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Metabolites Analysis in Mouse Brain Using the Image Analysis Function of IMAGEREVEAL™ MS

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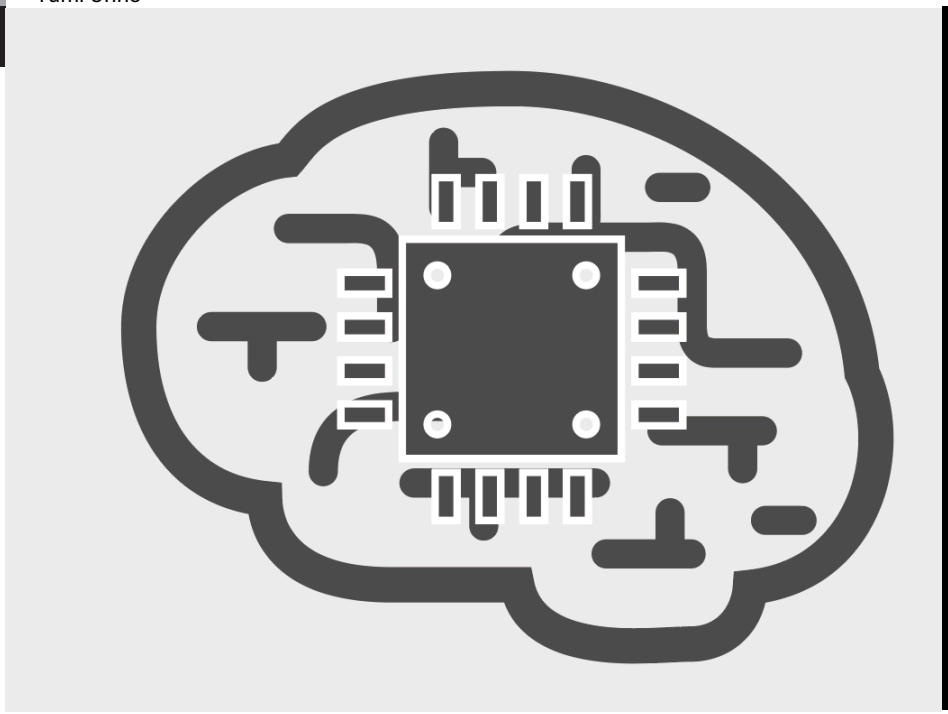

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■ Abstract

In recent years, matrix assisted laser desorption/ ionization-mass spectrometry imaging (MALDI-MSI) has improved instrumental performance, enabling the acquisition of a large number of peaks at a time. Until the mid-2000s, those obtained peaks were manually analyzed since MALDI-MSI was first reported in 1997. These days, however, various analysis methods that utilize statistical analysis techniques have been proposed.

In this application note, MALDI-MSI was performed using iMScope™ QT equipped with a quadrupole time-of-flight mass spectrometer, and IMAGEREVEAL MS, a dedicated MSI software, was used for the analysis. iMScope QT can acquire data with high mass resolution and high mass accuracy. IMAGEREVEAL MS also allows even inexperienced users to perform statistical analysis of the obtained data easily and quickly. This application note describes one method for efficiently analyzing signals obtained from mouse brain sections using iMScope QT in combination with IMAGEREVEAL MS.

1. Introduction

MALDI-MSI has been applied to various fields as a new molecular visualization technique, and its applications have been described in previous Application Notes[1-3]. In recent years, a variety of instruments have been put on the market, and Shimadzu Corporation introduced the iMScope QT, which is based on the LCMS™-9050 quadrupole time-of-flight mass spectrometer (Q-TOF) and equipped with an atmospheric pressure MALDI ion source and an optical microscope. The iMScope QT features high-speed measurement, high mass resolution, and high mass accuracy due to temperature control of the TOF section. With these features, the iMScope QT can perform not only targeted measurements but also non-targeted analysis, i.e., it can detect a large number of peaks over a wide *m/z* range and acquire visualization information for a variety of molecules at once.

MALDI-MSI data has multidimensional information consisting of the position where the laser irradiation was performed and the *m/z* of the peaks in the mass spectrum obtained there. When MALDI-MSI was first reported, manual analysis was the mainstream method for analyzing such data, but now software that implements various statistical analysis methods can be used to perform such analysis.

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In particular, there are cases where there is no prior information such as region-of-interest (ROI), and the researchers want to know which signals show characteristic distribution across the entire sectioned sample from the obtained mass spectrum to aid in further analysis. The flow of such analysis is shown in Figure 1. This type of analysis is classified as "Image Analysis". Image analysis includes hierarchical clustering analysis (HCA) to present and classify various localizations, segmentation and similar image extraction to find characteristic distribution patterns (or specific distribution patterns).

In this application note, we introduce one method of mouse brain analysis that combines this segmentation with Shimadzu's proprietary method of similar image extraction.

2. Experiments

2-1 Mouse brain section preparation and matrix application

Frozen mouse brains were purchased from Funakoshi (Tokyo, Japan). The purchased frozen mouse brains were sectioned at 8 µm thickness at -20 °C using a cryostat (CM1950; Leica, Nussloch, Germany). The prepared sections were attached to indium-tin oxide (ITO) coated glass slides (100 Ω/m2 without anti-peel coating; Matsunami Glass, Osaka, Japan). α-cyano-4-hydroxycinnamic acid (CHCA) purchased from Merck (Maryland, USA) was used as the matrix. We also used a cluster peak of 2,5-dihydroxybenzoic acid (DHB, Merck, Maryland, USA) as the mass calibration reagent. Matrix was deposited on ITO glass slides loaded with tissue sections using the iMLayer™ matrix deposition system (Shimadzu Corporation, Kyoto, Japan) shown in Figure 2A. The Matrix deposition conditions were set at a sublimation temperature of 250 °C and 0.7 µm thickness.

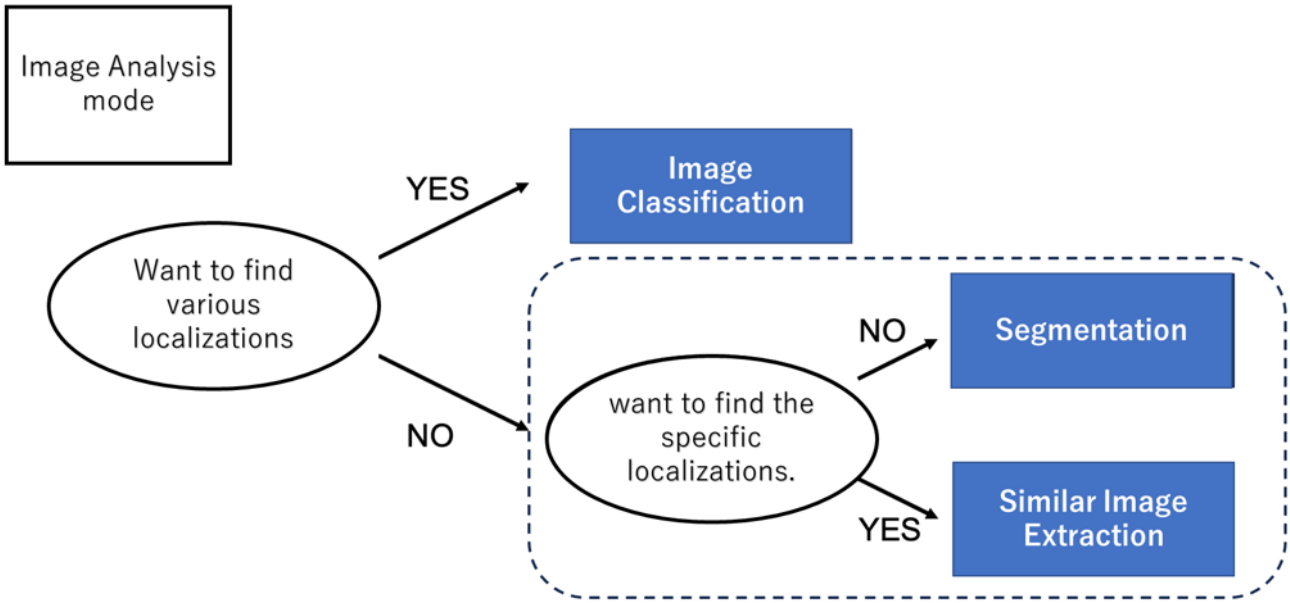

Figure 1. Image analysis mode in IMAGEREVEAL MS

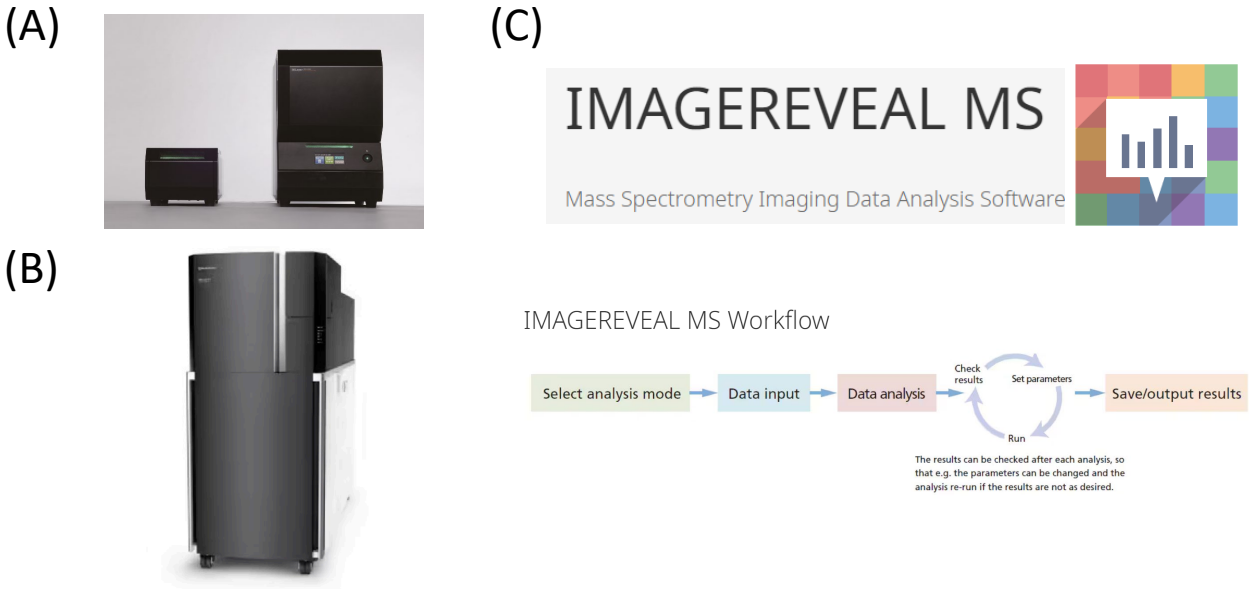

Figure 2. (A) iMLayer™ , (B) iMScope™ QT and (C) IMAGEREVEAL™ MS

2-2 MALDI-MSI analysis

MALDI-MSI analysis was performed using an iMScope QT (Shimadzu Corporation) equipped with an atmospheric pressure matrix-assisted laser desorption/ionization quadrupole time-of-flight mass spectrometer (Figure 2B). All mass spectra were acquired in positive ion mode in the *m/z* 100-210 range. The laser intensity was set to 65.0, the laser irradiation diameter to 2 (both in arbitrary units), and the detector voltage to 2.1 kV. Mass calibration was performed using DHB cluster peaks detected in the *m/z* 100-600 range. Post-analysis data analysis was performed by IMAGEREVEAL MS (Shimadzu Corporation, Fig. 2C) and the UMAP application created in Python4).

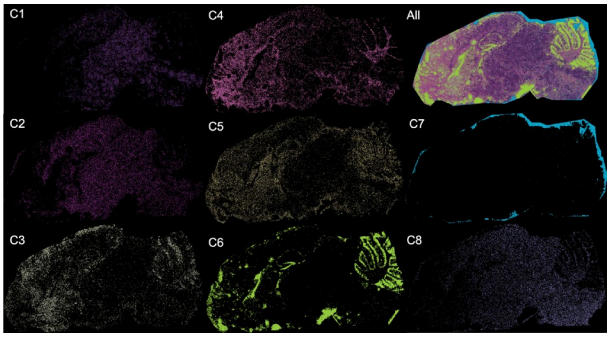

Figure 3. Imaging data obtained under the conditions of this experiment were separated into eight segments by UMAP

3. Results and Discussion

3-1 t-SNE and UMAP as Dimensional Condensation Methods.

While UMAP (Uniform Manifold Approximation and Projection) was used in this application note, t-SNE (t-distributed Stochastic Neighbor Embedding) is known as a similar method[5]. Both t-SNE and UMAP are dimensionality reduction methods that aim to embed high-dimensional data in a low-dimensional space. In other words, they are used to reduce multidimensional information ["location information of many mass spectra" times "number of peaks in each mass spectrum"] to two or three dimensions for visual handling. This dimensionality reduction facilitates tasks such as data visualization and clustering (also called segmentation). The following is a brief description of each method.

1. t-SNE (t-distributed Stochastic Neighbor Embedding):

t-SNE can be described as a method of embedding high-dimensional data into a low-dimensional space while preserving similarity. t-SNE is a dimensionality reduction method used primarily for data clustering and visualization. It is characterized by the following three features.

- Similar data points tend to be located close together in low-dimensional space.
- Cluster features are well preserved to maintain proximity within high-dimensional data.
- It is more robust than other methods against noise and outliers.

t-SNE is mainly effective for embedding data in 2D and 3D low-dimensional spaces, but it is known to be computationally expensive and tends to increase computation time for high-dimensional data.

2. UMAP (Uniform Manifold Approximation and Projection):

UMAP is another dimensionality reduction method that embeds high-dimensional data in a low-dimensional space and has similar goals to those of t-SNE. However, it has several different features. The three features are as follows.

- UMAP calculates faster than t-SNE, so it is easy to apply to large data sets.
- It can preserve the local structure while also preserving the overall structure well.
- It can better preserve distance relationships for high-dimensional data.

UMAP is widely used for analysis, visualization, and clustering of high-dimensional data. It is particularly effective for large data sets and is known to be computationally more efficient than t-SNE.

From the above, while either method can be selected depending on the characteristics of the data and the purpose, UMAP was chosen for this application note because of its low computational cost and its recent preference for high-dimensional data visualization and segmentation.

3-2 Segmentation Results Using UMAP

Figure 3 shows the imaging data obtained between *m/z* 100 and 210 separated into eight segments, using UMAP. All segments superimposed on the image are shown on the image labeled "All" Each segment from C1 to C8 shows a characteristic distribution. For example, C1 depicts the midbrain region, C3 the striatum. Among these segments, C7 detects ions distributed outside the tissue, suggesting that it is mainly composed of signals derived from CHCA matrix. Among these segments, C6 shows the clearest distribution, distinctly depicting the hippocampus and cerebellar granular layer. In the present analysis, the segments were divided into 8 segments, but the number of segments to be separated is arbitrary in this type of segmentation. There is much debate as to the optimal number of segments to separate, and there are attempts to automatically calculate the optimal number of clusters.

3-3 Similar Image Extraction to retrieve characteristic *m/z* from segmentation results.

Without going into details, it is known that UMAP (and t-SNE) loses mass (*m/z*) information in the dimensionality reduction process.

In other words, after the calculation, the mass information of the ions that make up the distinctly characterized distribution is lost, so while UMAP and t-SNE can show what the obtained mass spectrum has in terms of its characteristic distribution, they have the disadvantage of losing information for identifying the molecular species of the ions that make up that distribution.

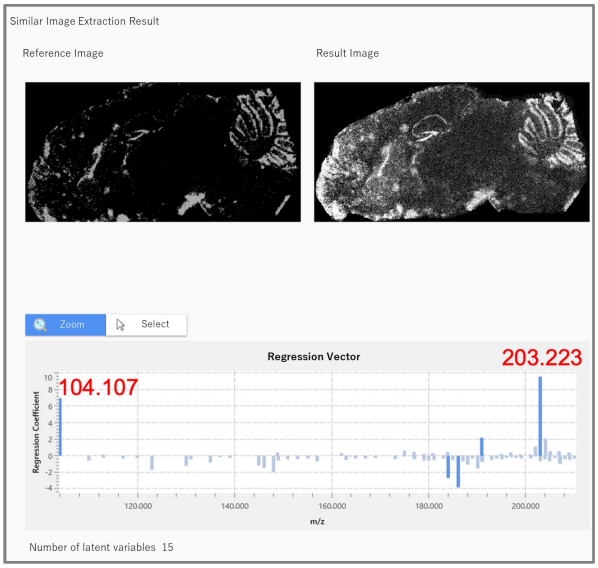

Figure 4. Result after similar image extraction

To solve this situation, IMAGEREVEAL MS provides a feature called similar image extraction. The similarity image extraction is a function that automatically presents the *m/z* of peaks that show a distribution similar to the distribution given in advance. Results generated from similar image extraction are shown in Fig. 4 and Fig. 5. Figure 4 shows the screen immediately after the calculation. Using the C6 distribution as a reference image, the *m/z* of the peaks showing a similar distribution is returned in the form of a score with *m/z* on the horizontal axis and Regression Coefficient on the vertical axis. A peak with a positive regression coefficient indicates that the distribution is similar to the C6 distribution, and the higher the value, the more similar it is. On the other hand, a negative value for the Regression Coefficient means that the *m/z* is distributed inversely to C6. In this experiment, the regression coefficient of the peaks at *m/z* 104.107 and 203.223 was positive and large in value.

To identify this peak, a search was then conducted in a database known as HMDB (https://hmdb.ca/). LC-MS search was used with positive ions, proton adduct as the ion species, and an *m/z* tolerance of ±0.005, taking advantage of the high mass accuracy of the iMScope QT. As a result, *m/z* 104.107 was found to be choline, and *m/z* 203.223 was found to be spermine (Figure 5). Figure 5 shows that spermine shows almost the same distribution as C6. On the other hand, choline seems to represent some features of the C6 distribution (spotty localization and arrowhead position localization).

4. Conclusion

In this application note, among the methods for analyzing MSI data obtained with iMScope QT with IMAGEREVEAL MS, we applied the combined analysis method of segmentation and similar image extraction to the analysis of mouse brain metabolites.

While segmentation is a powerful tool for extracting characteristic distributions, it has the disadvantage that *m/z* information in the mass spectrum is lost, making molecular identification impossible. However, by compensating for this disadvantage with the similarity image extraction function, we were able to identify these peaks as spermine and choline based on the mass information of the peaks that showed similar distributions to the characteristic distributions representing the hippocampus and cerebellar granular layer obtained by segmentation. This series of methods will be very useful in future situations where peaks showing characteristic distributions are extracted from a large number of mass spectral peaks.

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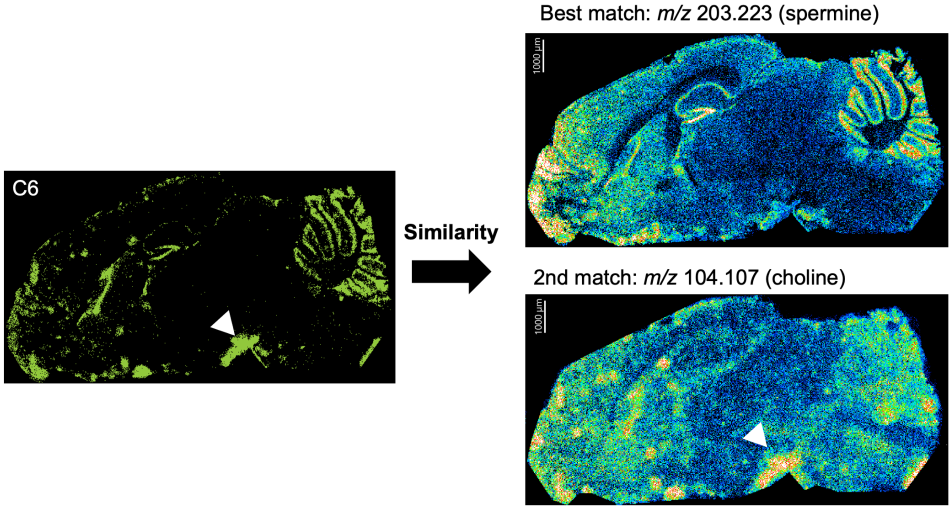

Figure 5. Distribution derived from *m/z* showing distribution similar to that of C6

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