SpatialOMx® tissue analysis couples MALDI imaging with in-depth 4D-Lipidomics

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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 hexosylceramides (HexCer), can give new insights into the role of lipid metabolism within the brain and can play a pivotal role in understanding disease progression.

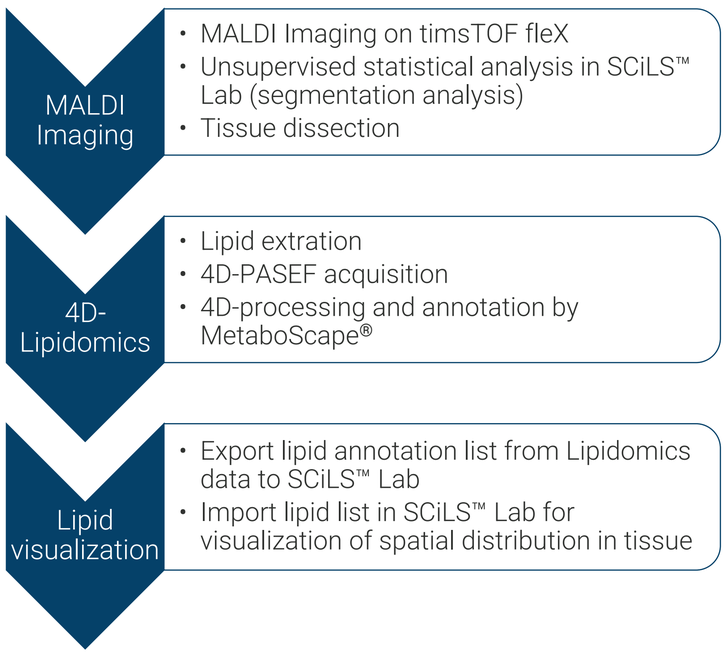


Figure 1. Schematic of SpatialOMx workflow listing the detailed steps

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® 2023. 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 separately and extracted with an extraction solvent 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 vacuum concentrator (~10 min) and finally reconstituted with a 9:1 mixture of MeOH: dichloromethane (DCM). LCMS 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: 1M ammonium formate) + 0.1% FA and a C18 Intensity Solo 100 x 2.1 mm column. As a quality control (QC) 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

MALDI Imaging and Lipid Visualization

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 gray matter in the brain (Figure 2A and 2B below).

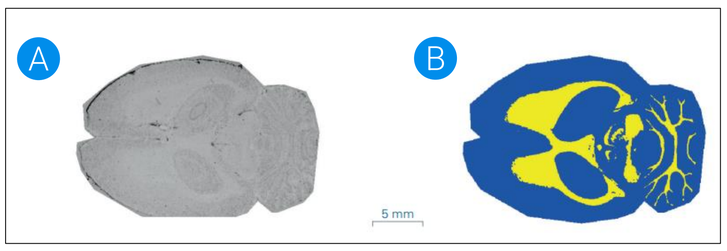


Figure 2. (A) Optical image of a rat brain and (B) results from an unsupervised statistical segmentation analysis in SCiLS™ Lab showing two distinct regions

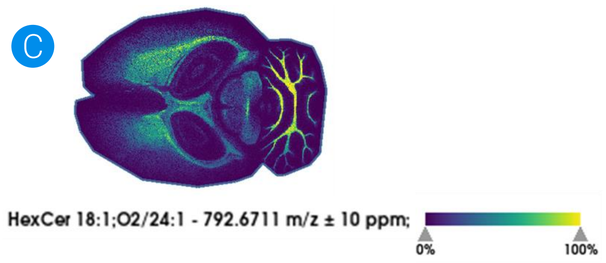


Figure 2. (C) MALDI image of HexCer 18:1;O_2/24:1 after the annotated lipids were imported back to SCiLS™

The two regions (yellow and blue) were excised out with a razor and extracted to obtain deeper MS/MS and collision cross section (CCS) measurements, in addition to accurate mass (*m/z*) and isotope distribution for lipid annotation.

4D-Lipidomics and Lipid Annotation

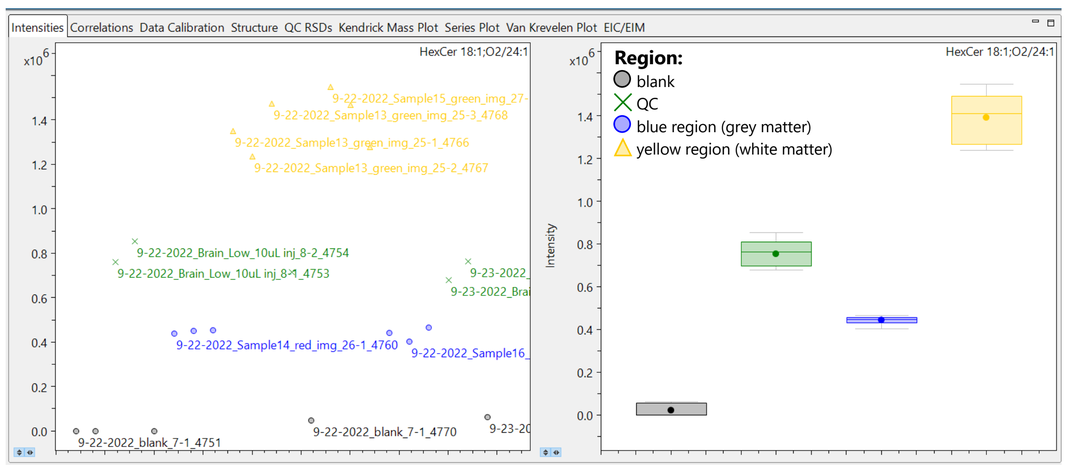


Figure 3. The peak intensity from a total of 22 sample injections (5 blanks, 5 QCs, triplicates from yellow/blue regions from each of the 2 tissue slices) is displayed. The left panel displays peak intensity from each individual sample injection, whilst the right panel shows grouped analyses for blank, QC, blue region, and yellow region, respectively.

The results indicate that HexCer 18:1;O_2/24:1 has greater relative abundance in white matter (yellow) and lower relative abundance in gray matter (blue) in the excised brain tissues. The distribution of HexCer 18:1;O_2/24:1 observed in white matter of the MALDI brain tissue image in 2C is consistent with high abundance in yellow region as observed in LC-MS/MS analysis in Figure 2. Approximately 100 Lipids in 11 lipid classes were annotated using rule-based lipid annotations.

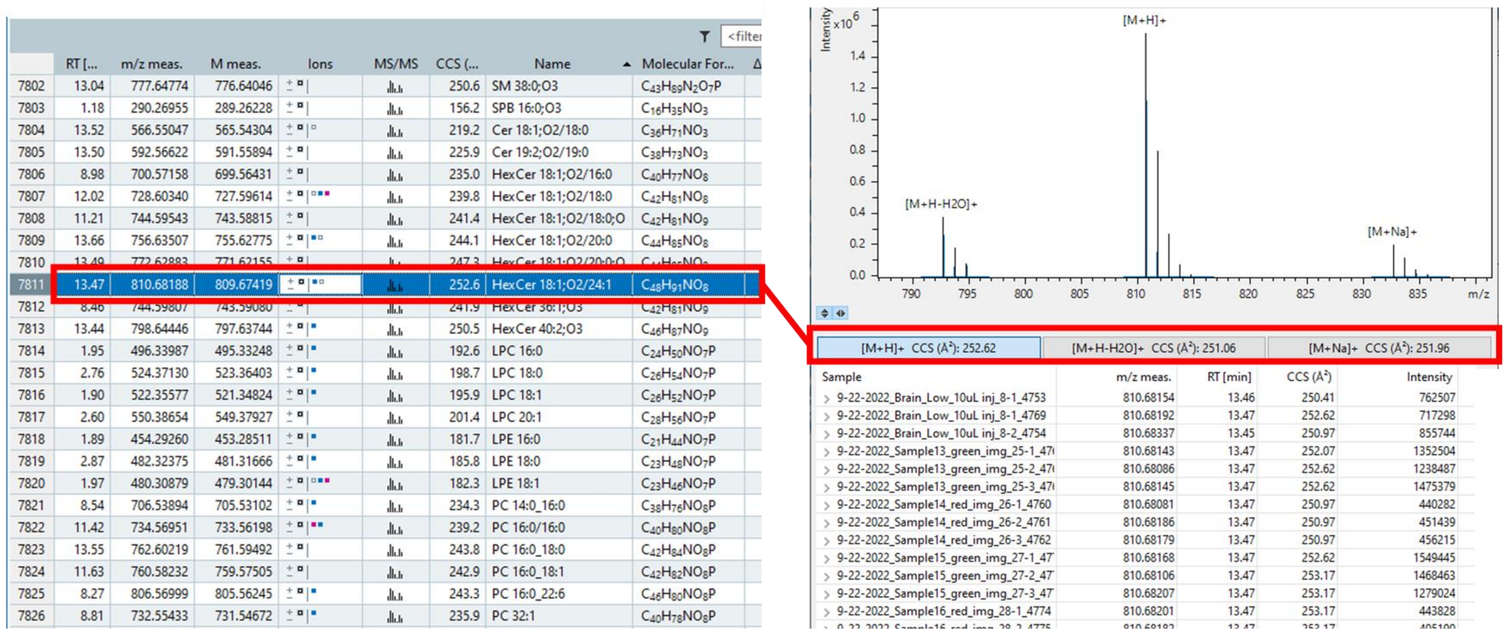
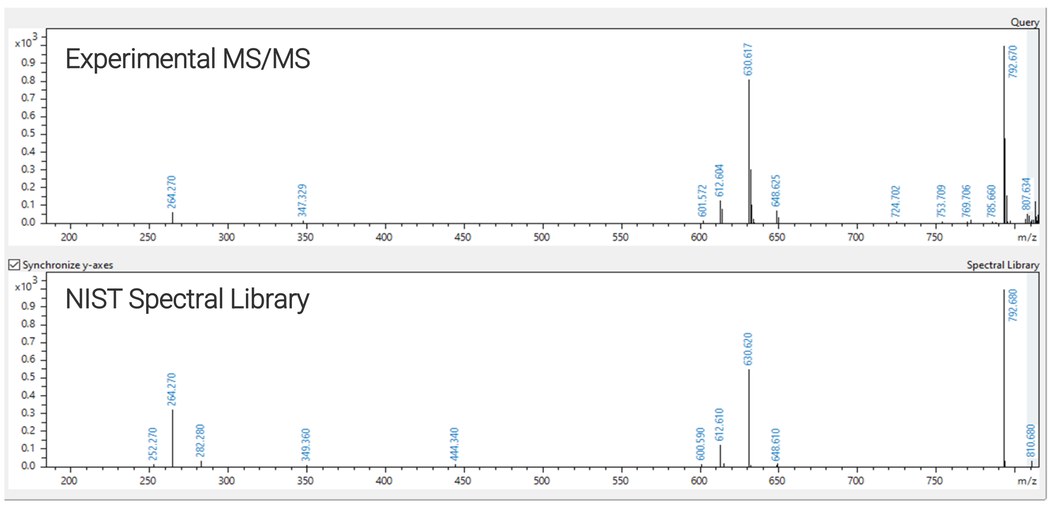


Figure 4. 4D-Lipidomics annotation results in MetaboScape: Feature table (left panel) highlighting details of HexCer 18:1;O_2/24:1, including protonation, sodium adduct, and water loss, from each sample data file.



MS/MS experiments were also performed using PASEF and compared to the NIST 2020 MS/MS Spectral Library which contains spectra of ~30k compounds. Shown in Fig. 5 is HexCer 18:1;O_2/24:1 as compared to the spectral library, demonstrating fragment *m/z* matches. This correlates with the MS/MS match score of 877/1000.

Figure. 5 MS/MS spectra of HexCer 18:1; O_2/24:1 from the measured (top) sample and the NIST 2020 MS/MS Spectral library (bottom)

Conclusion

- An easy pipeline that was implemented where MALDI imaging spatially guided more in depth lipidomics experiments.
- Integration of data acquired from SCiLS™ and MetaboScape® allows for visualization of annotated compounds.

SpatialOMx combining MALDI Imaging and 4D-Omics on timsTOF fleX