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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Poster: ThP 552
Abstract: 312475

Purpose

- 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 (STP^2) 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 the well characterized 5XFAD and SAA mouse models of AD were labelled with an intra-peritoneal injection of 0.5 mg/kg of methoxy-X04. Twenty-four (24) hours after injection, mice were euthanized with 0.1 mL/10 g ketamine-xylazine and perfused with 1× PBS and 4% PFA. Intact brains were then dissected and post-fixed in 4% PFA for 12–24 h at 4°C. Samples were embedded in an agarose block and polymerized in an embedding matrix to provide stability for sectioning.

STP^2 Imaging

Brain samples were sectioned serially with either 50 or 100 um thickness and imaged with a TissueCyte Serial Two-Photon Plus (STP^2) system **(Figure 1)**. With an excitation wavelength of 780 nm, autofluorescence of anatomical features could be visualized within channel 1 (emission spectrum > 560 nm) and channel 2 (500-550 nm). Methoxy-X04 labelling was visualized primarily within channel 3 (< 500 nm).

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 **(Figure 2)**.

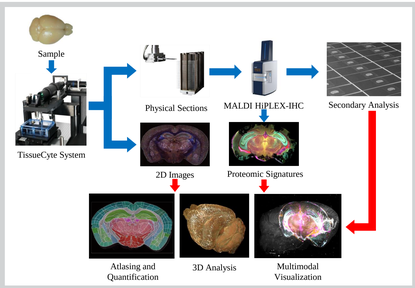

Figure 1. TissueVision's Serial Two-Photon Plus (STP^2) pipeline, enhanced by secondary processing with Bruker Daltonics IMS and AmberGen MALDI HiPLEX-IHC.

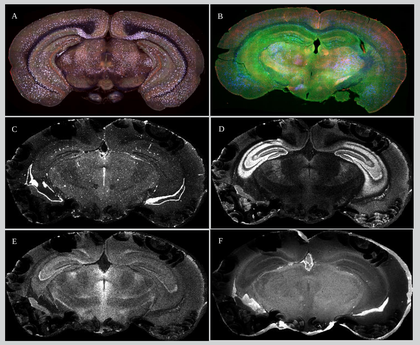

Figure 2. STP^2 and secondary analysis images.
(a): STP^2 with autofluorescence (red; green) and plaques (blue).
(b): Secondary analysis with DyLight-594 lectin (red), IBA-1 (green), and Methoxy-X04 (blue).
(c-f): MALDI HiPLEX-IHC ion images of (c) GLUT-1, (d) SNCA, (e) pTau, and (f) Myelin.

Results

Atlas Registration

Each brain was registered to the Allen Common Coordinate Framework (CCFv3) average template brain, and custom image analysis algorithms were developed to compute the density of Methoxy-X04 labelled plaques across samples for all regions annotated in the Allen Mouse Brain Reference Atlas **(Figure 3)**.

Figure 3. STP^2 section with registered atlas overlay. Plaque signal density is computed per atlas region. Region and quantitative results for the hippocampal formation are highlighted.

Plaque Analysis

The analysis of plaque distribution in the 5XFAD and SAA mouse models revealed distinct spatial-temporal changes across the whole-brain datasets. STP^2 imaging, combined with CCFv3 mapping, allowed for targeted evaluation of Aβ plaques and a comparison of regional molecular changes occurring with age or treatment. Using data from 4 time points, we are able to observe a progressive increase in plaque accumulation in regions such as the hippocampal formation, thalamus, and cortical subplate, providing a baseline for targeted additional analysis **(Figure 4)**.

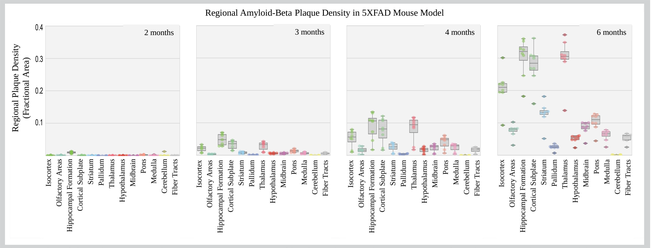

Figure 4. Plots of plaque density in major brain regions across time points of female 5XFAD mice, demonstrating a significant density accumulation (* p < 0.05).

Proteomic Analysis

Using AmberGen MALDI HiPLEX-IHC staining, fourteen (14) protein signatures, including Aβ42, GLUT-1, pTau, Myelin, Neurogranin, SNCA, and Synapsin, were imaged using mass spectrometry for a set of extracted brain sections. Ion images for each molecule were aligned to the corresponding STP^2 imaged section through a semi-automated registration pipeline **(Figure 5)**. 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 6)**. Proteomic signatures can be integrated within two-photon volumetric data to understand the full 3D spatial context of these molecules in the brain **(Figure 7)**.

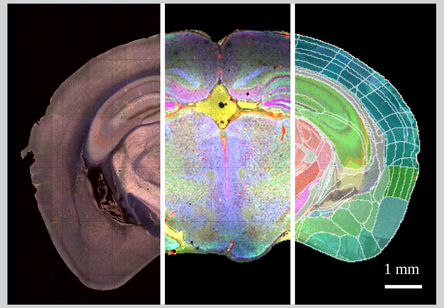

Figure 5. STP^2 imaged section (left) with aligned MALDI HiPLEX-IHC proteomic signatures (center) and mapped regions from the Allen Brain Atlas (right).

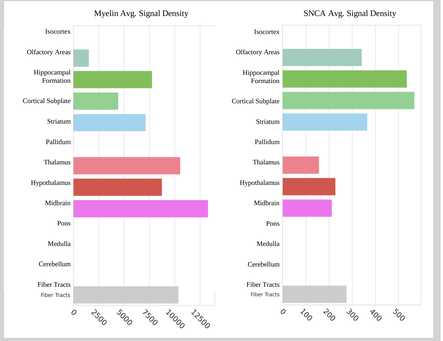

Figure 6. Average signal density for Myelin (left) and SNCA (right) within the processed MALDI HiPLEX-IHC sections for major brain regions.

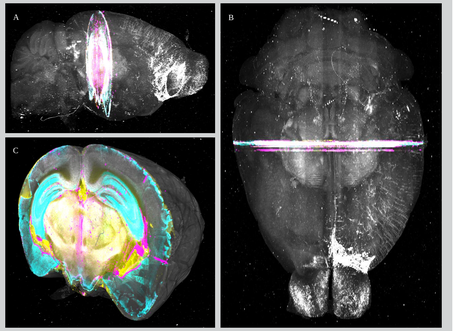

Figure 7. Integrated view of MALDI HiPLEX-IHC data with STP^2 3D volume.
(a): Sagittal view of sample with MALDI sections highlighted in context.
(b): Transverse view of sample with MALDI sections highlighted in context.
(c): Coronal view of sample, digitally sliced at first MALDI section.

Conclusions

- We demonstrated the capacity to map the progression of AD pathology in the 5XFAD and SAA 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 STP^2 data gives spatial context to features identified within targeted anatomical areas.
- This novel technology has great promise for quantifying the spatial-temporal Aβ plaque efficacy of AD animal models, and for producing translatable pre-clinical AD data for drug discovery. The high sensitivity and precision of the STP^2 platform can benefit region-specific disease progression compared to standard laboratory approaches.