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

Mark Townes1, Paul Murray1, Lukasz Migas2, Émmanuelle Caçaia2, Emmy Hoyles1, Richard Chapman1, Michael Baby1, Jeff Brown2
1 Waters Corporation, Wilmslow, United Kingdom; 2 University of Manchester, Manchester, United Kingdom

Introduction

Imaging mass spectrometry using MALDI is an established technique that allows 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 and process these data sets increases. Continued hardware enhancements, such as a faster sample stage/motorized computer system, 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 method for improving the sample stage movement of the sample carrier plate whilst maintaining high scan synchronization. Scanning the stage speed, laser beam diameter and desired size ensures that the acquisition rate is independent of image resolution.

Methods

In this study, we utilized the Waters Resources Enabled Software (WRES) to allow an in-situ standard acquisition methodology. WRES allows direct control of certain aspects of the instrument functionality, so the operator is able to optimize the entire MALTTOF acquisition process; from imaging in a continuous raster mode for both a “hybrid” and “super raster” movement of the sample stage to imaging in a “serpentine” mode.

The speed of the sample stage/motorized computer system can be set independent of the detector rate. The samples were found to be imaged with high quality and at high speed, with a maximum sample stage speed of 20 scans per second. Images at 20 scans per second were achieved without a reduction in imaging quality.

MS images were acquired using a Waters Sprint G2-QDE with a new 1.0 mm single pass with accelerated electron optics source. The samples were prepared from the tissue sections using standard cryo-cooling, rapid freeze and freeze-drying techniques. The laser was set to fire for the whole line in order to visualize the additional extra distance to achieve a pixel size of ~60 µm.

Images of varying resolutions (10-100 µm) can be obtained using the WRES software. These features include; easy to access scripts and a large script based format. Scripts were written to perform imaging in a continuous raster mode for both a “hybrid” and “super raster” movement of the sample stage to imaging in a “serpentine” mode.

Initial investigations were conducted on the applicability of high speed imaging with ion mobility separation. These features include; easy to access scripts and a large script based format. Scripts were written to perform imaging in a continuous raster mode for both a “hybrid” and “super raster” movement of the sample stage to imaging in a “serpentine” mode.

RESULTS

MS Imaging speed

The effect of MS imaging speed (scans per second) was investigated to assess the ability to raster the sample stage efficiently. Figure 1 shows an example of an 826.6 m/z ion image. The stage speed was increased from 5 to 20 scans per second while keeping the laser fixed. The images were acquired with a pixel size of 20 µm. Figure 1 shows that MS images at higher stage speeds (100 and 200 scans per second) resulted in a disruption of the ion mobility separation (data not shown). Figure 1 shows the results of 5 scans/second. The image was acquired in vertical serpentine mode at 20 scans per second. The images were acquired at 20 scans per second with a 20 µm pixel size and <25 µm laser focus.

Figure 1: Study of the effect of acquisition speed on a fixed pixel size. Images acquired with a 10 µm pixel size using horizontal left-to-right type raster mode, system indicates speeds per second. Laser was fixed. A) 50 µm pixel size, B) 60 µm pixel size, C) 30 µm pixel size, D) 20 µm pixel size.

MS 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, it can be seen that the distribution of the product ions correlate with the expected distribution for this peak.

Figure 2: 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 overheads.

Figure 3: Extracted spectra from each of the images in figure 2. Spectra were acquired between the center of each image and centroided.

Low and high resolution imaging with adjustable laser focus

MS imaging at high resolutions resulted in a significant challenge compared to traditional raster imaging. As the stage speed is significantly higher, Figure 4 shows the results of a 500 µm pixel size image at 20 scans per second. The fast stage movement and low pixel size resulted in a disruption of the ion mobility separation (data not shown). Figure 4 shows the results of 5 scans/second. Laser was fixed. Images of varying resolutions (10-100 µm) can be obtained using the WRES software. These features include; easy to access scripts and a large script based format. Scripts were written to perform imaging in a continuous raster mode for both a “hybrid” and “super raster” movement of the sample stage to imaging in a “serpentine” mode.

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 overheads.

Ions in tissue sections mounted on non conductive glass slides were both prepared by spray coating with recrystallized α-cyano-4-hydroxycinnamic acid (CHCA) 30-70 µm. The nebulising gas was set to 1.52bar. All images displayed using Waters High Definition Imaging 1.4.

Introduction of a new sample stage/motorized computer system 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 method for improving the sample stage movement of the sample carrier plate whilst maintaining high scan synchronization. Scanning the stage speed, laser beam diameter and desired size ensures that the acquisition rate is independent of image resolution.

Future work

• Improvement of stage and acquisition synchronization to enable a reduction in image acquisition overheads.

• Investigating the reduced intensity for improved duty cycle and sensitivity.

• Further investigation of optimal settings for high speed imaging with ion mobility separation.

• MS imaging can be acquired at 20 scans per second with a pixel size of 20 µm and <25 µm laser focus.

• Ion mobility imaging at 10 scans per second

Initial investigations were conducted on the applicability of high speed imaging with ion mobility separation. These features include; easy to access scripts and a large script based format. Scripts were written to perform imaging in a continuous raster mode for both a “hybrid” and “super raster” movement of the sample stage to imaging in a “serpentine” mode.

Figure 6: Assessment of the performance of MS/MS at high rate of acquisition. Protect m/z 864.6 & m/z 863.6 (m/z 761.7, m/z 863.6) moved toward laser focus and ion mobility. Laser focused to ~10-15 µm. 18 scans per second, min Max 1.0 mm Max 2.3 mm

Figure 7: High 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 overheads.

• Ion mobility imaging can potentially be acquired at a rate of 10 scans per second.

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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.

• An appropriate imaging direction with regard to tissue orientation can further reduce imaging acquisition time overheads.

• Images of varying resolutions (10-100 µm) can be acquired at the same rate of 20 scans per second.

• MS/MS imaging can be acquired at 20 scans per second.

• Ion mobility imaging can potentially be acquired at a rate of 10 scans per second.

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Figure 5. High 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 overheads.

Figure 8. Top—constructed montage image in figure 7, Bottom—extracted montage for m/z 866.5 A—60 µm B—45 µm C—30 µm D—25 µm