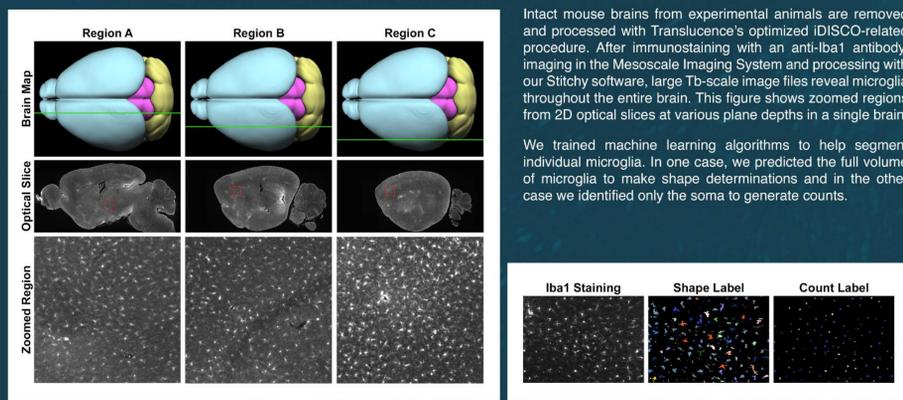# WHOLE BRAIN MEASUREMENT OF PLAQUES, MICROGLIA, α-SYNUCLEIN AND TYROSINE HYDROXYLASE IN AD AND PD MODEL MICE

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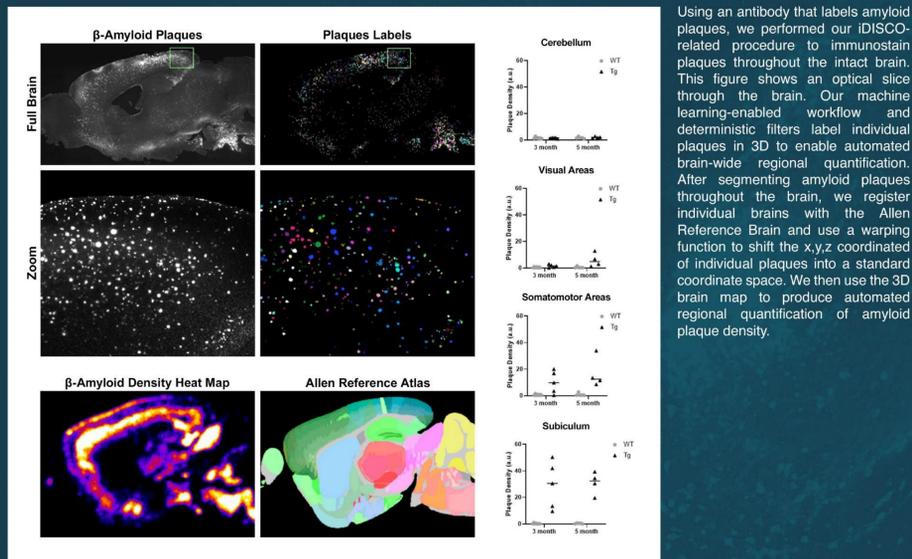
Introduction

- Alzheimer's disease and Parkinson's disease involve complex, brain-wide pathology, highlighting the need for more comprehensive tools to evaluate disease-related changes across the intact brain.
- Traditional 2D histology is constrained to thin sections and select regions, offering only a partial and potentially biased assessment of disease-associated biology.
- 3D tissue clearing combined with light-sheet imaging enables whole-brain visualization at cellular resolution across intact tissues.
- When paired with AI-powered analysis, this approach enables unbiased quantification of amyloid plaques, microglial activation, TH-positive staining, and α-synuclein cell bodies and fibrils across hundreds of brain regions.
- In this poster, we demonstrate how this pipeline has been adapted for AD and PD mouse models. In AD (5xFAD) mice, we segment microglia, plaque-associated microglia, and amyloid plaques. In PD models (MPTP, and PFF-injected mice), we use TH and α-synuclein immunostaining to map dopaminergic degeneration and α-synuclein pathology across the intact brain.

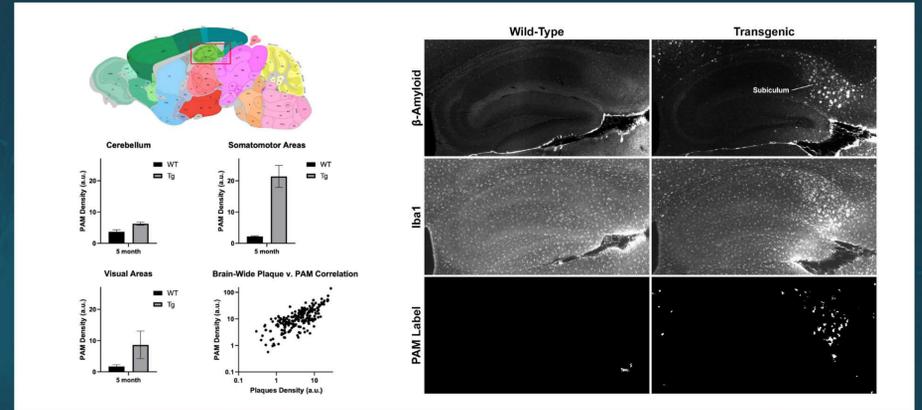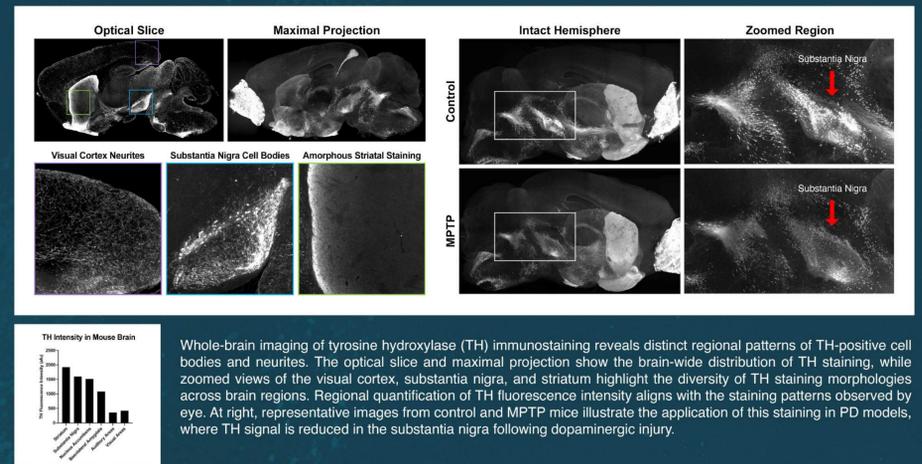## Whole-Brain Immunostaining of Iba1(+) Microglia

Intact mouse brains from experimental animals are removed and processed wth Translucence's optimized iDISCO-related procedure. After immunostaining with an anti-Iba1 antibody, imaging in the Mesoscale Imaging System and processing with our Stitchy software, large Tb-scale image files reveal microglia throughout the entire brain. This figure shows zoomed regions from 2D optical slices at various plane depths in a single brain.

We trained machine learning algorithms to help segment individual microglia. In one case, we predicted the full volume of microglia to make shape determinations and in the other case we identified only the soma to generate counts.

Brain-Wide Regional Quantification of β-Amyloid Plaques in 5xFAD Mouse Brains

Using an antibody that labels amyloid plaques, we performed our iDISCO-related procedure to immunostain plaques throughout the intact brain. This figure shows an optical slice through the brain. Our machine learning-enabled workflow and deterministic filters label individual plaques in 3D to enable automated brain-wide regional quantification. After segmenting amyloid plaques throughout the brain, we register individual brains with the Allen Reference Brain and use a warping function to shift the x,y,z coordinated of individual plaques into a standard coordinate space. We then use the 3D brain map to produce automated regional quantification of amyloid plaque density.
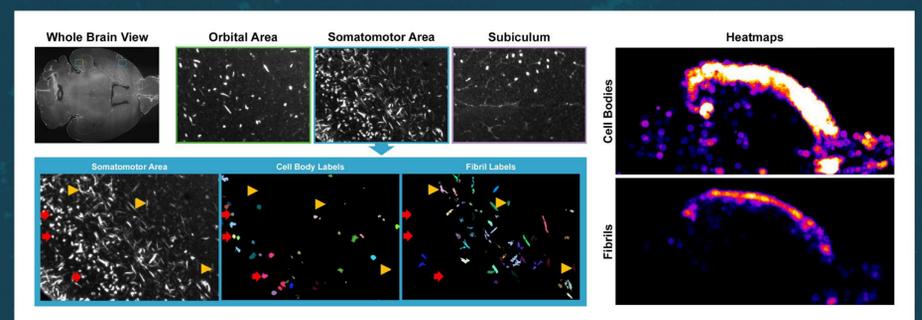
Colocalization of Microglia and Amyloid Plaques in 5xFAD Mice

In WT mice, individual homeostatic microglia are spaced out and have a ramified morphology with processes surveying the surrounding region. In diseased and inflamed states, activated microglia take on a condensed morphology. In 5xFAD Tg mice, microglia move to colocalize with β-amyloid plaques. Our automated methods can identify and count plaques and plaque-associated microglia (PAM) throughout the brain.

Brain-Wide Quantification of Plaque-Associated Microglia

In 5xFAD mice, Iba1(+) microglia colocalize with Amyloid plaques and become activated, retracting processes and taking on a distinct shape not seen in WT mouse brains. We trained our AI-powered workflows to selectively detect these activated plaque-associated microglia (PAMs) and not the majority of the Iba1(+) microglia in the brain that are in a homeostatic state. The PAM levels in Cerebellum, Visual and Somatomotor Areas are consistent with the Amyloid levels detected in the same mice. Across 100's of brain area the data from our Amyloid and PAM whole-brain quantification workflows are strongly correlated.

Whole-Brain Quantification of Tyrosine Hydroxylase in a PD Model

Whole-brain imaging of tyrosine hydroxylase (TH) immunostaining reveals distinct regional patterns of TH-positive cell bodies and neurites. The optical slice and maximal projection show the brain-wide distribution of TH staining, while zoomed views of the visual cortex, substantia nigra, and striatum highlight the diversity of TH staining morphologies across brain regions. Regional quantification of TH fluorescence intensity aligns with the staining patterns observed by eye. At right, representative images from control and MPTP mice illustrate the application of this staining in PD models, where TH signal is reduced in the substantia nigra following dopaminergic injury.

Whole-Brain Quantification of α-Synuclein Pathology in a PFF Model

Whole-brain detection of α-synuclein pathology in PFF-treated mice. A whole-brain overview shows widespread α-synuclein immunoreactivity, with zoomed views of the orbital area and subiculum illustrating dense regional deposits of α-synuclein+ cell bodies and fibrils. In the somatomotor area, machine-learning workflows separately label α-synuclein-positive cell bodies and fibrillar aggregates in the same fluorescence channel. Atlas-aligned heatmaps depict the spatial density of α-synuclein-positive cell bodies and fibrils, enabling region-specific assessment of pathology burden across the intact brain.

Summary

- In 5xFAD mice, whole-brain immunostaining and quantification revealed widespread β-amyloid plaque pathology across anatomically distinct brain regions.
- Iba1 quantification showed that microglia colocalize with amyloid plaques and adopt an activated morphology, enabling selective detection and quantification of plaque-associated microglia (PAMs).
- In PD models, whole-brain TH immunostaining revealed diverse regional patterns of TH-positive cell bodies and neurites and demonstrated reduced TH signal in injured animals relative to controls.
- Whole-brain α-synuclein immunolabeling in PFF-treated mice combined with AI-powered quantification of α-synuclein-positive cell bodies and fibrils, highlighted the regional distribution of PD pathology.
- Together, these data demonstrate the value of our integrated tissue clearing, light-sheet imaging, and AI-powered analysis pipeline for unbiased whole-brain measurement of amyloid pathology, microglial activation, dopaminergic degeneration, and α-synuclein aggregation.