SpatialOMx® analysis allows for the specific identification of lipid species paving the way for accurate flux analysis

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Introduction

Disease is a cellular process and *in situ* analysis of tissue provides the most direct and sensitive access to changes occurring within the cells. However, when samples are homogenized to extract information, their spatial information is lost. Mass Spectrometry Imaging is a powerful tool that enables untargeted analysis of a variety of samples to determine the spatial distribution of thousands of molecular species. SpatialOMx® is the use of MALDI Imaging to identify and target specific regions in a tissue section for deeper 4D-omics experiments and can easily be performed on the Bruker timsTOF fleX instrument which features an ESI/MALDI dual ion source. Here we present the SpatialOMx workflow in combination with 4D-lipidomics to identify lipids in the brain as shown in Figure 1 below. The up- or down-regulation of lipids, for example Hexaceramides (HexCer), can give new insights into the role of lipid metabolism within the brain and can play a pivotal role in understanding disease progression.

MALDI Imaging
- MALDI Imaging on timsTOF fleX
- Unsupervised statistical analysis in SCiLS™ Lab (Segmentation analysis)
- Tissue dissection

4D-Lipidomics
- Lipid extration
- 4D-PASEF acquisition
- 4D-processing and annotation by MetaboScape®

Lipid visualization
- Export lipid annotation list from Lipidomics data to SCiLS™ Lab
- Import lipid list in SCiLS™ Lab for visualization of spatial distribution in tissue

Figure 1. Schematic of SpatialOMx workflow listing the detailed steps

Methods

A frozen rat brain was sectioned at 10 µm and mounted on Bruker IntelliSlides® and sprayed with DHAP. The samples were analyzed on a timsTOF fleX in positive ion mode at 50 µm lateral spatial resolution. Data processing was done in SCiLS™ Lab 2023a and MetaboScape® 2023a. Following data acquisition, segmentation analysis revealed two distinct regions that were then used for the omics experiment. The two distinct regions from two consecutive brain sections were excised out separately and extracted with an extraction buffer consisting of tert-methyl butyl ether (MTBE) (400 µL) + methanol (MeOH) (80 µL) + water (H_2O) (200 µL). The mixture was vortexed and then sonicated for 10 min. Following centrifugation at 3000 rpm for 15 min, 200 µL of the supernatant was removed then dried using a vacufuge (~10 min) and finally reconstituted with a 9:1 mixture of MeOH: dichloromethane (DCM). LC-MS analysis was performed by injecting 10 µl with a gradient of mobile phase A (600:390:10 Acetonitrile (ACN):H_2O (1 M Ammonium formate) + 0.1% Formic acid (FA); B (900:90:10 Isoproponal (IPA): ACN: 1 M ammonium formate) + 0.1% FA and a C18 Intensity solo2 100 x 2.1 mm column. As a QC (Quality Control) check, a "bulk" sample was prepared by extracting an entire brain section and different volumes of the sample were injected to determine the LOD of the various lipid species. The samples were analyzed in triplicates.

Results

Data analysis was performed in SCiLS Lab 2023a and MetaboScape 2023. Segmentation analysis showed two distinct regions (yellow and blue), corresponding to the white and grey matter in the brain (Figure 2a and 2b below).

Figure 2. Optical image (A) of a rat brain and results from an unsupervised statistical analysis of segmentation in SCiLS Lab showing two distinct regions

The two regions (yellow and blue) were excised out with a razor and extracted to obtain deeper MS/MS and Collision cross section (CCS) information, in addition to accurate mass. Over 600 features were annotated from the spectral libraries available and around 100 lipids were annotated using rule-based lipid annotations in 11 lipid classes (Figure 3 below).

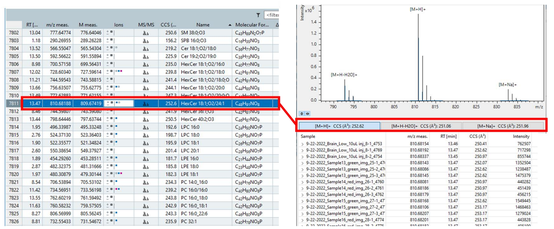


Figure 3. Visualization of 4D Omics result in MetaboScape: Feature table (left panel) and compound details of HexCer 18:1;O2/24:1, including protonation, sodium adduction, and water loss, from each sample data file.

MS/MS experiments were also performed on select mases and compared to NIST 2020 MS/MS Spectral Library which contains spectra of ~30k compounds (Figure 4 below).

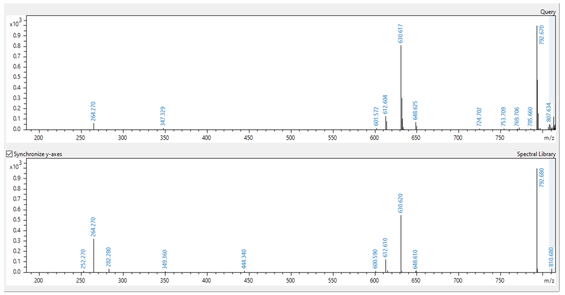
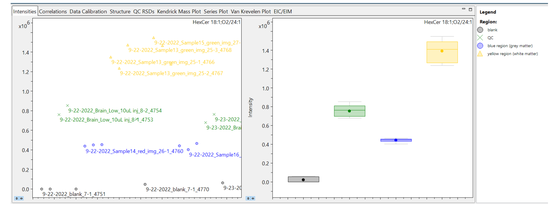


Fig. 4 MS/MS spectra of HexCer 18:1; O2/24:1 from the measured (top) sample and the NIST 2020 MS/MS Spectral library (bottom)

The peak intensity from a total of 22 sample injections (5 blanks, 5 QCs, triplicates from yellow/blue regions from each of the 2 tissue slices) is displayed in Figure 5a. The left panel displays peak intensity from each individual sample injection, whilst the right panel shows grouped analyses for blank, QC, blue region, and yellow region, respectively.



The result indicates that HexCer 18:1;O_2/24:1 has distinguishable amounts in different regions in brain tissue. The image of HexCer 18:1;O2/24:1 is displayed in Figure 5b. The distribution of HexCer 18:1;O2 /24:1 majorly in white matter is consistent with high abundance in yellow region as observed in LC-MS/MS analysis.

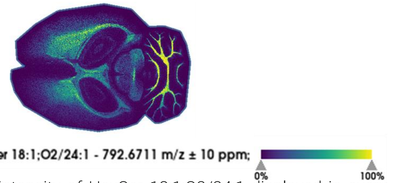


Fig. 5 Peak intensity of HexCer 18:1;O2/24:1 displayed in every sample injection (left) and in groups (right) (A). MALDI image of HexCer 18:1;O2/24:1 after the annotated lipids were imported back to SCiLS™

Conclusion

- An easy pipeline that can be implemented is presented here where MALDI imaging can spatially guide the user for more in depth lipid omics experiments.
- Combination of data acquired from SCiLS and MetaboScape allows for visualization of annotated compounds.

Imaging Mass Spectrometry; Method Development