Practical Method for Molecular Imaging with Single-Cell Resolution and Very High Speed

Marvin Vestal¹, Evgenia Dobrinskikh², Lingjun Li³, Xiaoxin Wang³, Christina Vestal¹, Sicheng Li¹, Kenneth Parker¹, Jeff Spraggins⁴, and Richard Caprioli⁴.

Outline

1. Ideal Mass Spectrometer for MALDI MSI
2. Proteins in Islets of Langerhans
3. Lipids in Rat Brain
4. Status

This work focuses on the introduction of a new linear MALDI-TOF mass spectrometer that provides both high spatial resolution and high speed.
Ideal Mass Spectrometer for MALDI MSI

• Satisfy the 4S-Criteria for performance*
  • Speed
  • Specificity
  • Spatial Resolution
  • Sensitivity

• 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

Total ion current images covering the range 2-20 kDa at 10 μm spatial resolution from all nine tissue sections from the obese, lean, and diabetic rats.

These tissues were chosen because earlier work had confirmed that images of pancreatic tissues were dominated by insulin and glucagon corresponding to the beta and alpha cells of the islets of Langerhans.

Since it was known that these cells were about 10 μm in diameter, the present work was undertaken to determine if single cells could be detected by imaging the tissue with 10 μm spatial resolution.

Three tissue sections from each rat were imaged; these range in area from 2 to 3 cm², and a total of 16 million spectra were acquired and analyzed in less than 24 hours.
Expanded view of the beta cells producing insulin and the alpha cells producing glucagon in four islets of Langerhans from obese rat. Mass spectra are average over the islets shown.
Images of one diabetic rat tissue section acquired by 100 μm raster of a 10 μm laser beam operating at 5 kHz. 33,000 spectra averaged over 50 laser shots were generated by acquiring mass spectra over the range 2-20 kDa with each spectrum representing a 50x100 μm pixel. Time to acquire, store, and process the data was less than 15 minutes, and additional time to produce the images shown was about 1 minute each.
Image of portion of diabetic rat tissue acquired at 10 μm resolution indicated by rectangle in center panel of Figure 4. Area imaged is 8.7 mm² comprising 86,700 pixels at 10 laser shots/pixel acquired in 5 minutes at effective rate of 300 pixels/s.
Expanded view of insulin MH$^+$ averaged over islets of Langerhans compared with theoretical isotope envelopes at 1000 resolving power.

All rats

control mouse

diabetic mouse

Almost all Insulin-2

Insulin-2 / Insulin-1 = 0.5

Insulin-2 / Insulin-1 = 1
Lipids in Rat Brain
Optical image from on board camera

10 μm spatial resolution
5 kHz laser
10 shots/pixel
5 mm/s scan
500 pixels/s acquired
300 pixels/s saved
3000 mass resolution
+/-0.1 Da mass accuracy
Expanded on next slide

10 μm

726.5 green

718.5 red
Expanded sections of images from previous slide

Single pixels
This plate contained 3 serial sections of rat brain each about 3 cm² in area. All were imaged in one day using a 5 kHz laser with 5 μm diameter laser, with a 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
722 red
726 green
10 μm
Ideal Mass Spectrometer for MALDI MSI

• Satisfy the 7S-Criteria for performance
  
  • Speed – 300 pixels/s, 5kHz, 10 shots/pixel

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<th>Spatial Resolution(μm)</th>
<th>Sample used(%)</th>
<th>min/cm²</th>
<th>pixels/cm²</th>
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• Trade-off between sensitivity, speed and size at lower spatial resolution (5 μm laser beam)

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• Survey scan prior to high spatial resolution (5 kHz, 5 mm/s)

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• Stability-1 billion laser shots since last cleaning 3 weeks ago
• Simplicity-no tuning required
Present Status and Future Prospects

The linear MALDI-TOF instrument excels in all but specificity

High mass resolution MALDI-TOF and MS-MS with same high speed and sensitivity required to provide elemental formula and structure of lipids imaged

Full 3D image of lipids is technically feasible with 20 μm spatial resolution for both positive and negative ions

Next step is to establish lipid signature for each cell detected in an image

Comparison of lipid signatures between control and purported disease state or trauma may provide a powerful tool for detection, diagnosis and treatment.
20 um  m/z 715.5 green, 728.5 red, 766.5 white  single pixels