High-Performance MALDI-TOF Imaging Mass Spectrometry of Proteins and Peptides with Single-Cell Resolution and Sensitivity

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Introduction

A new high performance linear MALDI-TOF mass spectrometer is employed to image spatial distribution of proteins in pancreatic tissue samples. 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 is described in more detail in a separate poster.

Method

The present work represents one of the first tests of this system. This work 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. Three tissue segments from the pancreas of each rat were freeze mounted on ITO coated glass slides and analyzed using sinapinic acid matrix deposited by sublimation followed by rehydration to provide matrix crystals nominally 10 μm in size. Spectra were acquired and saved over the mass range 2000-20,000 Da at an effective acquisition rate of 300 pixels/s. Images of whole pancreatic tissue sections were generated at spatial resolution of 10 μm. The area of these sections ranged from 1 to 4 cm^2. The time to produce an image at 10 μm spatial resolution was typically 1 hour/cm^2.

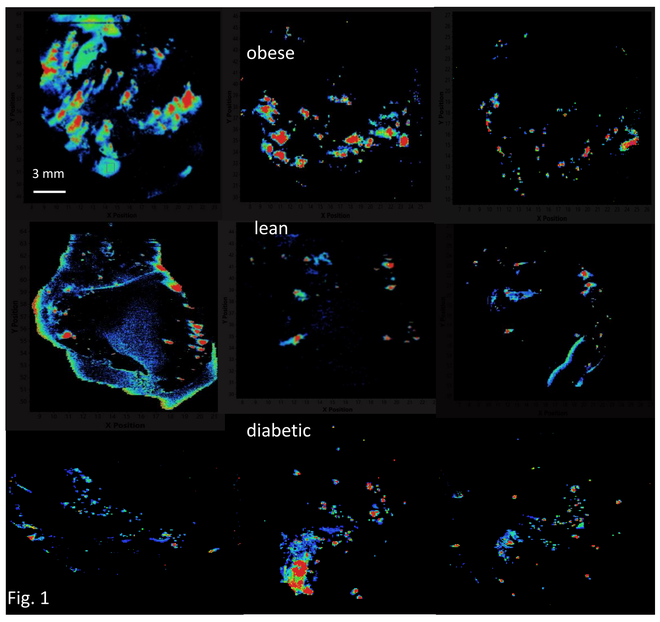


Fig. 1

Results

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 are shown in Figure 1. These range in area from 1.2 to 3 cm^2, and a total of 16 million spectra were acquired and analyzed in less than 24 hours. These images are dominated by insulin at m/z 5800 and glucagon at m/z 3484 corresponding to the beta and alpha cells of the islets of Langerhans. The images shown were generated by setting a minimum intensity for the masses included, thus rejecting a low level matrix background at low mass and other proteins with low intensity distributed over the sample. An example of an expanded view of the beta cells producing insulin and the alpha cells producing glucagon in one islet of Langerhans is shown in Figure 2 for obese rat. Similar views of beta cells (insulin) and alpha cells (glucagon) for all of the islets detected in all three rats may be generated by expanding the appropriate region of the image.

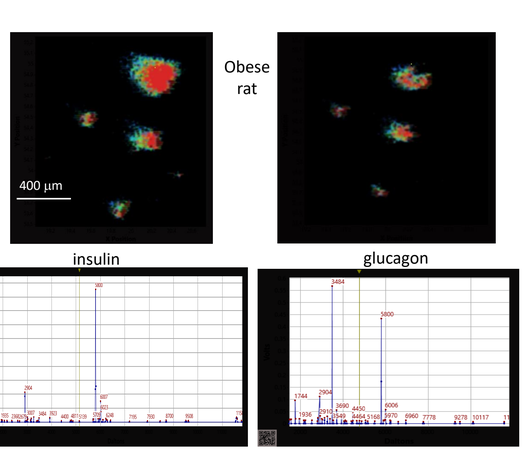


Fig. 2

Figure 3 shows a small portion of a one-dimensional display of the intensities of m/z 3484 (glucagon) and 5800 (insulin) across a selected islet of Langerhans in lean rat. The spectra displayed show that some 10 μm pixels correspond to a single beta cell.

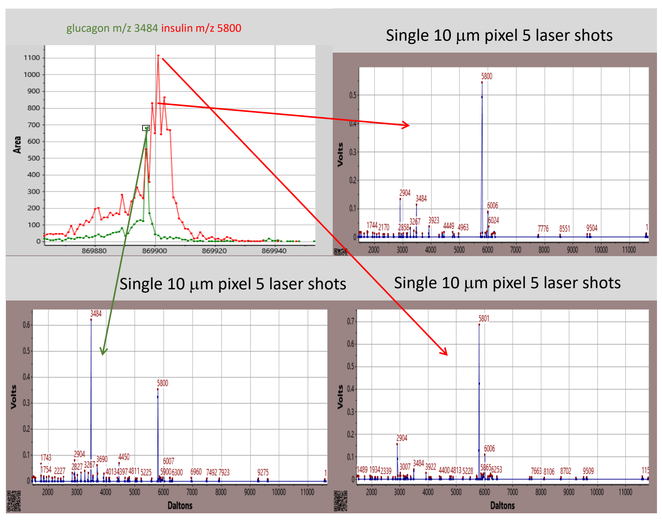


Fig. 3

Figure 4 displays the intensities of m/z 3484 (glucagon) and 5800 (insulin) over one tissue section for obese and lean rat. These images correspond to the total ion images at the left end of Figure 1.

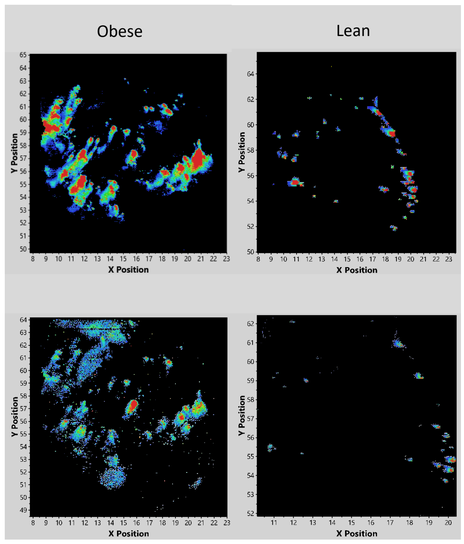


Fig. 4

Figure 5 is an expanded view of the m/z 3484 image for one islet of obese and lean rat. The single pixel spectra shown are for one of the more intense pixels in the image that are high in glucagon but still show significant intensity of insulin.

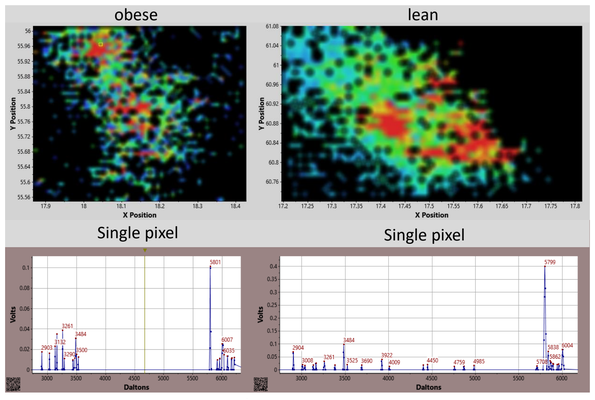


Fig. 5

Protein images of all the tissues studied have been generated with 10 μm spatial resolution employing matrix deposition by sublimation and rehydration. The large files generated in this work have challenged our ability to store the complete results on our server. Ten days of operation produced about 100 million spectra from about 100 cm^2 of tissue. This overwhelmed the storage capacity of our server with 5 terabytes of data. By saving only peaks this was reduced to 3 terabytes. But when full images of large tissue sections are displayed, there is almost no discernible difference between images acquired at 10 μm spatial resolution and those acquired at 100 μm resolution as shown in Figure 6. The latter can be acquired and used to produce an image at a rate of 3 min/cm^2 with 10,000 spectra/cm^2. These can be used to identify regions of interest and images at 10 μm resolution can be generated at a rate of 3 min/mm^2. Images generated by expanded views of areas of interest selected from full sections acquired at 10 μm resolution are compared with similar areas acquired at 10 μm resolution following selection from full images acquired at 100 μm resolution for both protein and lipid imaging all of the tissues available.

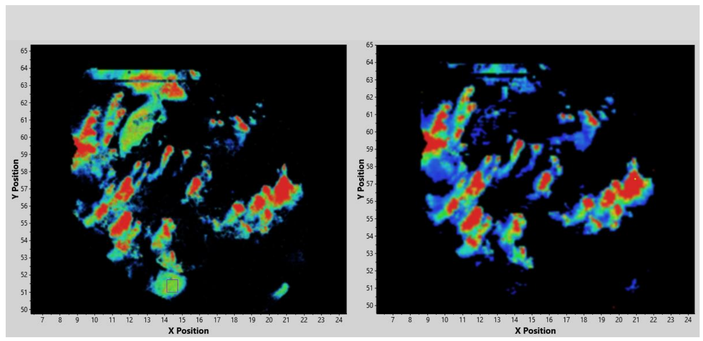


Fig. 6

1,591,692 spectra
10 μm resolution
1.6 hours to acquire
1 hour to analyze masses
10 minutes to generate image

15,592 spectra
100 μm resolution
3 minutes to acquire
30 seconds to generate image

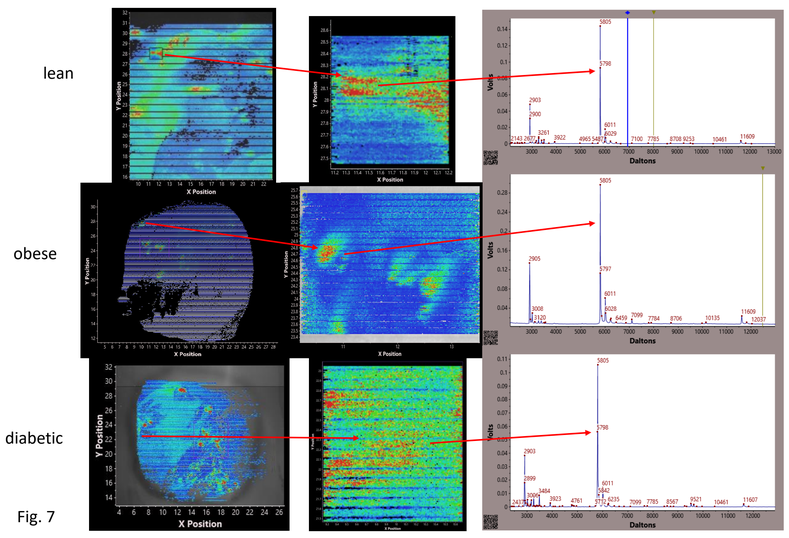


Fig. 7

Figure 7. Left panel full TIC image 2-20 kDA for one tissue segment for each rat imaged at 100 μm resolution. Center panel is image of region selected from the first at 10 μm resolution. Mass spectra shown are the integrated spectra over a selected islet of Langerhans. Major peaks in all cases are singly and doubly charged insulin and insulin dimer.

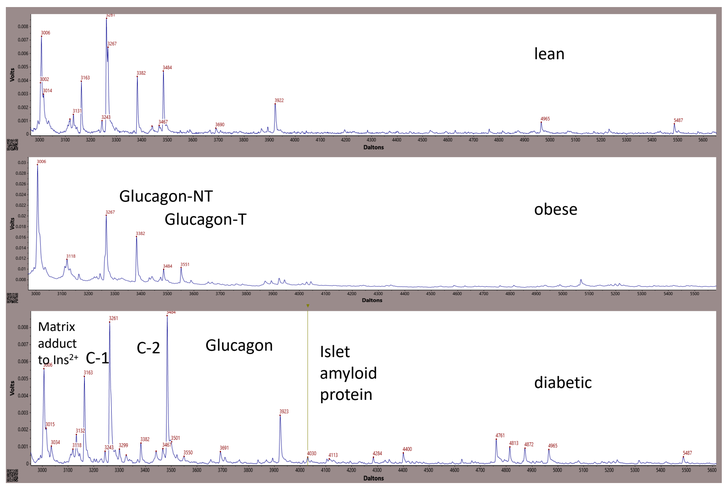


Fig. 8

Figure 8. Expansion of these spectra in range 3000-5700 Da between doubly and singly charged insulin. All of the identified peaks are detected in lean rat. In the obese rat glucagon is detected at lower intensity, but the truncated glucagons are similar to the lean rat while the C-peptides and the Islet amyloid protein are not detected. The diabetic rat is similar to the lean rat except that the truncated glucagons are weaker and there are several peaks in the 4 kDa range that are not detected in the other rats.

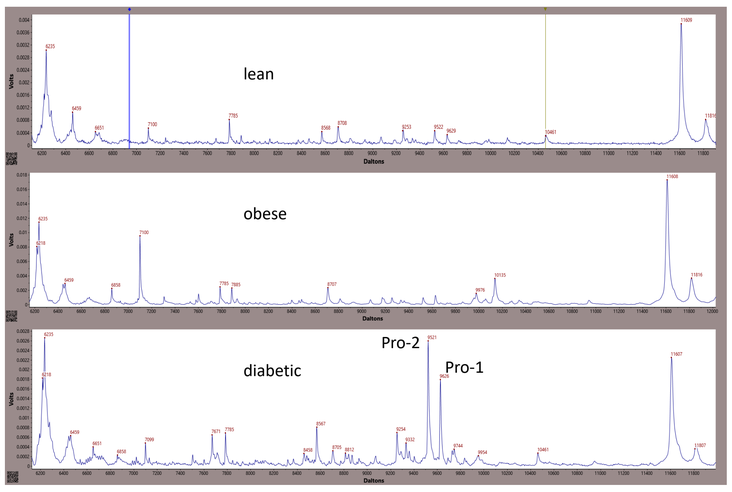


Fig. 9

Figure 9. Expansion of these spectra in range 6100-13000 Da above singly charged insulin and including the insulin dimer. Spectrum for the diabetic rat is similar to that for the lean rat except for the much higher intensity of the peaks corresponding to pro-insulin one and two.

Two insulin genes in rat: Insulin 1 and Insulin 2

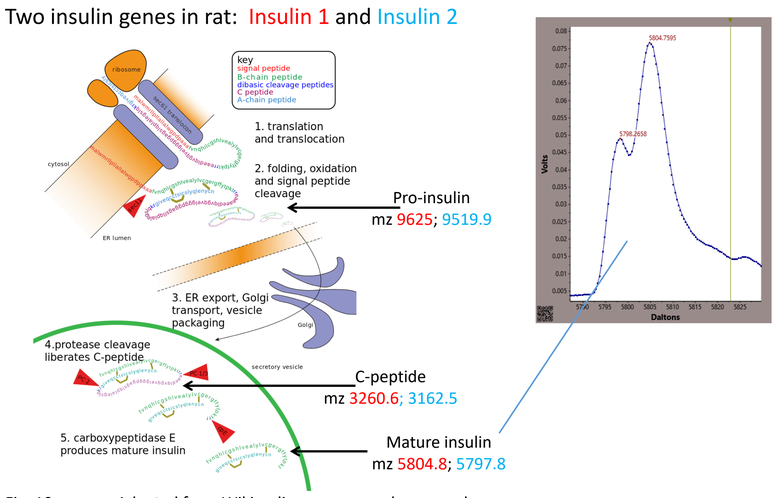
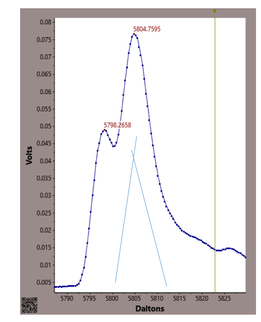


Fig. 10 Adapted from Wikipedia; sequences shown are human

Figure 10 shows a cartoon illustrating insulin production and several of the masses detected. The presence of both insulins is demonstrated by the expanded view of the singly charged insulin detected in this work. Earlier work on rat and mouse have generally used a m/z value of 5800 which is the average of the true values.

Relative intensity of major peaks in part per thousand of total ion current				
	m/z	lean	diabetic	obese
insulin 2 Cpeptide	3163	4.4	10.3	1.8
insulin 1 Cpeptide	3261	0	17.3	1.8
glucagon-NT	3267	41.8	0	6.5
glucagon-T	3382	33.1	1	5
glucagon	3484	39.9	10.9	1.7
islet amyloid peptide	3922	4.9	16.8	1.7
?	4965	2.5	5.2	0
?	7784	0	0	11
?	7884	2.2	1.8	8.8
?	8566	1.2	11.5	0
insulin 2 intact	9520	1.2	13.6	0
insulin 1 intact	9625	0.9	8.8	1.6
?	10461	1.5	1.4	6.8
insulin 2	5998	36	24	14
insulin 1	5805	449.4	477.3	494.9
total insulin		485	503	508

Fig. 11



The wavelet peak detection is excellent at detecting partially resolved peaks, but is not very accurate at determining relative intensity.
Approximate deconvolution indicates expression of insulin 2 is about ¼ that of insulin 1. There is no indication that this ratio is significantly different for the different rats used in this work.

Table in Figure 11 shows the relative intensities of the major peaks in these spectra. The total intensity of the insulin peaks including the doubly charged and the dimer (not included in the table) is about 2/3 of the total ion current for all 3 rats.

Pro-insulin sequences

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        Signal                    B chain                    C peptide                  A chain
R1-MALWMRFLPLLALLVLWEPKPAQA FVKQHLCGPHLVEALYLVCGERGFFYTPKS RR EVEDPQVPQLELGGGPEAGDLQTLALEVARQ KR GIVDQCCTSICSLYQLENYCN

R2-MALWIRFLPLLALLILWEPRPAQA FVKQHLCGSHLVEALYLVCGERGFFYTPMS RR EVEDPQVAQLELGGGPGAGDLQTLALEVARQ KR GIVDQCCTSICSLYQLENYCN

Hu-MALWMRLLPLLALLALWGPDPAAA FVNQHLCGSHLVEALYLVCGERGFFYTPKT RR EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ KR GIVEQCCTSICSLYQLENYCN

Red: Rat insulin 1 specific; Green: Rat insulin 2 specific;  Blue: Human insulin specific
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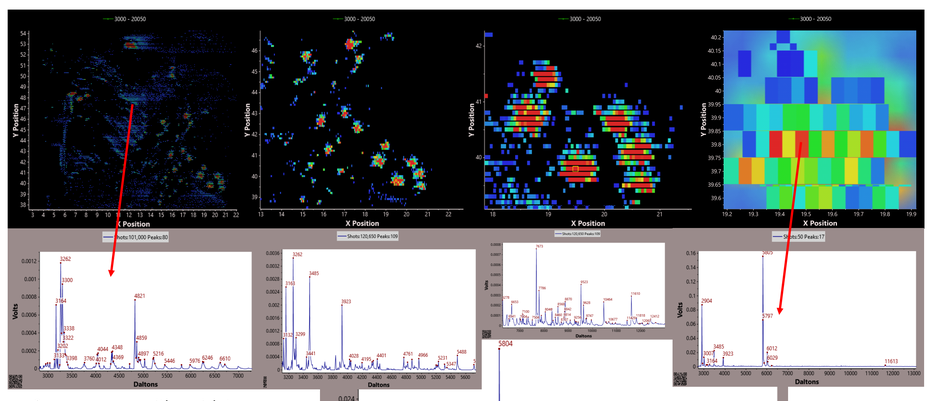

Fig. 12 Diabetic rat 100 μm resolution

Figure 12. Total ion current 3-20 kDa image of one tissue section of diabetic rat at 100 μm resolution showing successive expansions of regions including islets of Langerhans. Spectra include a single pixel with high intensity; sum of spectra over all islets, calculated by summing all spectra in which insulin is detected; and sum of spectra over a region where insulin is not detected.

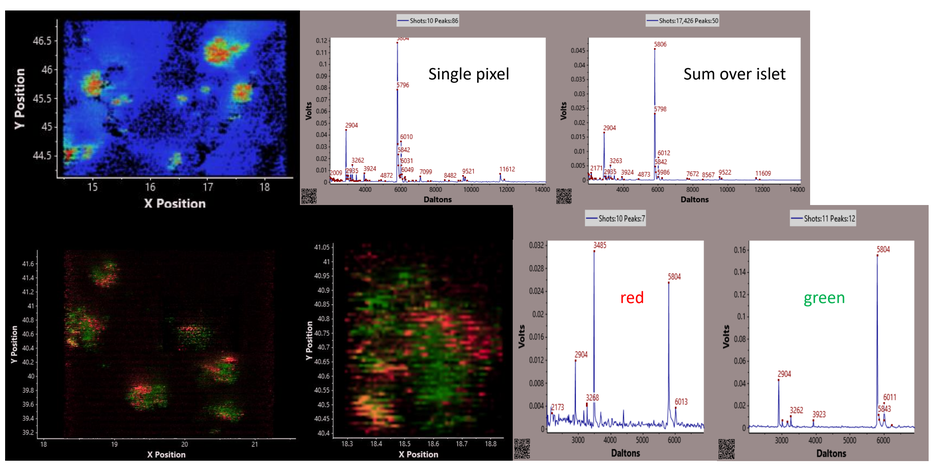

Fig. 13 10 μm images of selected areas

Figure 13. Two examples of images at 10 μm resolution for m/z 3484 (glucagon) and 5704 (insulin) for diabetic rat show glucagon at higher intensity around the outside of the islet

Conclusions

- Lean and diabetic rat islets are smaller than obese rat islets and show some separation between insulin and glucagon.
- Obese islets tend to be larger and low level of glucagon is distributed over same areas as insulin.
- Spectra from diabetic rat are similar to that of lean rat except that truncated glucagons are lower and several additional peaks are detected.
- Files generated by imaging proteins in large tissue sections at 10 μm resolution are too large to export to user or other imaging software.
- This work has demonstrated that imaging of 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.
- Time required to produce initial image is reduced by a factor of 100, and multiple high resolution images can be generated for regions of interest as required.
- In all cases the files are small enough (typically 40,000 spectra or less) that they may be exported, processed, and analyzed efficiently.

Acknowledgement
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References

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