Imaging Beyond the Slide : Novel Whole-Brain Serial Two-Photon Imaging and Spatial Proteomics in Pre-Clinical Alzheimer's Disease Animal Models

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Introduction

Many molecular-level studies of disease and homeostasis require physiological contextualization through imaging to gain insight into biomarker relevance. However, in most cases for ex-vivo molecular imaging, this visualization occurs in two-dimensions as three-dimensional techniques are not widely available.

Here we show application of 3D proteomics imaging in Alzheimer's disease models of brains to underscore the importance of protein location.

Methods

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. Samples were embedded in an agarose block and polymerized in an embedding matrix to provide stability for sectioning. Brain samples were sectioned serially with either 50 or 100 um thickness and imaged with a TissueCyte Serial Two-Photon Plus (STP2) system (Figure 1). Additional sections were analyzed using AmberGen MALDI HiPLEX-IHC and Bruker;s timsTOF fleX imaging platform for multiplex proteomic analysis .

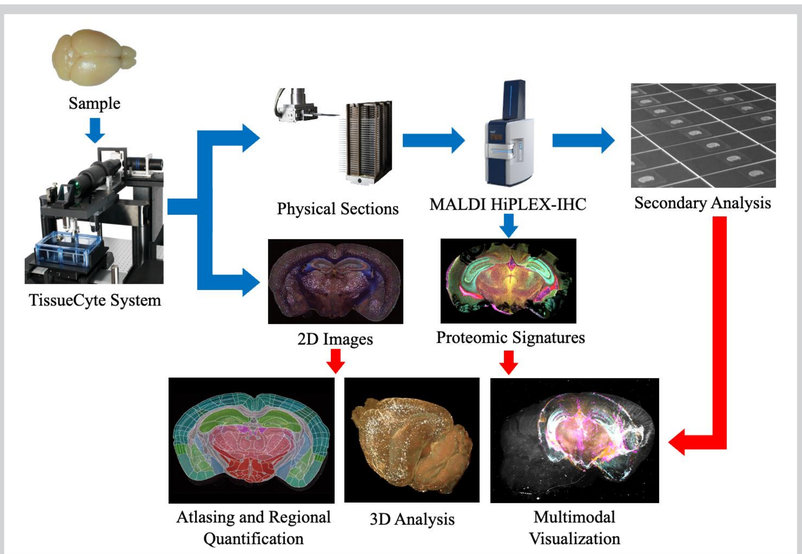

Figure 1. TissueVision's Serial Two-Photon Plus (STP[2]) pipeline, enhanced by secondary processing with Bruker Daltonics IMS and AmberGen MALDI HiPLEX-IHC.

Results

- 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).

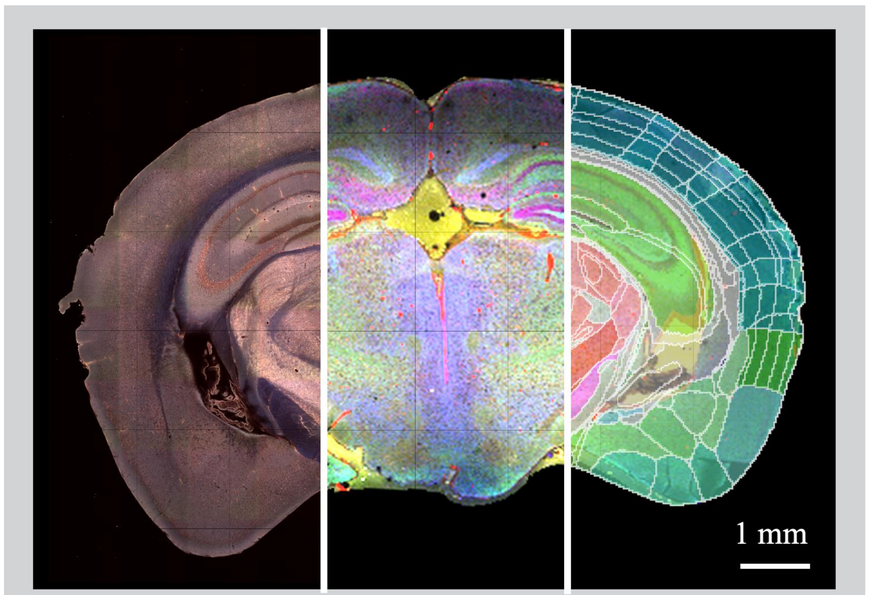

Figure 2. STP[2] imaged section (left) with aligned MALDI HiPLEX-IHC proteomic signatures (center) and mapped regions from the Allen Brain Atlas (right).

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 (timsTOF fleX) 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 2). 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 3) Proteomic signatures can be integrated within two-photon volumetric data to understand the full 3D spatial context of these molecules in the brain (Figure 4).

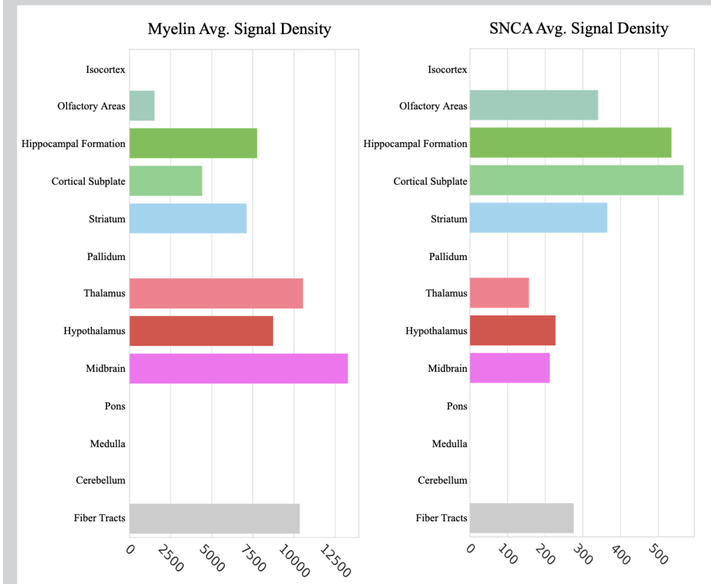

Figure 3. Average signal density for Myelin (left) and SNCA (right) within the processed MALDI HiPLEX-IHC sections for major brain regions.

Summary

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 Imaging Mass Spectrometry 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.

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 STP2 platform can benefit region-specific disease progression compared to standard laboratory approaches.

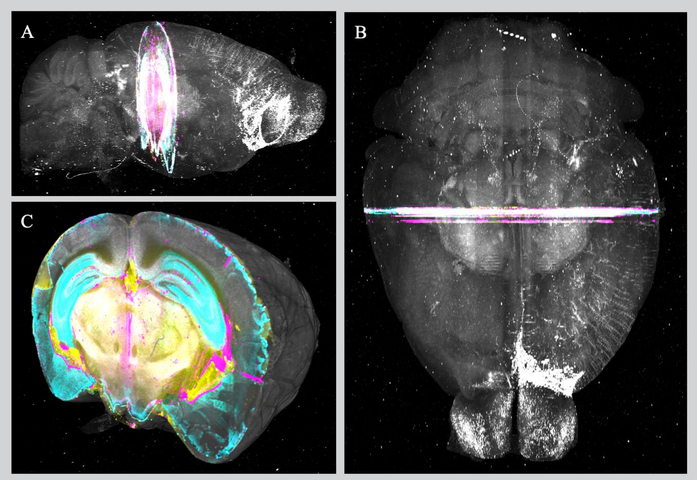

Figure 4. Integrated view of MALDI HiPLEX-IHC data with STP[2] 3D volume.
(a): Sagittal view of sample with MALDI sections highlighted in context.
(b): Transverse view of sample with MALDI sections highlighted in context.
(c): Coronal view of sample, digitally sliced at first MALDI section.