AN INVESTIGATION OF MALDI IMAGING WITH HIGHER SPEED SAMPLE STAGE "RASTERING" FROM AN ION MOBILITY ENABLED Q-TOF MASS SPECTROMETER

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INTRODUCTION

Imaging mass spectrometry using MALDI is an established technique to determine the localization of specific m/z species within tissue sections. As the demand for increased spatial resolution drives the trend to larger data sets, the time required to acquire these also increases. Continued hardware enhancements, such as faster lasers and computer processors aid in reducing the time demands. Improvements in acquisition methods and data handling can also reduce the time spent obtaining the data. Here, we evaluate a new acquisition method, involving faster point-point movement of the sample carrier plate whilst maintaining pixel synchronisation. Scaling the stage speed, laser beam diameter and desired pixel size ensures that the acquisition rate is independent of image resolution.

METHODS

In this study we have utilised the Waters Research Enabled Software (WREnS) to allow a non standard acquisition methodology. WREnS allows direct control of certain aspects of the instrument functionality via the input of commands in a script based format. Scripts were written to perform imaging in a continuous raster mode for both a "typewriter" and "serpentine" movement (1) with either a horizontal or vertical directionality. The scripts also calculated the necessary over shoot to ensure that the stage was at the correct velocity when acquiring the image. The laser could be fired for the whole line or just the region to be imaged.

MS images were acquired using a Waters Synapt G2-S with a prototype MALDI source with features designed specifically for imaging applications. These features include; easy to access ion optics which can be cleaned without venting the instrument, a variable laser focus ~15 µm to ~150 µm and a high precision stage. Data were acquired to assess the effects of directionality, acquisition rate and laser focus on the data.

In addition MS/MS and ion mobility MS images were acquired using a Waters Synapt G2-S*i* with a commercial MALDI source to observe the effect of high pixel acquisition rates on these modalities (figure 6 and figure 7).

Both sources used the same 2.5KHz Solid state Nd:YAG laser (λ = 355 nm)

Sample preparation

12µm thick mouse sagittal brain sections and mouse testis tissue sections mounted on non conductive glass slides were both prepared by spray coating with recrystallized α-cyano-4-hydroxycinnamic acid (CHCA). 5 mg/ml CHCA in 70:30 Acetonitrile : 0.2%TFA was applied using a SunCollect sprayer from SunChrom. An initial two layers were sprayed at 15 µl/min followed by 33 layers for the mouse brain sections and 25 layers for the mouse testis sample. Line spacing was 1.5mm using speed medium 1. The nebulising gas was set to 1.75bar.

All images displayed using Waters High Definition Imaging 1.4.

RESULTS

Imaging Directionality

An assessment of the effect of image acquisition direction has been made, the results for which can be seen in figure 1. From figure 1 A and B (acquired in typewriter) it can be seen that whilst there are some aberrations due to minor synchronicity effects, the direction of acquisition has no significant effect on the quality or sensitivity of the data. These results mean that: 1) a serpentine raster, which decreases acquisition time by removing the carriage return is a viable option, as seen in figure 1 C and D, and 2) an appropriate direction of acquisition can be selected based on the orientation of the tissue section. All images were acquired at 20 scans per second. In Figure 1 D the selection of a vertical direction resulted in an 8% decrease in acquisition time compared to Figure 1 C with a horizontal directionality with an average of 16.5 pixels per second for C and 18 pixels per second for D.

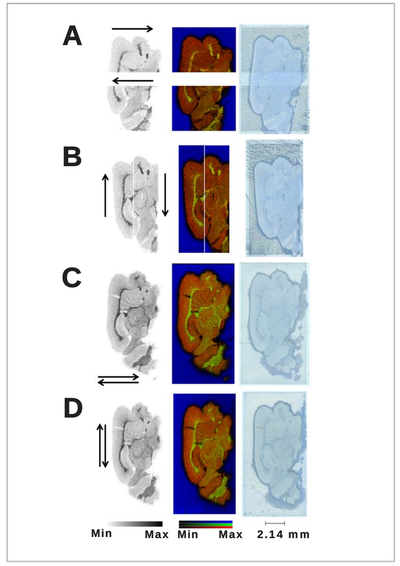

Figure 1. Study of the effect of acquisition direction on signal response on mouse brain tissue. Arrows indicate direction of acquisition A) horizontal typewriter, B) vertical typewriter, C) horizontal serpentine, D) vertical serpentine. Left: m/z 826.6 PC (36:1) K+, Centre: color overlay [Red m/z 798.5 PC (34:1) K+, Green m/z 826.6 PC (36:1) K+, Blue m/z 656.1 matrix peak], Right: scan of tissue post acquisition. Images were acquired at 20 scans per second with a 50 µm pixel size and <25 µm laser focus.

Imaging speed

The effect of imaging speed (scans per second) was investigated to assess the image quality achievable and the effect on sensitivity. Figure 2 shows the results of cross-sectional imaging runs at scan rates from 5 to 40 scans per second (1 pixel = 1 scan cycle). Figure 2 shows that at scan rates from 5 to 40 the image resolution is maintained with only a slight loss of synchronicity at higher rates. Figure 3 shows extracted spectra for each image in figure 2. Whilst the spectrum quality is maintained, some loss of intensity occurs at higher scan rates. This is a consequence of a reduction in the number of laser shots per pixel, and a decreasing duty cycle due to the fixed interscan delay.

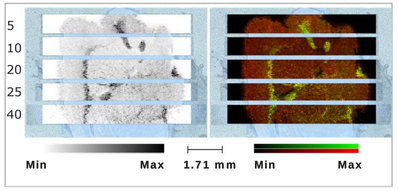

Figure 2. Study of the effect of acquisition speed at a fixed pixel size. Images acquired with a 50 µm pixel size using horizontal left to right type writer mode, numbers indicate scans per second. Left image m/z 826.6, Right, composite image (red m/z 798.5, green 826.6). The laser was set to fire for the whole line in order to visualize the additional extra distance to accelerate the stage to higher velocities. Images on individual intensity scales.

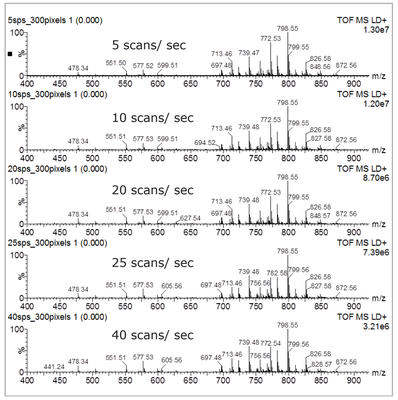

Figure 3. Spectra extracted from each of the images shown in figure 2. A 3x100 pixel area through the centre of each image was combined.

Low and high resolution imaging with adjustable laser focus

MS imaging at low resolution and high pixel rates represents a significant challenge compared to high resolution imaging, as the stage speed is significantly higher. Figure 4 shows the results of a 100 µm pixel image performed at 20 scans per second. The stage speed for this image was 2,000 µm/sec. It can be seen that despite the increased stage speed, the image quality was not compromised, in comparison to the 50 µm images in figure 1. To maximize sensitivity, the laser focus was expanded to ~90 µm, increasing the desorption area whilst preventing oversampling.

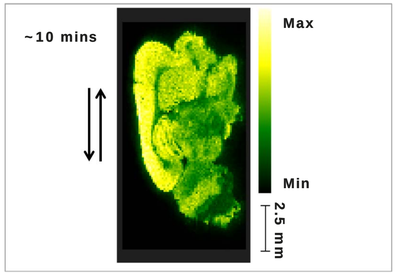

Figure 4. Low resolution image of mouse brain m/z 713.5, image acquired in vertical serpentine mode at 20 scans per second. Laser focus expanded to ~90 µm spot size. Total acquisition time ~10 mins.

Figure 5 shows the results of 10µm pixel imaging at 20 scans per second on a sample of mouse testis. The laser was refocused by altering the position of the lens. The tissue substructure was clearly visible with signals relating to tubule/lumen, smooth muscle and interstitial space.

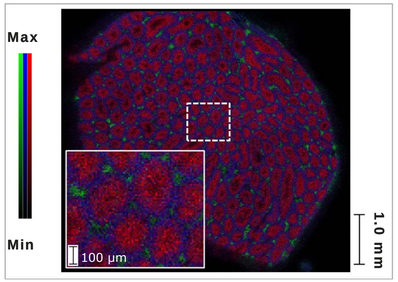

Figure 5. High resolution image of mouse testis. Image was acquired with a 10 µm pixel size at 20 scans per second in horizontal serpentine mode. Lipid signals selected to highlight tissue sub structure (Red m/z 846.5 Tubule with central Lumen, Blue m/z 798.5 Smooth muscle layer and Green m/z 826.6 interstitial space). Laser focused to ~10-15 µm.

MS/MS imaging at 20 scans per second

An investigation was performed looking at the ability to perform tandem mass spectrometry imaging at 20 scans per second. Figure 6 shows images for product ions generated from the fragmentation of PC (36:1)K+ (m/z 826.6). The images show the distribution of a potassium adduct of cyclic 1,2-phosphodiester ion (m/z 163.0) and the neutral loss of trimethylamine (m/z 767.5). Comparing the images to those in figure 1 it can be seen that the distribution of the product ions correlate with the expected distribution for this peak.

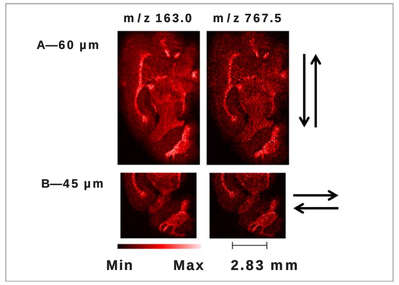

Figure 6. Assessment of the performance of MS/MS at high rate of acquisition. Precursor m/z 826.6 PC (36:1) K^+. Left - m/z 163.0 (cyclic 1,2-phosphodiester ion (K^+)), Right - m/z 767.5 (neutral loss of trimethylamine). A) 60 µm pixel size vertical serpentine, B) 45 µm pixel size horizontal serpentine. Both data sets acquired at 20 scans per second.

Ion mobility imaging at 10 scans per second

Initial investigations were conducted on the applicability of high speed imaging with ion mobility separation. Whilst an attempt was made to image at 20 scans per second this resulted in a disruption of the ion mobility separation (data not shown). Data acquired at 10 scans per second (figure 7) showed some minor aliasing (transfer Twave and pusher phasing) but the ion mobility separation was maintained (figure 8). Future work will look to optimize the ion mobility settings, in particular the Twave velocities.

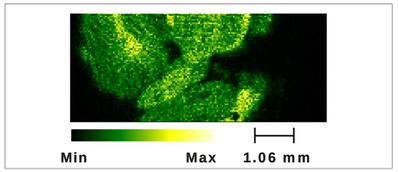

Figure 7. High acquisition rate ion mobility MS imaging. Image showing m/z 826.6 dt bin 105.0 acquired at 10 scans per second in a mouse brain cross section. 45 µm pixel size.

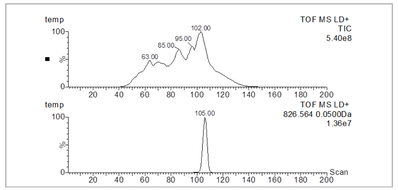

Figure 8. Top— combined mobilogram for image in figure 7, Bottom— extracted mobilogram for m/z 826.6

Future work

- Improvement of stage and acquisition synchronization to enable a reduction in image acquisition overhead.
- Investigate reducing the interscan delay to improve duty cycle and sensitivity.
- Further investigation of optimal settings for high acquisition rate ion mobility MS to remove aliasing with a potential for phase locking using a wideband enhancement acquisition mode.

CONCLUSION

- **Direction of continuous raster imaging had no significant effect on image quality or sensitivity.**
- **A serpentine raster can be employed to reduce imaging acquisition time overheads.**
- **Selecting an appropriate imaging direction with regard to tissue orientation can further reduce imaging acquisition time over head.**
- **An acquisition rate of 20 scans per second in MS-Tof mode can easily be achieved with the potential to be increased.**
- **Images of varying resolutions (10-100 µm) can be acquired at the same rate of 20 scans per second.**
- **MS/MS images can be acquired at 20 Scans per second.**
- **Ion mobility images can potentially be acquired at a rate of 10 scans per second.**

Acknowledgements

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References

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