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Laser Beam Filtration for High Spatial Resolution MALDI Imaging Mass Spectrometry

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Abstract

We describe an easy and inexpensive way to provide a highly defined Gaussian shaped laser spot on target of 5 μm diameter for Imaging Mass Spectrometry using a commercial MALDI TOF instrument that is designed to produce a 20 μm diameter laser beam on target at its lowest setting. A 25 μm pinhole filter on a swivel arm was installed in the laser beam optics outside the vacuum ion source chamber so it is easily flipped into or out of the beam as desired by the operator. The resulting ion images at 5 μm spatial resolution are sharp since the satellite secondary laser beam maxima have been removed by the filter. Ion images are shown to demonstrate the performance and are compared to the method of oversampling to achieve higher spatial resolution when only a larger laser beam spot on target is available.

Introduction

For MALDI Imaging Mass Spectrometry [1], high spatial resolution ($<10\ \mu\text{m}$) is required in some applications but are not achievable with current off the shelf commercial instruments. There are several custom experimental setups allowing for high spatial resolutions, based on two main approaches: the microscope mode where data from relatively large areas of a sample are acquired in parallel [2] and the microprobe mode where data acquisition for the image is performed pixel by pixel [3-5]. The microprobe mode is most often used because of a higher ion per pixel sensitivity, and instrumental simplicity and lower cost. For the microprobe mode of operation, the final spatial resolution of the MALDI MS image is a combination of several factors that influences the size of the pixels but is mainly influenced by the diameter of the laser beam on target and the pitch of the ablation areas, i.e., the spacing of the ablated spots. Practically, there are two approaches for achieving high resolution images. The first and most effective approach is the use lenses or other optic devices to produce the desired laser beam size on target, and the second is oversampling [6] using a much larger laser beam that is larger than the pixel size desired. Oversampling is useful when it is not practical or possible to optically narrow the laser beam but suffers the disadvantage that all of the sample in a spot must be completely ablated before moving the beam a fraction of the beam diameter to irradiate new sample and also as a consequence, increases data acquisition time.

To be able to achieve optimal focusing conditions for the smallest possible laser spot, a high quality laser beam is required. Previously there were several attempts of using optical single mode fibers as a tool to achieve a clean Gaussian beam profile and therefore a small beam

spot size [7]. One major disadvantage of this method is the fragility of the input fiber tip that is easily damaged by focused laser radiation and that coupling the laser energy to a single mode fiber leads to extreme loss of laser energy. The optical fiber under channeled pulsed laser radiation develops non-linear dynamic and static changes of its properties. To overcome these disadvantages, the interaction of the laser radiation with optical elements at high energy density must be minimized. In the current work, we present a method of spatial filtration using a pinhole that circumvents these issues and is relatively inexpensive to install in commercial instruments.

Methods

The commercially available MALDI MS Autoflex Speed linear and Autoflex TOF/TOF (Bruker Daltonics) were used in these experiments. Ceramic pinholes 25, 30, 40, and 50 μm models HP-3/8-DISC-CER were purchased from Lenox Laser (Glen Arm, MD). The pinhole was mounted on flipping holder to be able to switch the spatial filtration mechanically in or out of the laser beam from outside the vacuum system. The optics setup was mounted in a 30/60 mm cage using opto-mechanical components from Thorlabs (Newton, NJ).

The laser beam characteristics were studied by examination of ablation patterns in the DHB matrix layer prepared by matrix deposition on standard ITO-coated glass slides by sublimation under 60 mTorr vacuum [8]. Ablated patterns were analyzed by optical microscopy (Olympus BX-50) in bright field mode. Arrays of ablated spots at 200 μm pitch were generated using the instrument's minimum parameter laser spot setting in the FlexImaging instrument control software. At this setting, the elements in the SmartBeam were not moving during ablation from laser shot to shot, giving an irregular ablation area about 20 μm in total diameter.

For imaging, the samples were prepared as follows. 1,5-Diaminonaphthalene (DAN), chloroform, methanol were from Sigma. Indium tin oxide (ITO) coated glass slides were from Delta Technologies. Mouse brain sections were cut at 5 μm thickness, thaw mounted on ITO glass slides, and left to dryness in a vacuum desiccator for 4 hours. The section was then sublimated with DAN at 0.15-0.20mg/cm² under 60 mTorr, 110°C for 7 min. The coated section was treated with vapor consisting of chloroform and methanol (3:1). This step was found to significantly increase the signal to noise level of peaks in the mass spectrum and enable high spatial resolution imaging at 5 μm . Briefly, the slide with DAN coated section was attached to a stainless steel plate (Applied Biosystems) and the plate was fixed on the top part of a petri dish (100 mm diameter $\times$ 15 mm deep, Fisher, 3160101) with a heat conductive copper tape (Electron Microscopy Sciences, 77801) and left in the 85°C oven for 2 min. The bottom part of the petri dish was placed with a piece of filter paper, on which 30 μL of chloroform and methanol (3:1) was pipetted. The top and bottom of the petri dish were sealed together with tape (Fisher, 35901R). This petri dish was left in the 85°C for 2 minutes. The slide then was taken out to dry and kept in a vacuum desiccator to avoid oxidation.

Results and Discussion

In some MALDI MS instruments spatial gradient patterns across the laser beam are introduced to increase the instrument efficiency. For example, in instruments equipped with SmartBeam (Bruker), a laser beam modulator [9] is used to generate a pattern comprising a set of laser spots. When high spatial resolution for imaging MS is necessary, the laser beam has to be focused to a minimum possible spot. Due to geometry limitations of the ion source, the focusing optics in these instruments approaches the diffraction limit. The diffraction

restrictions can be minimized according to the formula (1), where it can be seen that besides minimizing the $f/D(0)$ ratio, the beam quality parameter, M^2 should also be minimized. For example, to be able to achieve a spot size $5\text{ }\mu\text{m}$, the M^2 should be below 2, which characterizes a beam shape close to clean Gaussian.

Spatial filtration in laser optics in general is widely used to achieve a clean Gaussian laser beam profile [10, 11]. Figure 1, panel A shows the scheme for a spatial filter, typically comprising 2 lenses and a pinhole between the lenses. The focusing conditions used to optimize the laser spot on a target and also to optimize parameters of a spatial filter can be assessed by a simple general formula. A laser beam of initial diameter $D(0)$ can be focused by a lens with focal length f , having no aberrations and limited only by diffraction, to the laser beam spot diameter $D(f)$:

$$D(f) = (4\lambda f M^2) / (\pi D(0)),$$

where λ - wavelength of laser, f - focal length of focusing lens, $D(0)$ - input beam diameter, and M^2 is a parameter of the beam quality, i.e., $M^2=1.1$ for an ideal Gaussian beam profile. According to formula (1) and taking into consideration the output laser beam convergence parameters in the instruments, the theoretical ablation area should have a diameter $D(f)$ near $10\text{ }\mu\text{m}$ and therefore a pinhole of $10\text{--}15\text{ }\mu\text{m}$ in diameter could be ideally used for filtration in the case of an ideal Gaussian beam and diffraction limited optics.

For laser beam filtration, a pinhole, located at the focal plane of the focusing lens, acts as a spatial filter, transmitting only the central part of the laser beam, containing low spatial frequencies. When the second lens converts the expanding beam back to the parallel beam, the low spatial frequencies compose a uniform beam, while small features of the original beam, contained in the high spatial frequencies, are cut off by pinhole [10]. To improve the shape of the main beam, the pinhole diameter ideally should be smaller than the unfiltered Gaussian laser spot diameter, in some cases near 0.9. In practice, the diameter for the pinhole filter is typically chosen to minimize loss of laser energy and to cut off secondary effects (for example, satellite spots and rings). Thus, theoretically a pinhole diameter is 1.5 times larger than the beam spot diameter in equation (1) will suffice. In practice, our experiments with several different pinhole diameters in a selected instrument showed that a $25\text{ }\mu\text{m}$ pinhole filter was most effective.

The spatial filtration principle is a simple and inexpensive to cut off satellite laser spots and shape the beam on target, keeping the Gaussian beam profile of the central laser beam. This method does not require introducing any extensive modifications to the instrument since the pinhole filter can be easily installed and removed (Figure 1, panel B). Without the pinhole, the “minimum” laser spot settings of the instrument control software, on target the laser beam consists of a central spot together with several smaller satellite spots with an overall diameter of the envelope of this group of spots of approximately $20\text{ }\mu\text{m}$ (Figure 1, panel C). To estimate the effect of the satellite spots on mass spectra acquired, the following experiment was conducted. First, a mass spectrum was acquired with the pinhole in the optic path of a Bruker Autoflex linear TOF instrument, choosing a sufficient number of laser shots to burn off all matrix material. This spectrum was collected from the central laser spot area from the Figure 1, panel C, right. The corresponding spectrum is shown in Figure 1, panel D, top. Second, the pinhole was flipped out of the laser beam and the spectrum was acquired with the same number of shots, i.e., since the central part was burned off, the spectrum was derived from the satellite spot area shown the Figure 1, panel C, left. The acquired spectrum is shown in Figure 1, panel D, middle. Different spectral resolutions, observed for mass spectra collected with a filtered and unfiltered laser beam (Figure 1, panel D, top and

middle) was caused by a different laser energy at the target before and after filtration. To insure that the spot was not shifted due to mechanical displacements by moving the pinhole or translation stage, the pinhole was flipped in again and spectrum acquired. As expected, there was no signal, except noise, as shown in Figure 1, panel D, bottom. This experiment demonstrates that the signal being collected was from the satellite spot area. In a raster pattern, this effect produces significant signal carry over to the next pixels leading to a decrease in spatial resolution, sensitivity and dynamic range. Furthermore, we demonstrate that although it is possible to obtain images at 5 μm using oversampling with a larger unfiltered laser beam, the filtered beam without oversampling produces clearer images with finer details with significantly fewer laser shots.

The ability to flip the filter out of the laser optic path since it is outside the vacuum system allows direct comparison of images from a section on a single slide. Images of lipids were acquired with spatial filtration using a 25 μm pinhole in a Bruker Autoflex TOF/TOF instrument in the reflectron mode and compared to images acquired using the same instrumental parameters but with spatial filter flipped out of the laser beam path. Figure 1S (Supplemental material section) shows the MS images in negative ion mode of similar areas from serial sections of mouse cerebellum. The MS images are shown for 3 ions as an RGB mix, overlaid on co-registered optical DIC microscopy images of the tissue. The MS image in Figure 1S, panel A acquired with oversampling and without the filter, required a 5 times (or more) laser shots to ablate all the sample relative to the filtered beam (Figure 1S, panel B) with the latter showing greater detail. Further, Figures 1S, panel C and 1S, panel D show magnified images for m/z 888 in grayscale where again a more detailed image is produced using the filtered laser beam. The grayscale images in Figures 1S, panel C and 1S, panel D were drawn using the “voxel” option instead of “interpolate” in the “Display settings” of the BioMap software to best show the raw acquired data. The original data with the filtered laser beam has significantly less pixel to pixel noise and wider dynamic range. From the non-normalized mass spectra shown in Figure 1S, panel F, the spectrum obtained with the spatial filter gave higher signal to noise ratios from 52 peaks (using a picking program with criteria $s/n=10$ and relative intensity threshold 10%) compared to the spectrum obtained without the filter where 35 peaks were detected under the same program settings.

MS images for lipids in the positive ion mode using a filter pinhole to give 5 μm spatial resolution were compared to optical H&E stained images and these are shown in Figure 2A. A corresponding mass spectrum is shown in Figure 2B. A high level of detail in the MS image in positive ion mode allowed visualization of Purkinje cells and some of their surrounding dendrites, as well as axon fibers and granular cells.

Conclusions

Spatial filtration of a laser beam in MALDI MS instruments allows true 5 μm spatial resolution for commercial MALDI imaging MS instruments equipped with larger laser beam spots. Implementation of a ceramic pinhole spatial filter is a practical, low cost method of modifying an instrument to achieve higher spatial resolution than its original design specification with the ease of flipping the spatial filter in and out the laser beam path as desired. The proposed concept is suitable not only for the instruments, used in the work described in this application note, but for any MALDI MS instrument.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Panel A: Scheme of a laser beam spatial filter with the laser light propagating from left to right. Prior to pinhole spatial filtration, the laser beam profile contains noise and additional intensity maxima. After filtration, the secondary spatial beam components are cut off, resulting in a much cleaner Gaussian beam profile transmitted to the target. The Gaussian beam waist diameter, $D(f)$ is typically smaller than a pinhole diameter, d . Panel B: A 25 μm pinhole filter installed in Bruker Autoflex MALDI TOF instrument, showing the filter in the laser optic path (right) and flipped out of the path (left). The setup is similar for both TOF/TOF and the linear instrument. Panel C: Left - ablation pattern using the lowest beam diameter setting on an Autoflex TOF/TOF instrument. Right - ablation area using a 25 μm pinhole filter with the same instrument settings. Notice that the secondary ablation areas have been eliminated by the filter and the central laser beam is sharper on target. Panel D, from top to the bottom: spectrum acquired with pinhole in the laser path and burning out all matrix material; spectrum acquired from the same location, but with the pinhole out of the laser path, demonstrating a signal collected from the satellite spots; spectrum from the same location, but with the pinhole in the laser path again, demonstrating no mechanical displacement.

Figure 2.

Panel A; The lefthand image in Panel A is a brightfield optical microscope image of the serial H&E stained section and to the right are four MS images in positive ion mode from an area of mouse cerebellum acquired at 5 μm spatial resolution with a 25 μm pinhole laser beam filter Panel B, the average mass spectrum over the area imaged.