# HIGH RESOLUTION IMAGING PLATFORM INCORPORATING MALDI AND DESI

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## INTRODUCTION

**One key issue in imaging mass spectrometry (MSI) is analyte identification, particularly with regards to small molecules (metabolites/lipids). This is due to the abundance of molecular species with similar masses within a small mass range and no chromatographic separation. One way to improve compound identification confidence is through increased mass resolution and mass accuracy. To achieve this with a trapping style instrument requires a long scan duration. Here we demonstrate a multi-reflecting QTof instrument providing a mass resolution of up to 200,000 FWHM and a mass accuracy <500ppb with a scan speed of up to 10 Hz. The instrument supports both matrix assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI) sources, specifically designed for imaging applications.**

## METHODS

### Sample preparation

Porcine liver and Mouse brain tissues were sectioned on a cryostat (Leica™) at -20°C then stored at –80°C (18µm for DESI and 12µm for MALDI). For the MALDI experiments, samples were sprayed with DHB using a HTX™ M5 sprayer. However for DESI experiments, samples were used without further sample preparation.

### Data acquisition

Data were acquired using a multi-reflecting QTof instrument (SELECT SERIES™ MRT) figure 1. For MALDI, the effect of laser focusing and laser attenuation as well as data acquisition rate were explored, in positive ionization mode.

For DESI experiments, a DESI XS source utilizing a heated transfer line (HTL) and high performance sprayer were used. Solvent (95:5 MeOH:Water) was supplied using a ACQUITY™ M Class binary solvent manager at a flow rate of 1.5 µL/min. Data were acquired in negative ionization mode. The HTL was set to 450°C which significantly increases negative mode sensitivity.

### Data processing

Data were initially processed and visualized in HDI™ 1.6 and exported to MassLynx™ 4.2 software for spectral interrogation. In addition, data were imported into Lipostar™ MSI for lipid database searching against the LipdMaps structural Database. Due to the high resolving power and mass accuracy of the MRT, a search tolerance of 0.8ppm was used.

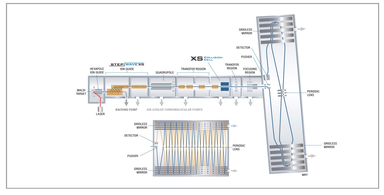

*Figure 1. Schematic of SELECT SERIES MRT showing multi reflecting ToF analyzer.*

## RESULTS

### MALDI

The MALDI source uses a continuous raster to allow imaging at up to 10 pixels per second. A two part attenuation system allows for a large range of laser focusing between >130 and <10µm. At high laser focus, a secondary fixed attenuator can be enabled to increase the dynamic range of the primary attenuator. The ablation region on tissue is a function of the lens position and the laser attenuation. Across the lens position scale, the optimum attenuation has been determined for the DHB matrix applied on liver tissue, by adjusting the attenuation to minimize laser induced fragmentation whilst maximizing signal.

In figure 2, the results of investigation in to the lens position at optimized attenuation can be seen. The resulting ablation widths have been measured optically to create a look up table for attenuation *vs.* lens position for a desired pixel size.

To assess the effectiveness of this table as well as the effect of scan speed, a series of images at different resolutions have been performed and a 4.5 mm area summed, the number of shots per pixel was kept the same by adjusting the laser repetition rate (figure 3). Finally a series of images at 10 s/sec at pixel sizes from 100-10µm (figures 4-8) were generated using optimized values to asses the instruments performance with a wide range of pixel sizes whilst minimizing under or over sampling.

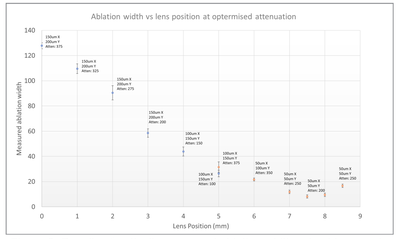


*Figure 2. Results of optically measured ablation widths at set lens position with optimized attenuation values. **Blue second attenuator disabled**, **orange enabled**. X and y pixel size with optimal attenuation noted on graph.*

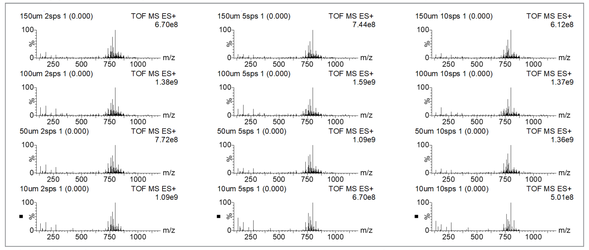


*Figure 3. Investigation of the effect of scan rate and image resolution versus signal response from liver with optimized attenuation and laser focus for MALDI +ive mode images. Left to right—2 s/sec, 5 s/sec, 10 s/sec. Top to bottom— 150, 100, 50 and 10 µm pixel size. Each spectra is a sum of a 4.5mm area of Liver tissue. The number of pixels increase from 900 (150µm)– 202,500 (10µm). The laser repetition rate was adjusted for approximately 200 shots per pixel. Beyond biological variation the signal response for the given area is mostly independent of scan speed and resolution due to the appropriate adjustment of the laser focus an attenuation.*

In addition the instrument performances in terms of mass resolution and accuracy at 10 µm were examined (figure 8).The mass resolution was found to be >191,000 with a mass accuracy <500ppb ( >200ppb for the selected images)

### DESI

In addition to the MALDI source, the SELECT SERIES MRT is also compatible with the DESI XS allowing DESI imaging to be performed. Here the effect of scan rate has been assessed for negative mode imaging of a mouse brain sample (Figure 9) . Images were performed at 5 and 10 s/sec. No discernable effect was noted in image quality, mass resolution or mass accuracy. Unlike the MALDI results where a fixed number of shots were performed regardless of scan speed, there was a proportional decrease in signal response, as would be expected due to the continuous sampling of the DESI source and therefore reduction in sampling time with increased scan speed.

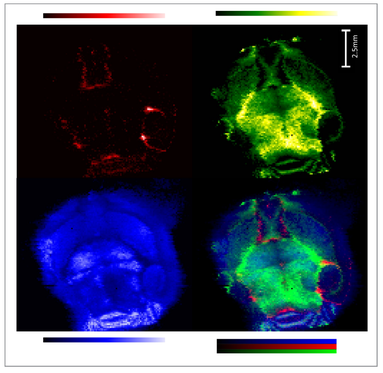


*Figure 4. **100µm** MALDI +ive mode image of mouse brain at 10 scans per second. Red- m/z 741.5304; Green- m/z 866.6483 ; Blue– m/z 844.5253; Laser focus: 2.0 mm. Attenuation: 275. Secondary attenuator: disabled. Laser rep rate: 1Khz.*

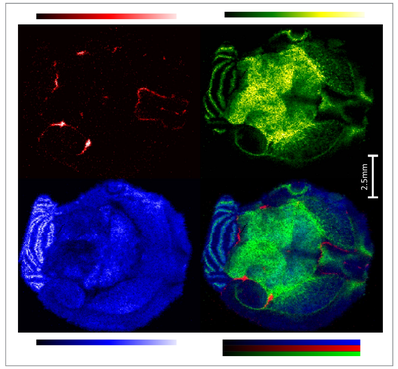


*Figure 5. **50 µm** MALDI +ive mode image of mouse brain at 10 scans per second. Red- m/z 741.5304; Green- m/z 866.6483 ; Blue– m/z 844.5253; Laser focus: 4.0 mm. Attenuation: 250. Secondary attenuator: disabled. Laser rep rate: 1Khz.*

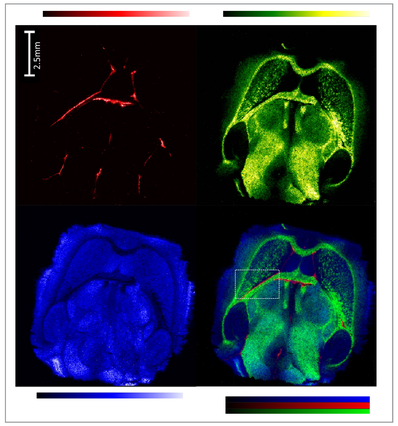


*Figure 6. **20 µm** MALDI +ive mode image of mouse brain at 10 scans per second. Red- m/z 741.5304; Green- m/z 866.6483 ; Blue– m/z 844.5253; Laser focus: 6.0mm. Attenuation: 325. Secondary attenuator: enabled. Laser rep rate: 1Khz. White box is the area expansion shown in figure 7.*

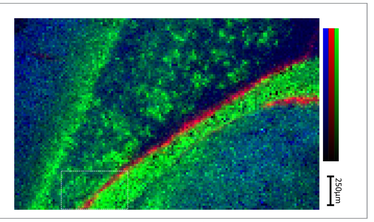


*Figure 7. Expansion of indicated area in figure 6. Three color overlay **20 µm** image or mouse brain at 10 scans per second. Red- m/z 741.5304; Green- m/z 866.6483 ; Blue– m/z 844.5253; Laser focus: 6.0mm. Attenuation: 325. Secondary attenuator: enabled. Laser rep rate: 1Khz. Single pixel width feature can be observed in area indicated—bottom left.*

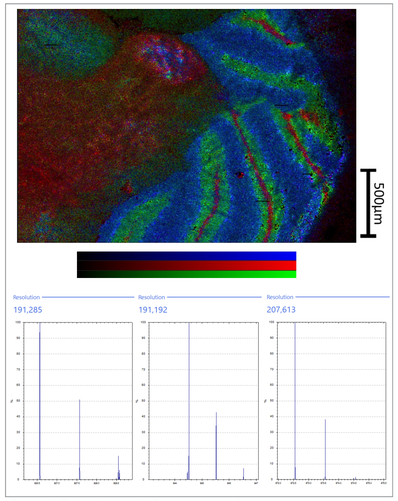


*Figure 8. Top - **10 µm** MALDI +ive image of mouse brain subsection at 10 scans per second. Green- m/z 844.52521 Red- m/z 826.57227; Blue- m/z 872.55646; Laser focus: 6.3 mm. Attenuation: 200. Secondary attenuator: enabled. Laser rep rate: 1Khz.*

*Bottom– Results of resolution calculations for an approximately 2500 pixel region from image in top showing a resolving power FWHM in excess of 190,000.*

*Lipid maps data base searching of the masses allows for tentative identifications — m/z 844.52521 PC(38:6) K+ (-118ppb), m/z 826.57227 PC (36:1) K+ (+12ppb), m/z 872.55646 PC(40:6) K+ (-172ppb);*

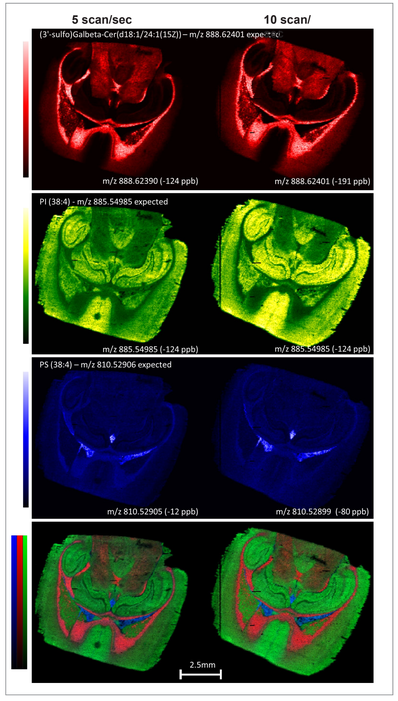


*Figure 9. **50 µm** DESI -ive mode image of mouse brain at 5 (left) and 10 (right) scans per second. Red- m/z 888.624; Green- m/z 865.54985 ; Blue– m/z 810.52899. The increase in speed from 5 s/sec to 10s/sec (right)had no discernable effect on the image quality or mass accuracy of the instrument (tentative identification and ppb error displayed on image. The HTL was set to 450°C.*

## CONCLUSION

- **The SELECT SERIES MRT allows both MALDI and DESI imaging on a single high performance platform.**
- **Optimized laser focusing and attenuation allow for MALDI images to be performed at 10-130µm pixel size with minimal over sampling maximizing signal response.**
- **Data can be acquired at 10 scans per second irrespective of the pixel size.**
- **The scan speed used has no effect on the instrument performance in either MALDI or DESI, allowing for mass a Mass resolution >200,000 FWHM with mass accuracy's >500ppb.**

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