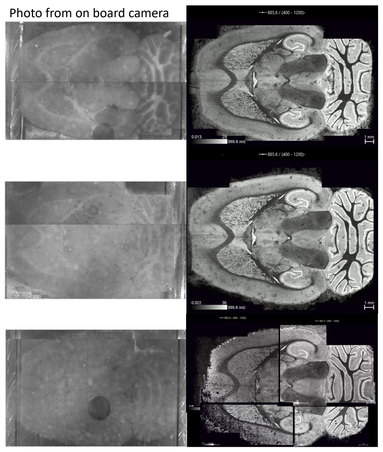
The Seven S Criteria for Evaluating Performance of MALDI Mass Spectrometer for MSI

Marvin Vestal, Christina Vestal, Sicheng Li, Kenneth Parker. SimulTOF Systems Division Virgin Instruments Corp.

Rats! – From head to tail at 10um spatial resolution – Brain

Photo from on board camera



This plate contained 3 serial sections of rat brain each about 3 cm² in area.
All were imaged in one day using 5 kHz laser with 5um diameter laser, with net rate of 300 pixels/s at 10 shots/pixel.

First segment
20 µm spatial resolution, rastering with horizontal motion of **10** mm/s
659,519 pixels saved in 39 minutes, 6% of sample used

Second segment
10 µm spatial resolution, rastering with horizontal motion of **5** mm/s
2,813,271 pixels saved in 2 hours and 48 minutes, 25% of sample used

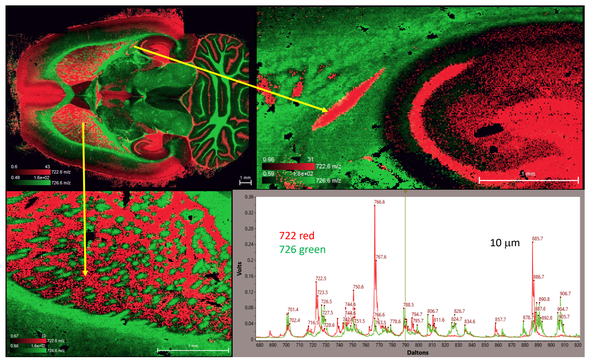
Third segment
5 µm spatial resolution, rastering with horizontal motion of **2.5** mm/s
Split into 4 sections
First section 3,691,614 pixels saved in 3 hours and 10 minutes
Second section 3,515,258 pixels saved in 2 hours and 54 minutes
Third section 1,767,795 pixels saved 1 hour and 28 minutes
Fourth section 1,486,208 pixels saved in 1 hour and 18 minutes
10,460,875 pixels saved in 8 hours and 50 minutes, 100% of sample used

Total time: 12 hours and 7 minutes;
Total Pixels: 14,000,000
140 million laser shots

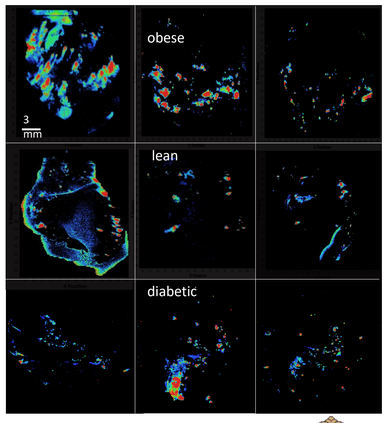
Plate provided by Jeff Spraggins and Richard Caprioli with sublimated DAN matrix

VANDERBILT UNIVERSITY

Expanded view showing images at 10 um spatial resolution

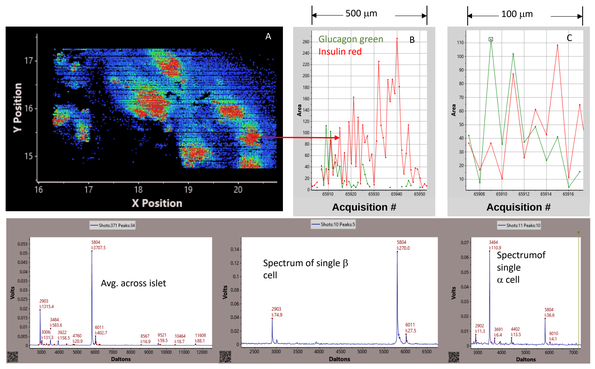


Rats! – Pancreas



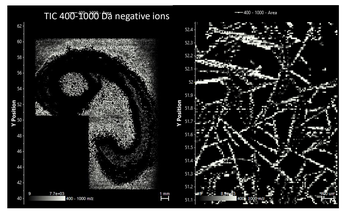
10 um images of nine rat pancreas segments showing high intensity for isles of Langerhans. Sample prepared and provided by Lingjun Li and associates

WISCONSIN UNIVERSITY OF WISCONSIN–MADISON



Expanded view of a portion of one pancreas section and spectra of individual pixels showing variation of insulin and glucagon in one islet of Langerhans. This show single cell resolution with signal detected at all 10 um pixels. The variation in intensity from pixel to pixel is larger than expected statistically and may be due either to variation in the protein concentration or to variations in the matrix thickness.

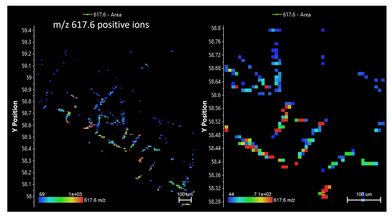
Rats! – Small Intestines



Rat small intestine DHB matrix sample prepared and provided by Lennart Huizing

M4I Maastricht University

Ion intensities much higher in positive ion mode with DHB but negative ion imaging is still possible but with reduced dynamic range



Ideal Mass Spectrometer for all MALDI not just MSI

- Satisfy the 4S-Criteria for performance*
  - Speed
  - Specificity
  - Spatial Resolution
  - Sensitivity Dynamic Range!!
- We propose to add 3 more
  - Stability
  - Simplicity
  - Size (of tissue sample and data files)
- Our focus is on the instrument but sample preparation and matrix application are vital for success of any instrument platform
- The linear MALDI-TOF instrument excels in all but specificity
  - ***Speed****: up to 300 pixels/s*
  - ***Specificity****: up to 50,000 mass resolving power; 1 ppm mass accuracy*
  - ***Spatial Resolution****: 5-20 um under computer control*
  - ***Sensitivity****: 10x higher than any competitor*
  - ***Stability:*** *up to 1 billion laser shots before cleaning; 1 trillion shots before service*
  - ***Simplicity:*** *fully automated*
  - ***Size:*** *very large samples with data files up to 5 million spectra analyzed efficiently*
- *Reflector SPEEDI-TOF and SPEEDI-Q-TOF provide Specificity*

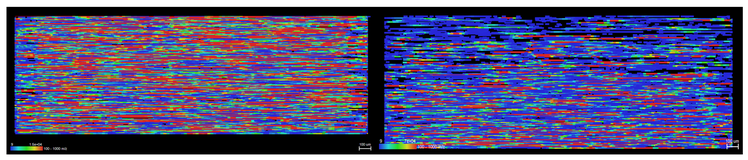
What determines sensitivity in mass spectrometry?

- Concentration
- Recovery
- Ionization efficiency
- Detection efficiency
- Chemical noise (for samples at low relative concentration)
  - Specificity—High mass resolution, accurate mass, MS-MS
  - Purification
    - Separation by LC or CE
    - Affinity capture

Sample preparation and matrix deposition are the limiting factors in obtaining high quality images at high spatial resolution and we are indebted to our colleagues cited above that provided these excellent samples. Our work is focused on the limitations imposed by the instrumentation

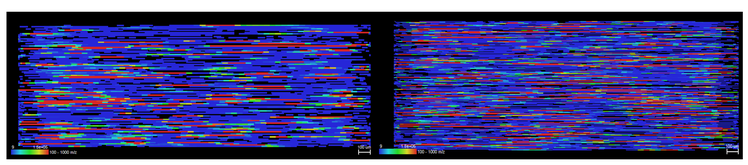
Our initial results show that ***higher spatial resolution does not invariably limit sensitivity,*** since in some cases the decrease in ablation area may be overcome by higher ionization and detection efficiency for the molecules irradiated. Here we provide quantitative data on the sensitivity of MALDI expressed as ions detected as functions of laser beam diameter, laser fluence, shots summed per spectrum, and spatial resolution. These results clearly depend on the nature of the sample and the matrix application. These initial studies focused on positive ions from α-Cyano-4-hydroxycinnamic acid (CHCA) with matrix deposition by sublimation on standard ITO coated glass plates.

Data were generated for effective laser diameters of 2.5, 5, 10, and 20 um, and effective pixel sizes of 5, 10, and 20 um using a laser frequency of 5 kHz and a lateral motion speeds of 1.25, 2.5, 5, and 10 mm/s.



5 µm raster, laser beam eff. dia 3 µm, 0.15 µJ fluence

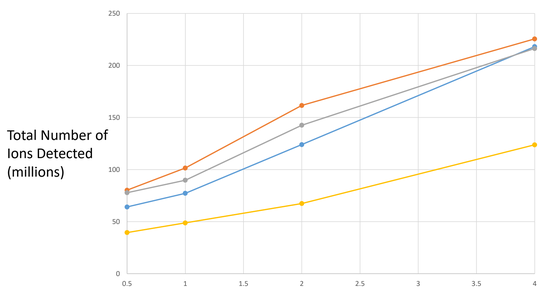
5 µm raster, laser beam eff. dia 5 µm, 0.175 µJ fluence



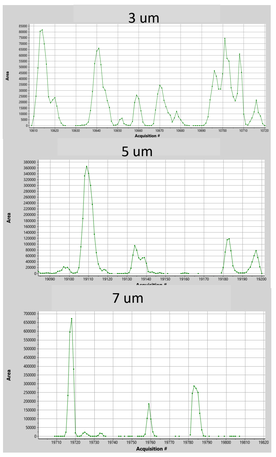
10 µm raster, laser beam eff. dia 7 µm, 0.225 µJ fluence

5 µm raster, laser beam eff. dia 7 µm, 0.225 µJ fluence

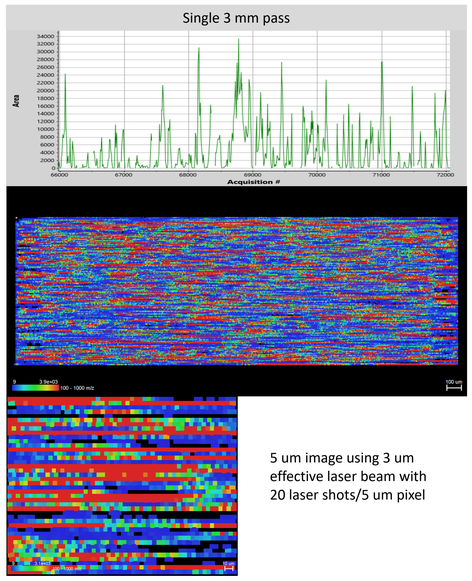
Images of the regions scanned in one set of experiments. These were generated by summing 100 laser shots per pixel and scanning speed of 1.25 mm/s corresponding to 4 laser shots/um.



Example of results from this study. The instrument includes a neutral density filter that transmits 5% of the incident beam; thus the fluence delivered to the sample is 5% of the fluence indicated by the laser readout. The effective laser diameter is taken as 10% of the $1/e^2$ width calculated for the optical system. For each effective laser diameter the fluence was adjusted to give approximately the same average intensity for the effective laser diameters are somewhat smaller than estimated.



TIC for one 3 mm pass across these samples. We were surprised that our matrix deposition was not as uniform as needed for high quality imaging.



5 um image using 3 um effective laser beam with 20 laser shots/5 um pixel

Conclusions and future work

In earlier work we have established that imaging with 5 um spatial resolution is practical at effective speed in excess of 300 pixels/s. The present work illustrates once again that uniform matrix deposition is vital for generating high quality images, and the present work shows a method for evaluating the quality of the matrix deposition without using valuable sample. We have initiated a study to quantitatively determine the trade-offs between spatial resolution and detection sensitivity for samples present at low surface concentrations. We are also developing a high resolution MALDI instrument that will provide Specificity without sacrificing the other six S's demonstrated with the linear instrument.

References

1. Schulz, Becker, Groseclose, Schadt, and Hopf; "Advanced MALDI mass spectrometry imaging in pharmaceutical research and drug development"; Current Opinion in Biotechnology 2019, 55:51-59.
2. Marvin Vestal, Christina Vestal, Sicheng Li, Kenneth Parker; "Molecular imaging of biological samples with sub-cellular spatial resolution and high sensitivity; SimulTOF Systems Application Note 19-1; 2019.
3. Marvin Vestal, Lingjun Li, Evgenia Dobrinskikh, Yatao Shi, Bowen Wang, Xudong Shi, Sicheng Li ,Christina Vestal, Kenneth Parker; "Rapid MALDI-TOF molecular imaging: Instrument enhancements and their practical consequences"; Journal of Mass Spectrometry, Special Issue – Research Article, July 2019.

View Online