabolites^{28,29} as well as xenobiotics^{30–32} in targeted or untargeted MSI methods.

Two pulses of the mid-IR OPO laser are required to achieve complete and reproducible desorption and optimal response from IR-MALDESI analysis of tissue cryosections tens of micrometers thick²⁷. To capture ions generated from each voxel probed during IR-MALDESI MSI, the ion accumulation time must be sufficiently long to encompass both laser desorption events with additional time for ion transfer and synchronization overhead. Hence, requisite ion inject time is inversely proportional to laser repetition rate, with a 20 Hz repetition rate mid-IR laser requiring more than 100 ms to accumulate ions generated from two laser pulses. As with other successful pulsed infrared MSI approaches⁹, it is desirable to fix the number of pulses and the inject time during MSI acquisition at individual voxels, and as a result methods to control trapped ion populations like such as AGC cannot be utilized. AGC uses a short pre-scan to dynamically set an ion accumulation time in order to maintain the trapped ion population at a target number and reduce space charging and saturation effects. In the Q Exactive, ions are accumulated in an RF-only storage trap (C-trap) before being injected into the Orbitrap for a single FT acquisition. The C-trap can be filled with roughly 10^6 charges³³, below the capacity of the Orbitrap itself³⁴, and typical ion accumulation times with AGC on tend to be on the order of milliseconds for a typical LC-MS analysis.

While measurement mass accuracy (MMA) of the mass spectrometer can be maintained during MSI in the absence of AGC through the use of lock-mass correction³³, the duration of ion accumulation required for MSI using typical mid-IR lasers is expected to degrade targeted ion abundance either as a result of reduced trapping efficiency or due to losses associated with space-charging of accumulated ions as the flux of sample-derived and ambient ions exceed the charge limits of the trap. In this work, we have developed a next-generation IR-MALDESI imaging source coupled to a Q-Exactive Plus mass spectrometer and investigate the gains in IR-MALDESI analyte response that can be attained by reduction of ion accumulation time offered by a higher repetition rate (100 Hz) pulsed mid-IR laser source. A comparison is made between response to ions introduced continuously and those that are generated through the pulsed IR-MALDESI mechanism. Results are presented for both targeted and untargeted studies of biological tissues.

EXPERIMENTAL

Materials

HPLC grade methanol and water were purchased from Burdick and Jackson (Muskegon, MI, USA). Formic acid, acetic acid, and lamivudine (3TC) were purchased from Sigma Aldrich (St. Louis, MO, USA). Emtricitabine (FTC) was obtained through the NIH AIDS Reagent Program. All materials were used without further purification.

Human cervical tissues were obtained from the University of North Carolina Tissue Procurement Facility through UNC IRB #09-0921 with written informed consent and incubated in a solution of 100 $\mu\text{g/mL}$ FTC for 24 hours as previously described³⁰. Mouse liver and hen ovaries were obtained from the NCSU College of Veterinary Medicine and School of Poultry Science, respectively. Animals were managed in accordance with the Institute for Laboratory Animal Research Guide and all husbandry practices were approved by North Carolina State University Institutional Animal Care and Use Committee (IACUC). All tissues were sectioned into 10- μm thick sections using a Leica CM1950 cryomicrotome (Buffalo Grove, IL, USA) and thaw mounted onto precleaned glass microscope slides or slides uniformly coated with an internal standard³¹.

IR-MALDESI Source

A more advanced IR-MALDESI source for mass spectrometry imaging system has been developed for the determination of analyte distribution within biological matrices. Based on the design from previous work²⁴, this next-generation ionization source has been developed that incorporates a new high rep IR laser, proportional-integral-derivative (PID) control of temperature and relative humidity (RH), and a high-precision, high-speed translation stage. Details of the new design components are described below. Within the fully enclosed source, a matrix layer of ice is carefully deposited onto the sample by adjusting the relative humidity within the source and the sample temperature. Incorporation of two new devices now allows for better control over ice deposition. First, power applied to the Peltier thermoelectric cooling element is automatically regulated using a TC-48-20 (TE Technology, Inc., Traverse City, MI) controller using pulse-width modulation. Second, relative humidity within the source chamber is monitored by an iSeries Humidity and Temperature controller (CNiTH-i32; Omega Engineering) that can provide PID control to an actuated valve supplying a flow of dry N_2 gas. Two-dimensional sample translation is achieved by stacked, motion controlled linear translation stages. A high-precision and high-speed linear stage (GTS70; Newport Corporation, Irvine, CA, USA) is used along the x-axis to allow rapid transit between voxel positions at 100 nm resolution, with a slower motion controller (LTA-HS; Newport Corporation, Irvine, CA, USA) used for translation along the y-axis. A high repetition optical parametric oscillator (OPO) laser (IR Opolette HR; Opotek, Carlsbad, CA) is used to generate $\lambda = 2.94 \mu\text{m}$ mid-infrared laser emission with a pulse duration of 9 ns for excitation of the asymmetric O-H stretch in water. The diode-pumped IR Opolette HR can operate at a 100 Hz repetition rate, whereas the previously employed Q-switched IR Opolette was limited to 20 Hz²⁴. Two metallic silver mirrors steer the output beam through a pair of CaF_2 lenses (PCCM2506CI-075 and PCX-25.4CI-400; Lambda Research Optics, Costa Mesa, CA) for beam expansion and collimation before it is focused with an uncoated CaF_2 plano-convex lens (PCX-25.4CI-75) to a diameter of $\sim 200 \mu\text{m}$, as evaluated by burn spots on thermal paper. Pulse energy at the sample stage was measured to be 1.05 mJ/pulse (Nova 2; Ophir, Jerusalem, Israel), corresponding to a laser fluence of 3.35 J/cm^2 distributed over the measured beam waist, and can be adjusted with a motorized variable attenuator. All experiments were conducted at full laser fluence unless otherwise noted.

Mass Spectrometer

The IR-MALDESI imaging source was fully synchronized with a Thermo Fisher Scientific Q Exactive Plus mass spectrometer (Bremen, Germany). The Q Exactive Plus was operated in full scan mode with a mass range m/z 150–600 for small molecule analysis and m/z 250–1000 for lipid analysis, with mass resolving power set to 140,000 (FWHM, m/z 200). Since AGC is turned off during IR-MALDESI experiments, lock masses were utilized in the control software to achieve parts per million mass accuracy³³. For positive ionization mode two peaks of an ambient ion, diisooctyl phthalate, at 391.2843 $[M+H]^+$ and 413.2662 $[M+Na]^+$ were used as lock masses. The peaks of palmitic acid at 255.2329 $[M-H]^+$ and stearic acid at 283.2643 $[M-H]^+$ were used as lock masses in negative ionization mode.

IR-MALDESI Imaging

As has been described previously²⁷, slide-mounted tissue sections were placed onto the Peltier stage and a controlled layer of ice was deposited on the surface. Source geometry was matched to parameter values previously found to yield optimal response from biological tissues when using an ice matrix²⁷, and no further optimization of this geometry for the next-generation source has yet been undertaken. Two laser pulses were used to completely ablate the 10- μ m thick tissue cryosection for each voxel. Samples were translated 100 μ m between laser ablation events in an oversampling method such that the stage was moved a distance less than the desorption threshold of the laser resulting in a consistent volume of tissue desorbed³⁵. Imaging of a rectangular region of interest is performed in a left-right, top-down flyback pattern. The plume of neutral tissue material interacts with an orthogonal electrospray plume, where analytes partition into the electrospray droplets and ionize in an ESI-like fashion^{36,37}. For targeted, positive polarity experiments, a 50/50 (v/v) solution of methanol/water with 0.2% formic acid was used as the electrospray solvent. Untargeted lipid analysis was performed using a polarity switching method, where spectra are adjacent voxels are analyzed with alternating polarities^{38,39}. For polarity switching experiments, a 50/50 (v/v) solution of methanol/water with 1mM acetic acid was used as the electrospray solvent, which has been found to yield the best untargeted analyte detection in acquisition from each polarity in comparison to other solvent systems³⁹.

The IR-MALDESI MSI acquisition GUI allows for the laser repetition rate to be changed by selecting integer fractions of the 100 Hz diode pump duty cycle for OPO excitation. Based on selected laser repetition rate, C-trap inject time is fixed according to t (ms) = $2n + 10$ to allow collection of ions generated from two laser pulses with a laser period of n milliseconds and an additional 10 ms overhead to ensure synchronization of stage, laser, and mass spectrometer for each scan. To evaluate the effect of laser frequency and trap time on ion abundance, OPO excitation was performed using integer fractions (1–5) of the 100 Hz pump diode for IR OPO repetition rates of 100, 50, 33, 25, and 20 Hz with corresponding C-trap accumulation times of 30, 50, 70, 90, and 110 ms. A timing schematic for 20 and 100 Hz scenarios illustrating temporal overlap between signaling of laser firing and C-trap accumulation can be seen in Figure 1.

Data Analysis

The .raw files obtained from the Q Exactive Plus instrument were converted to the mzML file format with the MSConvert software from Proteowizard⁴⁰, using a polarity filter to separate the positive and negative acquisitions for polarity switching experiments. The mzML files were then converted to the imzML file format using imzMLConverter⁴¹ and processed using MSiReader⁴². In order to demonstrate the quality of the raw data, ion images were neither interpolated nor normalized (unless specified). All ion maps were created with a mass tolerance of 5 ppm, and sample dimensions are indicated by scale bars. Supervised analysis of the MSI data was performed by MSiReader for untargeted discovery of ions associated with tissue. In this approach, the software averages voxel spectra over a region of interest specified by the user and identifies unique peaks associated with this region relative to a user-specified reference region, chosen to be off-tissue. In a left-to-right and top-to-bottom oversampling regime, the tissue sample volume of the first row and column imaged is larger than that of the remainder of the region of interest. These data have been excluded from analysis. Putative identification of ions selected by MSiReader based on exact mass has been performed where possible using the LIPID MAPS⁴³ and METLIN structure databases⁴⁴. No further targeted MS/MS analysis was undertaken to confirm these assignments, and as a result no attempt is made to distinguish structural isomers. These ions are identified by their molecular formula and lipid class.

RESULTS AND DISCUSSION

C-trap accumulation time with ions from multiple sources

In MSI analysis of biological materials by IR-MALDESI, analytical targets may include endogenous metabolites and therapeutic xenobiotics. Additionally, the mass spectra also include ions from ambient compounds, despite the IR-MALDESI source enclosure being purged with dry nitrogen to a constant RH. The effect of laser repetition rate and corresponding C-trap accumulation time on analyte ion abundance was evaluated initially for a representative ion from each of these classes during IR-MALDESI MSI of cervical tissue incubated in the antiretroviral drug FTC. Composed of a single stratified squamous epithelium cell type, cervical tissue represents a near-uniform lipid substrate and drug penetration was assumed to be uniform in tissue following 24 hour incubation. Tissue cryosections from this model were analyzed by IR-MALDESI in discrete regions of 2 mm × 2 mm (20 × 20 voxels) using five laser ablation repetition rates (100, 50, 33, 25 and 20 Hz) with matching C-trap accumulation times (30, 50, 70, 90, 110 ms). Resulting ion maps, shown in Figure 2A, illustrate that analyte ion abundance increased as C-trap accumulation time was reduced for: 1) the total ion current (TIC) over m/z 150–600 range; 2) polydimethylcyclsiloxane (PDMS, $[(CH_3)_2SiO]_5$), a ubiquitous ambient compound used in many personal products and considered a common interferent in mass spectrometry⁴⁵; 3) cholesterol, an endogenous sterol; and 4) FTC, incubated xenobiotic.

Average abundances for these ions were evaluated for each region of interest using MSiReader and normalized by the response at 100 Hz for each compound to allow the inter-comparison of trends with C-trap accumulation time (Figure 2B). PDMS and other ambient compounds remain present in the IR-MALDESI source during imaging where they are

ionized by the electrospray and sampled into the Q Exactive Plus continuously over the period of C-trap ion accumulation. The average abundance of this ambient compound increases nearly linearly over the accumulation time range. Analytes associated with tissue that are ablated and ionized via the pulsed IR-MALDESI mechanism exhibit a greater dependence on the laser repetition rate and trap time. Response to cholesterol, which commonly represents the most abundant tissue-related peak evaluated by IR-MALDESI, increases 6-fold as accumulation time is reduced from 110 ms to 30 ms. The average ion abundance of FTC, an order of magnitude lower than that of cholesterol, increases by 2 orders of magnitude over the range of accumulation times. The TIC follows a similar response to cholesterol rather than ambient ions, suggesting that TIC is significantly comprised of tissue-derived ions.

The relative contribution of C-trap accumulation time, based on laser repetition rate, to the observed gains in ion abundance is considered next. With typical thermal relaxation time in tissue on the order of milliseconds for length scales of the laser spot^{46,47}, the laser ablation of tissue is expected to take place under a condition of total thermal confinement with no diffusion of heat over the laser pulse duration of 9 ns. The confined interaction preferentially imparts laser energy to explosive tissue ablation, leaving minimal remnant heat to contribute to improved ablation efficiency over the duty cycles under consideration in this work.³³ It is conjectured that the gains in ion abundance observed may be a result of minimizing ion loss due to space charging within the C-trap. This hypothesis is supported indirectly by the fact that the instrumental response to ambient ions injected continuously to the trap during ion accumulation decays to a lesser extent than the response to ions created by pulsed laser ablation/post-ionization, which must be trapped for longer periods of time before analysis. While it is beyond the scope of the current work to formally decouple effects attributable separately to laser duty cycle and ion inject time, it is nevertheless clear that their combination results in ion abundance and sensitivity gains during IR-MALDESI MSI that are anticipated to translate to lower limits of detection and better visualization of low abundance analyte distributions. The following sections summarize evaluation of the effect of C-trap inject time on IR-MALDESI response in both untargeted and targeted analyses, comparing laser repetition rates of 100Hz and 20 Hz.

Untargeted analysis of endogenous lipids

Lipid profiles provide valuable information for understanding the biological basis of disease. Alterations in lipid metabolism have been linked to several diseases such as hypertension, diabetes, and cancer^{48,49}. MSI lipidomics have been proposed to detect region-specific lipid signature of cancerous cellular regions in tissue⁵⁰ and in the determination of tumor margins⁵¹.

A homogeneous tissue sample, mouse liver, was initially used to investigate untargeted analysis of biological materials. Lipids natively present were evaluated from a tissue section in positive ion mode over the mass range m/z 150–600. For each of the two laser repetition rates considered, nine 1 mm $\times$ 1 mm (10 $\times$ 10 voxels) regions of interest were analyzed at laser powers ranging from 10% to 100% to optimize MSI response of the next-generation IR-MALDESI source, though it should be stressed that a systematic optimization of

experimental parameters remains to be performed. Representative ion maps associated with carnitine and cholesterol are shown in Figure 3A, systematically indicating higher lipid ion abundance at the higher laser repetition rate and shorter ion accumulation window. While these maps indicate that optimal laser fluence exhibits some analyte specificity, as was recently demonstrated³⁷, the highest response across all tissue-related analytes is achieved at 100% laser power. This condition has been used in all subsequent MSI experiments. Mass excess plots of all tissue-related peaks discovered by MSiReader relative to an off-tissue reference region can be seen in Figure 3B for all regions imaged at 100 Hz and 20 Hz. Untargeted analysis of lipids associated with tissue over this mass range resulted in almost a two-fold increase in analytes identified using the 100 Hz repetition rate than when imaging at 20 Hz (416 ions versus 246 ions, respectively). Ion abundance of these species ranged from approximately one to three orders of magnitude higher at 100Hz than 20 Hz.

The hen ovary, representing the only animal model for the spontaneous development of ovarian cancer^{52,53}, was selected as a highly heterogeneous substrate for untargeted lipid analysis. An immature follicle in a hen ovarian tissue sample was analyzed in an untargeted manner on serial tissue sections using 20 and 100 Hz repetition rates (Figure 4A). Experiments were conducted in a polarity switching mode over the mass range m/z 250–1000 using a prescribed acquisition method alternating the acquisition polarity between adjacent voxels to increase instrumental response across lipid classes consisting of a range of polar headgroup compositions that can be preferentially ionized in either positive or negative ionization mode. The electrospray solvent composition and the concentration of modifier added were optimized in order to minimize the bias of the solvent for one polarity over the other and also to ensure a comprehensive lipid coverage in both polarities (data not shown). Representative ion maps of the follicle in +ESI (m/z 369.3516, cholesterol) and –ESI (m/z 289.2174, putatively assigned nonadecadiynoic acid) are shown in Figure 4B and 4C, respectively. As with the mouse liver, IR-MALDESI ion abundances for tissue-related ions were higher when imaging at the higher laser repetition rate and shorter C-trap accumulation time. The heterogeneous distribution of less abundant ions such as the fatty acid (Figure 4C) around the follicle nucleus relative to other regions of the ovarian tissue is more readily discerned under these conditions since the low analyte abundance remains above the limit of detection (LOD). At a repetition rate of 20 Hz, the fatty acid can only be detected in the follicular wall, whereas at 100 Hz the analyte can be resolved in the surrounding tissue structure. Hence, the added sensitivity of the higher laser repetition rate and shorter C-trap accumulation time offered a more accurate mapping of analyte distribution. This sensitivity gain also resulted in a two-fold increase in the number of identified tissue-related peaks: A total of 368 tissue-specific ions (272 in +ve, and 96 in –ve) were identified at 20 Hz, whereas 777 tissue-specific ions (567 in +ve, and 210 in –ve) were identified at 100 Hz. All tissue-specific ions detected using a repetition rate of 20 Hz were present in the dataset collected using a repetition rate of 100 Hz and their ion abundance was lower by at least an order of magnitude.

By improving the accuracy of analyte distribution for compounds with low ion abundance and increasing the number of potential biomarker candidates identified, IR-MALDESI MSI using the higher repetition rate and reduced ion accumulation time is expected to increase the likelihood for successful biomarker discovery from untargeted analysis of lipids.

Targeted Analysis of Xenobiotics

To evaluate drug efficacy, it is necessary to quantify drug concentration at key anatomical sites of action. In order to elucidate areas of analyte concentration that may arise due to biological variability of a sample, minimizing instrumental variability is imperative. As has been demonstrated recently³¹, normalization strategies relating the response of an analyte of interest to that of a uniformly deposited internal standard with similar desorption and ionization efficiency can significantly reduce the IR-MALDESI voxel-to-voxel variability.

The FTC-incubated cervical tissue model was used to determine the analytical variability associated with MSI of a uniformly distributed analyte to determine the best experimental conditions for evaluating xenobiotic distributions. Serial sections of the FTC-incubated cervical tissue were mounted onto a glass slide uniformly sprayed with the FTC analogue 3TC, which is also an HIV antiretroviral drug, and then imaged consecutively at 20Hz and 100 Hz. As in the discrete regions of interest shown in Figure 2, the ion abundance of FTC distributed over an entire tissue section increased two orders of magnitude when using the 100 Hz laser repetition rate and 30 ms accumulation time relative to imaging with the laser operating at a 20 Hz repetition rate (Figure 5, top). Additionally, the higher ion abundance of the internal standard, 3TC, in each voxel measured at 100 Hz imaging yields a more effective normalization of FTC ion abundances as quantified by the reduced variability per-voxel. With this normalization, relative standard deviations (%RSD) of incubated FTC to 3TC decreased from 103% to 28% for 110 and 30 ms inject time, respectively (Fig 5, bottom). This reduced variability per-voxel is critically important in determining accurate absolute concentrations of analyte within small tissue microenvironments encountered in heterogeneous tissues.

CONCLUSIONS

A next-generation IR-MALDESI source has been designed and constructed, incorporating a mid-infrared OPO laser capable of operating at a higher repetition rate than the IR-MALDESI prototype. The higher repetition rate reduces the duration of time required to fire multiple laser shots required for complete desorption of material from each voxel of a biological sample, thereby shrinking the window of ion accumulation time in the C-trap of the Q Exactive Plus during mass spectrometry imaging. Reducing the ion inject time was shown to result in gains in measured ion abundance for ions derived from continuous and pulsed mechanisms.

Enhancement is significantly greater for ions generated by IR-MALDESI associated with biological material, resulting in up to 3 orders of magnitude greater ion abundance for certain analytes. This approach has multiple advantages for both untargeted and targeted analysis of biological tissues by MSI. For untargeted studies, the increased analyte response results in identification of greater number of tissue-specific metabolites that may represent key identifiers and biomarkers for evaluation of discrete tissue health or systems biology.

Additionally, the heterogeneous distribution of less abundant analytes can be more precisely evaluated. As shown for ARV-incubated tissue, increased instrumental response toward both an incubated xenobiotic and a matched internal standard was achieved with the higher

repetition rate laser, facilitating a reduction in ion accumulation time. All gains in detectability observed in untargated and targated analyses are expected to increase as higher repetition rate mid-IR lasers become available, since the current ion accumulation time of 30 ms still represents a window that is an order of magnitude longer than those for dynamically controlled ion populations such as in typical LC-MS/MS analysis. This significantly reduced the voxel-to-voxel variability when normalizing the xenobiotic response by that of the internal standard, which improves per-voxel analyte quantification. The advantages detailed by this work are expected to be applicable to other MSI approaches utilizing pulsed infrared lasers.

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Figure 1.

Timing diagram of key IR-MALDESI events during MSI associated with pulsed sample desorption by the mid-IR laser and analysis of ions by the Q Exactive Plus mass spectrometer. For each acquisition event, C-trap accumulation time is sufficiently long to accumulate ions generated from two laser pulses and scales inversely with laser repetition rate.

Figure 2.

Dependence of Q Exactive Plus C-trap accumulation time on ambient and tissue-specific ion abundance during IR-MALDESI MSI analysis of ARV-incubated human cervical tissue. **(A)** MSI ion maps indicate increased ion abundance as C-trap inject time is reduced for: total ion current (150–600 m/z); ambient polydimethylcyclsiloxane (PDMS); endogenous cholesterol; and, xenobiotic emtricitabine (FTC). **(B)** Normalized ion abundance for each analyte, including 95% confidence limit, showing 2–100 fold increase when reducing accumulation time from 110 ms (previous limiting conditions with 20 Hz laser) to 30 ms (100 Hz laser).

Figure 3.

Laser fluence and C-trap accumulation dependence on endogenous lipid response from mouse liver tissue. **(A)** Representative ion maps of cholesterol (m/z 369.3516, $[M-H_2O+H]^+$) and carnitine (m/z 162.1125, $[M+H]^+$). **(B)** Mass excess of all tissue-specific peaks (+ESI, black dots) overlaid on mass excess distribution of lipids from LIPID MAPS Structure Database⁴³, with greater discovery of tissue related peaks at 100 Hz repetition rate.

Figure 4.

Untargeted lipid analysis of hen ovary. (A) Optical images of the tissue sections and the ROIs analyzed using 100 Hz (left) and 20 Hz (right) repetition rates, along with ion maps of (B) cholesterol (m/z 369.3516, $[M-H_2O+H^+]^+$) and (C) Nonadecadiynoic acid (m/z 289.2174, $[M-H^+]^-$). (D) Mass excess of all tissue-specific peaks (+ESI, black dots; -ESI, white dots) overlaid on mass excess distribution of lipids generated from LIPID MAPS Structure Database⁴³. The plots indicate that all of the tissue-specific peaks using 100 and 20 Hz repetition rates correspond to lipids across different classes, with greater discovery of tissue related peaks at 100 Hz repetition rate.

Figure 5.

Targeted analysis of emtricitabine (FTC). Top: Ion maps of FTC during imaging at 20 Hz and 100 Hz showing 100-fold increased absolute ion abundance under the latter conditions. Bottom: Normalized ion maps depicting the ratio of FTC:3TC, which was distributed uniformly onto the mounting slide as an internal standard. The higher abundance and reduced variability per spectrum of analyte and internal standard allows for normalization on a per-voxel level, improving %RSD from 103% to 28%.