SPATIAL CORRELATION COMBINED WITH HIERARCHAL CLUSTERING ANALYSIS FOR REDUCING COMPLEX MUTLI-DIMENSIONAL MALDI IMAGING DATASET

複雑な多次元 MALDI イメージングデータセットの解析を容易にする階層的クラスター解析と組み合わせた位置相関の利用

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Thanai Paxton[1], Hernando Olivos[2], Mark Towers[3], Kieran Neeson[3], Emmanuelle Claude[3]
[1]Nihon Waters, Tokyo, Japan, [2]Waters Corporation, Beverly, MA , [3]Waters Corporation, Manchester, United Kingdom

INTRODUCTION

Mass spectrometry imaging (MSI) allows for the correlation of spatial localization and chemical information directly from biological surfaces. Recently, it has allowed simultaneous visualisation of thousands of endogenous metabolites and lipids in an entire tissue section. An additional dimension, called high efficiency ion mobility separations (IMS), based on travelling wave (T-wave) technology is incorporated into the mass spectrometer to add specificity to the MALDI imaging experiment by separating ions based on their shape in the gas phase. This leads to the generation of several gigabytes of complex and high dimensional data from a single tissue experiment, resulting in the growing need for automated computerized processing.
Here, we present a novel MALDI imaging workflow that includes acquisition of the MALDI imaging data using ion mobility separation, processing of the raw data using High Definition Imaging software and a novel data spatial correlation approach using Pearson product-moment correlation statistical tool.

METHODS

Sample preparation

A thin section of a rat brain section, produced using a cryotome and was deposited on a none-conductive glass slide. Alpha-Cyano-4-hydroxycinnamic acid (CHCA) matrix was applied evenly to the sample in several coats using a TM-Sprayer sample preparation device.

Mass Spectrometry

Data were acquired using a SYNAPT HDMS G2 mass spectrometer (Figure 1) in MS mode with tri-wave ion guide optics to separate ions according to their ionic mobility in the gas phase. The mass range of acquisition was 100-1,000 Da where lipid species can be detected, as well as matrix species and small endogenous metabolites.

MALDI-MS

Laser:	solid state Nd:YAG laser (λ = 355 nm)
Pulse rate:	1000 Hz
Spatial resolution:	400 µm (lateral)
IMS pressure:	3.3 mBar
Wave height:	40 V
Wave velocity:	750 to 250 m/s

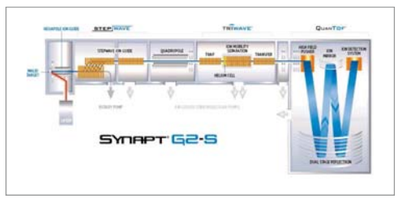

Figure 1. Schematic of the MALDI SYNAPT G2-S HDMS.

Data management

The obtained data sets were subsequently processed using High Definition Imaging 1.2 (HDI) MALDI software for detailed image analysis.

RESULTS

The acquired data set contains information from multiple dimensions, *i.e.* location (x,y coordinates), *m/z*, drift time and intensity. Moreover, the data acquired in continuum mode had reached 10.4 GB.

Data size reduction

The first step to reduce the large dataset was to process it by peak picking the data using the Apex3D algorithm in the *m/z* and drift time dimensions.

Ion distributions

The second step of the routine was to generate 2,000 ion distributions between *m/z* 600 to 1,000 containing x,y coordinate information, *i.e.* ion images, with a specific *m/z* window of 0.02 Da and drift time window of 0.054 ms. Those ion images are displayed in the Analysis tab of the HDI software as illustrated in Figure 2.

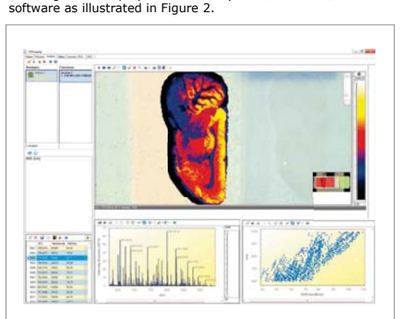

Figure 2. HDI 1.2 software Analysis tab displaying the ion image for lipid at m/z 772.52.

Cluster analysis

The third step is to carry out the Hierarchal Clustering by comparing the ion images between themselves and clustering by similarity. In Figure 3, the workflow is described that has been implemented into HDI 1.2 to create two clusters from all the ions images present in the processed dataset. Note that the comparison is not based on the MS spectra but on the ion images themselves using the Pearson-product moment algorithm.

Once the two K-means positions have been optimized, step 3 to 8 are user defined iterated n times. The results will be different for each iteration because of the randomness of the initial K-means position of step 3. The optimum cluster split result is identified from the iterations by the minimum summed distances to the respective cluster. In each new cluster, step 3 to 10 is repeated until all clusters contain a maximum of two ion images.

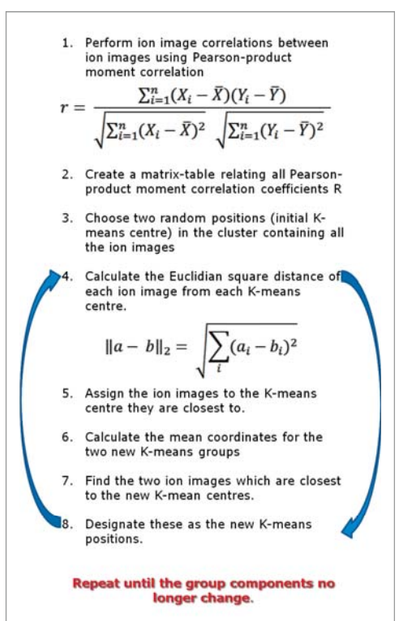

Figure 3. Workflow describing the Hierarchal Clustering in HDI 1.2 based on ion images similarities by K-medoid.

Rat brain lipid imaging

In this experiment, analyzing a thin section of a rat brain section, the most 1,000 intense ion images went through the Hierarchal clustering. The first split (cluster 2 and 3) allows for the differentiation of the lipid ion images with the matrix ion images where the pixels outside of the tissue were more intense compared to the pixels inside de tissue (Figure 4).

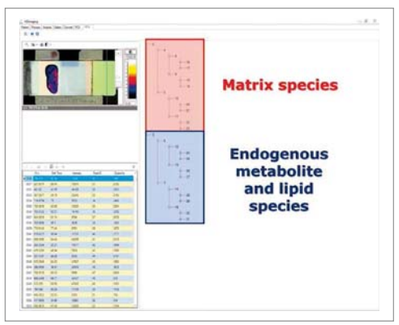

Figure 4. Dendogram result in the HCA tab of HDI 1.2 software rat brain imaging.

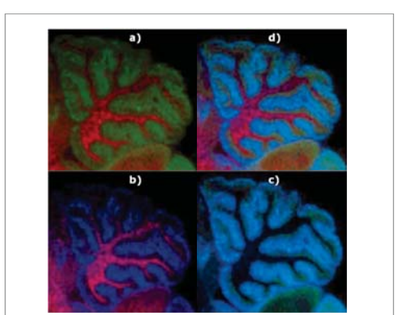

Figure 5. Overlay of three lipid ion images. Colour legend: Red: PC (36:1) K^+ (m/z: 826.5737); Green: PA (34:1) K^+ (m/z 713.4513); Blue: SM (36:1) K^+ (m/z 769.5621)
A) Red: PC (36:1) K^+ vs. Green: PA (34:1) K^+
B) Red: PC (36:1) K^+ vs. Blue: SM (36:1) K^+
C) Green: PA (34:1) K^+ vs. Blue: SM (36:1) K^+
D) Red: PC (36:1) K^+ vs. Green: PA (34:1) K^+ vs. Blue: SM (36:1) K^+

Figure 6 displays cluster 54, where lipid ions images were identified that are pronominally abundant in the grey matter of the Cerebellum, Thalamus, Hypothalamus and Cerebral Cortex of the brain. The next node shows correlation within the cerebellum of different lipids, i.e. 713.4513 Da (PA (34:1) K+) that are abundant in the molecular layer + the granular layer (cluster 106), whereas in cluster 107, the lipids i.e. 769.5621 Da (SM (36:1) K+) are more abundant in the molecular layer only as shown in Figure 5c.

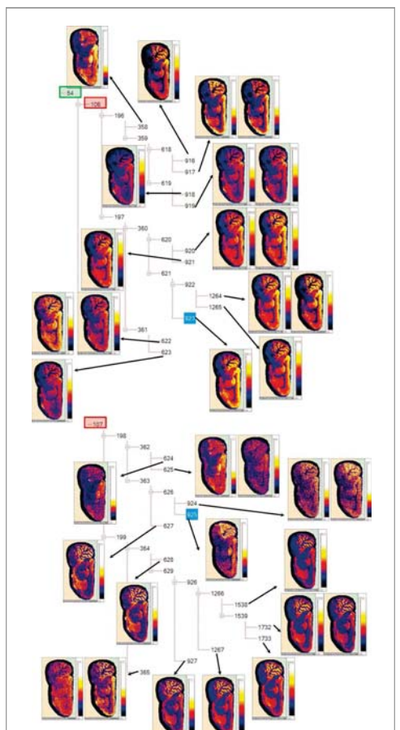

Figure 6. HCA results from cluster 54 with lipid ion images associated.

Figure 7 displays the dendogram details of cluster 63 where the ion images of the lipids were more abundant in the white matter of the Cerebellum, Corpus Callosum and Rhombencephalon, i.e. 826.5737 (PC (36:1) K+).

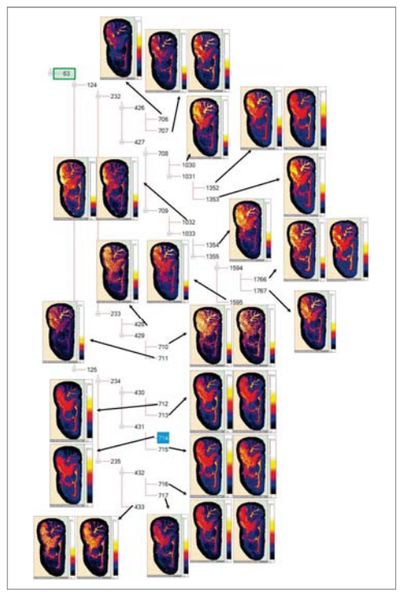

Figure 7. HCA results from cluster 63 with lipid ion images associated.

CONCLUSION

- **High Definition Imaging (HDI) 1.2 software provides a new hierarchical clustering process based on ion images comparison**
- **Matrix ion images were readily differentiated from endogenous ion images**
- **Lipids with similar localisation in rat brain were grouped as classes in the dendogram tree diagram**

Acknowledgements

This work was done in conjunction with David Geoffrey Rizzo from the Vanderbilt University Department of Chemistry.

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