DESI IMAGING AT VARYING ACQUISITION RATES WITH REAL TIME IMAGING DISPLAY FOR OPTIMIZED TISSUE IMAGING

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INTRODUCTION

Mass spectrometry imaging is a powerful analytical research tool to accurately localize molecules directly on a tissue section. To translate to a wider audience and a technology adopted in clinical research, workflows needs to be optimized with simple and robust sample preparation, faster acquisition rates and throughput, with improved informatics to assess the spatial and mass spectral information. This quality assessment would ideally be made "on-the-fly" or rapidly after acquisition.

Here we present the optimisation of a DESI imaging workflow with variation of acquisition speed (in both TOF and IMS mode) along with the real time display of the ion images to assess image quality.

METHODS

Tissue sections
Tissue sections analyzed were consecutive sections from porcine liver, mouse brain and kidney. The tissue samples were snap frozen in liquid nitrogen and stored at -80°C, prior to cryo-sectioning at 10-15 µm thickness.

Mass spectrometry
MS imaging experiments were performed using Waters Q-TOF mass spectrometers: Xevo G2-XS and SYNAPT G2-*Si*, the latter being equipped with a tri-wave ion mobility cell.
The 2D DESI stage (Prosolia, Indianapolis) was mounted on both mass spectrometers. DESI spray conditions were set at 1.5 µL/min, 98:2 MeOH:water with nebulising gas pressure of 5-7 bar.

Instrument settings

Polarity:	Negative and Positive ion mode
Mass range:	100 -1,200 *m/z*
IMS pressure:	3.1 mbar
Wave Height:	40 V
Wave Velocity:	1000 to 300 m/s

Scan rate (scan/s)	Stage speed (µm/sec)	Scan time (sec)
1	100	0.985
5	500	0.185
10	1,000	0.085
30	3,000	0.018

Data management
DESI imaging data were processed and visualized using High Definition Imaging 1.4 (HDI) software for detailed image analysis. DESI imaging data with Ion mobility separation (IMS) was assessed using DriftScope software. In-house developed prototype software was used to visualize DESI imaging data "on-the-fly".

RESULTS

DESI MS-TOF acquisition

Initial experiments were carried out on a SYNAPT G2-*Si* in TOF mode using a thin section of sagittal mouse brain. An optical image was taken, co-registered with HDI and used to define the area to be imaged. Three rectangular regions were defined longitudinally along the brain tissue section. The pixel size in the *x*-direction was defined by the speed of the stage movement and acquisition rate of mass spectra. The *y*-direction was defined by the distance between two lines of acquisition. All regions were imaged at a pixel size of 100 µm. To acquire the 3,000 pixels, it took 54 minutes at 1 scan/s, 9 minutes at 10 scans/s and 6 min at 30 scans/s. Figure 1,A) displays MS spectra combining 5 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 (FWHM) with the increased acquisition speed, due to oa-TOF mass analyser (figure 1,B).

Figure 2 displays the ion images for m/z 798.54 ($PC(34:1)K^+$) for the three experiments. Ion images are displayed A) not normalised and on the same intensity scale, B) not normalised using a different intensity scale, C) TIC normalised and on the same intensity scale, and D) TIC normalised and using a different intensity scale.

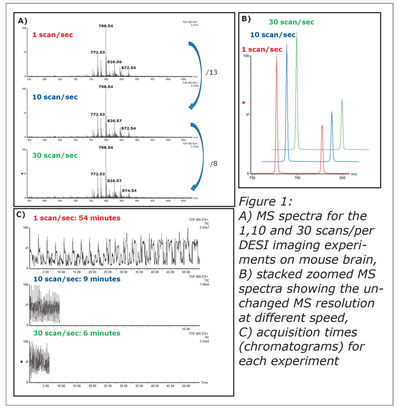


Figure 1: A) MS spectra for the 1,10 and 30 scans/per DESI imaging experiments on mouse brain, B) stacked zoomed MS spectra showing the unchanged MS resolution at different speed, C) acquisition times (chromatograms) for each experiment

To evaluate the quality of the image generated at different speeds, vertical lines using red ink were printed onto a piece of paper. In this experiment, three regions of 8 x 3 mm were defined and acquired at 2, 10 and 30 scans/sec with a pixel size of 50 um x 50 um. Results in figure 3 demonstrate that even at the highest speed there was no obvious smearing of the signal observed and the red lines from the 2 scans/s and 30 scans/s acquisition are both equally sharp.

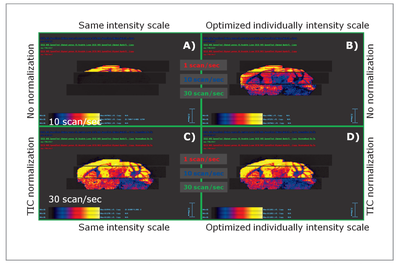


Figure 2: Ion images of m/z 798.54 A) not normalised and on the same intensity scale, B) not normalised and using different intensity scale, C) TIC normalised, on the same intensity scale, D) TIC normalised using a different intensity scale.

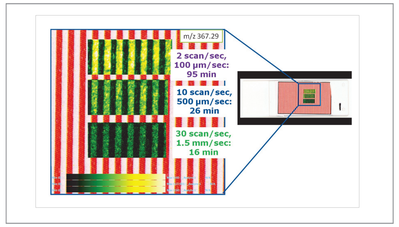
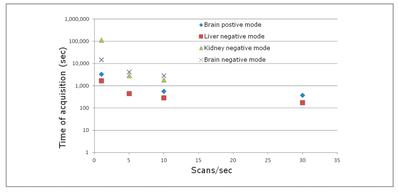


Figure 3: Printed red ink paper DESI MSI at various speed showing no blurring at higher speed of acquisition.

Further experiments were carried out on porcine liver, mouse brain and mouse kidney tissue sections at 1, 5,10, and 30 scans/ sec and results are shown in in graph 1 where scan/ sec vs. time of acquisition (log seconds) is displayed.
From these experiments 5 and 10 scans/sec would be the ideal situation between the gain in speed and the decrease in sensitivity.



Graph 1: Time of acquisition versus the scan rate of acquisition for four DESI imaging experiment (brain, liver and kidney).

DESI MS-TOF with ion mobility separation (IMS)

Experiments were conducted on high speed imaging combined with ion mobility separation. It was found that the IMS separation was disrupted at a speed at 20 scans/sec.
Speeds of 1, 5 and 10 scans/sec were selected and DESI imaging with IMS acquired in negative ion mode on the same mouse brain and kidney tissue sections.
As observed in TOF mode, the MS resolution was not affected by the change of acquisition speed. m/z 772.59 (PE(38:1)), 764.52 (PE(38:5)) and 909.55 (PI(40:6).

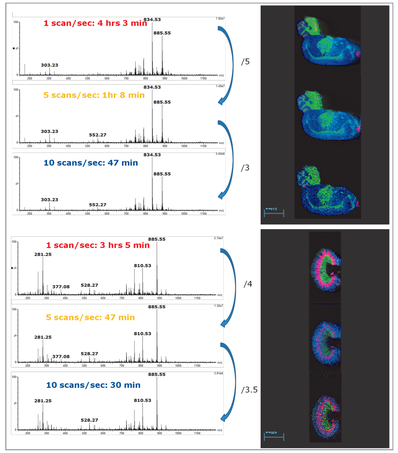


Figure 4: MS spectra and RGB (ion images m/z 772.59 (PE (38:1)), m/z 764.52 (PE(38:5)) and m/z 909.55 (PI(40:6)) results from DESI IMS imaging at 1, 5 and 10 scans/sec on mouse brain (upper) and kidney (lower) tissue sections.

Figure 4 shows the MS spectra for each experiment, along with the RGB of m/z 909.55 PI(40:6), m/z 788.55 PS(36:1) and m/z 834.58 ST(d18:1/C20:0) for the brain tissue section; and 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.

As expected, the intensity decreased proportionally with the increasing scan rate. However, IMS separation was maintained (figure 5) across the different acquisition speeds, up to 10 scans per second. Minor distortion due to a phasing de-synchronisation between the transfer Twave and TOF pusher slightly affected the quality of the images.

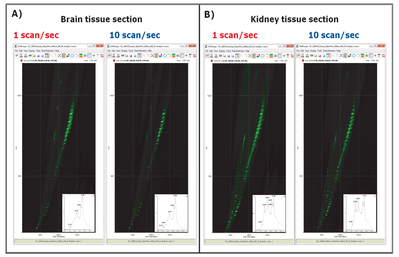


Figure 5: DriftScope mobilogram results from DESI IMS imaging at 1, 5 and 10 scans/sec on mouse brain and kidney tissue sections.

Multiple DESI MS/MS-TOF with ion mobility separation (IMS)

A further fast scanning method investigated with DESI imaging was multiplexed MS/MS with IMS where the precursors were CID fragmented in the transfer collision cell to distinguish normally isobaric species.
In this experiment, the quadrupole was set for 0.1517 sec per transition (m/z 917.5, 750.5, 834.6) (equal to 6 scans/sec).

With m/z 750.5 a singly and doubly charged precursor were fragmented (figure 6). Figure 6,A) shows that their drift time was very different and as fragmentation occurred after IMS separation, the fragments share the same drift time as their respective precursor ions. Driftscope software was able to generate precursor specific MS/MS spectra (figures 6,B and 6,C). In addition, the fragment distributions were different, showing two different lipids were present: PE(P-16:0/22:4) and PE(P-18:0/20:4). The identification of these lipids was based on www.lipidmaps.org.

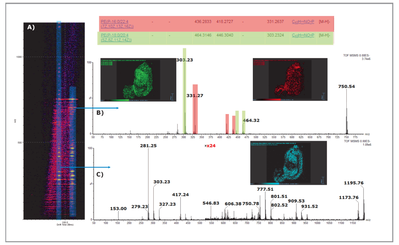


Figure 6: A) Driftscope 2D-plot drift time vs. m/z of the transfer fragmentation m/z 750.5; B) MS/MS of the singly charged species identified as PE(P-16:0/22:4) and PE(P-18:0/20:4) ; C) MS/MS of the doubly charged.

Fast DESI acquisition with "Real time" viewer

An experiment was also designed to demonstrate the use of in-house, prototype software, which displayed in real time up to 9 ion images of different m/z values to observe the progress of the DESI imaging experiment and assess the image quality of the experiment in real-time.

Figure 7 overviews a fast DESI imaging experiment, set-up at 10 scans/s in negative mode with a pixel size of 120 µm. In this case 4 ion images were displayed in real time (m/z 888.59, 774.51, 797.51 and 865.47).
The total experiment took less than 22 min. The data was automatically processed by HDI after the acquisition and the ion images generated demonstrate the same ion distribution as the real time ion images.

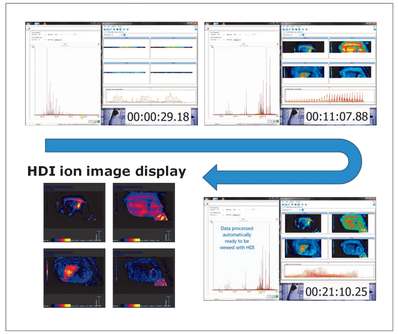


Figure 7: Screenshots of the fast DESI imaging experiment on mouse brain with the real-time display of m/z 888.59, 774.51, 797.5 and 865.47, along with the final ion images displayed in HDI.
To view the above as a movie, scan the QR code to the right.

CONCLUSION

- **DESI TOF imaging at 30 scans/sec was achieved using the SYNAPT G2-*Si* and Xevo G2-XS.**
- **Despite a decrease in sensitivity, the image quality and MS resolution were not affected by the increase in acquisition speed.**
- **It was demonstrated that DESI imaging at up to 10 scans/sec was feasible with ion mobility separation.**
- **Fast transfer MS/MS IMS DESI imaging allowed the generation of ion images for isobaric species at a scan rate of 6 scans/sec**
- **Combining fast DESI imaging with "Real time" viewer software allowed the visualisation of 4 m/z on the fly to assess the quality of the experiment**

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