A Novel 3D Imaging Pipeline for Analyzing Efficacy of Compounds on Amyloid-Beta Plaque Dynamics in Pre-clinical Alzheimer's Disease Animal Models

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Introduction

Amyloid-beta (Aβ) plaque deposition in the brain represents a significant hallmark of Alzheimer's Disease (AD). Standard laboratory approaches assessing Aβ lack the ability to provide region-specific quantitation of Aβ with high-throughput whole-organ imaging. Furthermore, many Aβ analyses require destructive homogenization of tissue, preventing secondary analysis. We developed a novel Serial Two-Photon Plus (STP²) pipeline to quantify Aβ plaque progression and depression as a function of brain region, resulting in indexed brain sections for secondary analysis using MALDI HiPLEX-IHC with imaging mass spectrometry (IMS).

Methods

Tissue Preparation: Aβ plaques in an AD mouse model at four time points (t= 2, 3, 4, 6 months; n= 8 male, 8 female) were labelled with an intra-peritoneal injection of 0.5 mg/kg of methoxy-X04. Samples were excised and embedded in an agarose block and polymerized in an embedding matrix to provide stability for sectioning in the TissueCyte Serial Two-Photon (STP²) pipeline **(Figure 1)**.

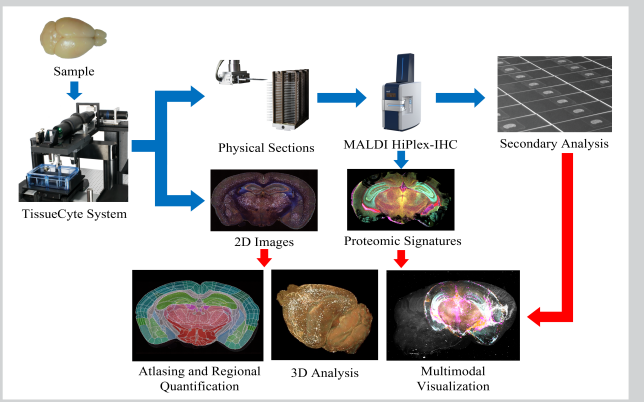

Figure 1. TissueVision's Serial Two-Photon Plus (STP²) pipeline.

STP² Imaging: Brain samples were sectioned serially with the STP² pipeline and mapped to the Allen Reference Atlas **(Figure 2)**. Imaging produced channels corresponding to autofluorescene (channel 1, channel 2) and methoxy-X04 (channel 3).

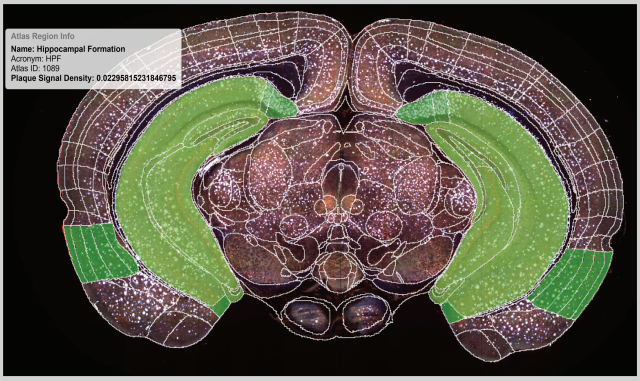

Figure 2. STP² section with registered atlas overlay. Plaque signal density is computed per atlas region. Region and quantitative results for the hippocampal formation are highlighted.

Secondary Imaging: Secondary analysis of DyLight-594 conjugated lectin for vasculature labelling (channel 1) and IBA-1 labelled microglia (channel 2) was performed on select physical sections. Additional sections were analyzed using AmberGen MALDI HiPLEX-IHC and Bruker Daltonics IMS for multiplex proteomic analysis.

Results

Plaque Analysis: The analysis of the methoxy-X04 tagged plaque distribution revealed distinct spatial-temporal changes across the whole-brain datasets of AD mouse models. STP² imaging, combined with Allen Reference Atlas CCFv3 mapping, allowed for targeted evaluation of Aβ plaques and a comparison of regional molecular changes occurring with age or treatment. Using data from the 4 time points, we are able to observe a clear progressive increase in plaque accumulation in regions such as the hippocampal formation, thalamus, and cortical subplate, providing a baseline for targeted additional analysis.

Proteomic Analysis: Using AmberGen MALDI HiPLEX-IHC, fourteen (14) protein signatures were measured for a set of extracted brain sections. Ion images for each molecule were aligned to the corresponding STP² imaged section through a semi-automated registration pipeline **(Figure 3)**. The average signal intensity of each ion was assessed quantitatively for all processed 2D sections and summarized across major 3D brain regions in the Allen CCF **(Figure 4)**. Proteomic signatures can be integrated within two-photon volumetric data to understand the full 3D spatial context of these molecules in the brain **(Figure 5)**.

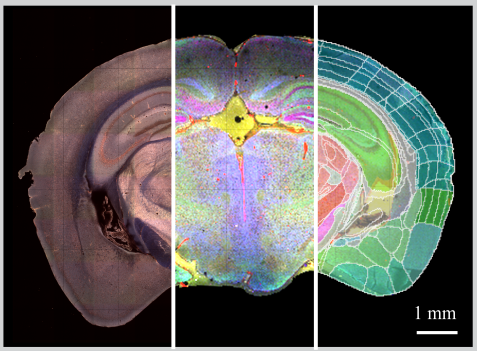

Figure 3. STP² imaged section (left) with aligned MALDI HiPLEX-IHC proteomic signatures (center) and mapped regions from the Allen Brain Atlas (right).

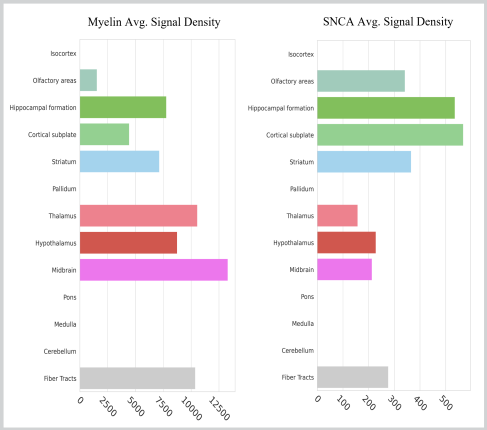

Figure 4. Average signal density for Myelin (left) and SNCA (right) within the processed MALDI HiPLEX-IHC sections for major brain regions.

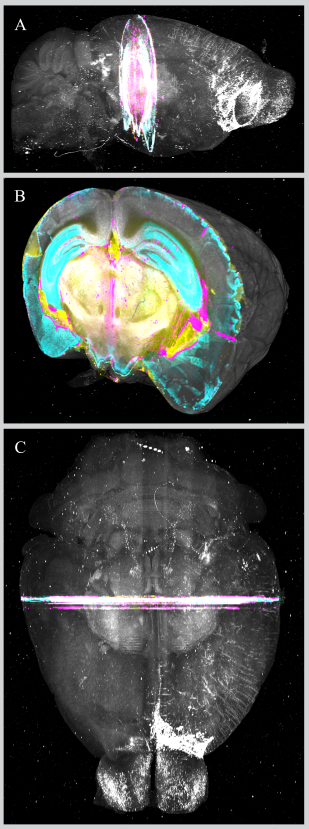

Figure 5. Integrated view of MALDI HiPLEX-IHC data with STP² 3D volume with (a) sagittal (b) coronal, and (c) transverse views.

Conclusion

We demonstrated the capacity to map the progression of AD pathology in AD mouse models by quantifying the density distributions of Aβ plaques at defined time points. This technique reveals the colocalization of vasculature and Aβ, providing a way to assess cerebral amyloid angiopathy (CAA).Multiplexed proteomic imaging using AmberGen's MALDI HiPLEX-IHC and Bruker Daltonics IMS expands the analytical capabilities of whole-organ imaging. The mapping of proteomic information and secondary analysis into the 3D volumetric STP² data gives spatial context to features identified within targeted anatomical areas. Overall, this novel technology has great promise for producing translatable pre-clinical animal disease model data for drug discovery.