

## High Throughput DESI Imaging for Optimized Analysis of Biological Tissues

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*For research use only. Not for use in diagnostic procedures.*

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

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### Abstract

This technology brief demonstrates the optimization of a DESI imaging workflow to allow high throughput sample analysis while obtaining tissue structural information.

### Benefits

Fast, high sensitivity DESI-MS allows for higher sample throughput, to assess spatial and mass spectral information.

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### Introduction

Mass spectrometry imaging is a powerful analytical research tool that enables the researcher to

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accurately localize molecules directly in a tissue section. Desorption electrospray ionization (DESI) is an ambient ionization technique that has gained popularity over the past few years due to the ease of sample preparation, the ESI-like spectra, and the non-destructive nature of the DESI technique. This is particularly advantageous in tissue imaging, where the same section can be submitted to DESI analysis followed by further investigation such as histological staining. One drawback of some current DESI systems has been the slow speed of acquisition, with a single imaging experiment sometimes taking several hours to acquire. Acquisition speed is typically limited by both the scan speed of the instrumentation and sensitivity (which drops proportionally to scan speed).

For the wider adoption of this technology, workflows will need to be optimized to allow for faster sample throughput, with improved informatics to assess spatial and mass spectral information.

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## Results and Discussion

The new Waters Sprayer on the Prosolia 2D DESI stage provides higher spatial resolution, sensitivity, and robustness. When combined with the Xevo G2-XS or SYNAPT G2-Si, which provide high sensitivity and fast scan rates, this allows a dramatic increase in acquisition speed, bringing MS imaging time down to minutes rather than hours. High quality lipid spectra can be obtained from tissue, even at high scan rates.

Initial experiments were carried out using a SYNAPT G2-Si in TOF mode. Figure 1 shows data from DESI imaging experiments conducted on mouse brain sections at 1, 10, and 30 scans per second. All regions were imaged at a pixel size of 100  $\mu\text{m}$  and 3,000 pixels were acquired.

This acquisition took 54 minutes at 1 scan per second, 9 minutes at 10 scans per second, and 6 minutes at 30 scans per second. Figure 1A displays MS spectra combining five lines of acquisitions where the tissue was present, demonstrating a decrease in signal intensity as the DESI scan acquisition rate increased. However, the MS resolution was maintained at >20,000 full width at half maximum (FWHM) (Figure 1B) with the increased acquisition speed, due to the orthogonal acceleration TOF geometry (Figure 1B).

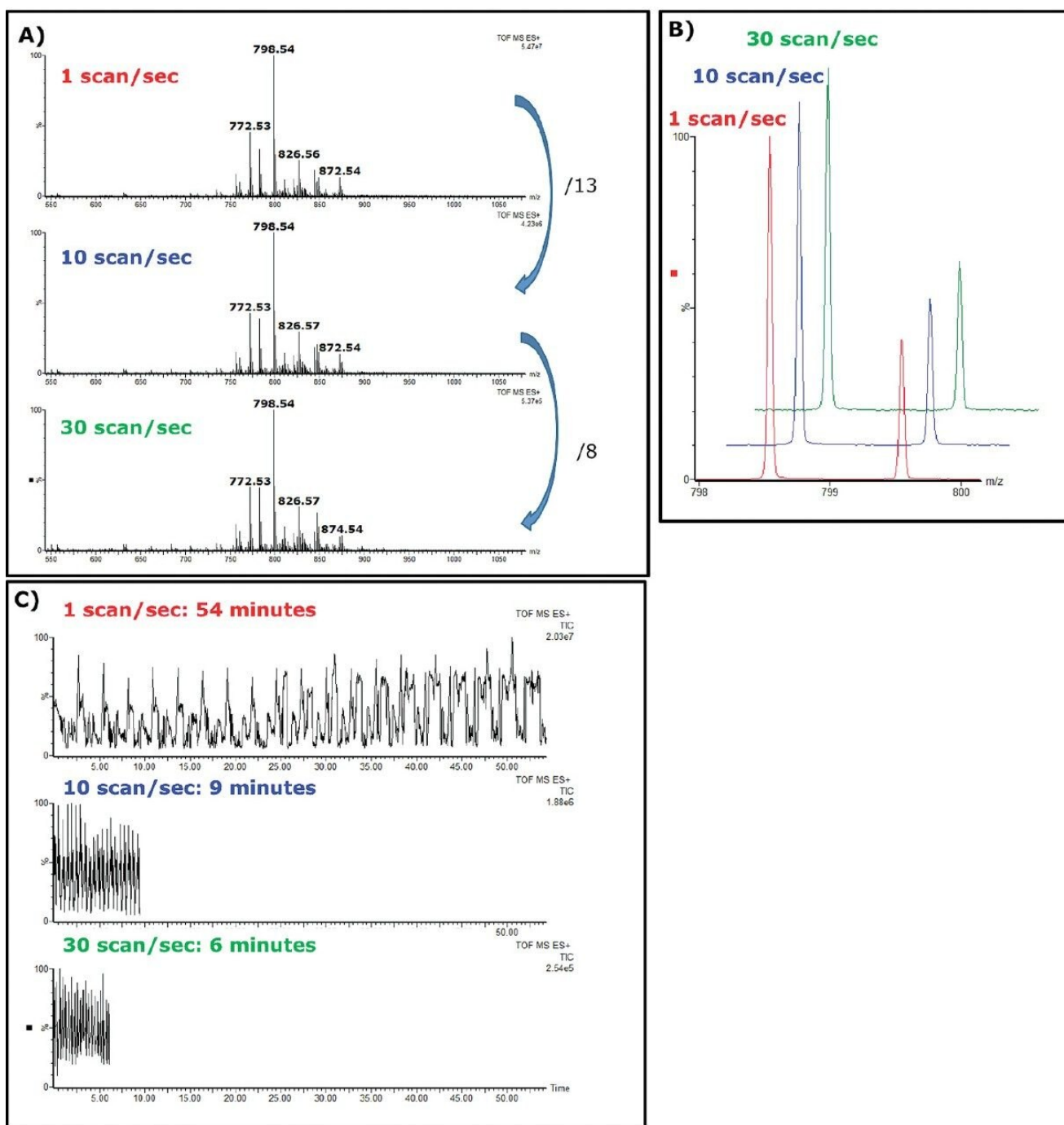


Figure 1. MS spectra for the 1, 10, and 30 scans/per DESI imaging experiments on mouse brain.

Comparison of mouse brain images acquired at 10 and 30 scans per second (Figure 2) shows that the

distribution of detected ions in the tissue is comparable and that there is no loss of spatial information at higher scan speed. K-means clustering of mouse brain data acquired at 5, 10, and 30 scans per second (Figure 3) shows comparable segmentation, suggesting that differences in the lipid spectra between distinct regions of the brain are conserved, even when intensity is reduced at very high scan speeds.

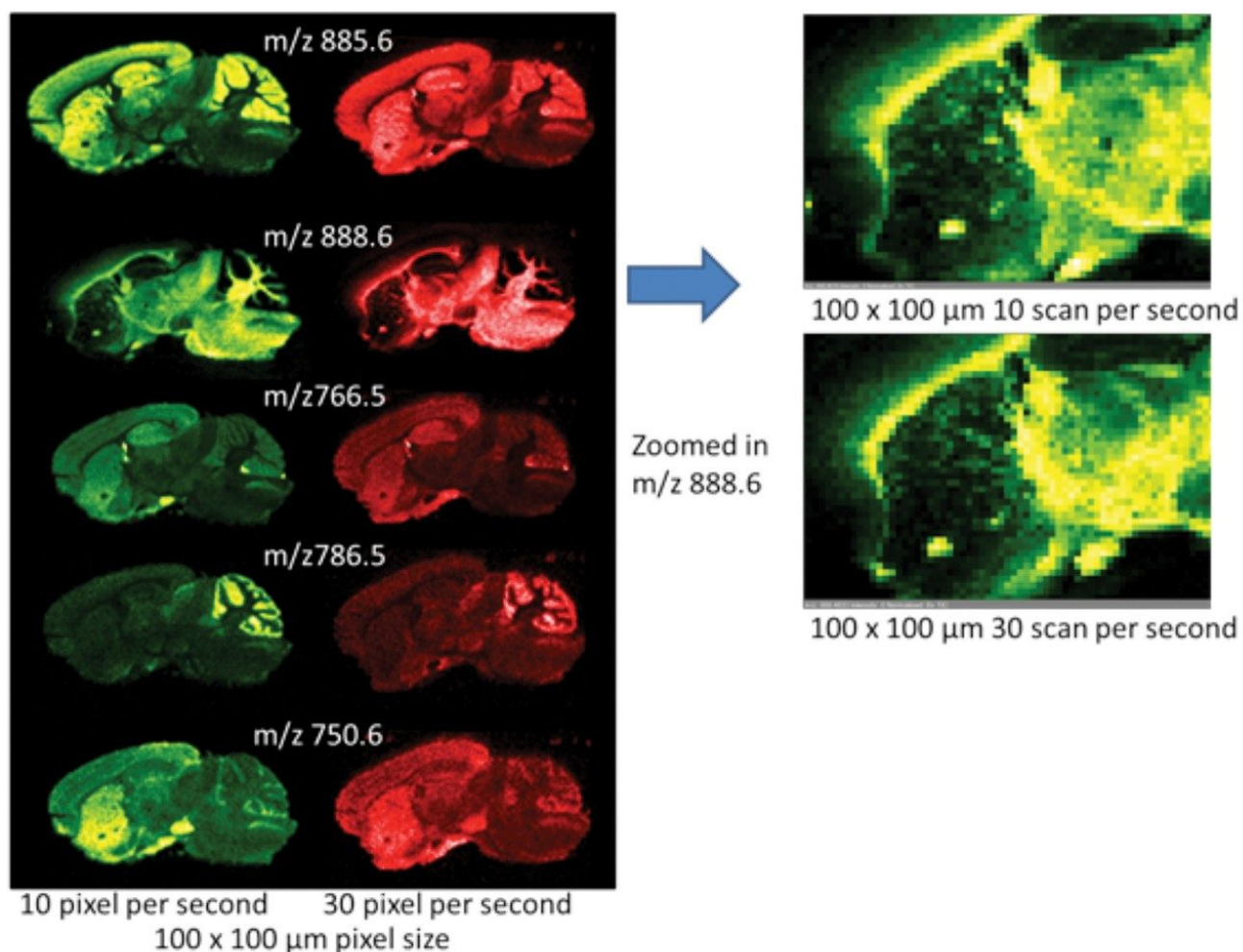
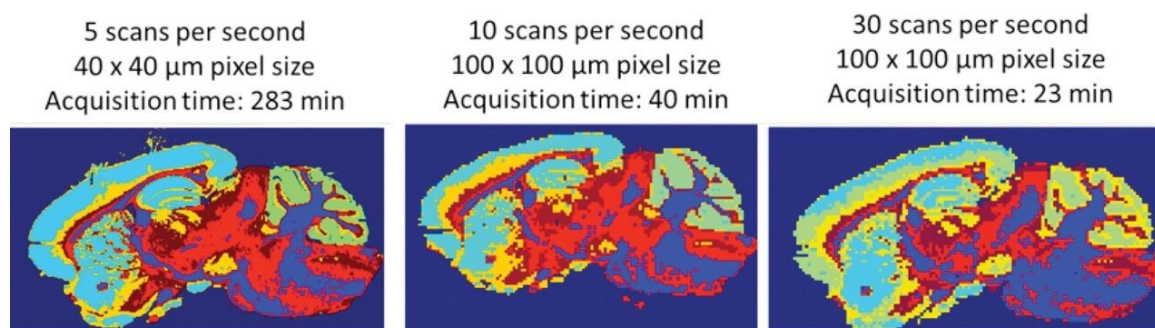


Figure 2. Comparison of selected ion images acquired at different scan speeds.



*Figure 3. K-means clustering of MS images of mouse brain acquired at different pixel sizes and scan speeds.*

Finally, to examine the use of ion mobility separation (IMS) at fast scan speeds, a further set of studies were conducted on a mouse kidney tissue section in IMS-TOF mode. Speeds of 1 and 10 scans per second were selected. Faster scan rates were found to disrupt the IMS, whereas the IMS separation was maintained across the different acquisition speeds up to 10 scans as demonstrated in Figure 4, displaying the RGB of  $m/z$  772.59 PE(38:1),  $m/z$  764.52 PE(38:5), and  $m/z$  909.55 PI(40:6) for the kidney tissue section (PE is phosphoethanolamine, PI is phosphoinositide).

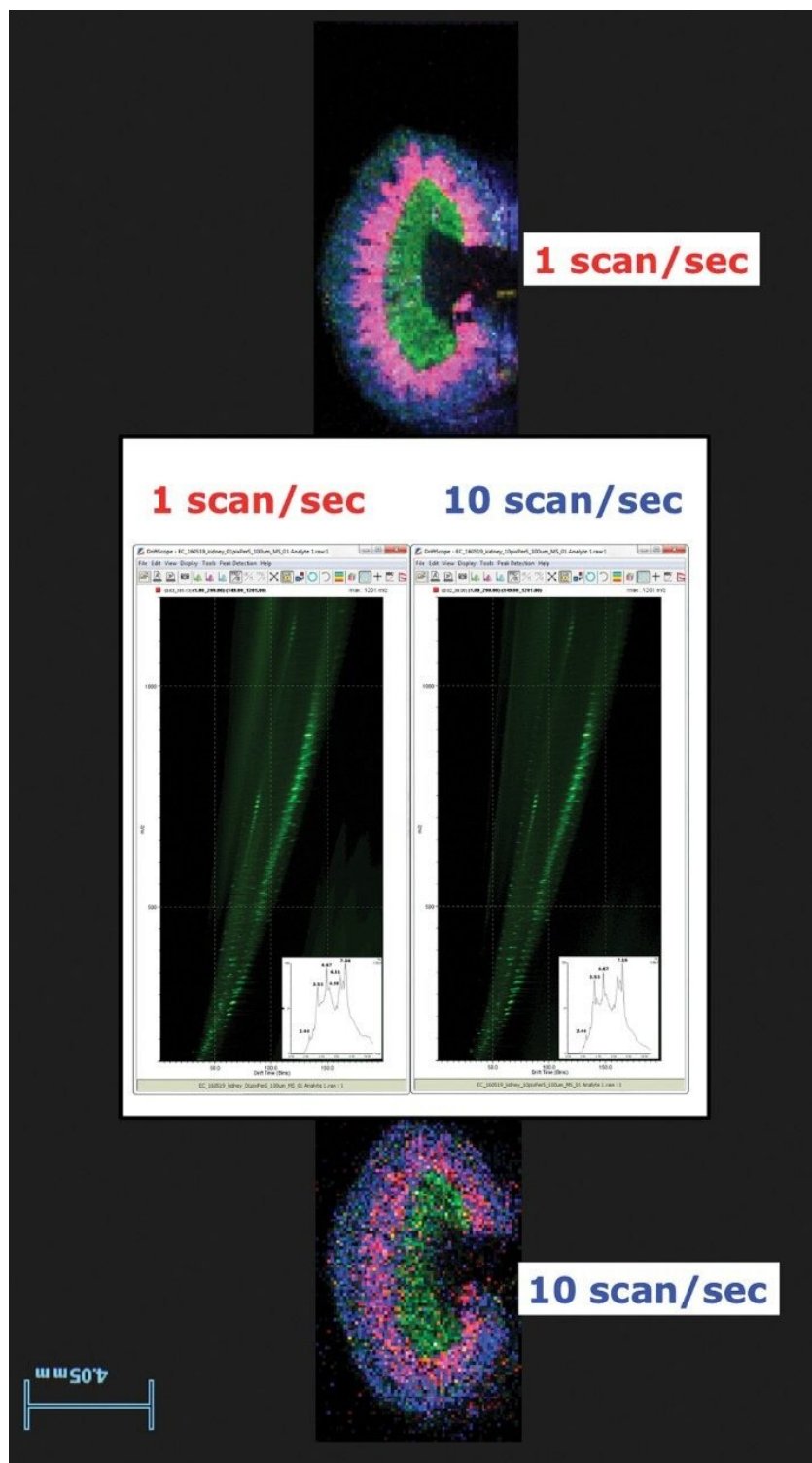


Figure 4. MS spectra and RGB (ion images  $m/z$  772.59 (PE(38:1)),  $m/z$

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764.52 (PE(38:5)), and  $m/z$  909.55 (PI(40:6)) results from DESI IMS imaging at 1 and 10 scans/sec on kidney tissue section.

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## Conclusion

Using the Waters approach for fast-DESI allows:

- Fast, high sensitivity DESI-MS imaging of tissue, with the potential for high throughput analysis.
- Conservation of MS resolution at higher acquisition rates.
- DESI imaging with ion mobility separation at up to 10 scans per second.

The increase in scan frequency, along with ion mobility separation, dramatically reduces image acquisition times, from several hours to tens of minutes, without significantly compromising data quality, making DESI a much more practical technique for routine lab analysis.

## Acknowledgements

1. We thank Wolfson Molecular Imaging Centre of University of Manchester for providing the mouse brain samples.

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## Featured Products

Mass Spectrometry Imaging <<https://www.waters.com/134833481>>

Xevo G2-XS QToF Quadrupole Time-of-Flight Mass Spectrometry <<https://www.waters.com/134798222>>

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