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Note d'application

Full Spectrum Molecular Imaging on the SYNAPT XS: Combined Imaging Techniques Yield Comprehensive Results

Emmanuelle Claude, Mark Towers, Joanne Ballantyne

Waters Corporation

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

Abstract

This application brief showcase the complementary nature of Full Spectrum Molecular Imaging on the SYNAPT XS, demonstrating the benefit of this updated fully integrated solution to MS imaging customers.

Benefits

The only HRMS platform to feature both fully integrated traditional MALDI and new and improved DESI XS sources, providing a flexible, comprehensive, and simple solution for holistic molecular MS imaging.

Introduction

Full Spectrum Molecular Imaging (FSMI) combines several analytical techniques in a single platform to probe a wide spectrum of spatially distributed molecular ions. This is used to understand molecular function and physiology or to monitor the distribution of drug compounds throughout a tissue or organism. Since 2015, the SYNAPT family platforms have employed this combined type of workflow to enable MS imaging customers to have the most comprehensive imaging solution possible for their wide-ranging discovery needs.

Full Spectrum Molecular Imaging systems utilize a combination of mass spectrometry techniques that are well suited to analyze particular types of molecules (peptides, lipids, small molecule metabolites, sugars, etc.). See Figure 3 for the relative benefits of MALDI and DESI analyses.

These techniques complement each other to deliver the most comprehensive level of information possible with MS imaging (see Figure 1 for FSMI workflow for tissue imaging).

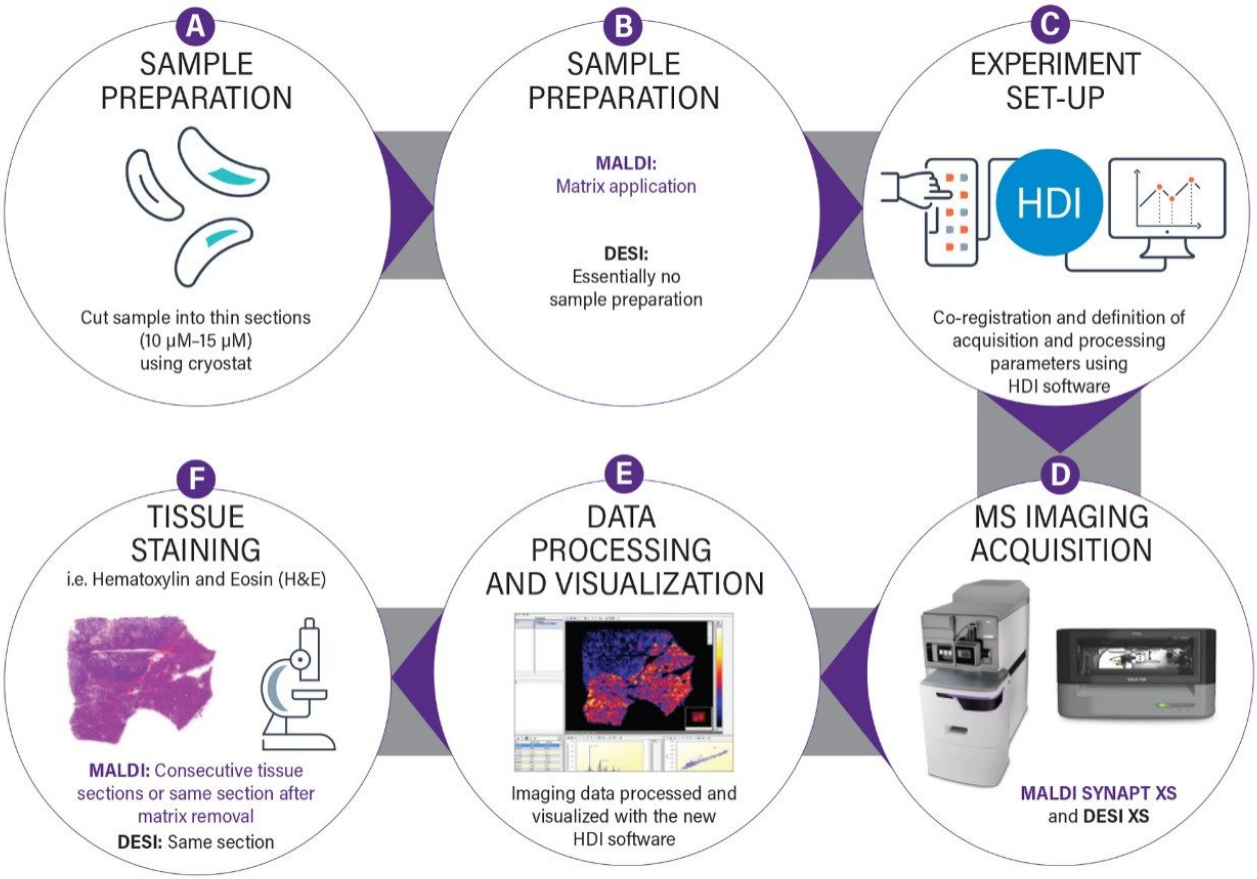

Figure 1. Full Spectrum Molecular Imaging workflow.

By integrating DESI, MALDI, and ion mobility mass spectrometry techniques with a single informatics solution, Waters Full Spectrum Molecular Imaging system provides detailed molecular information that exceeds any single MS imaging technique currently available.

- Discover, identify, and measure the spatial distribution of target or endogenous molecules
- Perform imaging studies without the need for labelled probes
- Effectively study a wide range of large and small molecules
- Extract the maximum level of information from a single sample
- Obtain definitive molecular distribution of key compounds

Results and Discussion

To augment MS imaging capabilities, we have significantly enhanced the FSMI system solution, both with the introduction of the SYNAPT XS, the state-of-the-art HRMS ion mobility-enabled mass spectrometer, and the new and improved DESI XS source. In addition to this, we have responded to customer requests by improving the Waters High Definition Imaging (HDI) Software with advances in data processing speed for large data sets, improving data alignment for HDMS^E data, and removing limits on the amount of data that can be processed and loaded into the software.

Benefits of the FSMI systems include:

- Enhanced sensitivity of the SYNAPT XS making it possible to analyse compounds at lower levels than ever before.
- Improved performance for labile species making it possible to more easily visualise spatial distributions of the most fragile of compounds.
- Inclusion of more analytical modes of analysis on the same platform (HDMS^E and SONAR) making characterization of compounds quicker and easier.
- Partially sealed DESI source housing that increases environmental stability and reduces atmospheric interference during the sample analysis.
- Side-on view of the DESI sprayer and the sample. This helps the user to locate the optimal spraying position for the DESI analysis more easily.
- Precision-manufactured DESI sprayer armature with a spring loaded arm encompassing caged roller bearings to give improved precision and durability for large scale acquisitions.
- Integrated DESI electronics and gas handling, meaning gases and voltages are automatically shut off at the end of the analysis. This is not only more environmentally friendly, but also saves on the cost of MS imaging consumables and prolongs the life of the DESI sprayer.
- Ability to set-up multiple tissue sections on a single slide and further slides in a single experiment, thus saving the user time.

Full Spectrum Molecular Imaging is only available on the SYNAPT family IMS-enabled MS system solutions. These systems can also be used as standard ESI-Tof instruments for other UPLC-MS based applications by

easily changing system configuration.

Figure 2 shows an example of FSMI data acquired on the SYNAPT XS highlighting complementary images generated from both MALDI and DESI data. These images highlight different lipid profiles between the cortex and the medulla of the kidney, which are DESI or MALDI analysis. It also emphasises that that small molecules such as metabolites are more easily detected with DESI than MALDI.

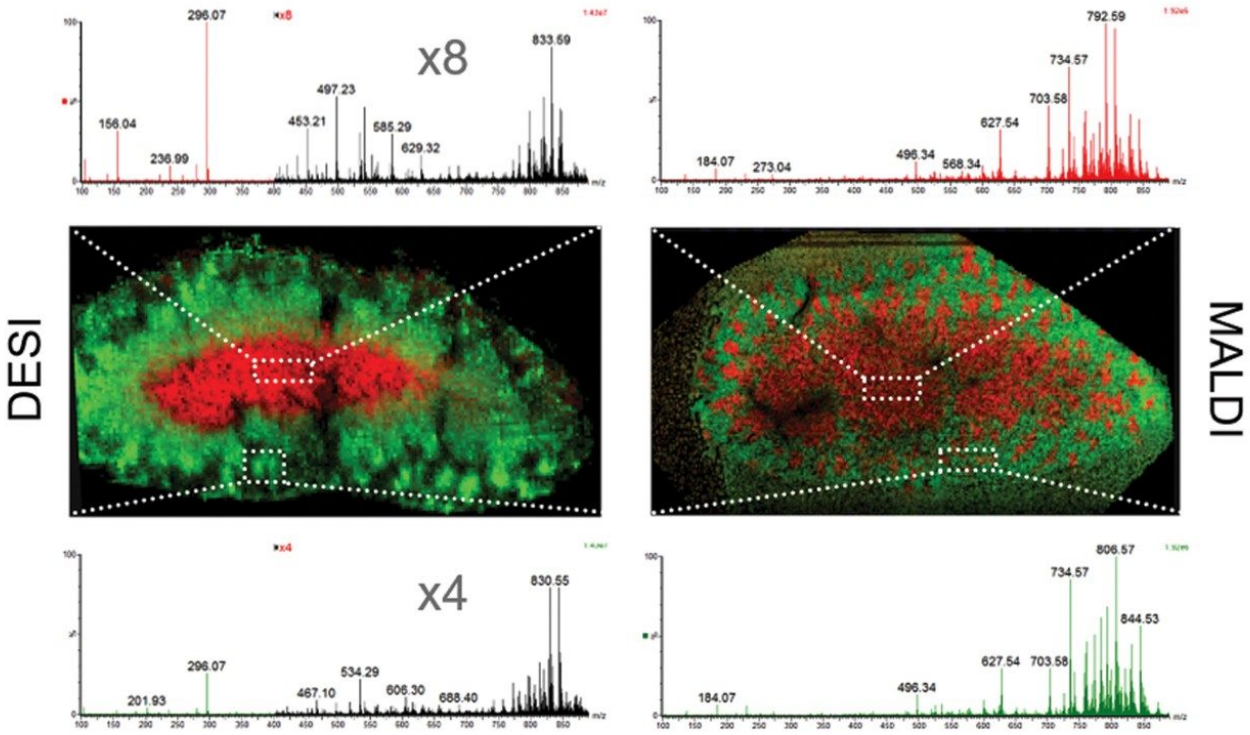

Figure 2. FSMI DESI and MALDI analysis between the cortex and the medulla of the kidney. Black spectrum areas have been magnified in the Masslynx spectrum view. Red and green spectra are from the medulla and the cortex of the kidney respectively.

MALDI	DESI
Intermediate to high vacuum	Ambient atmosphere
Small molecules (metabolites/ lipids) to peptides/proteins	Small molecules (metabolites/lipids)
Good sensitivity for small molecules	Good sensitivity for small molecules
Sample preparation step: application of matrix	No sample preparation
Semi-destructive technique	Non-destructive technique
Spatial resolution 5-10 microns to hundreds of microns	Spatial resolution 50 microns to hundreds of microns

Figure 3. Relative benefits of MALDI and DESI analyses. Green text displays advantages.

MS imaging publications also acknowledge the complementary nature of FSMI and have shown methods and workflows for complete comparison between the techniques and for their intra-technique variables such as polarity, MALDI matrix, and DESI solvent system.[1]

With the increased use of MS imaging in drug development,[2] which allows the mapping of drugs and metabolites within tissue, it is important to understand the complementary nature of DESI and MALDI ionization techniques. Figure 4 reports the results from a range of pharmaceutical compounds, analyzed by DESI and MALDI, on the same mass spectrometer. It highlights the power of having both ionization capabilities to increase molecular coverage. Differences in ionization efficiency, limit of detection (LOD) and ease of sample preparation were all observed.

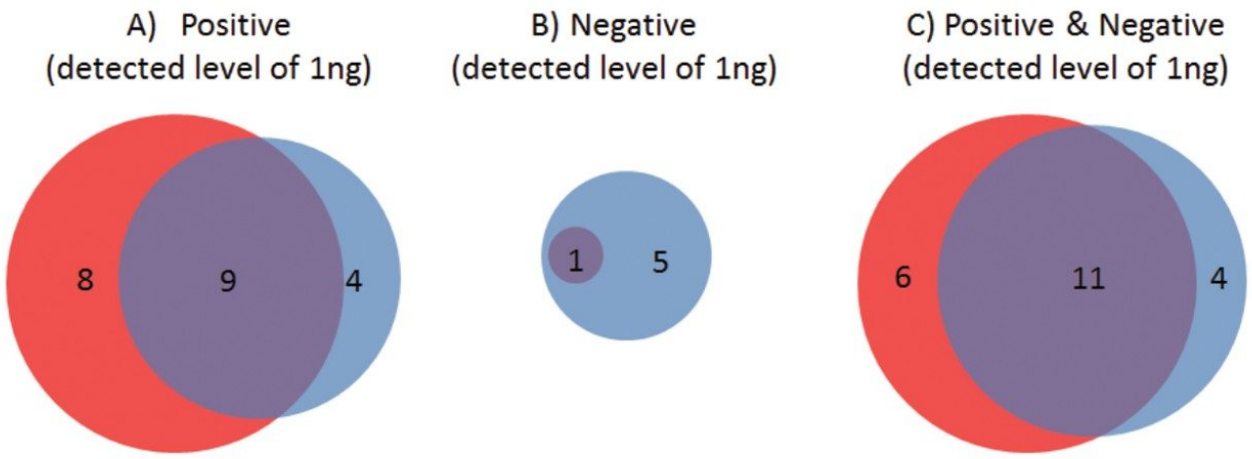

Figure 5. Summary of the screening results of a panel of 24 pharmaceutical compounds analyzed by DESI and MALDI on a SYNAPT mass spectrometer highlighting the number of compounds which were detected at 1 ng on glass slide: A) positive mode, B) negative mode, C) positive and negative combined. In purple, same level detected with MALDI and DESI. In red, better detection with MALDI. In blue, better detection with DESI.[3]

The relative individual benefits of the techniques incorporated into a Full Spectrum Molecular Imaging system shown in Figure 5, are:

DESI Imaging

DESI imaging utilizes a charged solvent spray to desorb ions directly from the surface of a sample without any sample preparation. Waters has taken traditional DESI imaging and enhanced its capabilities to provide new levels of usability and performance by adding innovation to this imaging technique. Some of the advantages of using DESI imaging include:

- Minimal sample preparation, not requiring a matrix
- Extraordinary results for lipid and small molecule imaging
- Enables multiple imaging experiments on the same sample (e.g. imaging in positive ion followed by negative ion mode and then using the same sample for H&E staining)

MALDI Imaging

MALDI imaging utilizes a laser based, direct ionization method to analyze molecules from a chemical matrix coated sample. MALDI imaging is considered the standard technique for imaging applications using MS. The technique provides:

- Excellent spatial resolution
- Ability to analyze a wide variety of molecules (with appropriate selection of matrix)
- Method of choice for images requiring ionization of larger molecules

Ion Mobility

Ion mobility can add another dimension of molecular separation to imaging studies. This technique is capable of resolving molecules for analysis based on their size and shape. Useful for removing interferences or isolating target molecules for more intense scrutiny, ion mobility is a unique method to enhance the analytical capabilities of an imaging system with even greater molecular discrimination. Ion mobility can be used to:

- Remove interfering molecules from an image
- Discriminate between closely structurally related molecules, e.g. lipids
- Isolate a particular type of target analyte

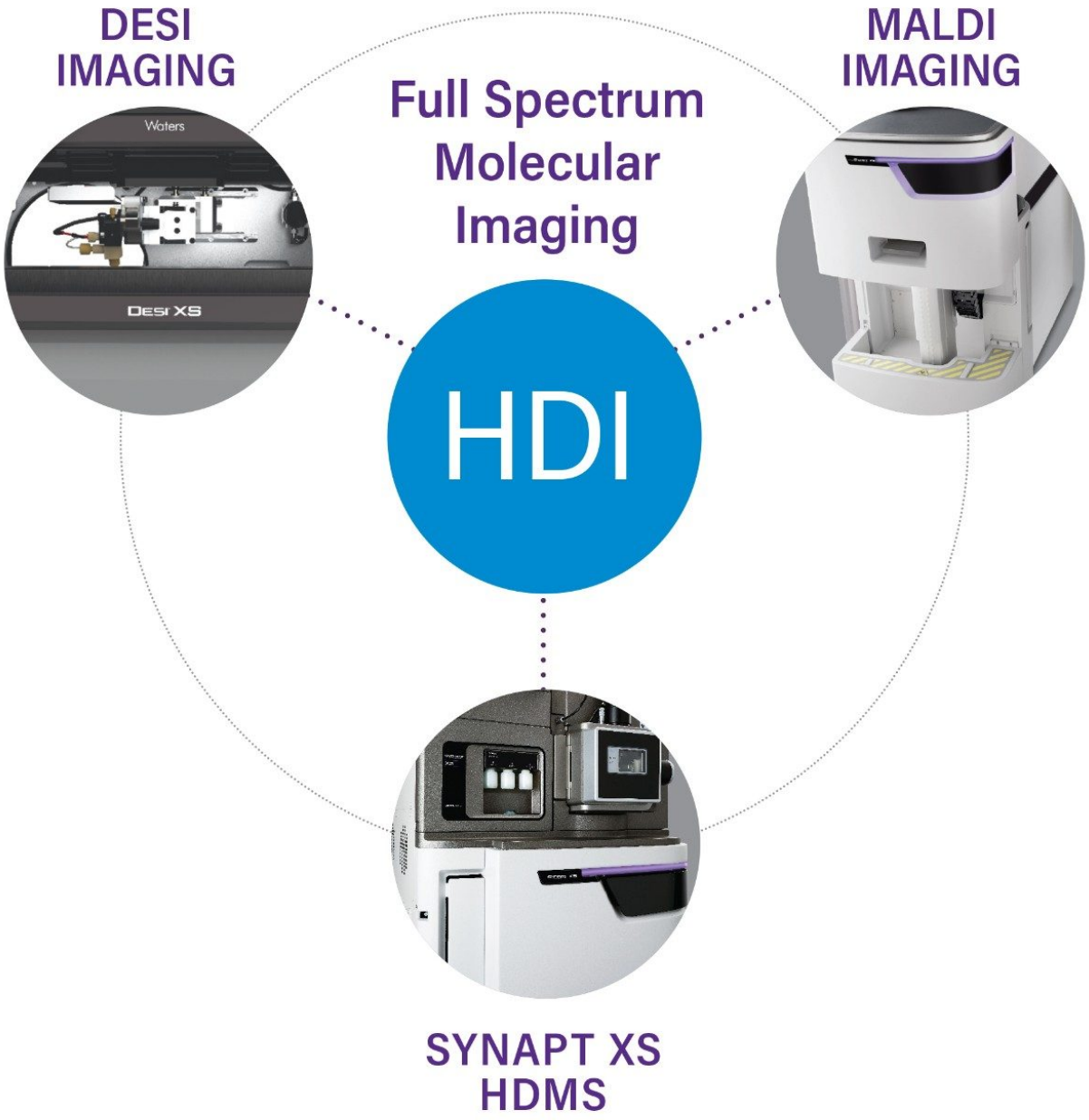

Figure 4. Components of the Full Spectrum Molecular Imaging system solution.

Conclusion

Full Spectrum Molecular Imaging capabilities allow visualization of wider range of molecular ions and provide key insights into the underlying mechanisms of cancer, cardiovascular, and neuro-degenerative diseases. In other studies, Full Spectrum Molecular Imaging can be used to identify different tissue types, differentiate diseased and normal tissue, and identify the boundary of healthy and diseased tissue based on their molecular composition. With the performance improvements obtained with the combination of SYNAPT XS and DESI XS, the Waters Full Spectrum Molecular Imaging solution delivers greater benefit than ever before for all imaging needs.

References

1. *Anal. Chem*. 2018, 90, 9, 5637-5645 Exploring Ion Suppression in Mass Spectrometry Imaging of a Heterogeneous Tissue. Date: February 20, 2018 https://doi.org/10.1021/acs.analchem.7b05005
2. Waters Application Note 720005947en. DESI-Mass Spectrometry Imaging Investigation of Discrete and Cassette Drug Dosed Tissues.
3. Evaluation of the Ionization Efficiency Between MALDI and DESI MSI for the Analysis of Pharmaceutical Compounds, 2018 poster. Emmanuelle Claude and Mark Towers.

Featured Products

SYNAPT XS High Resolution Mass Spectrometer <https://www.waters.com/135020928>

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

DESI XS <https://www.waters.com/135047466>

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