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Characterization of a Novel Miniaturized Burst-Mode Infrared Laser System for IR-MALDESI Mass Spectrometry Imaging

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Abstract

Laser systems are widely used in mass spectrometry as sample probes and ionization sources. Mid-infrared lasers are particularly suitable for analysis of high water content samples such as animal and plant tissues, using water as a resonantly excited sacrificial matrix. Commercially available mid-IR lasers have historically been bulky and expensive due to cooling requirements. This work presents a novel air-cooled miniature mid-IR laser with adjustable burst-mode output, and details an evaluation of its performance for mass spectrometry imaging. The miniature laser was found capable of generating sufficient energy for complete ablation of animal tissue in the context of an IR-MALDESI experiment with exogenously added ice matrix, yielding several hundred confident metabolite identifications.

Graphical Abstract

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Compliance with Ethical Standards

Use of Research Animals

This study utilized tissues sourced from animals managed in accordance with the Institute for Laboratory Animal Research Guide. All husbandry practices were approved by North Carolina State University Institutional Animal Care and Use Committee (IACUC).

Conflicts of Interest

J. Manni is president and owner of JGM Associates Inc. (JGMA) which makes the 2950 nm OPO laser used in these experiments. Certain aspects of the laser design are JGMA patent-pending. None of the remaining authors have any conflicts of interest.

The use of a novel miniature 2.94 micron burst-mode laser in IR-MALDESI allows for rapid and sensitive mass spectrometry imaging of a whole mouse.

Keywords

Mass spectrometry imaging; Infrared laser; IR-MALDESI; HRAM Mass Spectrometry

Introduction

The use of lasers to generate ions for mass spectrometry dates back to the 1960s when Honig and Woolston introduced what would later be known as laser desorption/ionization (LDI)(1, 2). However, significant fragmentation of molecular ions was observed in LDI experiments. In the late 1980s, it was discovered that mixing the analyte with an energy-absorbing matrix prior to laser desorption and ionization significantly reduced the fragmentation, allowing truly 'soft' ionization(3, 4). In matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, ions are formed in chemical reactions between the sample and matrix substance, before (in solution) or directly following (gas-phase) a sampling laser pulse(5). As a consequence, ionization efficiency depends heavily upon the choice of matrix and the method of matrix application.

Rather than relying on this mechanism for ion generation, it is possible to use a secondary excitation source to ionize neutrals desorbed by the sampling laser. This post-ionization approach was described by Beekman and coworkers in 1980(6), who successfully used a second laser beam to produce ions from laser ablated neutrals through resonant excitation. Other groups proved the utility of this method for the analysis of biomolecules(7, 8). More recently, Shiea and coworkers demonstrated the use of an electrospray plume for effective post-ionization in what they called electrospray-assisted laser desorption ionization (ELDI) (9). Sampson and coworkers brought this strategy closer to its MALDI roots by re-introducing the use of a resonantly absorbing matrix, a method known as matrix-assisted laser desorption electrospray ionization (MALDESI)(10).

In MALDESI, the sampling behavior of MALDI is combined with the ionization behavior of ESI(11), resulting in a very flexible ion source capable of analyzing a wide range of analytes. MALDESI has been shown to work well with a range of laser wavelengths for sampling, from typical MALDI ultraviolet lasers to lasers well into the far-infrared range ($\lambda = >10 \mu\text{m}$)(12). The mid-infrared (IR) range close to $3 \mu\text{m}$ has proven particularly useful for the analysis of biological samples such as animal or plant tissues, as endogenous or exogenous water can be used as an efficient energy-absorbing matrix, reducing the need for sample preparation(13). The method of relying entirely on the native mid-IR absorptivity of native water in the sample is often referred to as Laser Ablation Electrospray Ionization (LAESI)(14) rather than ELDI which was originally performed using a 337 nm N_2 laser. The use of lasers for sample introduction makes these ion sources inherently suitable for mass spectrometry imaging (MSI), where sampling is regularly spaced across a surface to produce a spatial ion map.(15)

Many commercially available IR lasers suitable as sample probes for mass spectrometry use an optical parametric oscillator (OPO) to frequency-shift a lower wavelength, such as those produced by Nd- or Er-based solid-state pulsed lasers. Such laser systems are typically tunable, allowing access to a number of wavelengths based on experimental needs. OPO-based lasers used to date have employed flashlamp-pumped Nd:YAG lasers that required closed-loop water-cooling, resulting in relatively large and expensive lasers that need periodic maintenance of the flashlamp and water cooling system. Here we demonstrate the use of a novel miniature, diode-pumped, conductively-cooled OPO laser for MSI instrumentation, suitable for IR-MALDESI, LAESI, ELDI and IR-MALDI applications.

Experimental

Materials Used

Nitrogen gas was purchased from ARC3 gases (Raleigh, NC, USA). LC-MS (Optima) grade methanol and water were purchased from Fisher Scientific (Pittsburgh, PA, USA). MS-grade formic and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Neonatal mouse samples were stored at -80°C prior to analysis, and sectioned into 25 μm sections on the day of analysis using a CM1950 cryomicrotome (Leica, Buffalo Grove, IL, USA).

Prototype burst-mode Laser

The “burst-mode” 2940 nm laser prototype was developed by JGM Associates, Inc. (Burlington, MA) and is shown in Figure 1. This laser is a miniaturized 1064 nm-pumped OPO that converts 1064 nm emission into 1664 nm (signal wave) and 2950 nm (idler wave emission). The 1064 nm laser is a diode-pumped, passively Q-switched solid-state Nd laser made by JGM Associates for handheld LIBS instruments (laser-induced breakdown spectroscopy). The 2950 nm emission is separated from the residual 1064 nm emission and 1664 nm emission using two dichroic mirrors inside the head; only the 2950 nm emission is emitted and used for laser desorption.

A temporal waveform for the 1664 nm pulse is shown in Figure 2a. This waveform was measured using an ET-3500 InGaAs photodetector (Electro-Optics Technology; Traverse City, MI) having a 25 ps rise/fall time, and a 1.5 GHz bandwidth LeCroy LC684 DXL oscilloscope (Teledyne-LeCroy Inc.; Chestnut Ridge, NY). The waveform for the 2950 nm pulse is expected to be similar, but we didn't have a detector fast enough to resolve the modulation on the pulse. Figure 2b shows the 2950 nm pulse measured using a PEM-10.6 photoelectromagnetic (PEM) detector (Boston Electronics; Brookline, MA) having a rise/fall time of 1 ns.

Since all of the 2950 nm pulse energy is delivered within a short time compared to the thermal time constant⁽¹⁶⁾ of a typical MSI irradiation area/volume (focal spot diameters in the 10 to 100 micron range), tissue ablation proceeds as if all of the pulse energy were delivered instantaneously. Pulse energy of each 2950 nm pulse is 500 μJ measured at the laser.

The laser generates bursts of such 2950 nm pulses which may include 1 to 15 pulses per burst (user defined) at a maximum burst rate of 4 Hz. Energy of the individual pulses in the burst are all the same, *e.g.*, a 10-pulse burst has total pulse energy of 5 mJ. During each burst, individual pulses are about 100 microseconds apart which means that laser pulse rate during each burst is 10 kHz. This inter-pulse period can be varied by adjusting peak current to the 1064 nm laser's diode-pump array.

The laser head includes a photodiode that provides a pulsed output signal that is synchronous with generation of the Q-switched 1064 nm laser pulse. (Generation of the 2950 nm pulse is coincident with 1064 nm pulse generation). JGM Associates indicates that the complete diode-pumped 2950 nm laser head can be packaged into a 2"W × 2"L × 0.9"H volume as shown in Figure 1. Certain aspects of this laser design are patent-pending.

Incorporation of Laser into Existing IR-MALDESI Platform

The burst-mode laser was integrated into an existing IR-MALDESI research instrument(13) with minimal modification. The laser head was mounted to the optical bench using pedestals (standoffs) that provided little or no conductive cooling into the optical bench. The laser head was essentially convectively air-cooled; a cooling fan was not used.

Control of fire order and synchronization to the ion injection on a Q Exactive Plus mass spectrometer (Thermo Scientific, Bremen, Germany) required the construction of an external control circuit, using an Arduino Uno prototyping platform (Arduino, Ivrea, Italy) to interface the system to an instrument PC, enabling easy adjustment of laser parameters. A complete schematic of the required electronics is provided as Figure 3.

Driving current for the laser pump was provided through a D100 laser driver board (Highland Technology; San Francisco, CA), which was set to provide a 150 A peak current pulse in response to a 5 V trigger signal. The number of pulses in each burst was in this way directly controlled by the duration of the trigger signal. The laser pulses were detected using a silicon photodiode (optically filtered to block diode-pump light) inside the laser head and the width and spacing were measured using a THS-720A oscilloscope (Tektronix, Beaverton, OR). Representative oscilloscope readings to confirm correct laser behavior are shown in Figure 4. The pulse frequency was determined to be 10 kHz (*ca.* 100 μs pulse spacing) when 150 A of peak diode pump current was used. The pulses representing the individual Q-switched 1064 nm pulses "ride" on a pedestal of detected 1064 nm laser fluorescence that is scattered around within the laser head, but none of this fluorescence pedestal emission is emitted (does not interact with the sample).

In a diode-pumped passively Q-switched laser, laser pulse rate during each burst of pulses is directly determined by the pumping current to the laser's diode pump array. A preliminary IR-MALDESI experiment (data not shown) showed no increase in performance when using currents of up to 200 A, so the lower current level of 150 A was selected for use in imaging experiments to minimize ohmic heating. The average energy per pulse was measured to 500 μJ at the laser head using an Ophir PE25-C pyroelectric sensor (Ophir Optonics Ltd.) and up to 14 pulses per burst could be fired at a continuous burst rate of 2 Hz for several hours without degradation in performance or buildup of heat. Over the course of several hours of

continuous firing at a fixed trigger pulse width, the pulse count per burst was found to drift slightly (± 1 pulse) as the laser reached thermal equilibrium. In order to use the laser for IR-MALDESI imaging with fixed ablation energy, a method for controlling the pulse count per burst was thus necessary. To enable reliable performance for the duration of an imaging experiment, as well as easy adjustment of energy output, the 5 V readout of the photodiode was fed back to the Arduino microcontroller, allowing on-demand triggering of the desired pulses-per-burst regardless of the thermal state of the laser head and diode-pump array.

Performance Evaluation

An imaging experiment was performed in order to evaluate the performance of the miniaturized laser. A 25 μm thick whole-body section of a 2 day old neonatal mouse was imaged in a 10 hour experiment. The laser beam was focused through a single 50 mm EFL (effective focal length) aspheric lens onto the sample stage, and the firing order was synchronized to the trigger signal of the Q Exactive Plus, run in low-latency handshake mode. Other than a gold-coated turning mirror, no other optics were used between the laser head and 50 mm EFL asphere lens.

An ice layer was deposited over the sample prior to analysis according to previously established protocol(13), stabilized by regulating the humidity of the enclosure using a nitrogen flow controlled by an OMEGA CNiTH-I3222-2 process controller (OMEGA Engineering Inc, Norwalk, CT). The imaging was performed using a previously developed polarity switching MSI method to maximize metabolome coverage(17).

The resulting mass spectra were converted from to .mzml format using the msconvert utility of the ProteoWizard software package(18), and from .mzml to to .imzml(19) format using imzMLConverter(20) for data analysis. The open-source software package MSiReader(21, 22) was used to produce all images shown in this work. The resulting dataset was uploaded to the Metaspace annotation platform(23) (<http://annotate.metaspace2020.com>) for automatic identification of metabolite signals.

Safety Considerations—The laser system used in this study is a class IV laser of sufficient energy flux to cause harm to humans upon exposure to a focused beam. To protect others working in the same laboratory space, a LAZ-R-SHROUD barrier (Rockwell Laser Industries, Cincinnati, OH) was used to enclose the working area at all times. The greatest risk of stray beam exposure was during alignment of the invisible IR laser beams, and during all such work, OD 5+ glasses were worn by all personnel in the zone of potential exposure. During normal operation the beam was fully enclosed in a 1" beam tube.

Results and Discussion

Validation of Basic Functionality

The output of the burst-mode laser was monitored through oscilloscope readings during the experiments. The correlation of the trigger signal and photodiode current in feedback controlled bursts was recorded, and representative oscilloscope readouts may be found in Figure 4. To use the laser with the Q Exactive Plus in low-latency handshake mode, the signal for acquisition start was synchronized to the laser trigger to within a microsecond.

The laser system was found to produce an oval beam profile approximately 50 μm X $\times$ 100 μm Y using the assigned dimensions of the stage motor axes. At optimal alignment, 50% transmission efficiency through the beam path (1 meter) was achieved, yielding a measured 220 μJ per pulse after the final optic. For imaging, 10 pulses per burst were used (2.2 mJ per burst at target, see Figure 5), which was found sufficient to completely ablate 25 μm thick tissue as shown in Figure 6B.

IR-MALDESI Imaging

The imaging datasets of whole-body neonatal mouse section were uploaded to Metaspace as *Mouse_Wholebody_3* and *Mouse_Wholebody_3_Negative*. Figure 6A shows two representative lipid distributions acquired in negative mode. At the time of writing, a Metaspace search against the Human Metabolome Database(24) with 10% FDR yields 266 identifications from the positive ion mode data, and 175 identifications from negative ion mode for a total of 441 metabolites. A previously uploaded set from an equivalent tissue section analyzed with a 20 Hz pulsed laser in positive ion mode only, also available on Metaspace as *mouse_body_2*, produced 414 identifications at 10% FDR.

Conclusions

A compact diode-pumped burst-mode laser was successfully incorporated into an existing instrument for mass spectrometry imaging. We have demonstrated the suitability of the laser system for full-thickness ablation and sampling of animal tissue in the context of IR-MALDESI. The burst-mode system yields images with quality comparable to that of previous work done with flashlamp pumped Nd:YAG pulsed lasers, but without the requirement for water cooling or large heatsinks. The reduction in size and weight of the laser allows for a substantial reduction in size of the imaging source, and the feedback controlled variable bursts provide a flexible means of adjusting the energy per shot.

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Figure 1.
Photograph of the prototype burst-mode laser system in 50 mm² package.

Figure 2.

Laser output measured on a LeCroy LC684DXL scope. **A)** 1664 nm pulse, output from ET-3500 photodetector **B)** 2950 nm pulse, output from PEM-10.6 photodetector

Figure 3.

Circuit diagram for laser controls. The burst-mode laser is monitored and triggered by two general-use logic pins (D2 and D4 in diagram). The Q Exactive is controlled through contact-closure channels as described in the latest version of the instrument operating manual.

Figure 4.

Oscilloscope confirmation of laser bursts controlled by active feedback. **A)** ten-pulse burst produced by $\sim 1100 \mu\text{s}$ long trigger signal. **B)** Single pulse produced by $\sim 200 \mu\text{s}$ trigger signal. For a user selectable pulse count N , the termination of the trigger pulse is synchronized to the N -th falling edge of the pulse train.

Figure 5.

Illustration of the IR-MALDESI test sequence used, showing a comparison between the pulse energy at sample of the burst-mode laser (10 pulses) compared to a single 3 mJ flashlamp pumped laser as used in previously published MALDESI imaging.

Figure 6.

A) Representative images of whole-body neonatal mouse in negative mode. Here showing raw signal from m/z 281.2486 and 331.2642 (2.5 ppm tolerance), identified as isomers of oleic and andrenic acid, respectively at <5% FDR (Metaspace, HMDB). **B)** Microscope image acquired with 10× magnification on a LMD7000 (Leica, Buffalo Grove, IL, USA), illustrating the approximately $50 \times 100 \mu\text{m}$ ovaloid shape of the ablation spots.