INTEGRATING QUANTITATIVE MASS SPECTROMETRY IMAGING WITH THE ALLEN MOUSE BRAIN ATLAS ENABLES QUANTIFICATION OF BRAIN REGION LIPIDOMES

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Introduction

Lipids are crucial to biological processes, influencing both homeostasis and driving disease. Mass spectrometry imaging (MSI) is a powerful tool for studying lipids, enabling detailed analysis of lipid species within tissue sections. However, analyte quantification in MSI is hindered by unequal matrix ion suppression. Traditionally, internal standards (ISs) of analyte analogues are used to address this. Given the varying ionisation efficiencies across lipid classes, class-specific ISs are essential. While lipidomic studies typically focus on single classes, we developed an on-tissue workflow using a custom multiclass IS mixture reflecting endogenous concentrations of 13 lipid classes. This approach enables quantitative (Q-)MSI of over 200 lipids using both MALDI and MALDI-2 simultaneously.

By integrating publicly available resources such as the Allen Mouse Brain Atlas (AMBA), we were able to map lipid species concentration across brain regions in mice with Parkinson's Disease (PD)-related mutations. Our methodology involved two sets of image registrations with a proprietary non-rigid registration pipeline, enabling extraction of ion intensities from pixels in each brain region for differential expression analysis.

AMBA not only streamlines the workflow but also enhances data analysis by linking to other datasets, reducing manual annotations, and enabling comprehensive cross-referencing.

Methods

Whole mouse brain samples from wild type (WT), heterozygous (HET) and homozygous (HOM) PD mutants were sectioned separately at UOW and M4i. The IS mixture, consisting of standards for 13 lipid classes, was deposited onto the tissue sections, followed by spray coating of the MALDI SPLASH mix (#330841, Avanti Polar Lipids) and MALDI matrix. Tissue sections were analysed using an Orbitrap Elite mass spectrometer (negative ion mode analysis: conventional MALDI; positive ion mode analysis: MALDI-2) and a MALDI-2 timsTOF fleX.

Maximum intensities of lipid species were extracted through binning of the spectra around the computed m/z value. Images were normalised through pixel-wise division of the original m/z image by the reference m/z image (IS), followed by multiplication by the corresponding IS concentration.

Mapping of Q-MSI data to the Allen Mouse Brain Atlas (AMBA)

This method (Fig.1) facilitated the mapping of regions of interest (ROIs) onto MSI data, enabling the extraction of ion intensities from pixels within each AMBA ROI and the subsequent calculation of mean lipid species concentrations across various anatomical regions within tissue sections. Digital H&E-stained whole slide microscopy images of adjacent tissue sections were co-registered to the best-match AMBA layer Nissl stain and to the MSI images using Aspect Analytics' proprietary landmark-based non-rigid registration tool. Dimensionality reduction of the MSI data through non-negative matrix factorization (NMF) generated composite images summarising ion intensities across the different m/z values.

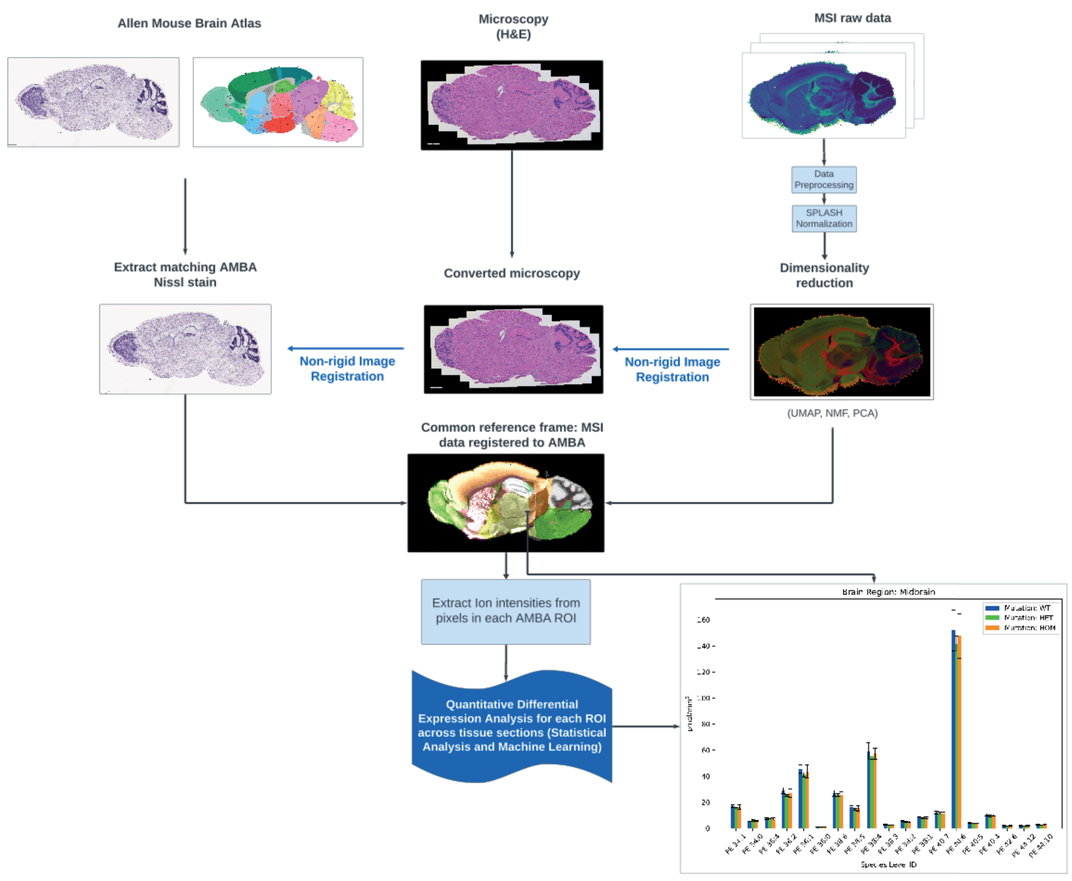

Fig. 1: Workflow illustrating the process of registering Q-MSI data with the AMBA.

References: [1] Vandenbosch M et al. Toward omics-scale quantitative mass spectrometry imaging of lipids in brain tissue using a multiclass internal standard mixture. Anal Chem 2023. [2] Eiersbrock F et al. Validation of MALDI-MS imaging data of selected membrane lipids in murine brain with and without laser postionization by quantitative nano-HPLC-MS using laser microdissection. Anal Bioanal Chem 2020. [3] Choi J et al. Comprehensive analysis of phospholipids in the brain, heart, kidney, and liver: brain phospholipids are least enriched with polyunsaturated fatty acids. Mol Cell Biochem 2018.

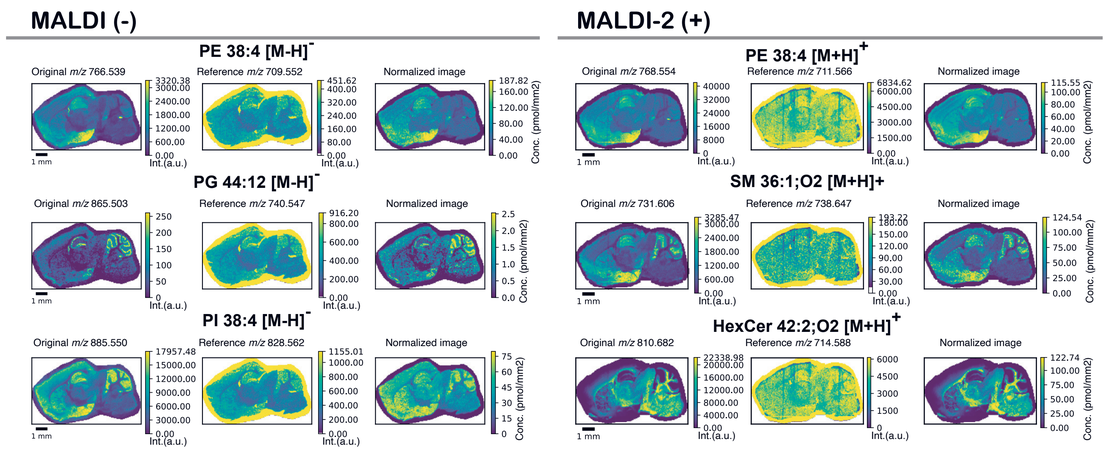

Fig. 2: Representative internal standard (IS) normalised ion images for a subset of the analysed lipid species detected with MALDI (left; negative ion mode) and MALDI-2 (right; positive ion mode). The images depict the impact that IS normalisation has on observed ion intensities across the mouse brain and in different ROIs. The three panels correspond to the original ion image (left), the class-specific IS distribution image (middle) and the IS normalised lipid distribution image (right).

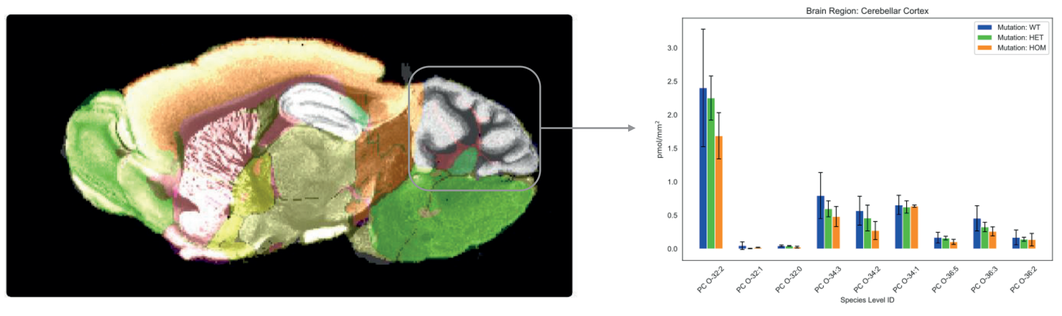

Fig. 3: Mean lipid concentration (pmol/mm2) for selected lipid species in WT, HET and HOM PD mutants across the cerebellar cortex acquired with the Orbitrap Elite in positive ion mode. Our method allows for the quantification of lipids, which can be cross-checked between studies. Each bar represents the mean concentration from n=3 biological replicates; error bars correspond to ± 1 SD.

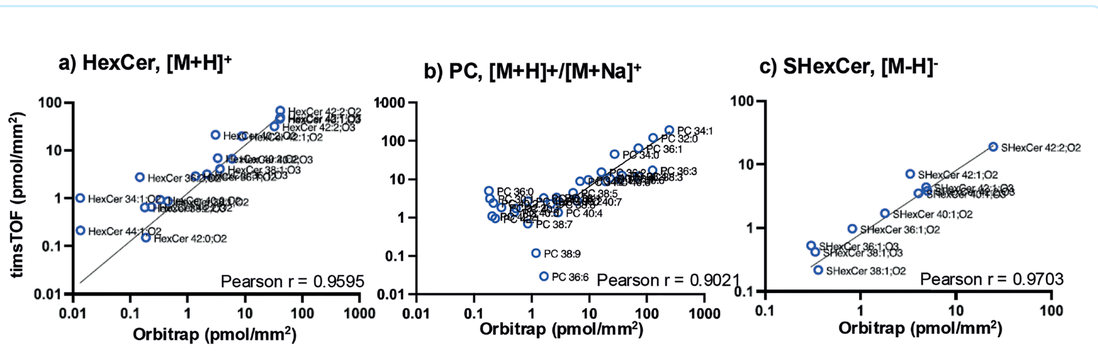

Fig. 4: Correlation analysis of Q-MSI data acquired with timsTOF and Orbitrap Elite for a subset of lipid species following averaging of all on-tissue pixels for each section. The high positive correlation (r > 0.9) in lipid concentrations across different instruments and labs corroborates the robustness of our analysis and demonstrates the potential of this method for normalisation of measurements in MSI. Each data point corresponds to the average of n=3 biological replicates measured on each system.

Conclusion

Our study successfully demonstrated the feasibility of Q-MSI for over 200 lipid species, significantly enhancing the application of MSI in spatial lipidomics[1]. This method enabled comparisons of quantitative lipid fingerprints across different brain regions, revealing subtle variations in lipid distribution that are often missed by standard normalisation methods. The findings were reproducible across different MS platforms and laboratories and are consistent with other bulk lipidomics studies, such as LCMS and shotgun lipidomics[2,3], confirming the robustness of our approach.