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Technical Note

Improved MALDI-MS imaging performance using continuous laser rastering

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Abstract

MALDI-MS imaging experiments conventionally use a ‘spot-to-spot’ approach in which spectra are acquired in a time-consuming ‘stop-and-go’ fashion. Here a linear rastering technique is introduced in which the laser is moved continuously in rows across the sample surface. Image acquisition time is decreased by a factor of ~ 2.5 to 5, with greater time savings at high image resolution. More efficient laser sampling of the tissue results in an improvement in sensitivity. High spatial resolution images (e.g. 50 µm × 50 µm) can be generated even with a larger laser spot (~ 100 µm)

Introduction

MALDI-MS imaging has recently emerged as a powerful technique for analyzing the spatial distribution of compounds in biological tissues. This technique offers several considerable advantages over existing bio-imaging methods (e.g. autoradiography), such as the high specificity of MS detection, minimal sample preparation, lack of requirement for radiolabeled compounds, and applicability to a wide variety of analytes. However, one of the key disadvantages of MALDI-MS imaging is the analysis time; depending on tissue size and image resolution, acquisitions can take up to several days for a single sample. This is largely due to the stop-and-go manner in which the laser is halted at each voxel to acquire data and subsequently moved on to the next.

Here we describe a new MALDI-MS imaging technique in which the laser is continuously rastered across the tissue without the conventional stop-and-go motion. This technique improves acquisition time by a factor of 2.5 – 5 while simultaneously improving sensitivity and allowing for high resolution images.

Materials and Methods

Instrumentation: All experiments were performed on a QSTAR® Elite Qq-ToF system (Applied Biosystems/MDS Analytical Technologies, Concord, ON) equipped with an oMALDI™ 2 source. A high repetition-rate Nd:YAG laser with elliptical 150 (h) × 100 (w) µm diameter spot was used, operated at 3.0 µJ/1000 Hz for all experiments. The ion source and laser were controlled by oMALDI™ Server 5.1 software. Data were analyzed using TissueView™ Software ver. 1.0 (Applied Biosystems/MDS Analytical Technologies, Concord, ON).

Sample Preparation: Coronal sections of rat brain were sliced using a Leica Cryostat CM 3050 S (Meyer Instruments, Houston, TX). Slices were made at 12 µm thickness at -16°C. Tissues were thaw-mounted directly on stainless steel Opti-TOF® MALDI plates and stored in a dessicator. Matrix (20 mg/mL a-cyanohydroxycinnamic acid) was applied using a pneumatic nebulizer; 60 coats were applied with 30 seconds drying time allowed between each coat.

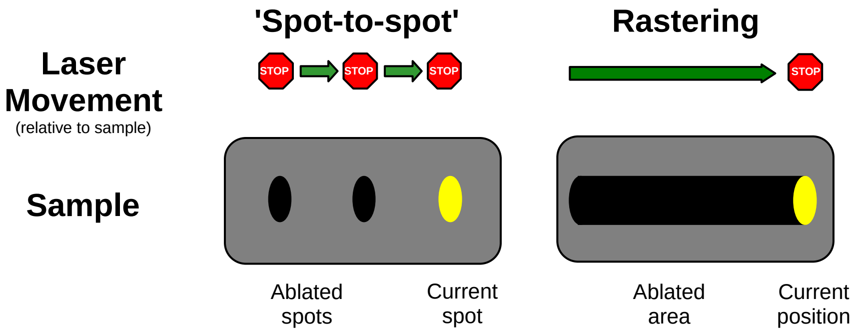


Figure 1: Schematic representation of conventional (left) and continuous rastering (right) acquisitions. During conventional MALDI MSI experiments data are acquired at discrete spots with laser and sample held stationary relative to each other for data accumulation at each voxel. In contrast, during rastering the laser is moved continuously relative to the sample surface, resulting in continuous ablation and data accumulation.

Analysis Time

During conventional MALDI-MS imaging experiments, the laser and sample are held stationary at each voxel for a pre-determined time period for MS (or MS/MS) data accumulation. After this time, the laser shutter is closed and the stage is moved to align the laser with the adjacent voxel to be analyzed, and so on until the entire area to be imaged has been covered. This time-consuming process results in extremely long run times, particularly for larger tissues at high image resolution. On the QSTAR® Elite system using a continuous rastering approach the laser is applied continuously across the sample surface and is only stopped at the end of each row (Figure 1). This results in considerably faster acquisition times compared to 'spot-to-spot' imaging, even when the MS accumulation time per-voxel is the same.

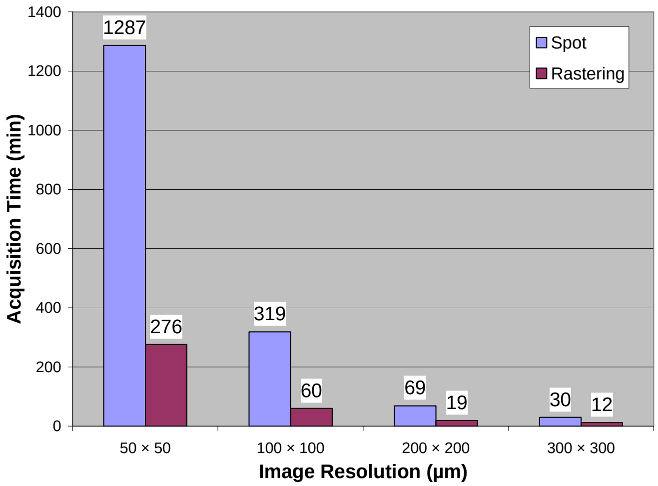
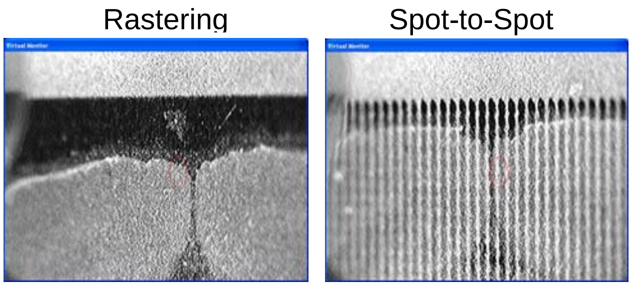


Figure 2: Comparison of acquisition time for MALDI-MS imaging of coronal rat brain sections. All samples were of similar dimensions (approximately 16 mm × 10 mm). For all samples the acquisition time per voxel was identical for both rastering and spot acquisitions to allow useful comparison. Raster imaging was considerably faster than spot imaging in all cases, with the greatest improvement shown at high image resolution (4.7-fold improvement at 50 × 50 µm).

Sensitivity

One of the current challenges for MALDI-MS imaging is the considerable signal suppression which is observed for compounds desorbed from tissue surfaces relative to those ionized directly from a MALDI plate. Depending on the analyte and the particular tissue being sampled, one can expect to experience a loss of signal up to ~1 order of magnitude. It is therefore important to maximize the surface area sampled by the laser to give the greatest possible signal at each voxel.

Most current MALDI-MS imaging instrumentation uses the spot-to-spot movement described above (Figure 1). At each voxel only a tissue surface area equal to the area of the laser spot itself is sampled. Often imaging experiments require a voxel size considerably larger than the laser spot dimensions, and thus a spot-to-spot approach results in a large amount of wasted sample surface area. This is demonstrated in Figure 3 (right panel) which clearly shows unablated matrix between each spot. It follows that spot-to-spot imaging data represents an incomplete sampling of the molecular content at each voxel and, furthermore, will be of reduced sensitivity.


Rastering

Spot-to-Spot

Figure 3: In-source images of rat brain sections after MSI analysis in rastering (left) and spot-to-spot (right) modes of acquisition at 200 µm × 200 µm voxel resolution. Rows of unablated matrix on the spot-to-spot sample clearly indicated unsampled tissue surface. With the rastering acquisition laser sampling of the tissue surface is near complete at this resolution.

Using continuous laser rastering on the QSTAR® Elite the laser sampling of the tissue surface is much more thorough than a spot-to-spot acquisition, and reaches 100% coverage at spatial resolutions greater than ~200 µm × 200 µm (Figure 3). This results in improved representation of the sample molecular population in the imaging data, and considerably improves the sensitivity. Figure 4 shows a side-by-side comparison of rastering and spot-to-spot imaging of phosphatidyl choline in rat brain tissues (adjacent tissue slices, coated with matrix simultaneously, and analyzed back-to-back). The enhanced sensitivity is quite apparent for lower resolution images (i.e. voxel size ~ 300 µm × 300 µm) where laser sampling of the tissue in spot-to-spot mode is particularly inefficient. For even lower resolution images (e.g. > 400 µm × 400 µm) maximum sensitivity can be realized by using the Dynamic Pixel Imaging feature which combines rastering and spot-to-spot approaches by continuous rastering of the laser in a serpentine pattern within each voxel area.

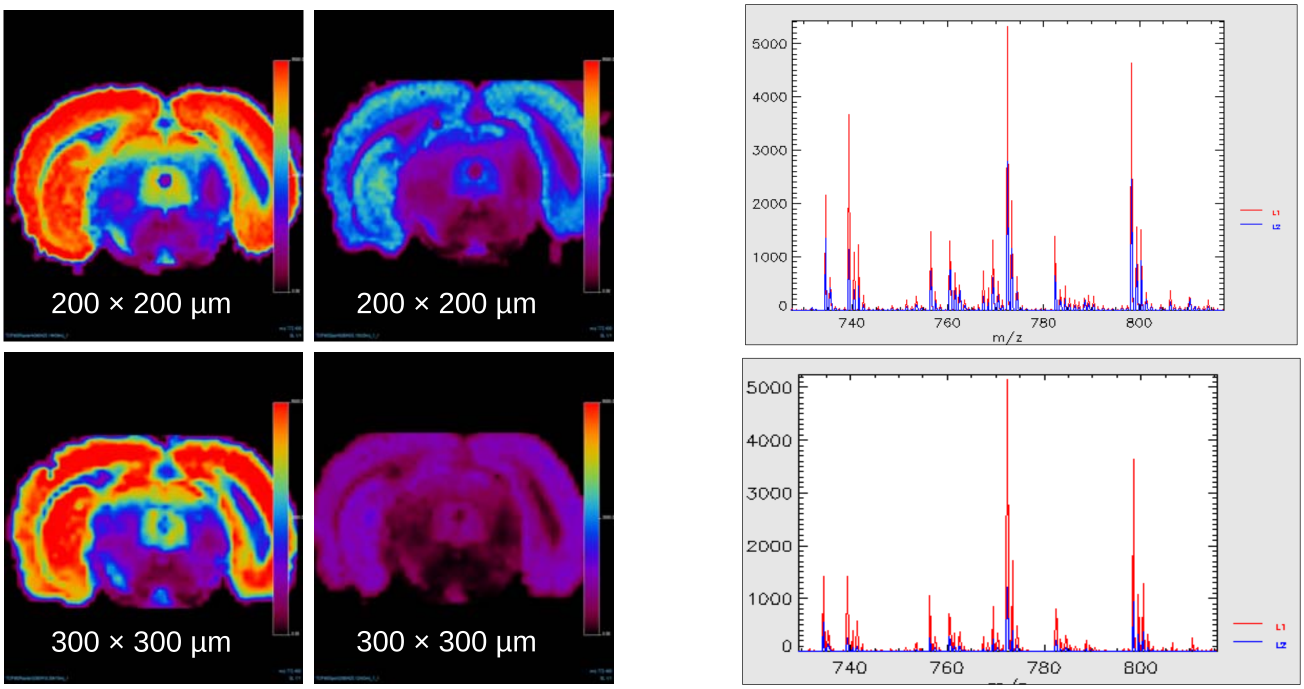


Figure 4: Signal intensity for phosphatidyl choline in rat brain (m/z 772.4) for continuous rastering (left images) and spot-to-spot (right images) acquisitions. Colour intensity scales are identical for both types of acquisitions to allow visual comparison. Overlaid spectra for rastering (red) and spot-to-spot (blue) acquisitions are shown on the right. For all analyses the rastering technique resulted in improved signal intensities for observed species. This effect is most pronounced at lower resolution where spot-to-spot imaging is least efficient (*i.e.* unsampled tissue area is larger).

Image Resolution

Oversampling: The limiting factor for image resolution is typically understood to be laser spot size. This perceived limitation can be overcome easily by oversampling[1]. With this technique a spot is first 'burned-out' by the laser so that no additional ions can be desorbed from that location; this is followed by moving the laser only a fraction of its width such that the new area from which ions are desorbed corresponds to only a fraction of the total laser spot area. Using oversampling it is possible to generate images on the QSTAR® Elite system with voxel resolutions of 50 µm × 50 µm.

High-Resolution Rastering: With continuous laser rastering, high resolution on the vertical axis can be obtained by oversampling (i.e. overlapping rows). In the horizontal direction, however, the effective minimum voxel size is determined by the distribution of ions desorbed along the entire laser width at any point in time during rastering. If ions are desorbed to a similar extent along the entire laser cross-section then the horizontal resolution is limited by the laser width (*i.e.* ions generated across the entire 100 µm laser spot will contribute significantly to the mass spectrum). Alternatively, if a large majority of ions are created at the front edge of the rastering laser, then the effective voxel width can be much less than the width of the laser (*i.e.* since much of the laser spot area will contribute relatively few ions to the mass spectrum).

At a given rastering rate and laser repetition frequency any position along the laser cross-section can be considered in terms of the number of laser shots to which it has been exposed:

Distance from front edge of laser = [# shots] × [Raster rate (µm s^{-1}) ÷ Laser rep. rate (s^{-1})] [Eq. 1]

Sample at the laser 'front' will have been exposed to fewer shots than sample at the 'rear' of the laser. Using Eq. 1, one can construct a profile of the quantity of ions desorbed as a function of position along the laser cross section by monitoring the signal at a particular location on tissue after it has already been pre-exposed to a varying number of laser shots. Here the desorption profiles for several ions in rat brain were determined (Figure 5).

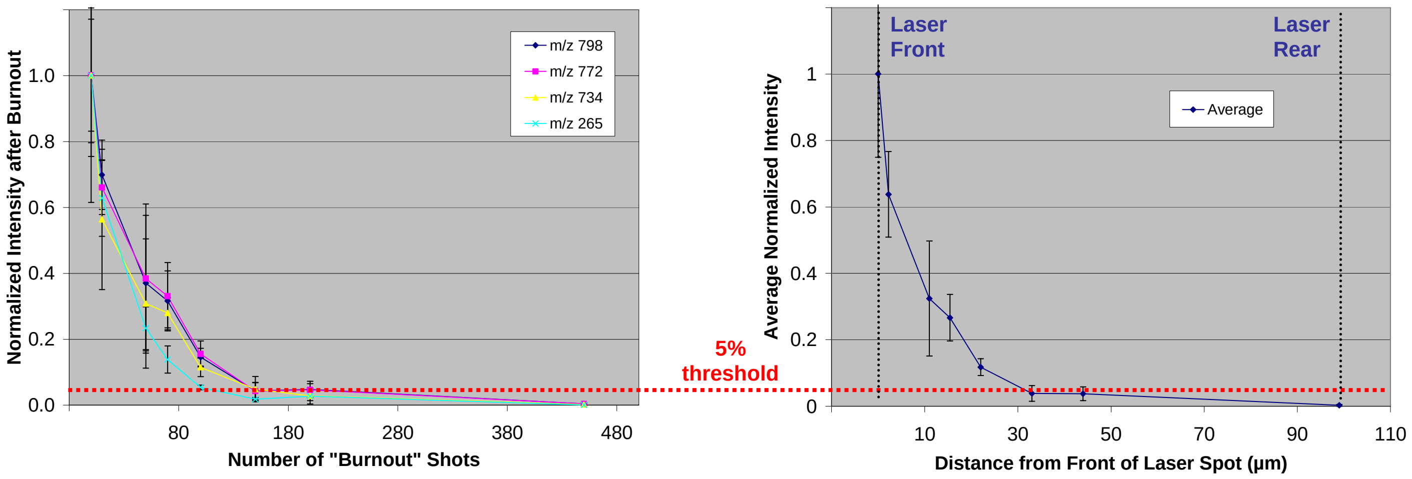


Figure 5: *Left:* Signal intensity of a selection ions on-tissue following pre-exposure to a specified number of "burnout shots". Using equation 1 (above) this data can be replotted to represent the flux of ions across the entire width laser (*Right*). Here it can be seen that the flux of ions drops to < 5% of the original intensity approximately 30 µm from the front of the laser. This represents the minimum voxel size under the experimental conditions.

Figure 5 (right) indicates that the signal falls below 5% of the initial intensity by ~ 30 µm from the front of the laser. Integration of the curve indicates that this area encompasses approximately 98% of the total ion current generated across the entire laser width. Consequently, images with 50 × 50 µm voxels are easily obtainable (Figure 6), and even higher resolutions can be achieved by using a slower rastering rate and/or a higher laser power setting. The laser power used here corresponds to only 30% of the maximum available on the high repetition rate Nd:YAG laser used with the QSTAR® Elite system; considerable additional power is available which would presumably result in a higher proportion of ions desorbed near the front-most portion of the laser during rastering, thereby improving voxel resolution.

Conclusions

Here it is demonstrated that continuous laser rastering is a notable improvement in many important ways over conventional spot-to-spot imaging. Specifically, analysis time was shown to be improved by up 5-fold on rat brain sections, with particular advantages at the highest image resolutions where conventional imaging is often prohibitively time-consuming. Considerable improvements in sensitivity are also realized owing to more efficient sampling of the tissue surface. Finally, it is shown that high resolution images can be obtained using continuous laser rastering. Here images with 50 µm × 50 µm voxels are shown but even higher resolutions are easily achievable on the QSTAR® Elite system.

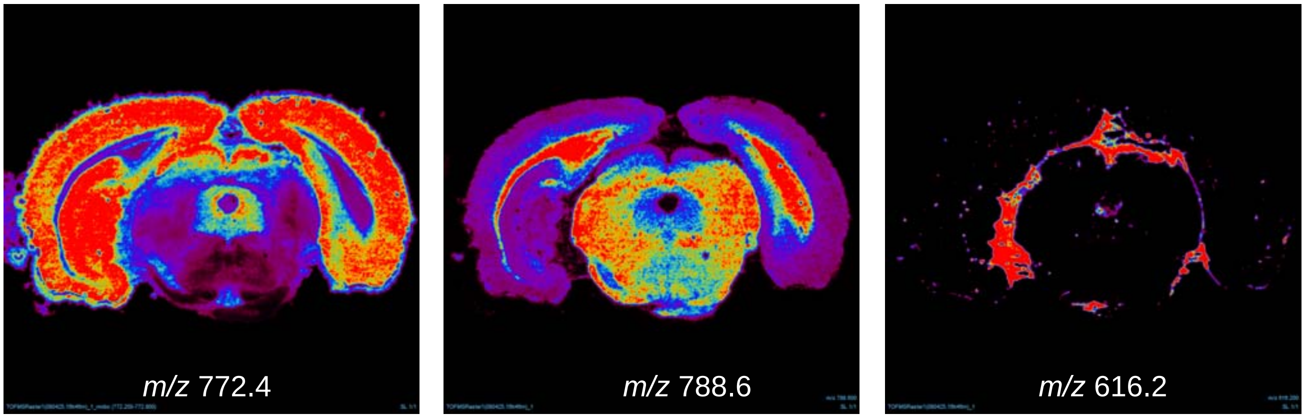


Figure 6: Rastering acquisition of coronal rat brain section at 50 µm × 50 µm voxel resolution. Selected ions chosen for display include lipids at m/z 772.4 and 788.6, and heme at m/z 616.2, showing the locations of blood vessels in the tissue section. Acquisition time was approximately 4.5 hrs.

References

1. Jurchen J. C., Rubakhin, S. S., and J. V. Sweedler, 2005, MALDI-MS imaging of features smaller than the size of the laser beam. J. Am. Soc. Mass Spectrom., 16:1654 – 1659.

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