

DESI XS with Xevo MRT Mass Spectrometer for MS Imaging

Accelerating imaging. Amplifying sensitivity. Advancing visualization.

INTRODUCTION

This product solution highlights the capabilities of the Xevo™ MRT mass spectrometer equipped with the DESI™ (Desorption Electrospray Ionization) XS source (Figure 1), demonstrating its ability to provide rapid acquisitions while maintaining high sensitivity for MS imaging. With combined high mass resolution and mass accuracy, your lab can achieve greater spectral quality, improved analyte detection, and higher confidence in compound identification. The data presented includes data acquired in both positive and negative ionization modes and compares various acquisition speeds.

THE POWER OF DESI XS

Analysis on the DESI XS source offers a powerful and versatile approach to mass spectrometry imaging, delivering a range of benefits that enhance a wide range of applications, including pharma, chemical materials, and pre-clinical research applications. One of the most significant advantages of DESI is its ability to perform ambient image acquisitions without the need for extensive sample preparation or matrix application. The direct sample analysis without the application of matrices makes DESI ionization particularly valuable for high spatial resolution acquisitions where application of a matrix could lead to analyte movement. Whilst simple, rapid sample preparation makes it ideal for real-time analysis applications.

The DESI XS source supports high spatial and high mass resolution imaging, currently offering analyses with pixel sizes as small as 5 μm , enabling single-cell image resolution. This level of detail allows researchers to visualize molecular distributions with exceptional fidelity, crucial for understanding complex biochemical processes and tissue heterogeneity. The integration of Microscope Mode within High-Definition Imaging (HDI™) Software further enhances efficiency by allowing rapid survey scans followed by targeted high-resolution re-acquisition of regions of interest [1].



Figure 1. Next-generation solution for MS imaging: DESI XS with Xevo MRT mass spectrometer.

BREAKTHROUGH COMBINATION DESI XS COMBINED WITH XEVO MRT MS

The Xevo MRT mass spectrometer, Figure 2, offers a breakthrough in high-resolution, high-speed untargeted MS Imaging. By combining up to 100,000 FWHM mass resolution with sub-ppm mass accuracy at acquisition rates up to 100 Hz, it is now possible to perform rapid MS Imaging at speed. Its multi-reflecting time-of-flight (MRT) technology ensures sharp, well defined analyte spectra across a broad mass range, allowing spectral separation of compounds with similar molecular weight such as isoforms, making it an ideal platform to combine with the DESI XS source for advanced MS Imaging.

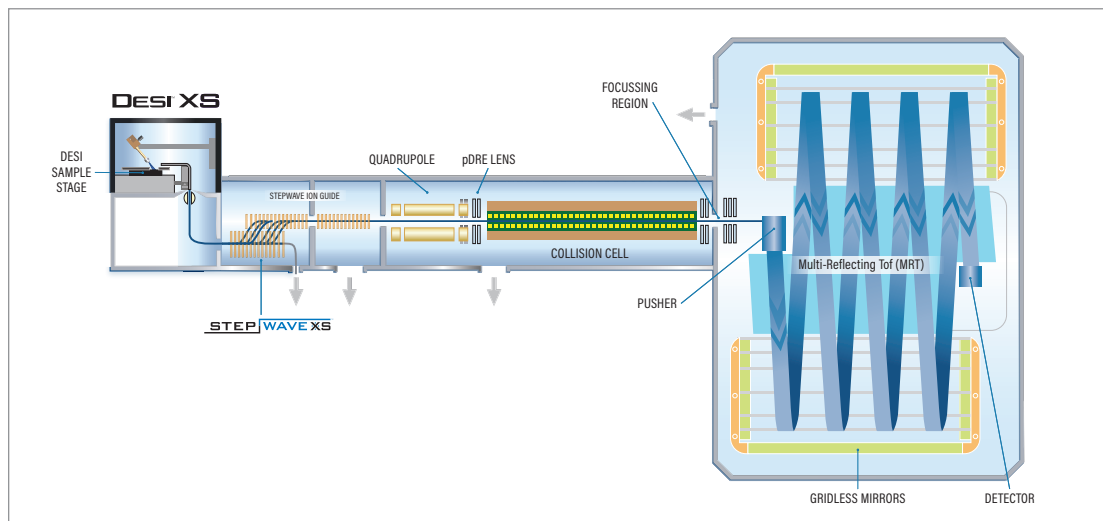


Figure 2. Schematic of DESI XS Xevo MRT MS Imaging solution.

METHODS

Mass spectrometry imaging was performed on porcine liver sectioned at 18 μm on to standard glass slides and imaged without further sample preparation using the DESI XS source fitted with a high-performance sprayer and heated transfer line.

The DESI source was combined with the Xevo MRT mass spectrometer optimally tuned for MS imaging. An ACQUITY[™] μBSM was employed with a low flow kit delivering a 95:5 methanol : water solvent at a delivery rate of 2 $\mu\text{L}/\text{min}$ (for 50 μm pixels) and 0.2 $\mu\text{L}/\text{min}$ (for 5 μm pixels). Data acquisition was performed at varying speeds (from 10 Hz to 100 Hz) and at a variety of spatial resolutions to evaluate system performance and image quality.

INSTRUMENT CONFIGURATION

- DESI XS source with high-performance sprayer (HPS) and heated transfer line (HTL)
- Xevo MRT MS tuned for MS Imaging
- μBSM ACQUITY solvent delivery with low flow kit including back pressure regulator delivering solvent at 2 to 0.2 $\mu\text{L}/\text{min}$
- Imaging software: HDI 1.9, MS Imaging software and waters_connect[™] instrument control

RESULTS

Positive and negative ionization mode imaging was conducted on several sequential sections of porcine liver at different acquisition speeds as follows:

Positive Ion Mode (Figure 3)

- Imaging of porcine liver sections at 50 μm spatial resolution.
- Acquisition speeds and image generation time:
 - 20 Hz: time 143.34 mins, image area 1.84×1.81 cm (132,854 pixels).
 - 100 Hz: time 70.07 mins, image area 2.5×1.9 cm (191,883 pixels).
- Speed increase: $\sim 2.7\times$ faster at 100 Hz (taking into consideration carriage return).
- Comparable spectral quality was maintained across acquisition speeds.

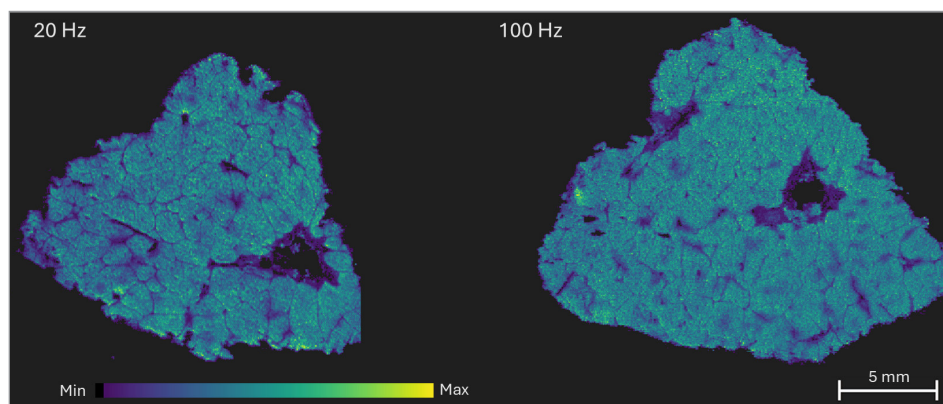


Figure 3. DESI XS Xevo MRT MS Imaging solution acquired sequential porcine liver sections acquired at both 20 Hz and 100 Hz respectively, showing hepatic portal vein with positive ion lipid extracted ion images. Acquired in MS mode at 50 μm spatial resolution and μBSM running at 2 $\mu\text{L min}$.

Negative Ion Mode (Figure 4)

- Imaging of porcine liver sections at 50 μm spatial resolution.
- Acquisition speeds and image generation time:
 - 20 Hz: time 173.70 mins, image area 2.035×1.995 cm (162,393 pixels).
 - 100 Hz: time 69.32 mins, image area 2.275×1.955 cm (177,905 pixels).
- Speed increase: $\sim 2.8\times$ faster at 100 Hz (taking into consideration carriage return).
- Comparable spectral quality was maintained across acquisition speeds.

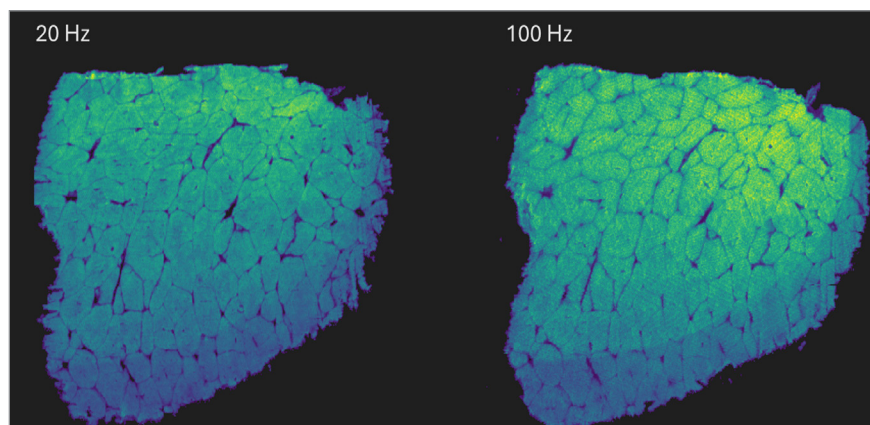


Figure 4. DESI XS Xevo MRT MS Imaging solution acquired sequential porcine liver sections acquired at both 20 Hz and 100 Hz respectively showing hepatic structure with negative ion lipid extracted ion images. Acquired in MS mode at 50 μm spatial resolution and μBSM running at 2 $\mu\text{L min}$.

CLEAR VISUALIZATION WITH FASTER TOTAL IMAGE ACQUISITION

The images acquired demonstrate clear visualization of hepatic portal and other common liver vein structures. Increasing acquisition speed from 20 Hz to 100 Hz resulted in a 2.7–2.8x reduction in total acquisition time, while maintaining spectral integrity (Figures 5 and 6).

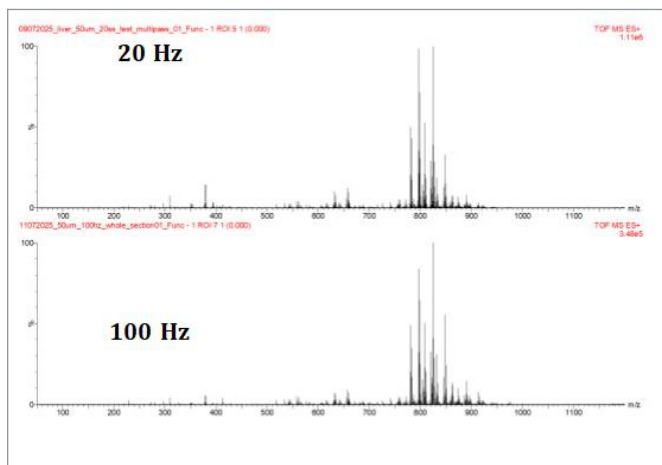


Figure 5. Positive mode analysis. Spectra show high similarity over the range of acquisition speeds. MS Signal intensity decreased as expected in line with increase in speed.

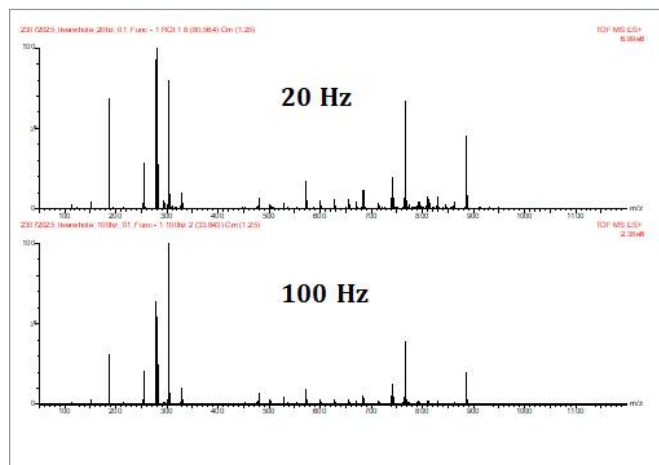


Figure 6. Negative mode analysis. Spectra show high similarity over the range of acquisition speeds. MS Signal intensity decreased as expected in line with increase in speed.

Although the spectral integrity was maintained, it is important to note that the signal intensity decreased proportionally with the increase in speed, with this overall reduction in signal at higher speeds. The top 1,000 processed analytes remained consistent, and therefore data quality remained unaffected by the higher acquisition speeds. This investigation into the effect of acquisition speed and spectral quality has historically been explored in more detail on an older instrument capable of analyses at a maximum of 30 Hz [2].

Examples of sub-10 μm resolution imaging at 20, 50, and 100 Hz utilizing the enhanced duty cycle mode (EDC) is also demonstrated on porcine liver (examples shown in Figure 7 are images generated at 5 μm pixel sizes). This highlights the potential of high-resolution DESI imaging at higher speeds than ever previously achieved. The optimal speed for the acquisition of sub-10 μm resolution imaging is the subject of further work.

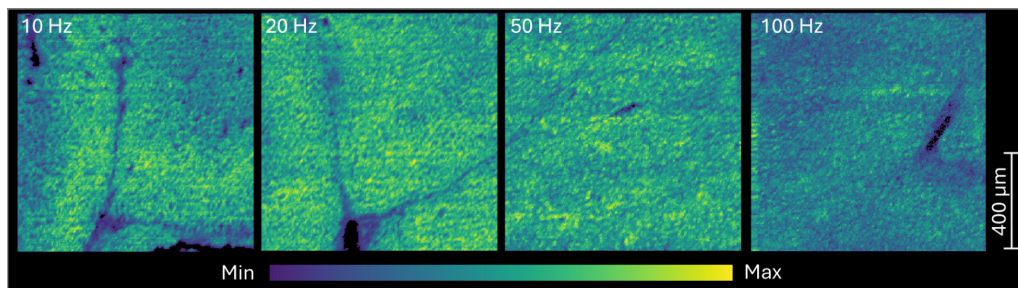


Figure 7. Negative mode analysis. Porcine liver data acquired at 10, 20, 50 and 100 pixels per second in at 5 μm pixel size on the DESI XS Xevo MRT Imaging solution (m/z 885.56) using enhanced duty cycle.

ADVANTAGES OF ENHANCED MASS RESOLUTION

With a rat brain 20 μm spatial MS imaging data, we explored the benefits in data quality achieved by the Xevo MRT MS (up to 100K FWHM) mass resolution (Figure 8). In the highlighted examples, the Xevo MRT MS delivers >80K mass resolution, compared to the 30K of the Xevo G3 QToF. This improvement in mass resolution is significant for direct analysis, as with no chromatographic separation, this technique leads to high molecular complexity. Improving mass resolution results in a more comprehensive spectral profile, greater number of analytes detected, and increased confidence in compound identification. In one example of lipid ions, the Xevo MRT MS was able to resolve two distinct lipid ions where the Xevo G3 QToF was only able to resolve a single peak. The two different lipid ions observed with the DESI XS Xevo MRT MS showed distinct differences in distribution in the brain. However, the Xevo G3 QToF at 30K resolution was not able to resolve them, showing an image which was an amalgamation of the two ions.

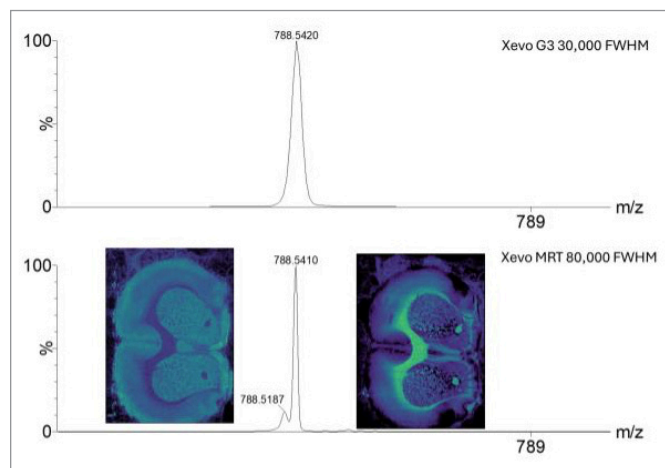


Figure 8. Xevo G3 vs Xevo MRT MS spectral resolution for DESI Imaging, extracted lipids – Showing increased mass resolution with the Xevo MRT mass spectrometer and differential biolocalization of separated ions using the DESI XS Xevo MRT MS system solution.

CONCLUSIONS

The integration of DESI XS source with the Xevo MRT mass spectrometer significantly enhances MS imaging acquisition speeds through the combined inherent sensitivity and rapid acquisition modes. The ability to maintain spectral quality at higher acquisition speeds supports its use for imaging workflows requiring analysis of larger tissue or surface areas in reasonable time frames. The additional mass resolution of the Xevo MRT mass spectrometer can help labs achieve clearer visualization of ions, with close m/z giving a deeper insight into the molecular complexity of a direct tissue analysis.

The DESI XS source with Xevo MRT mass spectrometer offers a powerful solution for MS imaging, combining speed, sensitivity, and high-quality spectral data. This combination is well-suited for advanced MS imaging research and pre-clinical research applications requiring detailed molecular imaging.

To learn more about how the DESI XS source and Xevo MRT MS can help with imaging workflows in your lab, speak to your Waters representative.

References

1. <https://www.waters.com/nextgen/global/library/application-notes/2024/single-cell-image-resolution-with-DESI-xs-coupled-to-a-xevo-g3-mass-spectrometer.html>
2. <https://www.waters.com/nextgen/global/library/application-notes/2024/routine-comprehensive-tissue-imaging-on-the-xevo-g3-qtof-mass-spectrometer-using-a-DESI-xs-source.html>

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