Distinct lipid profiles of hippocampal subregions and plaque microenvironments in an Alzheimer's Disease mouse model revealed by high-spatial resolution MALDI-imaging

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Introduction

Lipids are increasingly implicated in age-related neurodegenerative diseases, such as Alzheimer's disease (AD). Abnormal lipid accumulation was described by Alois Alzheimer in 1907; however, this observation was largely overlooked due to limited methodologies for studying lipids. Modern mass spectrometry imaging (MSI) enables characterization of lipids with high structural and spatial resolution. Recent advances such as routine sub-10-micron spatial resolution now enable deeper characterization of lipids in AD. Here, we used 5-micron spatial resolution MALDI-MSI to examine lipid accumulations associated with AD plaques in the hippocampus of the 5xFAD mouse model of AD. By registering the data to the Allen Mouse Brain Atlas, we associated MSI signals with fine morphological subregions that were previously unmappable at lower MSI resolutions.

Methods

Cryosections of 3 5xFAD and 3 C57BL/6 mouse brains (10um; 15 months; n=3/sex) were collected through the center of the hippocampal formation (HF) and thaw-mounted on superfrost slides. Autofluorescence images were obtained prior to MALDI-MSI analysis. Sections were coated with 2,5-DHA via sublimation and then recrystallised. Tissues were analysed using a timsTOF fleX (Bruker), in positive (MALDI-2) and negative (MALDI) mode at 5um spatial resolution. After MALDI-MSI analysis, tissues underwent H&E staining and digitised. MALDI-MSI and imaging data were analysed using Weave (Aspect Analytics). The data were spatially mapped using a non-rigid registration algorithm, and then integrated into a single combined dataset for downstream analysis and subsequent registration to the Allen Mouse Brain Atlas (AMBA). MALDI-MSI data underwent peak picking using a 15 ppm peak window. Peaks were extracted through aggregation of the maximum signal per tissue, filtered according to signal-to-noise ratio and the number of pixels expressing the signal. This resulted in ~1100 and ~2100 extracted features in negative and positive mode respectively.

Results

Reading direction

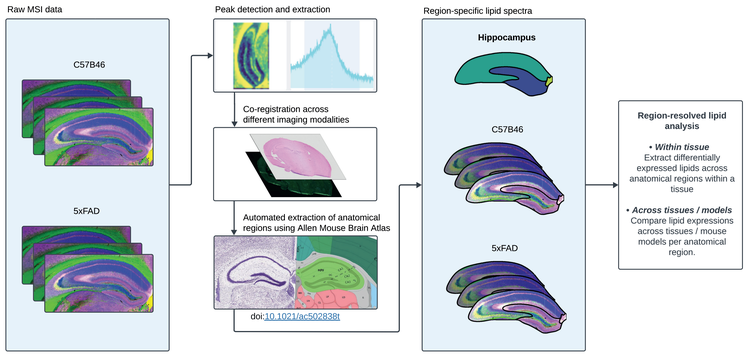


Figure 1: Workflow
Workflow illustrating the process of registering experimental MSI data with the different imaging modalities and AMBA.

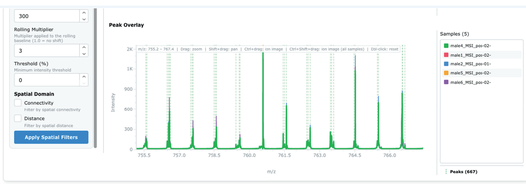
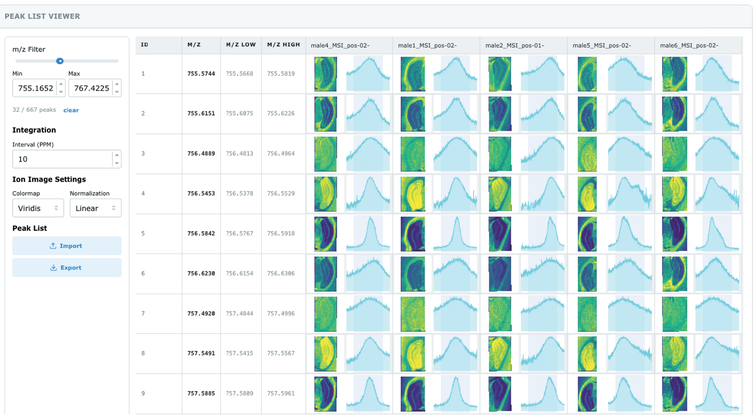
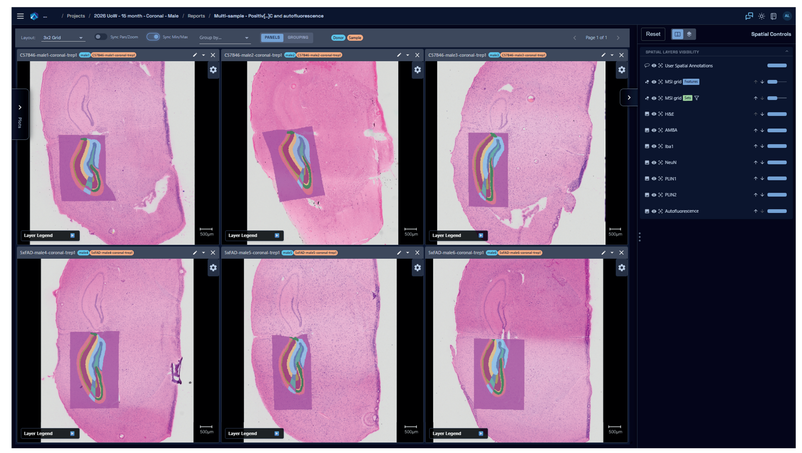


Figure 2: Untargeted peak detection in Weave.
Weave's novel untargeted peak picking tool provides efficient and in depth feature extraction for small and large scale projects. Users can inspect intensity distributions across peaks to evaluate signal consistency and biological relevance.





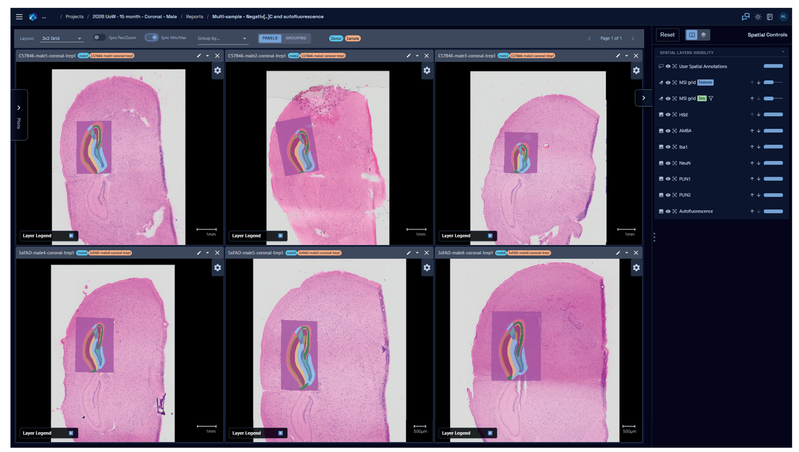


Figure 3: Web-based interactive reports to explore data on a cohort level.
Weave allows simultaneous viewing of a sample cohort with associated datasets. The cohort sample metadata is used to create intuitive layouts. All reports are web-based, which allows findings to be readily shared with study collaborators (e.g. neurobiologists, pathologists), facilitating collaborative analysis. These reports shows six samples; three C57Bl/6 control samples (top row) and three 5xFAD samples (bottom row) for (A) positive mode and (B) negative mode measurements, with AMBA annotations of the HF overlaid onto the sample H&E images.

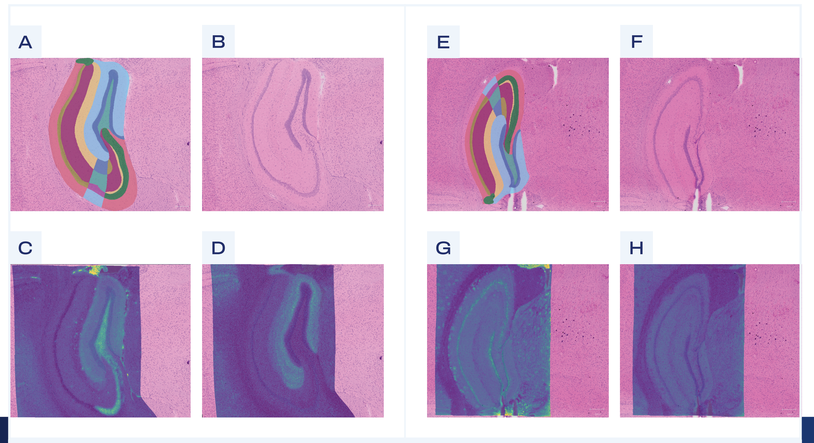


Figure 4: High-spatial resolution MALDI-MSI enables mapping of lipid signals to discrete histological regions.
Zoom of the the hippocampal formation (A & E) with and (B & F) without annotations from the AMBA overlaid.
(C) m/z 724.523 is most highly expressed in the molecular and polymorph layers of the dentate gyrus and the stratum lacunosum and pyramidal layers of the CA3.
(D) m/z 1283.815 is most highly expressed in the molecular layers of the dentate gyrus and the CA1 and CA2 strata lacunosum and moleulare.
(G) m/z 885.548 is expressed across the entire HF with highest expression in the granule cell layer of the dentate gyrus and pyramidal layers of the CA1 and CA2.
(H) In contrast, m/z 836.535 is expressed across the entire HF with low expression in the granule cell layer of the dentate gyrus and pyramidal layers of the CA1 and CA2.

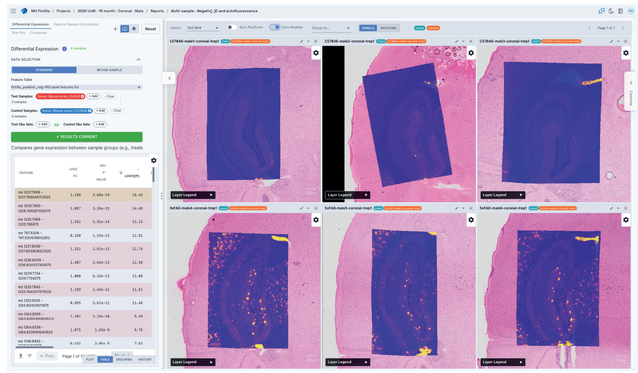


Figure 5: Amyloid-β plaque microenvironment of the HF in 5xFAD mice have a distinct lipid signature.
Comparison of control and AD mice identified a number of significantly differentially expressed lipids with punctate distribution patterns matching those of AD plaques.

Conclusion

- 5-micron MALDI-MSI resolves distinct lipid signatures in hippocampal subregions and plaque microenvironments in mouse model of AD.
- Weave's peak detection pipeline allows interactive, in-depth extraction of MSI features, scaling for large cohorts and datasets.
- Allen Mouse Brain Atlas integration allows direct, standardized comparison of lipid signals in fine-grained spatial regions, both within and across tissues and animals.

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