



SpatialOMx[®] lipid analysis of rat brain

MALDI Guided SpatialOMx[®] is a powerful technology that combines the spatial detection of analytes from tissue with a traditional solution-based omics approach.

Abstract

A horizontal rat brain tissue section was imaged at 50 μm lateral spatial resolution on the timsTOF flex. Following segmentation analysis in SCiLS[™] Lab 2023a, two distinct regions were noted, that correspond to anatomical regions of interest. These were then excised out with a razor, extracted with organic solvent and run by Bruker's 4D-Lipidomics[™] workflow using the Elute UHPLC coupled with the VIP-HESI-timsTOF platform. Over 130 lipids in 11 lipid classes were annotated following analysis in MetaboScape[®] 2023. The data was reimported into SCiLS Lab for complete spatial visualization of the annotated compounds.

Keywords:
MALDI Imaging,
SpatialOMx, brain lipids,
SCiLS Lab 2023a,
MetaboScape 2023

Introduction

Lipids are essential components of all tissues as constituents of the cell membrane and participate in a range of cellular functions including modulation of membrane protein signaling, signal transduction, cell motility and adhesion, and apoptosis. The Bruker timsTOF flex instrument features an ESI/MALDI dual ion source for seamless switching between ionization modes within seconds, providing access to an expanded application space. SpatialOMx[®] is the integration of MALDI Imaging to identify and target specific regions in a tissue section for deeper 4D-Omics analysis. The timsTOF platform includes the TIMS/PASEF[®] (trapped ion mobility spectrometry/Parallel Accumulation - Serial Fragmentation) technology which provides unmatched MS/MS acquisition speed, uncompromised sensitivity, and ultra-high resolution and delivers high-confident lipid annotation with retention time, MS, MS/MS fragmentation pattern, and CCS (collisional cross section). Here we describe a workflow that utilizes MALDI Imaging of lipids within a brain section to determine spectrally unique regions of the tissue in combination with traditional LC-MS techniques for more accurate annotation of the species identified.

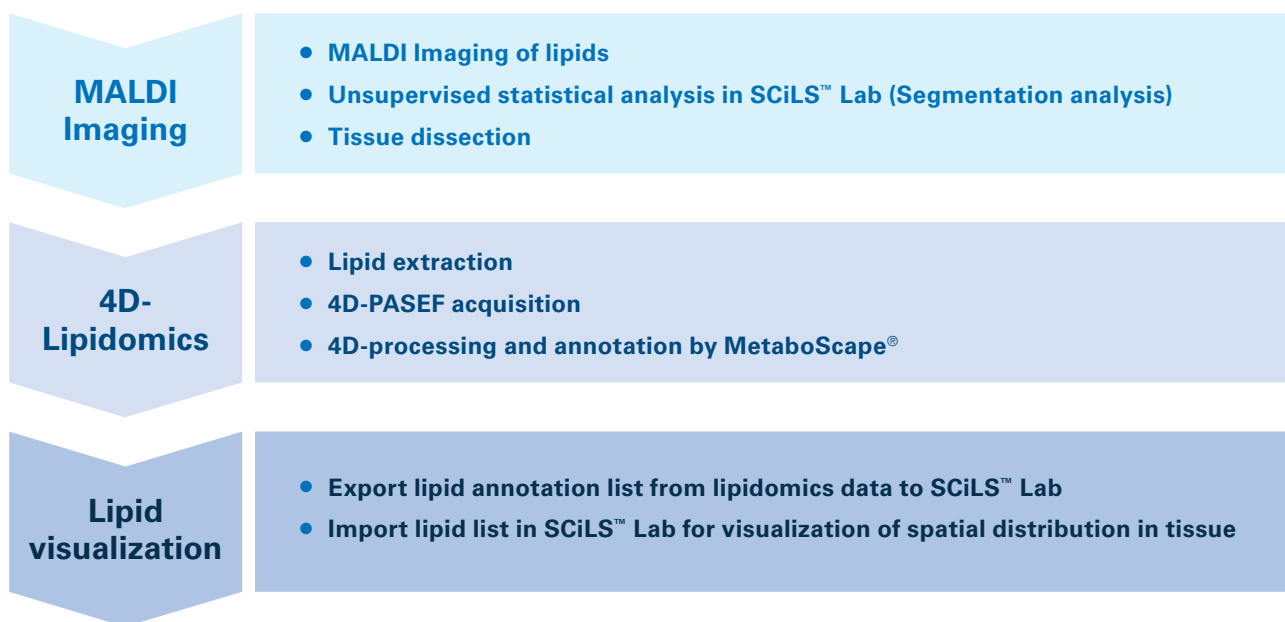


Figure 1
Schematic of SpatialOMx workflow listing the detailed steps.

Methods

Fresh frozen rat brain was sectioned at 10 μm and thaw-mounted on Bruker IntelliSlides®. DHAP matrix was sprayed on using an HTX M3 TM-sprayer, and the samples were analyzed on a timsTOF fleX in positive ion mode at 50 μm lateral resolution. All imaging data processing was done in SCiLS Lab 2023a. Following data acquisition, segmentation analysis revealed two distinct regions that were chosen for the continuing lipidomics experiment, representing the white and gray matter of the cerebellum. The two distinct regions from two consecutive brain sections were excised with a razor and lipids were extracted using a solvent consisting of methyl tert-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 at 4°C, 200 μL of the supernatant was removed then dried using a vacuum concentrator (~10 min) and reconstituted with a 9:1 mixture of MeOH:dichloromethane (DCM). The volume of the gray matter region (highlighted in blue, see Figure 2) is approximately three times larger than the white matter region (highlighted in yellow, see Figure 2) as estimated by the region mapper within SCiLS Lab based on the pixel count. Therefore, the reconstitution volume for the regions were 300 μL and 100 μL , respectively. Final LC-MS analysis was performed by injecting 10 μL on a C18 Intensity Solo 100 x 2.1 mm column using a 20 min gradient consisting of mobile phase A (600:390:10 acetonitrile (ACN): H_2O (1 M ammonium formate) + 0.1% formic acid (FA) and mobile phase B (900:90:10 isopropanol (IPA): ACN: 1 M ammonium formate) + 0.1% FA. The Elute UHPLC system is connected to a timsTOF Pro 2 via VIP-HESI (vacuum insulated probe – heated ESI) source. The default 4D-Lipidomics application method was used with PASEF enabled in positive mode using the same mass range as the imaging experiment. 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. All samples were analyzed in triplicate.

Results and discussion

Imaging data was used to perform a segmentation analysis to identify regions of interest (ROIs) in the rat brain tissue. Figure 2 shows the results from the segmentation analysis in SCiLS Lab, which identified two distinct regions that correlate to gray matter (blue) and white matter (yellow) in the cerebellum. The overall average spectrum for the whole region is also shown below.

Imaging data containing m/z and CCS information can be imported into MetaboScape and annotations obtained based on accurate mass, isotopic fine structure and ion mobility. This standard workflow can be enhanced when this information is combined with MS/MS and retention time, resulting in more confident annotations for distinct tissue regions. By excising the ROIs that were determined using MALDI Imaging, a focused set of lipids specific to gray and white matter could be analyzed via 4D-Lipidomics. Annotations were then mapped onto the brain tissue image to visualize relative lipid abundances specific to the cellular phenotypes of interest.

Lipidomics data were analyzed in MetaboScape 2023. Samples were grouped into cohorts: blue (gray matter), yellow (white matter), QC (bulk brain tissue) and blanks (MeOH). Each sample was calibrated at the beginning of each run by injecting a mixture of sodium formate (mass) and TuneMix (mobility), which was used for internal calibration during data processing. After peak picking, retention time alignment, deisotoping and deadducting, a feature table containing ~8,000 unique features was generated. Annotation was performed using the available Spectral Libraries in MetaboScape: MetaboBase[®] 3.0, the HMDB Metabolite Library 2.0, the NIST 2020 MS/MS Library, MS-DIAL LipidBlast Library and the rule-based lipid annotation workflow which adheres to the Lipid Standards Initiatives.

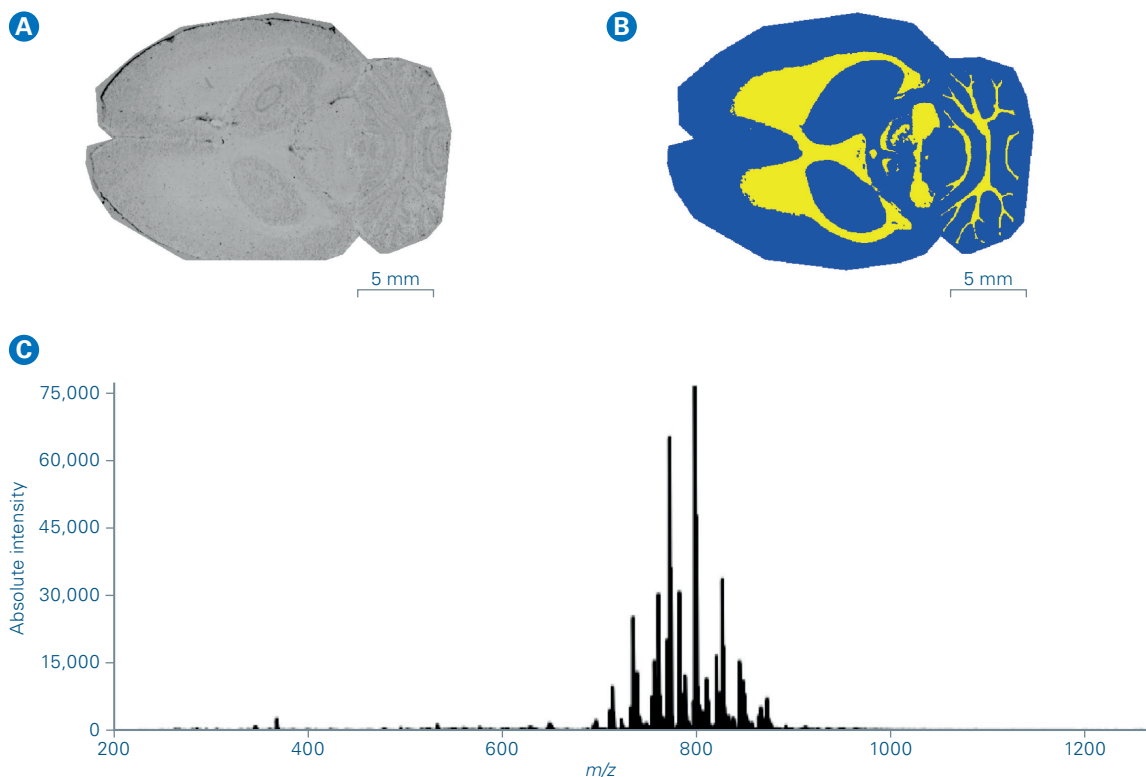


Figure 2

(A) Optical image of a rat brain sample and (B) results from an unsupervised statistical analysis of segmentation in SCiLS Lab showing two distinct regions. (C) The average spectrum of the entire region is shown in the bottom panel.

Figure 3 displays a portion of the MetaboScape feature table with the hexosylceramide HexCer 18:1;O₂/24:1 highlighted as an example lipid of interest in the SpatialOMx workflow. Data acquired for each feature is tabulated, such as retention time, accurate mass, grouped ions, MS/MS, CCS and compound annotations (left panel). Detailed information for each feature can be viewed, including ion isotope clusters of the protonated species as well as its adducts and neutral losses. CCS values for each ion species can be viewed as well as measured data from individual sample data files. In the example of HexCer 18:1;O₂/24:1, ion clusters were grouped into a single unique feature for [M+H]⁺, the water neutral loss and the sodium adduct.

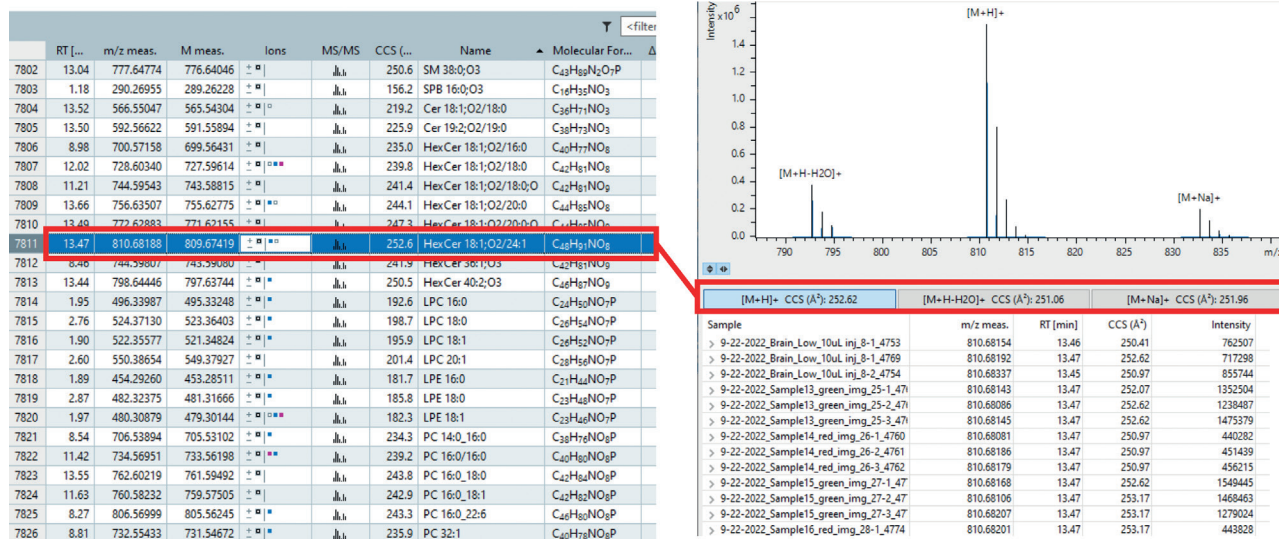


Figure 3

Visualization of 4D-Omics result in MetaboScape:

Feature table (left panel) and compound details (right panel) of HexCer 18:1;O₂/24:1, including the protonated molecular ion, the water neutral loss and the sodium adduct in each sample data file.

HexCer 18:1;O₂/24:1 was annotated from NIST 2020 MSMS Spectral Library which contains MS/MS spectra of ~30k compounds and the rule-based lipid annotation method. Figure 4 displays measured MS/MS spectrum of HexCer 18:1;O₂/24:1 (top) comparing to the spectrum from this Spectral Library (bottom). Comparison of the fragment ions gives a visual confirmation of the lipid assignment in addition to the MS/MS similarity score of 877 out of 1000.

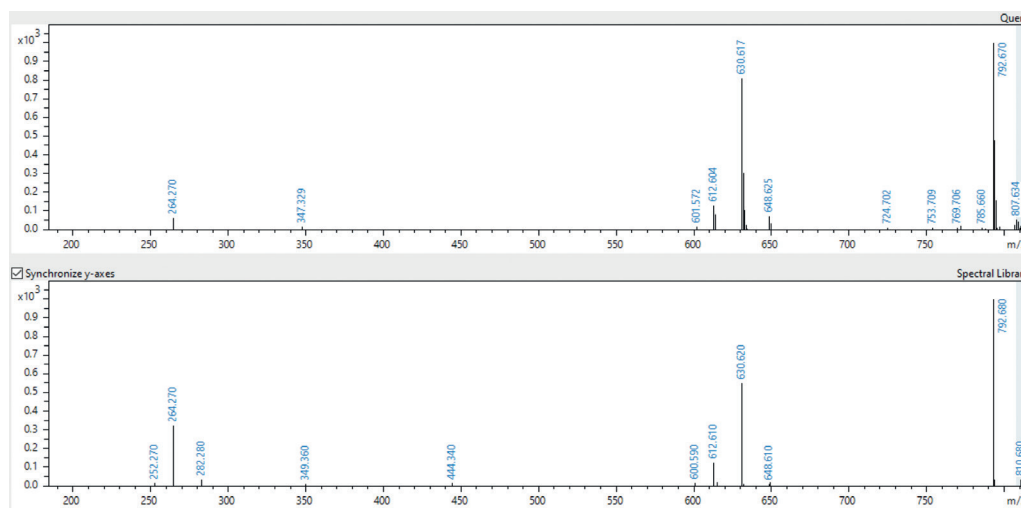


Figure 4

MS/MS spectra of HexCer 18:1;O₂/24:1 from measured (top) and NIST 2020 MSMS Spectral Library (bottom).

The relative abundance of HexCer 18:1;O₂/24:1 from the gray matter (blue) and white matter (yellow) can be seen in MetaboScape when the feature is highlighted in the feature table. 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 5B. The left panel displays peak intensity from each individual sample injection, while the right panel shows grouped analyses for blank, QC, blue region, and yellow region, respectively. The results indicate that HexCer 18:1;O₂/24:1 has a greater abundance in the white matter and a lower abundance in the gray matter. The QC samples sit nicely between the two sample cohorts while the blanks show negligible signal intensity, ensuring that the data analysis is relevant to the phenotypes of interest. A list of annotated lipids was exported from MetaboScape and imported back to SCiLS Lab to visualize the distribution of annotated compounds. The image of HexCer 18:1;O₂/24:1 is displayed in Figure 5A. The observed distribution of HexCer 18:1;O₂/24:1 is greater in white matter, which is consistent with higher abundance in yellow region as observed in LC-MS/MS analysis.

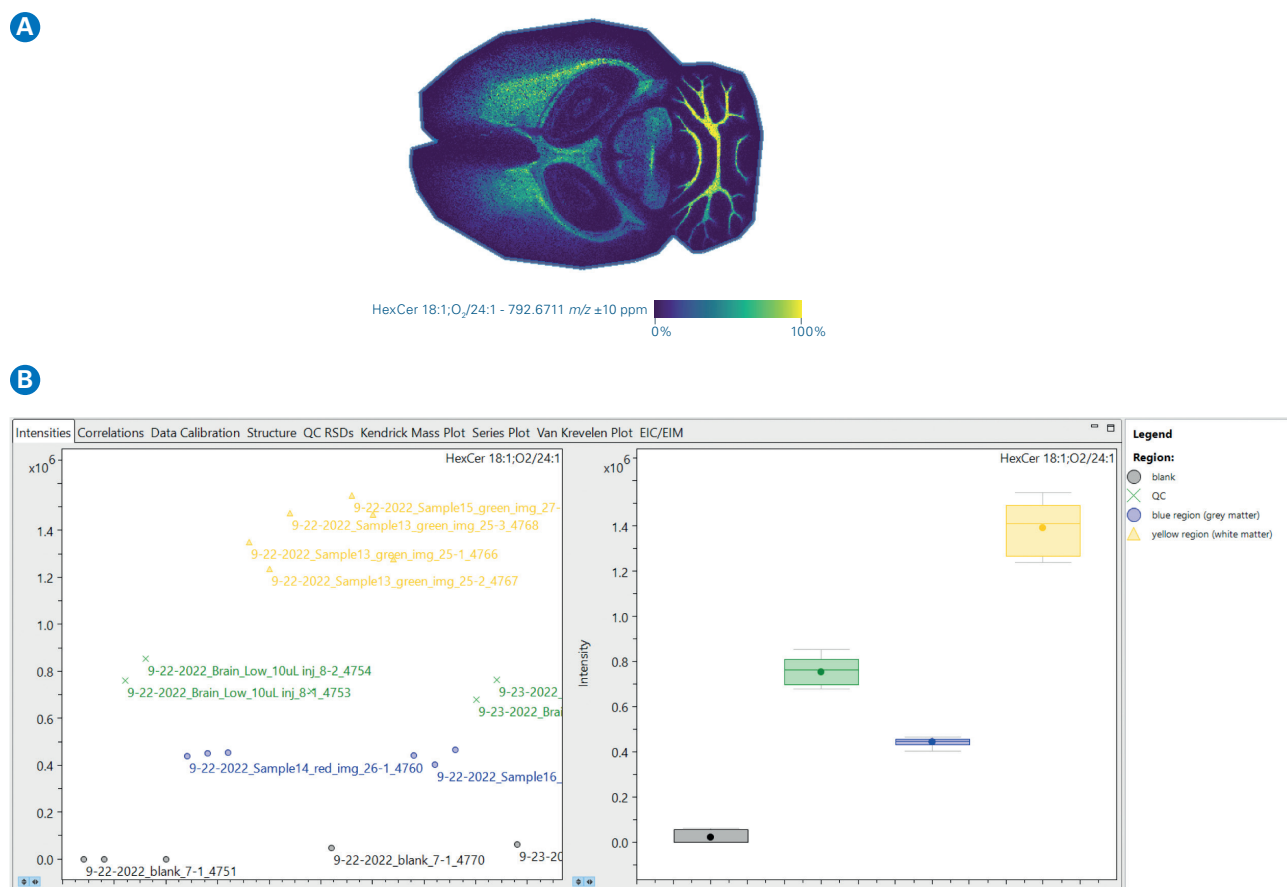


Figure 5

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

Some of the lipid species identified in the yellow region (white matter) include ceramides (Cer) and phosphatidylserines (PS) while the species identified in the blue region (gray matter) included sphingomyelins (SM) and lysophosphatidylcholines (LPC), examples of which are shown in Figure 6 below.

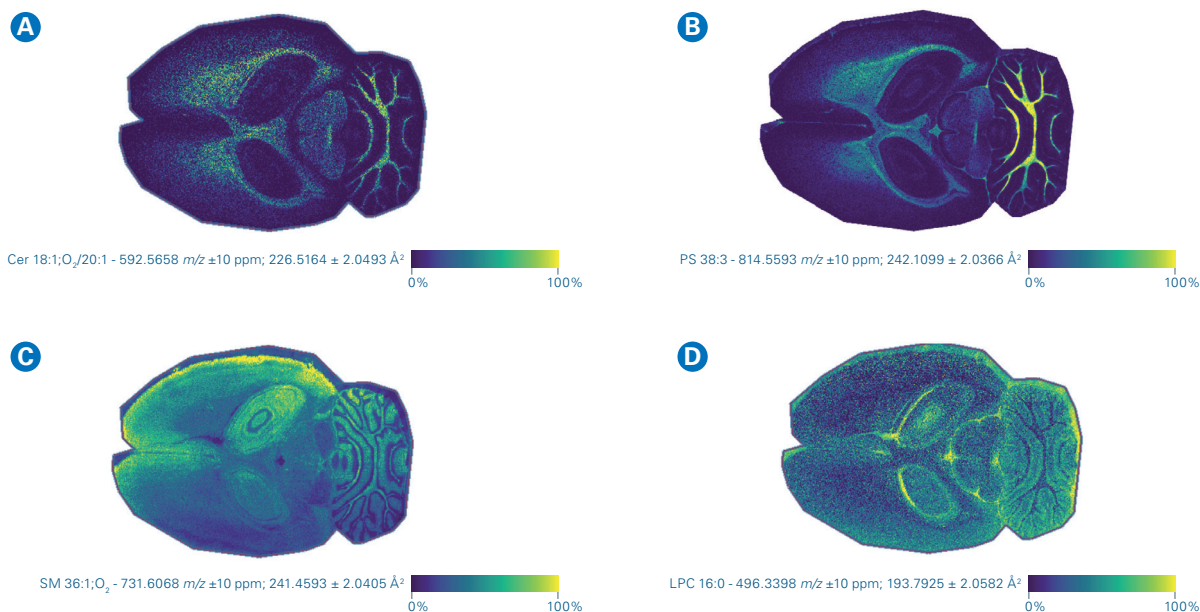


Figure 6
Spatial distribution of lipids like **A** ceramide and **B** phosphatidylserine within the yellow region and **C** sphingomyelin and **D** lysophosphatidylcholine within the blue region excised from a rat brain.

Conclusion

The spatial distribution of lipids within rat brain tissue sections was demonstrated using the SpatialOMx workflow. Segmentation analysis was used to identify ROIs within the tissue that are spectrally distinct, representing different cellular compositions. Segmentation is a critical workflow “first step” in identifying regions of histological or biological interest within a given study. 4D-Lipidomics featuring Metaboscape 2023 was used to identify differences in lipid abundance and perform Spectral Library and rule-based lipid annotation. These annotations were directly imported into the tissue image, where they correlated well with the identified brain segments of white and gray matter. The timsTOF fleX provides a robust and multiplexed platform for in-depth lipid characterization in MALDI tissue images using SpatialOMx.

Notes

* The experiment was repeated to determine the effect of washing on analyte detection by washing with 100% EtOH for 30 sec with gentle agitation.

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