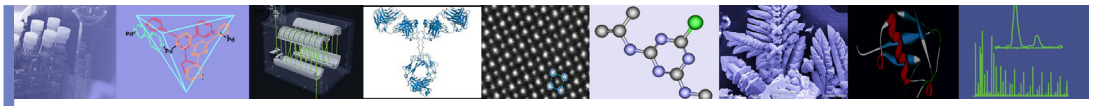

Applications Note

# SpiralTOF™

## High Mass Resolution MALDI-imaging MS Using JMS-S3000 SpiralTOF and msMicroImager

**Introduction**

Imaging mass spectrometry using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-Imaging) has been expanded during the last decade in biological applications, to assess the distribution of proteins, peptides, lipids, drugs, and metabolites in a tissue specimen. In MALDI-Imaging measurements, a laser irradiation point was scanned on a sample surface to acquire a mass spectrum at each point. Analyzing the mass spectra with two-dimensional position information, localization of compounds with inherent molecular weights can be visualized or the mass spectra for certain regions of interests (ROIs) can be created. The JMS-S3000 SpiralTOF (Fig. 1) is a MALDI-TOFMS, which utilizes the JEOL patented spiral ion optical system. It has a 5-10 times longer flight path than the typical reflectron type MALDI-TOFMS. As a result, it can achieve high mass-resolution to separate peaks that have the same nominal mass but have different exact masses (isobaric separation). On the other hand, there are some issues for analyzing high mass resolution and high lateral resolution MALDI-Imaging raw data with common imaging software options such as Biomap.

1. It is difficult to handle the large size raw data, especially for a large number of mass spectrum points.
2. It is difficult to use the detailed information from high mass resolution MALDI-Imaging by extracting mass images manually. Furthermore, peaks observed in the mass spectra cannot be identified by its origin, such as samples, matrix compounds or surface contaminations, before drawing the mass images.
3. A lack of function to overview a large number of mass images.

The JEOL msMicroImager software for high mass resolution MALDI-Imaging raw data was designed to resolve all of these issues.

a)


b)

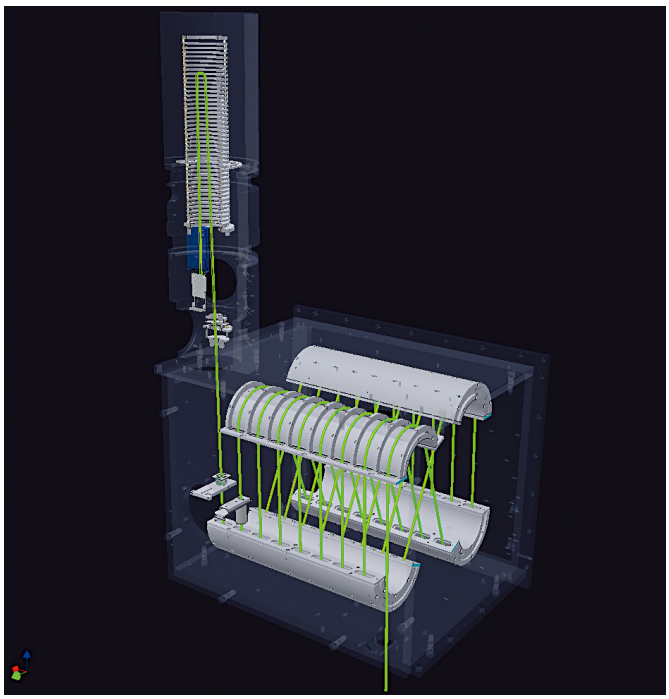

Fig. 1 a) Appearance of JMS-S3000 SpiralTOF and b) its spiral type ion optics



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**Experimental**

A mouse brain tissue section was placed on an ITO conductive glass slide plate. The matrix compound DHB was sprayed onto the surface of the tissue and then the sample was introduced into the mass spectrometer. The MS Imaging measurements were performed on the left half of the brain tissue section (5 mm×7 mm) with 40 µm spatial resolution. The sampling interval of data acquisition system was 0.5 ns, which included 170,000 mass data points in *m/z* 500-1000. The total pixel was 21,125 and raw data size was 14GB.

**Results and discussion**

**Handling large MALDI-Imaging data**

The size of high mass resolution and high lateral resolution mass imaging data can be quite large. The raw data used in this report was 14 GB. Consequently, it often took 10 sec to extract one image by accessing the data on external storage devices. The msMicroImager software has a function that improves the processing speed by storing the compressed data on the RAM. The data compression can be achieved by limiting the mass ranges, mass spectrum binning or pixel binning. Binning is a process to average intensities of several points to one point. For example 3 point mass spectrum binning and 2×2 pixel binning, the 14 GB data was compressed to 1GB. The mass images of *m/z* 868 without binning and 2×2 points pixel binning are shown in Fig. 2. This process has an advantage in extracting hundreds of mass images simultaneously, as described in next section.

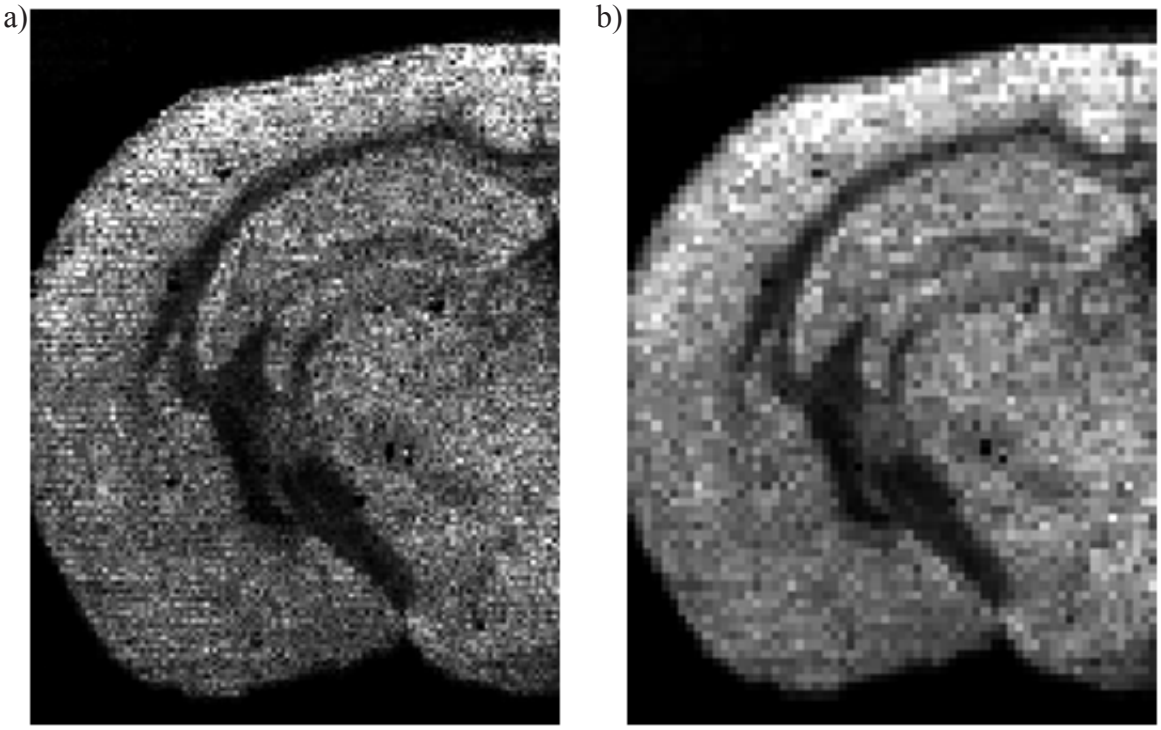
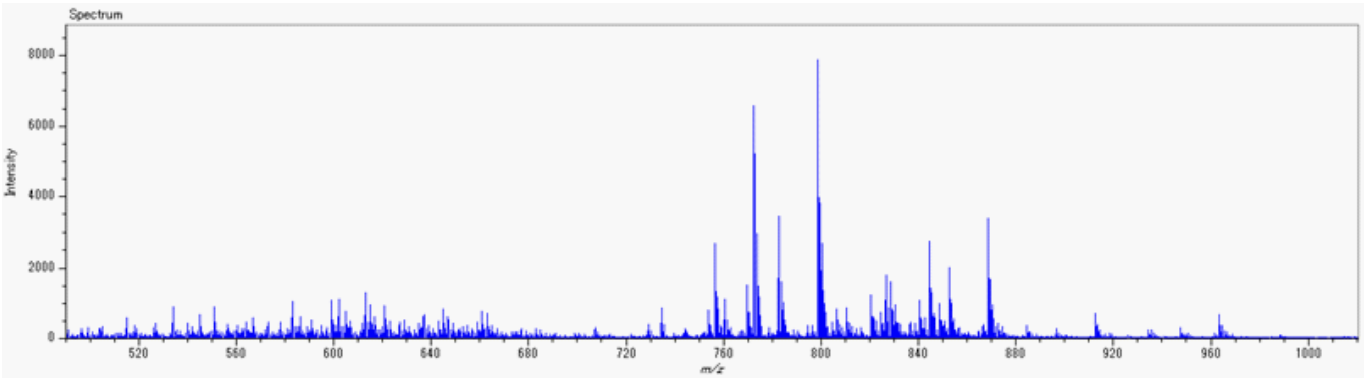



Fig. 2 a) The mass images at *m/z* 866 without binning process and b) after 2×2pixels binning process.

**Extracting large number of mass images**

An averaged mass spectrum of all pixels is shown in Fig. 3a.The enlarged mass spectrum at *m/z* 820-825 after 2×2 points pixel binning process was shown in Fig. 3b. The high mass resolution capability of the SpiralTOF is able to achieve isobaric peak separation even for minor components in the mass spectrum. In the case of MALDI-Imaging, a matrix compound is sprayed on the sample surface. The peaks observed in the mass spectrum were originated from target compounds, matrix compounds, and surface contamination. It is difficult to extract a number of mass images for the minor components manually by using common imaging MS software, because a manual extracting process includes several times of mass spectrum expanding. Furthermore, their origin cannot be identified before extracting the mass images. The msMicroimager software has two additional ways for extracting mass images beyond manual peak selection: extract mass images using i) import target peak list and ii) import peak list made by mass spectrum analysis software for SpiralTOF"msTornado Analysis". The green bands shown in Fig. 3b were selected peaks using the peak pick list from the msTronado Analysis software. Over 200 peaks can be selected between *m/z* 700-1000. The extracting time for these 200 mass images was only a few seconds after the binning process, where it took an hour without the binning process.

a)




b)

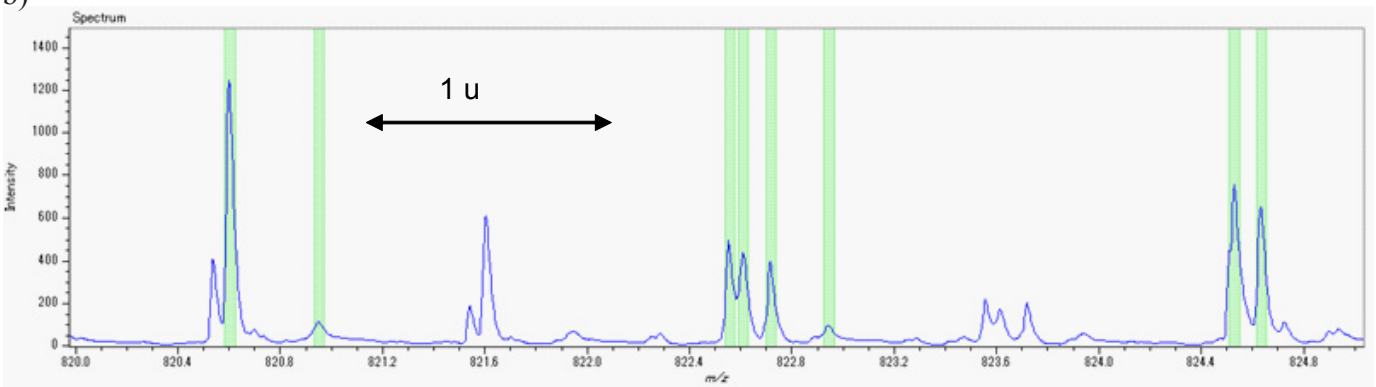



Fig. 3. a) Averaged mass spectrum of all measured pixel and b) an enlarged spectrum at *m/z* 820-825 after binning process.

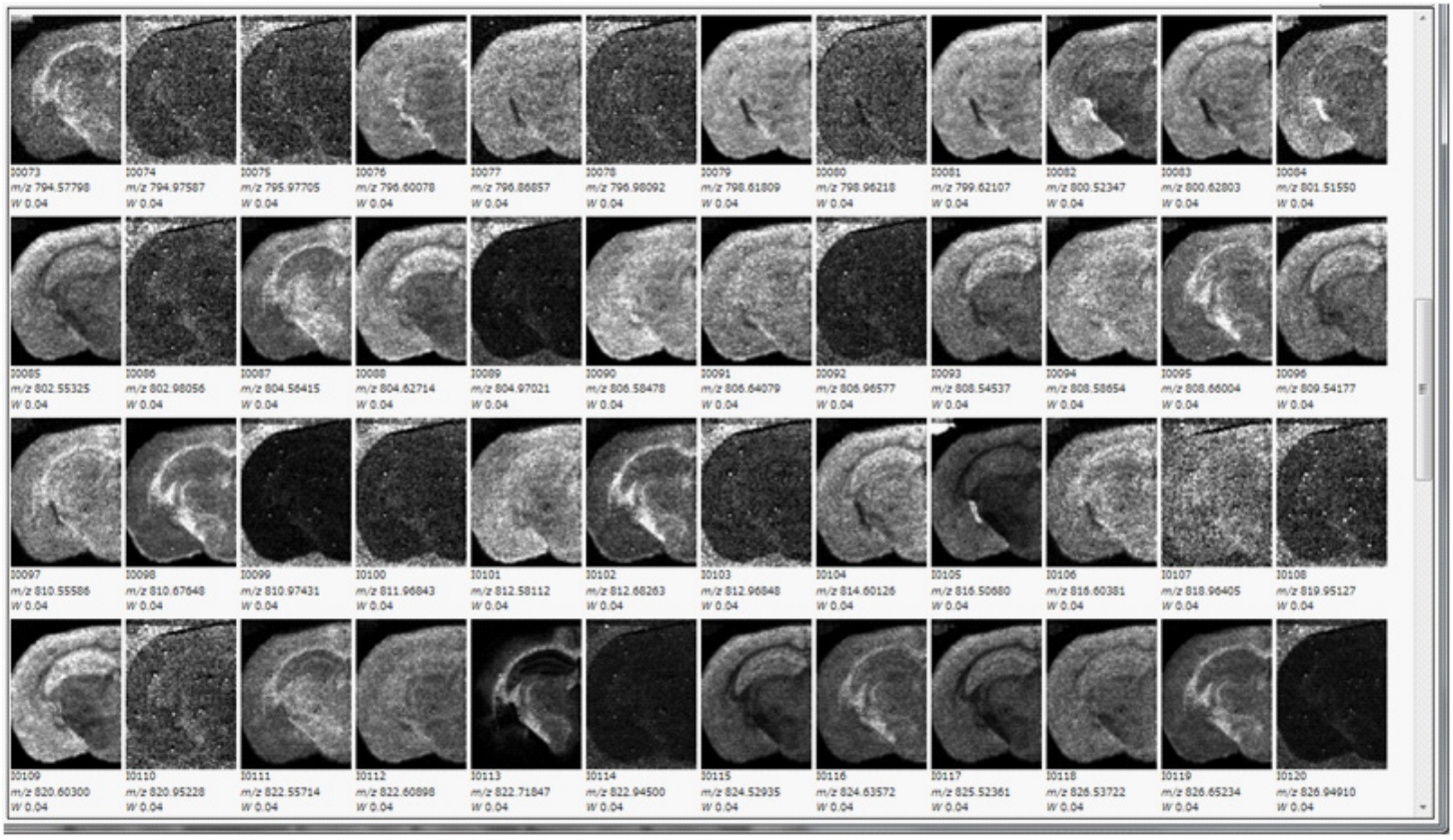



Fig. 4 Display a list of mass images extracted from high mass resolution imaging data.

**The overview of extracted mass images**

The msMicroImager is able to overview the extracting mass images. Fig. 4 shows a part of the 200 extracted mass images in the last section. The characteristic distribution can be found by looking at the mass images in order to guide a more detailed analysis of the sample.

**Conclusion**

MALDI-Imaging using SpiralTOF and msMicroImager make it easier to extract a number of mass images from large size raw data. The full use of the detailed information can be obtained from high mass-resolution and high lateral resolution MALDI-Imaging.