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Application Note

An Improved Workflow for DESI Imaging Mass Spectrometry Incorporating Waters High Definition Imaging Software

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the improvements in Waters High Definition Imaging (HDI) Software, which now provides a more intuitive user interface and streamlined acquisition process, including seamless integration of instrument control MassLynx Software. With these improvements, multiple experiments can be queued and the data can be processed automatically for review within HDI.

Benefits

New software acquisition approach streamlines DESI imaging workflow to maximize data acquisition.

Introduction

DESI (Desorption Electrospray Ionization) mass spectrometry is a simple and straightforward mass spectrometry imaging technique. It requires no sample preparation, is performed at ambient pressure, and provides good sensitivity for a range of compounds. Recent hardware improvements have made DESI a robust and stable technique, allowing DESI imaging experiments to be run at a spatial resolution in the region of 50 µm. Now the demand is on the MS acquisition system to ensure that maximum information can be obtained from each tissue sample. This requires fully automated sequential experiments – for example, the acquisition of multiple DESI imaging datasets from samples mounted on the same or adjacent glass slides. Without an integrated software solution however, sequential DESI imaging experiments can be challenging to set up, with multiple parameters to control.

Results and Discussion

New, improved workflow for DESI imaging setup.

An improved version of Waters High Definition Imaging Software – combined with the implementation of DESI imaging in MassLynx – has been developed to provide a single, streamlined, and user-friendly acquisition process.

This new DESI imaging workflow (Figure 1) consists of:

- Acquisition of an optical image (photograph or flat bed scan) of the sample – typically mounted on a conventional glass slide (Figure 1a).
- Placement of the sample slide onto one of the two DESI slide holders, or the large area microtitre plate holder (Figure 1b).
- Co-registration of the optical image with HDI to easily and accurately align the image with the sample stage (Figure 1c).
- Definition of the region to be imaged as well as the selection of the speed of the 2D-stage, with automatic MS scan speed calculation (Figure 1d).

- Selection of the mass spectrometry conditions: type of experiment (MS-Tof, MS/MS-Tof, MS-IMS, MS/MS-IMS, HDMS^E), mass range, collision energy, polarity, and mass analyzer mode (Figure 1d).
- Selection of the data processing parameters for automated processing (Figure 1d).

Once these simple steps are complete, the whole experiment is exported as a MassLynx sample list to be run directly on the system without any further input. MassLynx controls the stage of the Prosolia 2D DESI source during the imaging experiment – including the X/Y coordinates – acquiring all raw data into a single data file per image.

Following acquisition of the DESI imaging data, the raw data can be processed automatically (Figure 1e) such that it can be visualized in the Image Control section of HDI (Figure 1f), including the full integration of ion mobility separation, if present in the data. The DESI ion images and the optical image can also be easily overlaid.

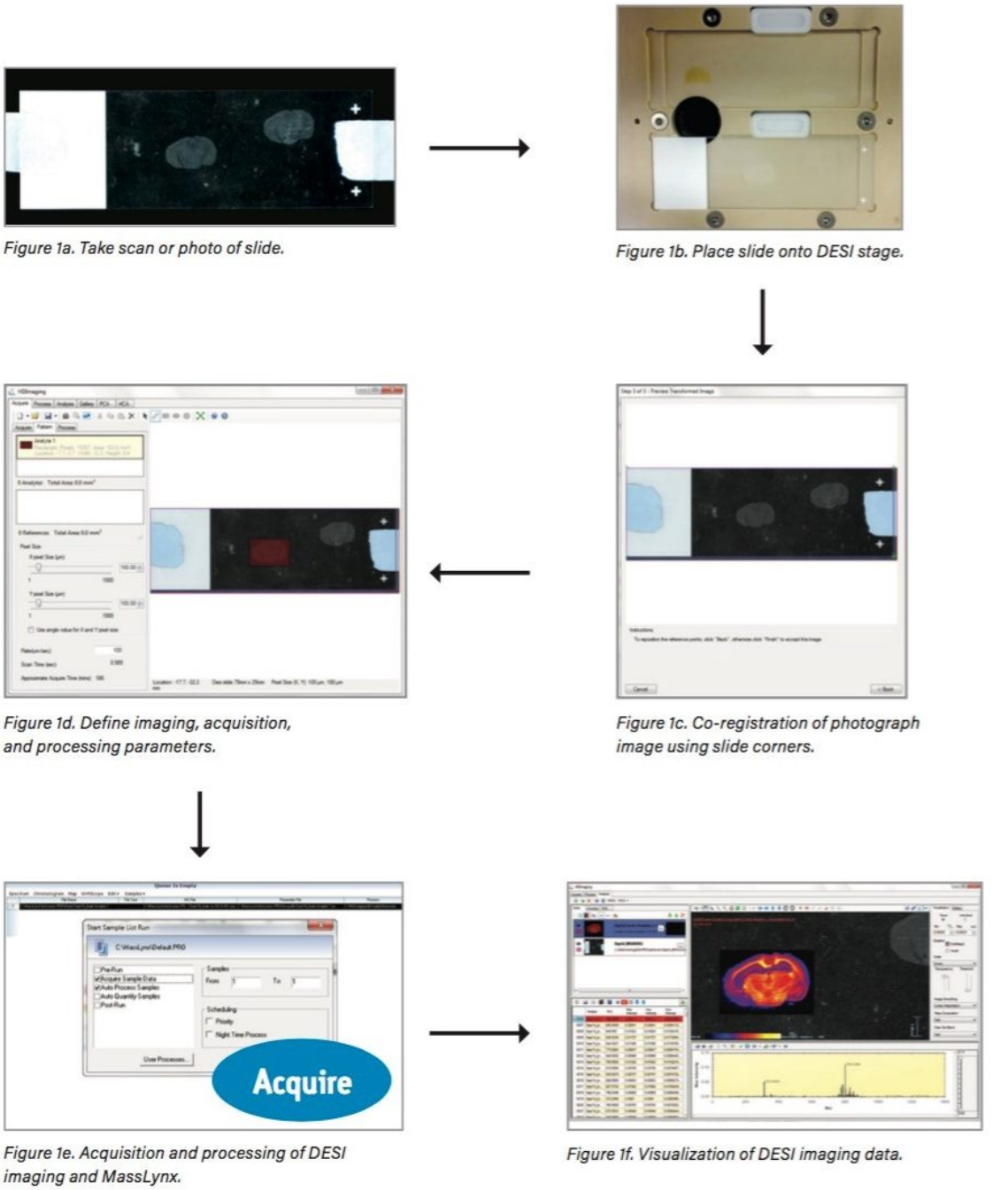

Figure 1a. Take scan or photo of slide.

Figure 1b. Place slide onto DESI stage.

Figure 1c. Co-registration of photograph image using slide corners.

Figure 1d. Define imaging, acquisition, and processing parameters.

Figure 1e. Acquisition and processing of DESI imaging and MassLynx.

Figure 1f. Visualization of DESI imaging data.

Figure 1. Improved DESI imaging workflow using High Definition Imaging (HDI) Software.

Batch Mode – acquiring multiple DESI imaging experiments

An additional advantage of this experimental approach is that multiple experiments can be queued up to run

within MassLynx. Therefore, a selection of samples can be analyzed without the need for user intervention.

In Figure 2, this approach has been used to analyze a mouse brain tissue section, first in negative ion mode, followed directly by positive ion mode of acquisition over the same area of the tissue. At the end of both experiments, the two datasets were automatically processed and ready to be reviewed by the user within HDI.

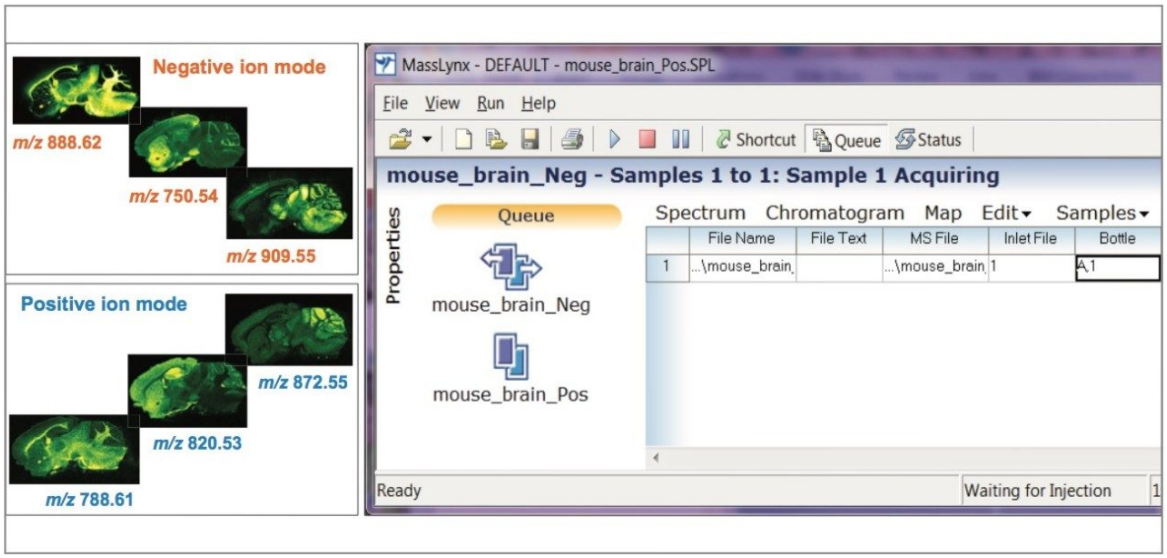

Figure 2. Multiple negative and positive ionization DESI imaging experiments on the same mouse brain tissue section, facilitated by the use of batch analyses through MassLynx sample list.

Another example is displayed in Figure 3, showing the workflow used on three samples of drug-treated mouse skin tissue sections placed onto one slide, and three samples of tissue sections from an untreated mouse placed onto a second slide. By mounting these slides into the two holders of the DESI source, six imaging experiments were run in sequential fashion, eliminating the need for user intervention and allowing a complete imaging MS study to be run overnight. With automated processing of the six raw datasets, it was possible to carry out unsupervised and supervised multivariate analyses – Principal Component Analysis (PCA) and Orthogonal Partial least squares Discriminant Analysis (OPLS-DA) – to extract the molecular differences between the treated and untreated skin tissue sections.

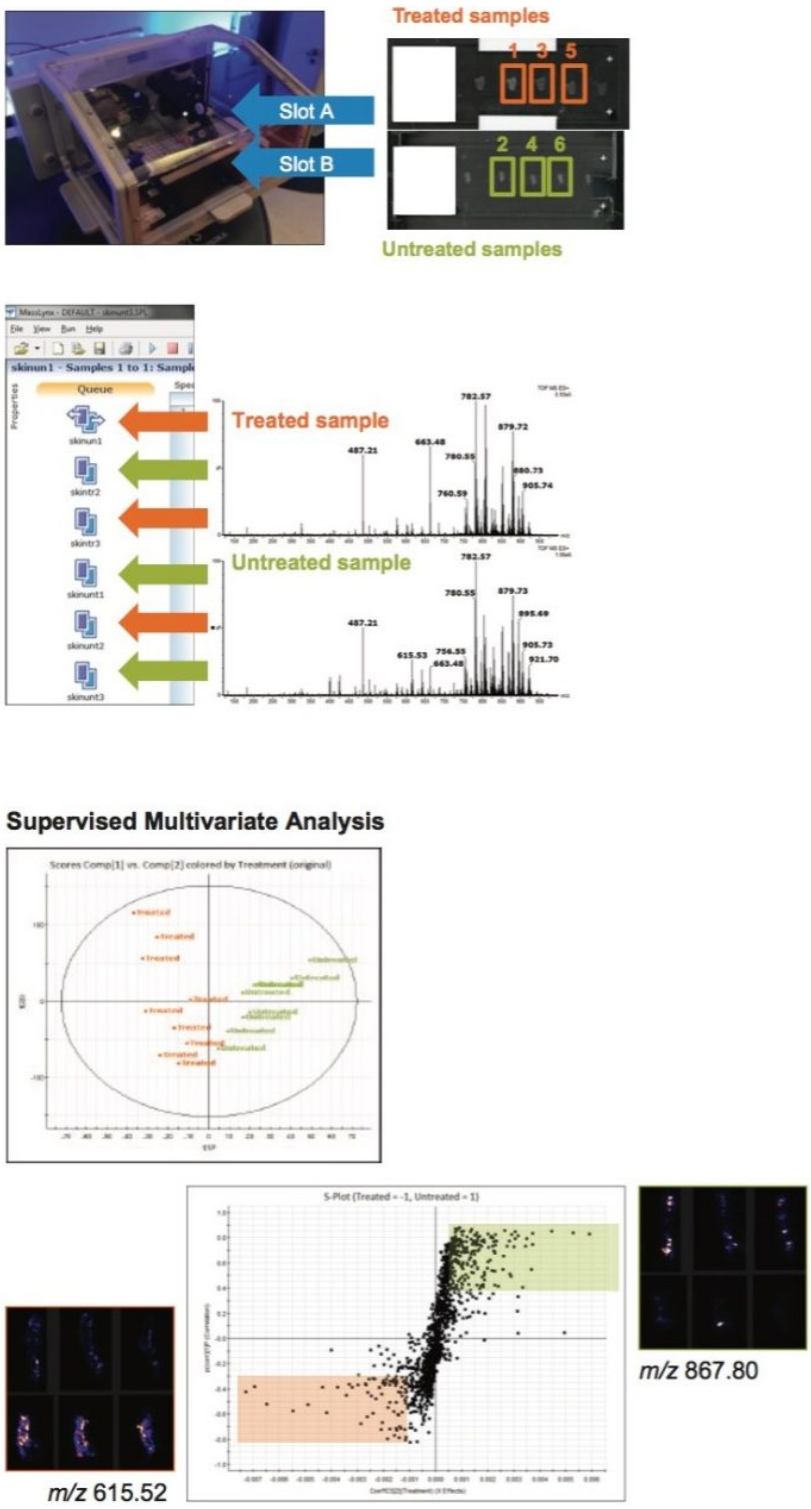

Figure 3. Multiple DESI imaging experiments of different tissue sections, facilitated by the use of batch analyses

through MassLynx sample list with multivariate analysis to determine the difference in endogenous molecules identified using the different conditions.

Conclusion

- Waters software improvements in HDI and MassLynx allow for a highly intuitive and rapid imaging experiment setup workflow for DESI imaging.
- Series of multiple DESI imaging experiments can be queued up and carried out overnight to maximize data collection and improve sample throughput.
- All improvements and software functionalities are implemented on the SYNAPT G2-S*i* and Xevo G2-XS mass spectrometers.

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High Definition Imaging (HDI) Software <https://www.waters.com/134833914>

MassLynx MS Software <https://www.waters.com/513662>

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