High-Performance MALDI-TOF Imaging Mass Spectrometer

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Introduction

A new high performance linear MALDI-TOF mass spectrometer is described. This instrument employs a new ion optics system with grounded ion source and efficient transfer and detection of ions over a broad mass range that provides very high sensitivity, precision, and dynamic range for both positive and negative ions. The instrument employs a solid-state Nd:YLF laser that provides 349 nm photons with fluence up to 30 μJ at 5kHz. The laser spot size is adjustable from 3 to 30 μm under computer control. A new 8 Bit High Speed Digitizer with an ADC sampling rate up to 2.5 GS/s on a single channel is employed. This digitizer features a PCI Express interface and optimized drivers that enable data transfer rates in excess 3.4GB/s. Software has been developed and validated that allow complete spectra to be acquired, stored and analyzed at very high rates. This software makes it possible to process the raw spectral data "on the fly" to carry out processes including smoothing, baseline correction, peak detection, and recalibration without sacrificing speed, making it practical to save peaks and discard the raw data. As a result the files are reduced in size, and time required for producing images is also reduced. We have demonstrated the ability to acquire and save single-shot spectra at a rate of 5000/s, but for many applications the effective speed is limited by the time required to process the large files generated from spectra covering a broad mass range. At present the maximum rate for processing and transferring to disk the large files generated for protein imaging is about 500 pixels/s. The 5 kHz laser allows averaging 5 laser pulses/pixel at acquisition rate of 1000 pixels/s or 10 laser pulses/pixel at acquisition rate of 500 pixels/s. The effective rates, including data transfer, are then 333 and 250 pixels/s for these two cases. Performance of this new instrument was evaluated for both protein and lipid imaging on a variety of biological tissues, including pancreas, kidney, and lung.

Initial Results

This project was initiated to evaluate the speed and performance of the new MALDI-TOF imaging technology described above. The first work was focused on evaluating the performance using a single laser shot per 10 μm pixel using a 10 μm diameter laser beam. These first results do not represent the ultimate speed that can be achieved but demonstrate the very high efficiency for ion production, transmission, and detection. The protein spectra were acquired by rastering the 10 μm laser at 2 mm/s at intervals of 10 μm, and the laser was operated at 200 Hz giving 1 laser shot per 10 μm pixel.

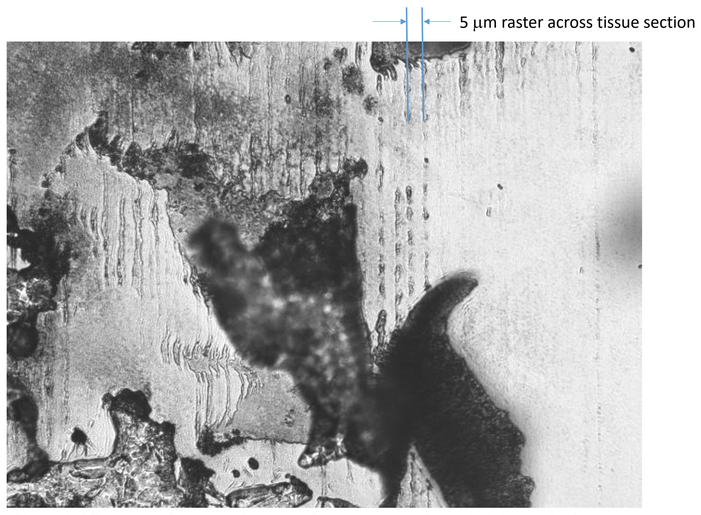

Figure 1. Microscope image of tissue section rastered at 5 μm. This image indicates that the effective laser beam diameter is approximately 4 μm.

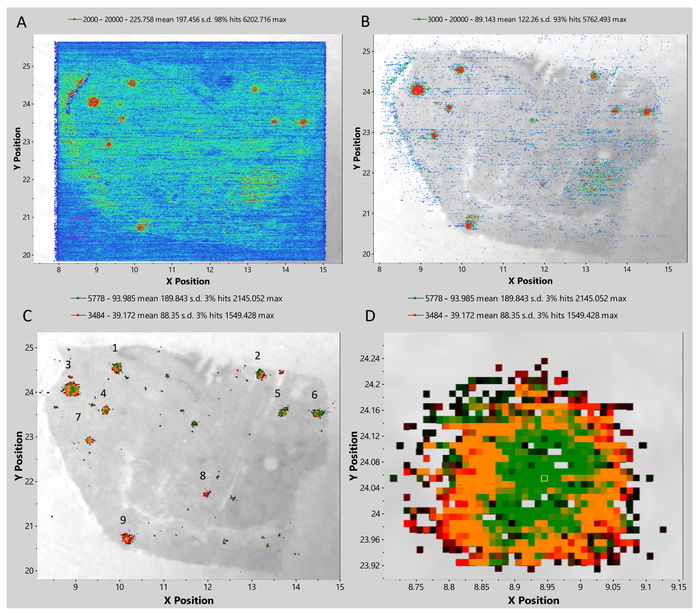

Figure 2. MALDI Images of porcine pancreas. **A** total ion current (TIC) for mass range 2-20 kDa with zero threshold with all 415,565 spectra displayed. Area imaged is 41.5565 mm^2 but the total area of the tissue section is approximately 22 mm^2 as shown by the camera image in **B** and **C**. The Islets of Langerhans are clearly visible as intense as intense spots in the TIC image. One of the larger islets spots is shown in the expanded view. Expanded image **D** of largest Isle at upper left in the overall image. The nine largest Islets of Langerhans are labeled in **C.**

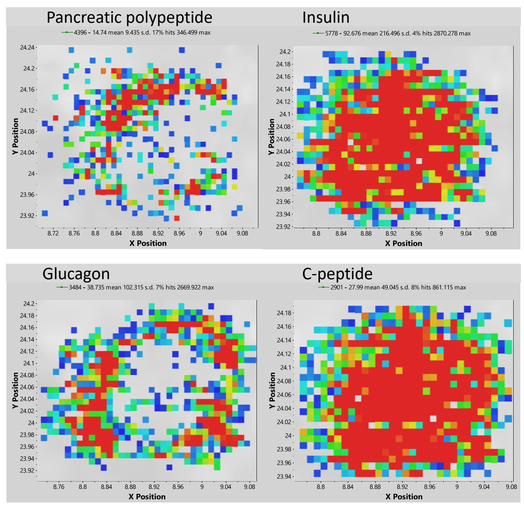

Figure 3. Heat maps of individual peptides – red high intensity, blue low

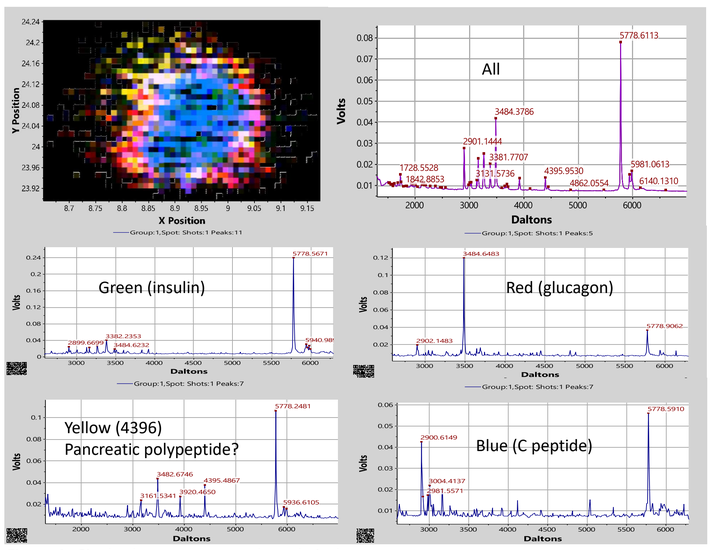

Figure 4. Single shot spectra on selected pixels

Method

Performance of this new instrument was evaluated for both protein and lipid imaging on a variety of biological tissues, including pancreas, kidney, and lung. One example, presented in another poster, employed Zucker Diabetic Fatty (ZDF) rats 16-weeks old purchased from Charles River and included one diabetic, one obese and one lean rat for MS image acquisition. The tissue sections for this study were from the large end of the pancreas and ranged in area from 1.2 cm^2 to more than 3 cm^2. Spectra were acquired by rastering the laser at 5 mm/s and saving 10 spectra/s. The time to produce an image at 10 μm spatial resolution was typically 1 hour/cm^2. This work demonstrates that imaging tissue sections as large as 4 cm^2 is possible, but may not be practical for proteins except in very special cases. A practical approach has been demonstrated that acquires initial images at lower resolution (e.g. 100 μm), and selects regions of interest from the initial image for imaging at 10 μm resolution. Since the laser diameter is 10 μm, 90% of the sample is available for high resolution imaging and the full spectra can be saved and efficiently processed to produce spectra with high precision and broad mass range.

Acquisition limits for 5 kHz laser and 10 mm/s motion

Resolution μm	Speed pixels/s	laser shots no./pixel	time req'd s/mm^2
100	100	50	1
50	200	25	2
20	500	10	5
10	1000	5	10
5	2000	2.5	20
2	5000	1	50
1	5000*	1	200

*limited by 5 kHz laser rate

Effective imaging speed is limited by time to transfer data to disc and time to process data to produce image, and these are determined by the number of time bins and the number and intensity of the peaks detected.

We have found that for imaging of proteins with acquisition speed of 500 pixels/s yields an effective imaging speed of about 300 pixels/s. Thus for 10 μm imaging we operate with motion speed of 5 mm/s and average 10 laser shots/pixel, and for 5 μm imaging we use 2.5 mm/s.

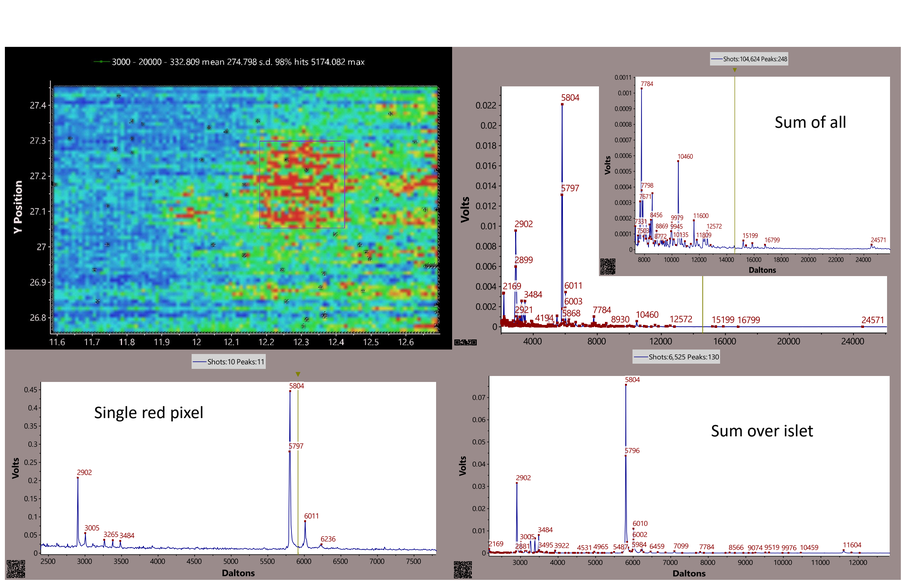

Figure 5. 10 μm heat map for TIC 3-20 kDa of a region including an islet of Langerhans selected from a tissue segment Initially imaged at 100 μm resolution. Spectra shown include the sum over the full section imaged at 10 μm, the sum over the islet, and the spectrum from a single pixel.

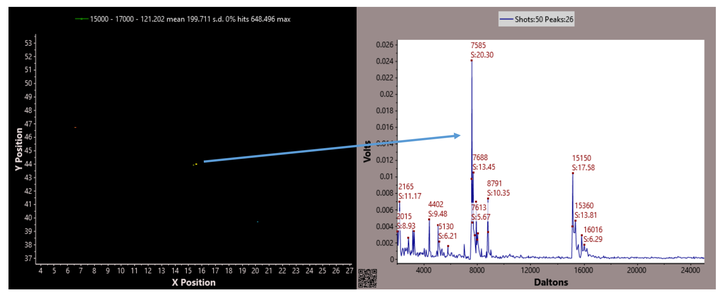

Figure 6. In this example hemoglobin was only detected in a few isolated pixels

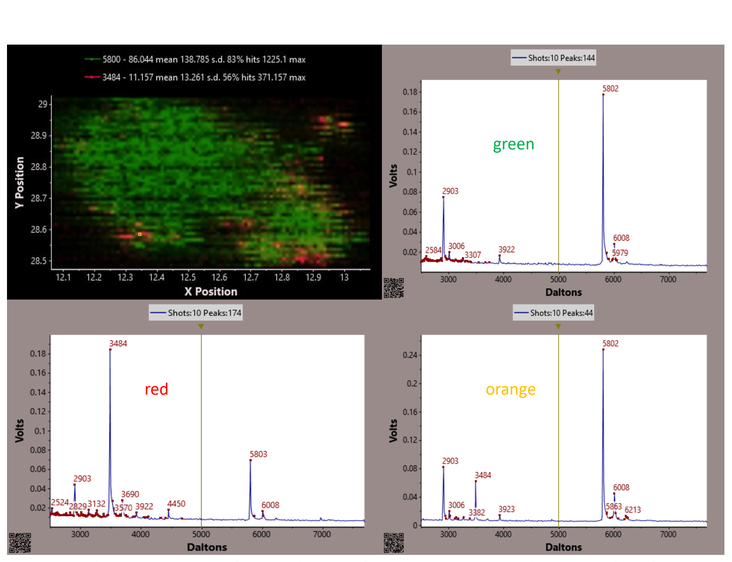

Figure 7. 10 μm Image of an islet from lean rat showing separation of glucagon and insulin

Acknowledgements: The porcine pancreas and mouse kidney tissue sections were supplied by Pierre-Maxence Vaysse and Ron Heeren of M4I and the rat pancreas sections by Lingjun Li and colleagues at U. of Wisconsin

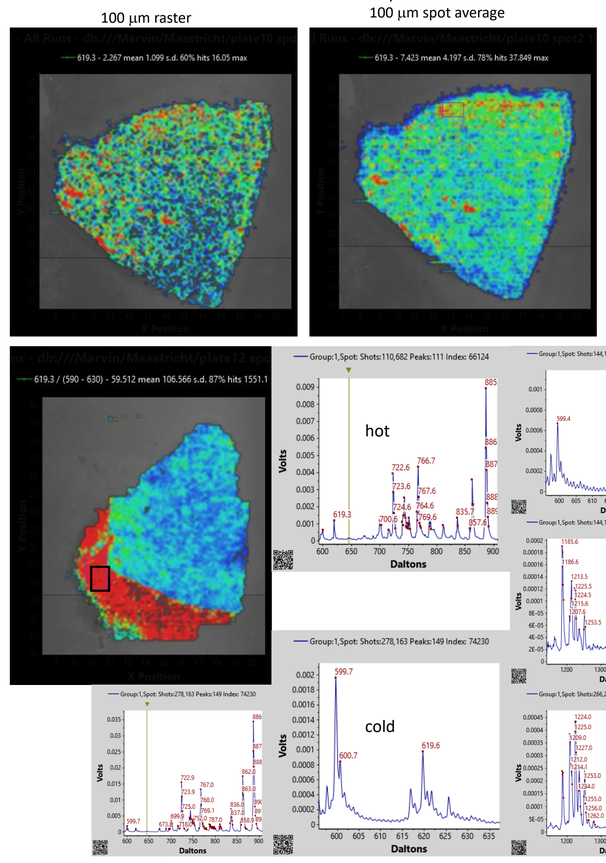

Figure 8. Examples of lipid imaging results on kidney sections supplied by M4i. Full images generated at 10 μm resolution in negative ion mode, m/z 300-1600 Da, with effective rate of 300 pixels/s.

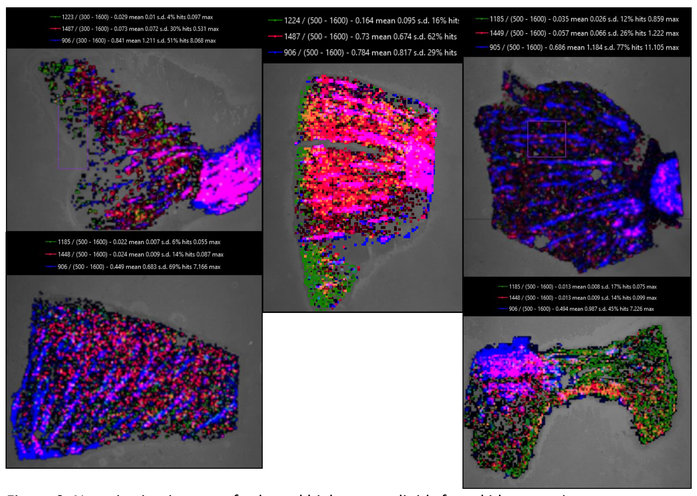

Figure 9. Negative ion images of selected higher mass lipids from kidney sections.

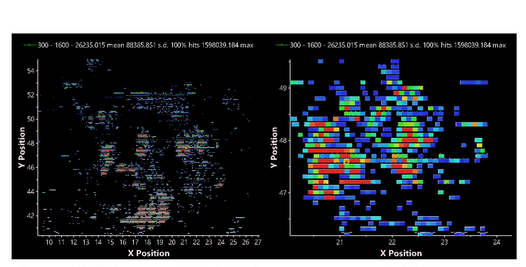

Figure 10. TIC image (300-1600 Da) from obese rat at 100 μm resolution

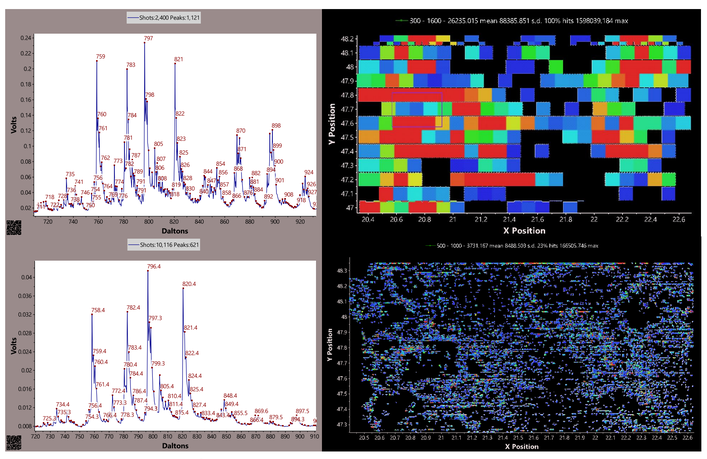

Figure 11. Comparison of images at 100 μm and 5 μm resolution

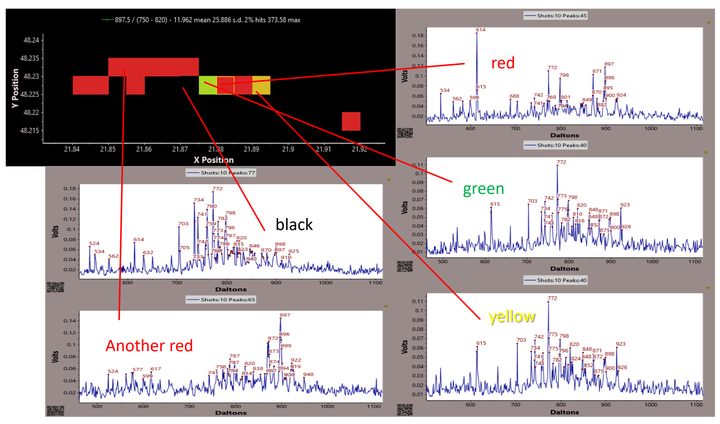

Figure 12. Spectra from single adjacent pixels in 5 μm image

Conclusions

Both protein and lipid imaging at 10 μm resolution and 300 pixels/s is possible but only practical in special cases

- Acquisition requires about 1 hour/cm^2 , and image generation may require another hour or more
- Data files are very large and difficult to export
- For detection of low abundance components, dynamic range and precision limited by relatively low number of ions detected/pixel.

Imaging at 100 μm resolution with 100 laser shots/pixel provides reproducible spectra with large dynamic range and excellent precision using 10 μm diameter laser beam

- Acquisition and image generation requires less than 5 minutes/cm^2
- Data files for 10,000 pixels/cm^2 are manageable and can be readily exported to third-party software
- High resolution (10 μm) images for regions of interest can still be generated since 90% of the sample is still available.

Imaging at 5 μm is practical for lipids and small molecules, but performance for proteins will depend on quality of matrix deposition.

Imaging at 50 μm resolution with both positive and negative ions can be done in less than 10 minutes/cm^2 (2 times 50 μm raster at 10 mm/s, 5kHz, 200 pixels/s, 25 shots/pixel)