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Technical Report

Lipid Analysis of a Mouse Brain by Statistical Analysis Software

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Abstract:

The most important element of MS imaging is how efficiently it can analyze the enormous amounts of data acquired by data acquisition. Imaging MS Solution Analysis is dedicated image analysis software for the iMScope that offers easy peak detection, Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Region of Interest (ROI) analysis. Peak detection and PCA were performed on MS imaging data of a mouse brain slice to obtain lipid groupings with the three different types of distribution characteristics that match the brain structures. It was demonstrated that overlaying a micrograph over these lipid distributions can visualize the localized distributions.

Keywords: MS imaging, statistical analysis, Imaging MS Solution, PCA, HCA, ROI, overlay

1. MS Imaging and Data Analysis

MS imaging involves using a mass spectrometer to capture the ionized materials generated by continuous irradiation by fine laser shots across the two-dimensional space of a tissue slice as a mass spectrum. Planar images are created from the *m/z*, positional information, and signal intensities in the obtained data.

The mass spectra obtained for the direct analysis of biological tissue have complex patterns containing several hundred to several thousand peaks. They differ significantly from the mass spectra obtained from normal separation and purification. As each peak offers its own two-dimensional positional information, a huge amount of information has to be handled. Automated analysis tools are required, as it is unrealistic to process this information manually. We have adopted statistical analysis methods to develop software that easily extracts the significant MS imaging data.

The iMScope imaging mass microscope can acquire up to 250 × 250-pixel mass spectra from each data acquisition at a resolution offering a minimum pitch of 5 µm and allowing data acquisition from extremely small regions. The iMScope is supplied with Imaging MS Solution Analysis dedicated image analysis software for accurate data analysis.

2. "Imaging MS Solution Analysis" Image Analysis Software

The "Imaging MS Solution Analysis" image analysis software used with the iMScope imaging mass microscope offers peak detection, Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Region of Interest (ROI) analysis. It accomplishes this diverse range of analyses without the need for tedious parameter setup of the analytical methods.

Principal Component Analysis (PCA) extracts the peak matrix from the mass spectrum and searches for principal components as characteristic patterns in the images. It detects values with a specific distribution and then searches for other values with a similar distribution.

Hierarchical Cluster Analysis (HCA) compares the *m/z* images to analyze similar *m/z* images as clusters (groups). The distance between clusters (mismatch factor) is used to evaluate the similarity for each cluster. The similarity can be confirmed by displaying the tree diagram and mismatch factor information.

PCA HCA ROI

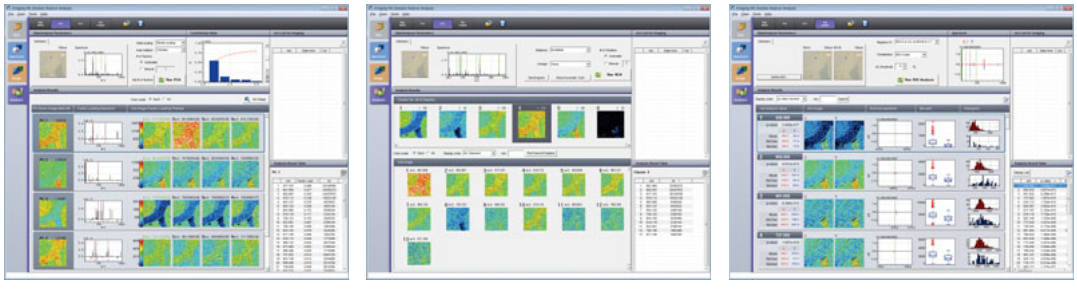

Fig. 1 Data Analysis Software

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"Imaging MS Solution Analysis" (cont.)

Region of Interest (ROI) analysis is performed to test for intensity differences between multiple designated ROI or inside and outside ROI in a single batch of imaging data. The p value indicates the probability of no intensity difference existing, with respect to the null hypothesis that no ion intensity difference exists. The smaller the p value, the higher the probability that an intensity difference exists; that is, whether each region specified as an ROI contains molecules with *m/z* values that have a characteristic distribution. For example, performing ROI analysis with separate ROI specified for cancerous and non-cancerous regions within the same tissue can detect molecules that are specific to the cancerous region.

This report introduces examples of PCA analysis of lipid-related molecules based on data acquired from MS imaging of a mouse brain slice using the iMScope.

3. MS Imaging of a Mouse Brain Slice

3-1. Analysis Conditions

Table 1 Target pharmaceuticals and method performance

Sample:	Mouse brain slice (approx. 10 µm thick)
Glass Slide:	ITO glass (Sigma Aldrich #578274)
Measuring Instrument:	iMScope
Matrix:	DHB (spray)
No. of Pixels:	110 × 213
Spatial Resolution:	50 µm
Measuring Range (*m/z*):	650.000 to 1000.000
Measuring Time:	7 minutes
Peak Picking:	Automatic (100 peaks) Excluding certain matrix peaks
PCA Analysis:	Automatic (default setting)

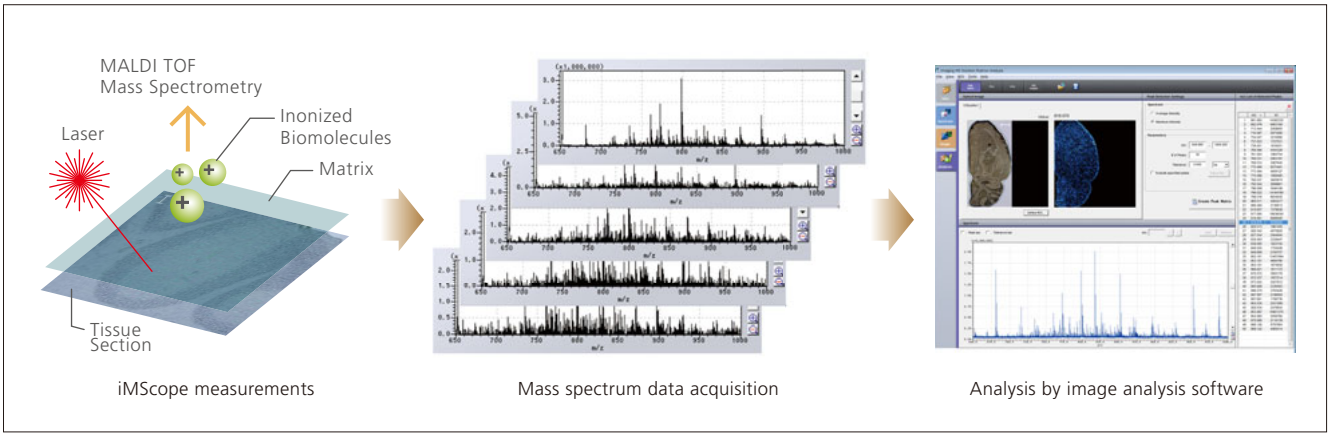

Fig. 2 Analysis Procedure

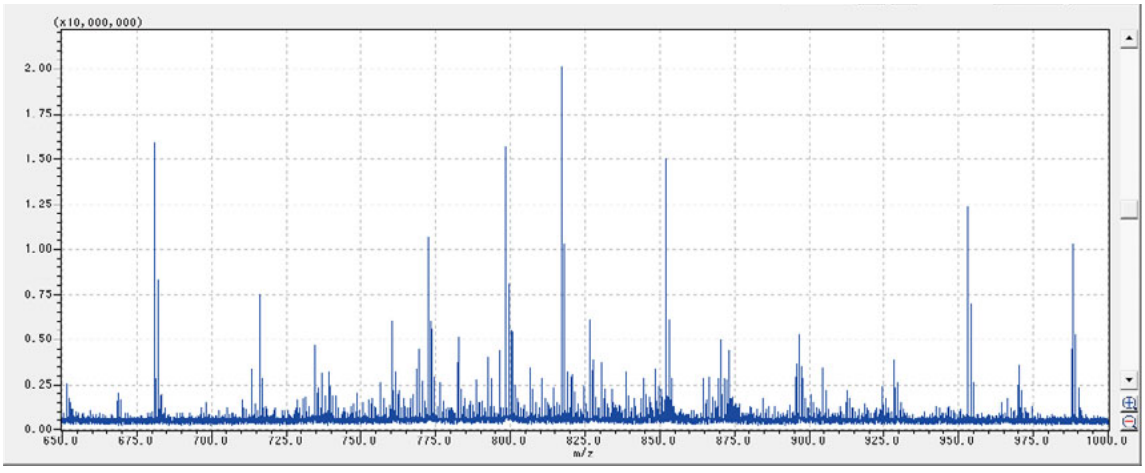

Fig. 3 Mass Spectrum of Entire Region (Max.)

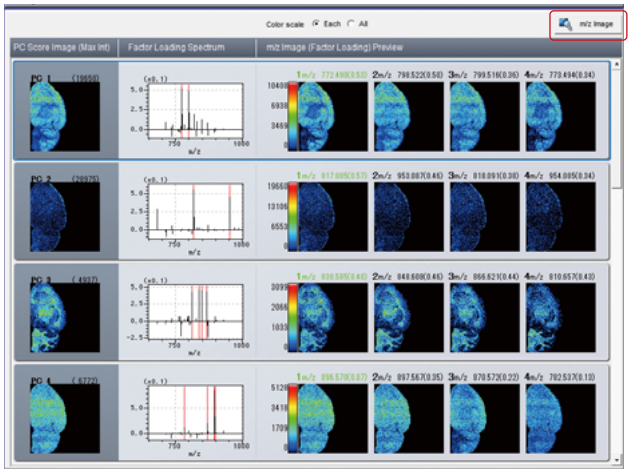

Fig. 4 PCA Analysis Results Window

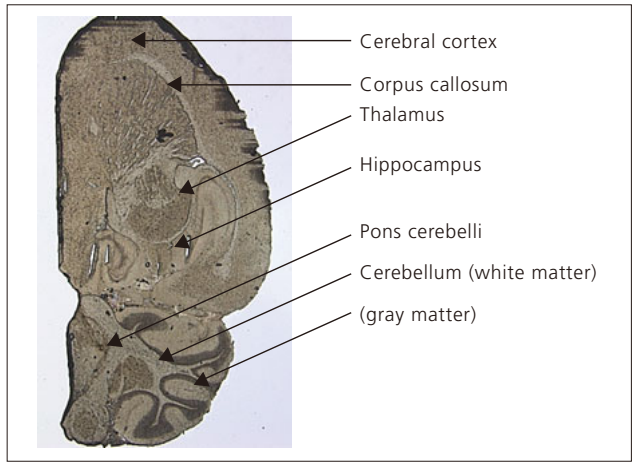

Fig. 5 Micrograph and Names of Brain Parts

3-2. MS Analysis and Imaging Analysis

This experiment was conducted according to section 3.1 Analysis Conditions and Fig. 2 Analysis Procedure. After MS analysis across the entire mouse brain slice, peak detection and PCA were automatically performed using the Imaging MS Solution Analysis software. (However, the number of peaks was changed to 100 from the default value of 50.) For peak detection, maximum intensity was selected to detect the highest peaks across the entire region (Fig. 3). PCA detected 37 principal components. Fig. 4 shows the top four principal components. All 37 components can be viewed by scrolling down using the scroll bar. The three components exhibiting the greatest correlation to the micrograph (Fig. 5) were selected. The *m/z* image display (circled top-right in Fig. 4) showed the images of the molecules (*m/z*) contained in each component and six sample images were displayed for each group (Fig. 6).

By associating these distribution patterns and the names of brain regions in the micrograph (Fig. 5), Group A shows the characteristic distributions for the corpus callosum, pons cerebelli, thalamus, and cerebellum (white matter). Group B shows the characteristic distributions for the cerebellum (gray matter) and hippocampus. Group C shows the characteristic distributions for the hippocampus and cerebral cortex (particularly near the frontal lobe).

A database search of the Human Metabolome Database (Ver. 3.0) estimated the respective *m/z* of each group to be a species of PE (phosphatidyl ethanolamine) or PC (phosphatidylcholine).

MS-MS analysis could be performed to identify the molecular species, but this was not done this time. iMScope offers a mode to displace the laser irradiation points by half a pitch on previously acquired area. This allows analysis of approximately the same region of the sample immediately after data acquisition has been completed.

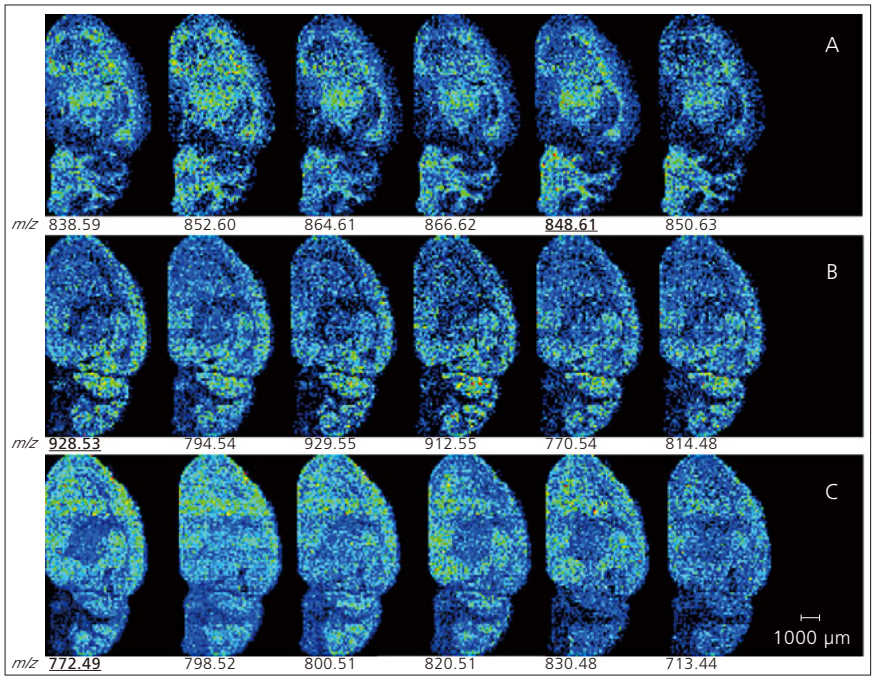

Fig. 6 Groups Obtained by PCA Analysis

3-3. Overlaying a Micrograph

Fig. 7 shows the micrograph overlaid on the characteristic localized molecular species in each region that was identified by PCA analysis. Section 3.2 described how the names of brain parts corresponding to the micrograph (Fig. 5) were estimated from the MS images in Fig. 6. However, providing images with the micrograph superimposed visually and directly reveals the localizations within each region. Although this analysis was performed at 50 µm spatial resolution, it is apparent that a good match was achieved between the molecular distributions and micrograph, even in regions with an extremely fine structure, such as the corpus callosum and hippocampus.

Imaging MS Solution Analysis software offers display functions for the overlay of optical images and *m/z* images and the overlay of multiple *m/z* images. It easily displays accurate overlay images, without the need for detailed positional adjustments. Similar overlay images are also available for MS-MS analysis data. Overlay images can be copied and easily pasted into a word processing or presentation software.

4. Conclusions

The Imaging MS Solution Analysis software offers automatic peak detection and three types of statistical analysis, including PCA, as support tools for data analysis. User settings can be performed to conduct more detailed analysis.

Both Imaging MS Solution Analysis software introduced in this report and Imaging MS Solution Acquisition software that easily acquires MS images, are supplied with the iMScope imaging mass microscope. This system allows analysis conditions to be set to perform up to 250 × 250-pixel data acquisition in about three hours. It permits stress-free data analysis even after acquisition of a large volume of data.

iMScope combines a microscope and a mass spectrometer. The Imaging MS Solution dedicated MS imaging software supports a wide range of customer requirements from observations of the analyzed object, to mass spectrometry and statistical analysis.

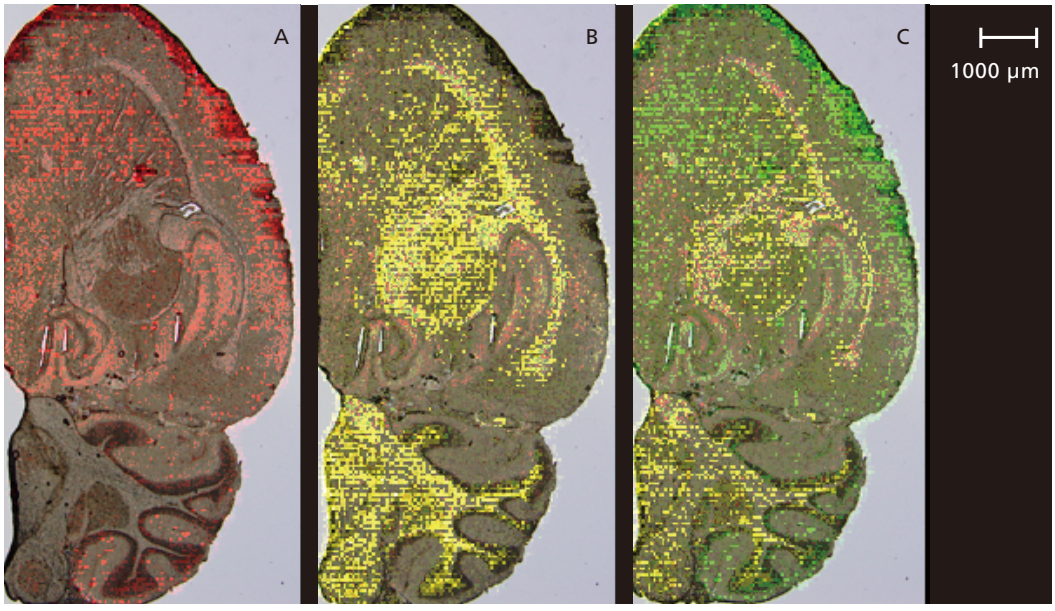

Fig. 7 Overlaid Optical Images
A: *m/z* 772.49, B: *m/z* 848.61, C: *m/z* Superimposing A (green, *m/z* 772.49) and B (yellow, *m/z* 848.61)

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