

Mass Spectrometry Imaging Using Desorption Electrospray Ionization

Introduction

Molecular imaging using mass spectrometry(1) allows correlations to be drawn between the chemical identity of each analyte and its spatial distribution at or near the sample surface. DESI-MS(2,3) is a new method for visualizing the distributions of many molecules directly from an intact tissue section, without the need for chemical labeling or prior chemical treatment to the tissue. By scanning the charged-droplet beam across the surface in a two-dimensional array of predefined points, molecular images are constructed by plotting the intensity of one or more of the ion signals derived from the surface as a function of position in the array.

Experimental Approach

Tissue imaging using DESI is typically performed on thin, histological sections between 4 and 20µm thickness. Flash frozen tissue is cut using a cryomicrotome and thaw mounted directly onto a microscope glass slide. A general DESI imaging workflow is represented in **Figure 1** (4).

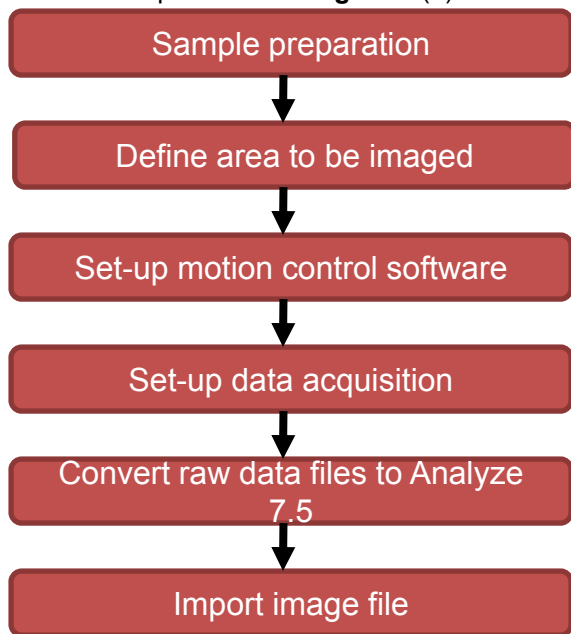


Fig. 1 Flow diagram of the DESI imaging workflow

Omni Spray® DESI

When using the Omni Spray Ion Source for imaging, the surface is scanned in one direction at a constant velocity (**Fig. 2**) (3-5). The surface is moved from left to right in the direction orthogonal to the inlet of the mass spectrometer, although the surface could also be moved from right to left. For imaging, the Omni Spray Ion Source is utilized in a continuous velocity (CV) scan mode. In the graphical user interface, the user defines the dimensions of the area to be imaged (as relative distances to the defined origin) and the surface velocity. The area to be imaged is then highlighted in the right panel (**Fig. 3**).

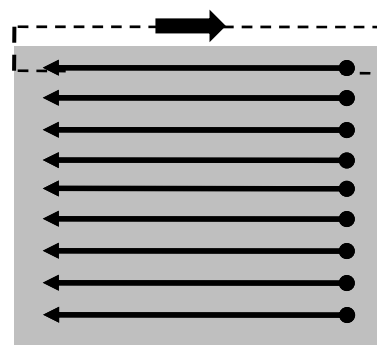


Fig. 2 Schematic representation of the motion pattern during data acquisition. The arrows represent the direction of the spray nozzle relative to the surface.

The DESI imaging experiment can be conducted in several operational modes: (i) full scan MS, (ii) product scan MS/MS or MSⁿ, or (iii) multiple reaction monitoring (MRM). Full scan MS allows simultaneous mapping of multiple ions while MS/MS imaging isolates a particular ion or set of ions over a narrow m/z range and monitors a characteristic fragment ion. Common experimental parameters for the Omni Spray® Ion Source are shown in **Table 1**.


Table 1. Omni Spray® Ion Source & Instrument Settings

Parameter	Setting
ES voltage	3-5 kV
Solvent flow rate	1.5-3 µl/min
Gas pressure	100-150 psi
Distance from tip to surface	1-2 mm
MS inlet temperature	300°C
Spray impact angle (α)	55-65°
Surface scan rate	Variable

Mass Spectrometry

A Thermo Fisher Scientific LTQ (San Jose, CA) linear ion trap mass spectrometer equipped with Xcalibur™ 2.0 data acquisition software or other mass spectrometer having an atmospheric pressure interface can be used. For DESI imaging the mass spectrometer’s atmospheric interface is equipped with an Extended Ion Transfer Capillary. Since the moving stage and the mass spectrometer work independently, a +5V TTL signal (or other mechanism) from the motion stage controller triggers the start of data acquisition at the beginning of each line scan. The data acquisition time per line, which is established in the data acquisition software, must be equal to the time that is required to perform one line scan at the defined surface velocity.

Data Conversion

FireFly  data conversion software allows transformation of raw data files into the Analyze 7.5 file format. A screenshot of the Graphical User Interface is shown in **Figure 4**. By converting raw data into Analyze 7.5, raw data can be visualized in BioMAP (Novartis), a universal software tool for data visualization and analysis. Raw data files are imported into FireFly and sorted sequentially by file name. For very large image sizes, the mass-to-charge data may be binned to reduce the image file size. Additionally, mass spectral scans in the x dimension may be averaged to increase the pixel width in the x dimension.

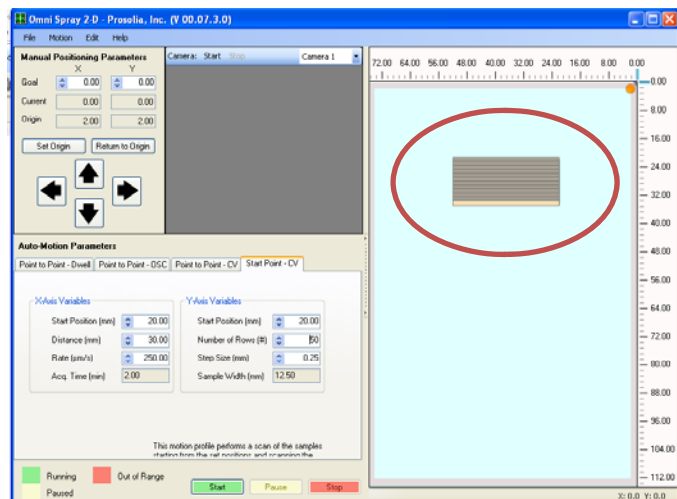


Fig 3. Screen shot of the Omni Spray motion control software. Parameters for imaging are entered into the designated text boxes under Auto Motion Parameters.

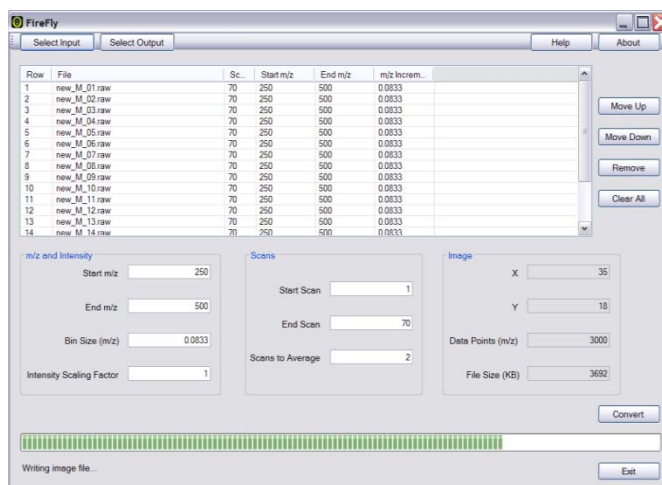


Fig 4. Screen shot of FireFly software for converting raw data into Analyze 7.5. (Powered by Indigo Biosystems)

Molecular Imaging Example

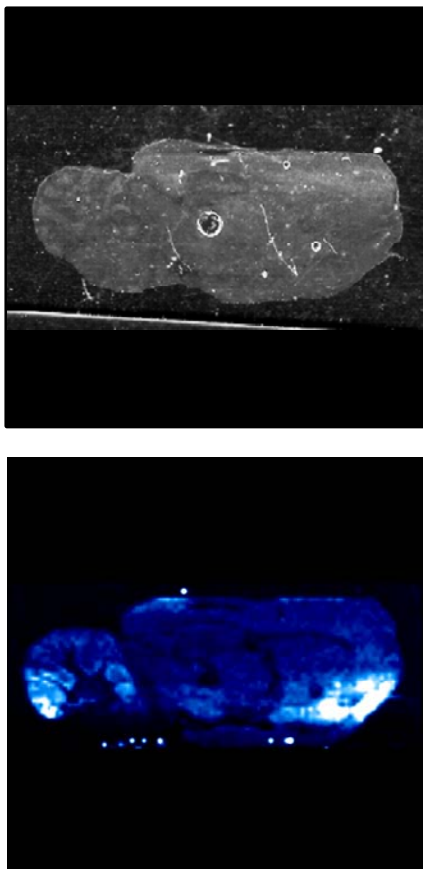


Fig 5. An example of a molecular image of m/z 772 recorded in positive ion mode of a 10 μ m sagittal rat brain section. Optical image (top) and molecular image (bottom). Pixels: 105 x 43. Pixel size: 245 μ m x 245 μ m.

Conclusions

The automated Omni Spray Ion Source enables molecular imaging of tissue samples of various sizes and shapes. The motion system has a sample capacity of up to 76 mm. by 114 mm. and has 10 μ m step resolution. The motion system is synched with the instrument vendor's data acquisition software.

FireFly data conversion software transforms your raw data into image files that are commonly viewed in BioMAP data visualization software.

MS imaging using DESI presents advantages over existing technologies used in bioanalysis, *viz.* 1) it does not require the use of radioactive labels; 2) it can be used to rapidly detect endogenous and exogenous compounds and display their spatial intensity distributions in two dimensions directly from untreated, intact tissue sections; and 3) it can reveal relative quantitative information. Considering all these features, this method promises broad applicability in pharmacology, toxicology and oncology. It has the advantage of limited sample preparation and, unlike traditional radiographic approaches used in pharmaceutical studies, it allows the simultaneous recording of molecular information for the drug molecule, its metabolites and endogenous compounds.

References

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