



Technical Note

A practical introduction to MALDI-MS imaging

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Introduction

MALDI mass spectrometric imaging (MSI) has become a powerful tool for determining the spatial distribution of compounds in biological tissues¹⁻³. This technique offers the speed and molecular specificity of MALDI-MS detection with the added dimensionality of spatial location. The power of MALDI-MSI is currently being realized in several fields including drug development, neuroscience, and cancer research. However, the potential applications for this technique span a much wider range, and include any work in which knowledge of the spatial arrangement or distribution of ionizable compounds is beneficial.

MALDI-MSI has several notable advantages over alternative techniques. Key among these is the applicability to a range of analytes, including small molecule drugs, metabolites, peptides, proteins, lipids, and any other compounds amenable to MALDI. Due to the high specificity of MS detection, it is straightforward to resolve a drug compound from its metabolites, which is generally not possible with methods that use a labeling approach.

It often comes as a surprise that the necessary steps for a MALDI-MSI experiment are quite simple,

especially when compared to alternative techniques such as autoradiography. The overall workflow is summarized in Figure 1. In brief, a sample (usually frozen animal tissue) is sectioned into ~10 – 20 µm thick slices and mounted onto a stainless steel MALDI plate. Matrix is applied over the entire sample area using one of several available coating techniques, and a two-dimensional array of MALDI spectra are acquired, with each spectrum associated with a location on the sample. Finally the data are visualized using specialized software such as TissueView™ 1.0 Software.

A general discussion of the steps required in an imaging experiment, from sample preparation to data processing, is presented here. Consideration is given to the practical and technical aspects of MALDI-MSI with the goal of informing those who are considering adding this capability to their arsenal.

Sample Preparation

In the case of biological tissues, once the tissue samples are harvested they should be stored in such a way as to preserve their original shape, and prevent delocalization of the molecular species within the tissues. This can be accomplished by first wrapping

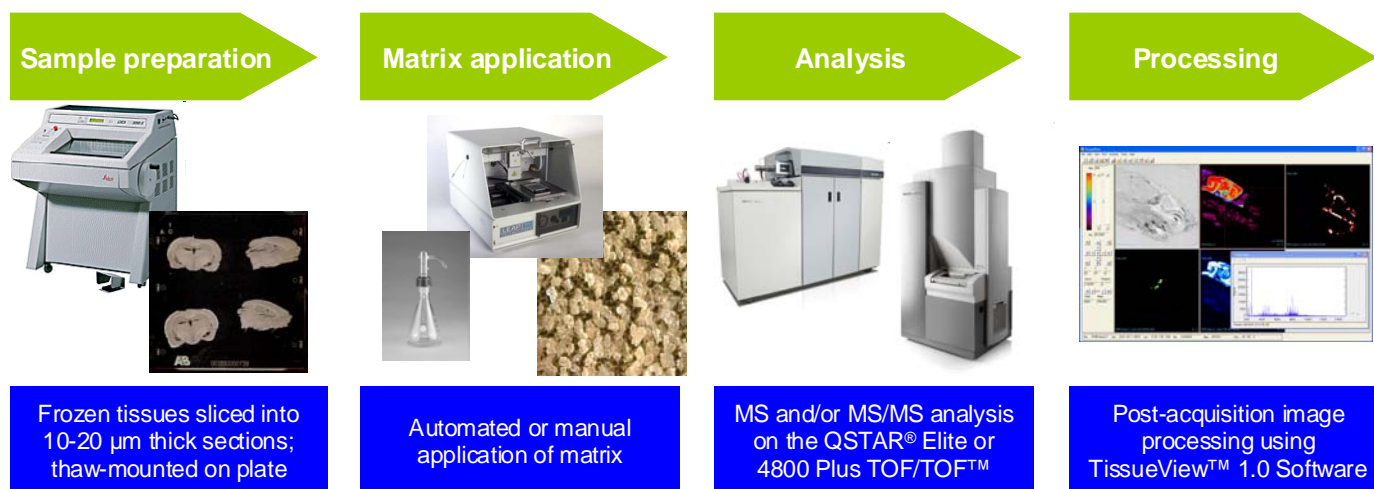


Figure 1: The MALDI MSI workflow

the harvested sample in aluminum foil and subsequently freezing the tissue using liquid nitrogen. Tissues can then be stored for up to a year at -80°C without degradation⁴.

To be compatible with MALDI analysis, frozen tissues must first be sliced into thin sections, usually performed using a cryostat device. This apparatus allows production of flat micrometer-thick slices (typically 10 to 20 μm) while maintaining the sample at sub-zero temperatures (-10 to -20°C). The 'flatness' of the tissue surface is important because highly irregular surfaces can result in poor mass calibration on axial instruments (e.g. TOF/TOF) and inaccurate location assignment on instruments with an off-axis laser (most other MALDI instruments). Once sliced, samples can be mounted on pre-cooled MALDI plates by manipulating the tissue to the desired position and applying a small amount of heat to the back side of the plate under the tissue (typically with the tip of the finger for smaller samples). No fixatives or adhesives are required for either the slicing or mounting procedures. Once the tissue slice is thaw-mounted on the plate it can be set aside in a desiccator to dry before matrix application.

Matrix Application

The application of matrix is a critical step in an MSI experiment. The goals of this process are two-fold: i) to extract the analyte from the tissue sample into the matrix solvent without causing lateral delocalization, and ii) to provide *uniform* coverage of matrix across the sample surface in a manner that allows formation of the finest possible crystals. These requirements can be met by applying the matrix solution in the form of a fine mist rather than the spotting fashion used for conventional MALDI experiments (Figure 2). Several methods can serve this purpose, including active spray techniques using a TLC sprayer, air brush or electrospray apparatus, or settling techniques such as sublimation⁵ or nebulization, which introduce the matrix in the airspace over the sample and allow it to settle passively on the tissue surface. Matrix application can be done manually with little expense (e.g. TLC sprayer) or in an automated fashion using commercial devices available from several manufacturers.

The choice of matrix compound is generally the same as that which would be used for the analyte in a non-imaging experiment. Typically α -cyano-hydroxy-cinnamic acid is used for peptides, lipids, and many small molecule drug compounds, sinapinic acid for proteins, and 2,5-dihydroxybenzoic acid for some small molecules and lipids.

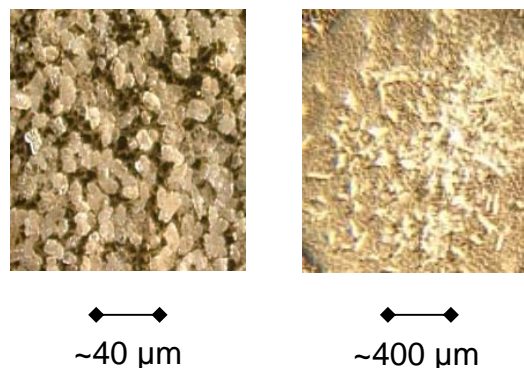


Figure 2: Matrix crystallization (sinapinic acid) using spray coating (left) and conventional spotting (right) techniques. Spray coating yields roughly spherical crystals on the order of 10 μm in diameter, whereas spotting results in considerably larger elongated crystals.

It should be noted that in some cases it is useful to pre-treat the tissue sample prior to matrix application. Particularly for protein imaging, it is common to rinse the mounted tissue section in ice-cold ethanol before the matrix is applied⁴. This serves to remove a significant amount of interfering/suppressing compounds, while preserving most of the proteins. For most peptide/small molecule imaging, however, a rinse is not necessary, and may delocalize or remove the analyte entirely.

Analysis

During an MSI acquisition the positioning stage is moved such that the laser is rastered across the sample, either continuously (QSTAR[®] Elite System), or one spot at a time (4800 Plus MALDI TOF/TOF[™] System and others) (Figure 3). At each position on the tissue a mass spectrum is acquired which is associated with that particular location. Ultimately a two-dimensional array of spectra is created covering the entire sample. Each point in the array is called a voxel; the area to be imaged, as well as the spacing between each voxel (*i.e.* the resolution of the resulting image) is user-defined prior to the acquisition. Voxels

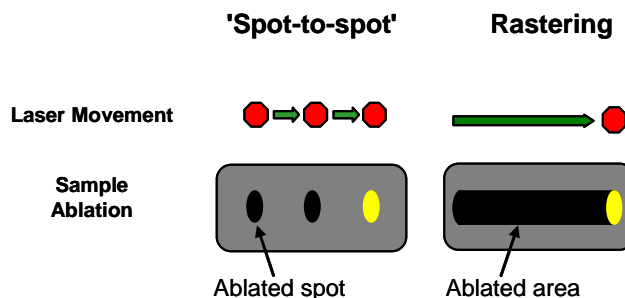


Figure 3: Schematic representation showing laser movement and sample ablation during spot-to-spot (most instruments) and continuous raster (QSTAR[®] Elite) imaging.

typically range from 50 × 50 μm up to 1 × 1 mm, with longer acquisition times required for higher resolutions.

The MSI acquisition generally takes on one of two forms depending on the goal of the analysis: MS profiling, or targeted analysis by MS/MS.

MS Profiling: This type of acquisition is typically used to survey a relatively broad spectrum of endogenous compounds. In this mode, a mass spectrum with a wide m/z range is acquired at each voxel position. An ion intensity map can then be generated for any m/z in the analyzed range, enabling simultaneous imaging of many compounds. However this wide coverage comes at the sacrifice of duty-cycle and specificity, and does not allow distinction between isobaric species.

Targeted Analysis: Due to the high complexity of tissue samples, in cases where the analytes are of known mass (e.g. a dosed drug) a targeted MS/MS approach is often used. For this type of experiment a product ion spectrum of the known precursor is acquired at each voxel. The resulting ion image is generated based on a known fragment ion. Ideally a

neat sample of the analyte is available for prior optimization of the MS/MS conditions, however, where that is not possible, MRM data/parameters from ESI-triple quadrupole experiments can be useful. In the case of proteomic imaging, a targeted MS/MS approach can be used to perform on-tissue sequencing and imaging of known peptides (either native peptides⁶ or tryptic peptides following an *in situ* digestion⁷).

Data Processing

Following the MSI acquisition data can be visualized using software such as TissueView™ 1.0 (Figure 4). This allows access to mass spectral data and generation of ion intensity maps for any peak in the detected m/z range. A mass spectrum for any location within the image can be displayed, as well as an average spectrum for a user-defined region of interest. Image manipulation tools allow overlaying of intensity maps for several ions, as well as the superpositioning of imaging data on to optical images for display of analyte distributions in the context of the entire sample. Mathematical tools are also included which allow a number of operations (e.g. subtraction,

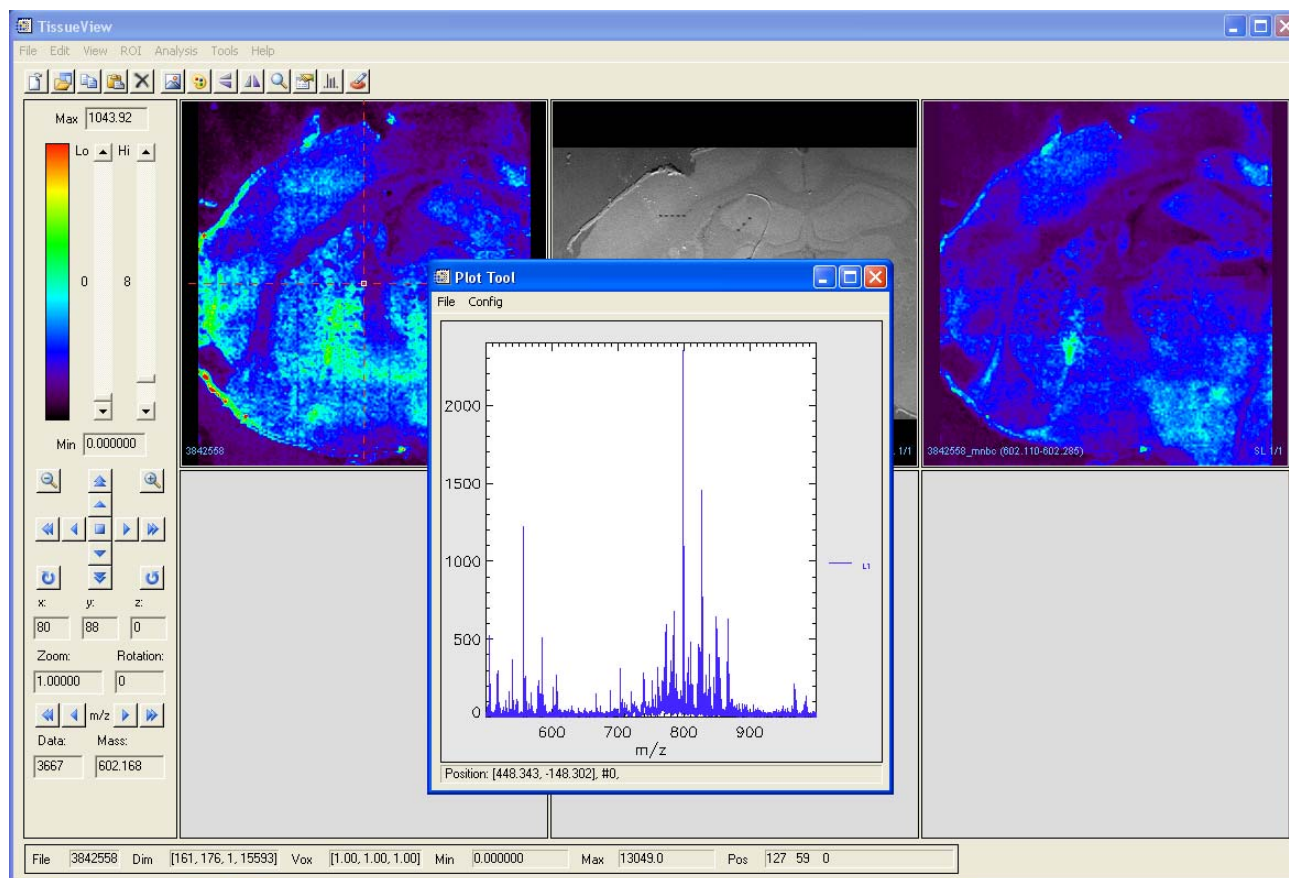


Figure 4: User interface for TissueView™ 1.0 Software. The Plot Tool (center) provides the link between mass spectral and spatial dimensions. This allows generation of images of any m/z in the mass spectrum, and, conversely, the mass spectrum of any location on-tissue.

division, baseline correction, *etc.*) to be applied to images, and statistical comparison within and between samples.

Conclusion

The workflow for a MALDI-MS imaging experiment has been discussed here with particular attention to practical considerations. The MSI workflow is straightforward, rapid, and inexpensive compared to alternative techniques. Mass spectrometric detection provides high sensitivity and selectivity, rapid acquisition, and the ability to detect simultaneously multiple analytes in a single imaging experiment. These unique advantages make MSI a powerful complement to established techniques in biomarker, clinical, and pharmaceutical research.

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