WHOLE BRAIN MEASUREMENT OF AMYLOID PLAQUES AND NEUROINFLAMMATION IN AD MODEL MICE

TRANSLUCENCE BIOSYSTEMS

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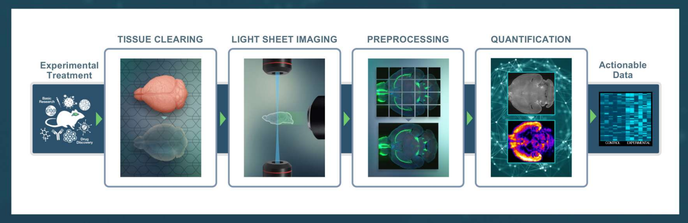
Introduction

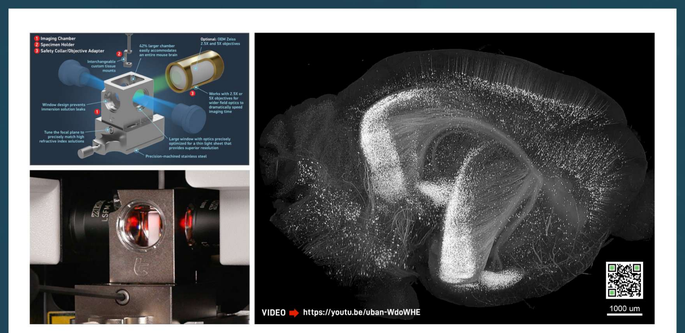
- Recent advances in optical clearing and light sheet imaging have opened exciting new avenues for brain-wide, cellular resolution immunostaining at the forefront of a dimensional shift from 2D to 3D histology.
- Traditional histological methods have been a mainstay of neuroscience research dating back more than 100 years.
- Despite great advances in tissue labeling and imaging technology, until very recently imaging more than a few hundred microns into a tissue has required slicing and mounting on slides.
- When looking for read-outs of genetic or pharmacological manipulations that affect the entire brain, this traditional focused approach is lacking, forcing researchers to look at specific brain regions of interest.
- To help solve this issue, we have developed an optimized iDISCO-based tissue clearing method and with our Mesoscale Imaging System for ZEISS Lightsheet microscopes, we can image cellular-resolution immunoreactivity across entire intact mouse brains in ~25 min.
- Further, our machine learning-enabled 3TK software identifies individual immunostained cells and objects throughout the brain and aligns them to the Allen Reference Atlas to produce an unbiased, regionalized read-out of cellular patterns across 100's brain areas.
- We have applied this technology to quantify microglia and β-amyloid plaques in the 5xFAD mouse model of Alzheimer's Disease.

Pipeline For the Generation of 3D Anatomics Data



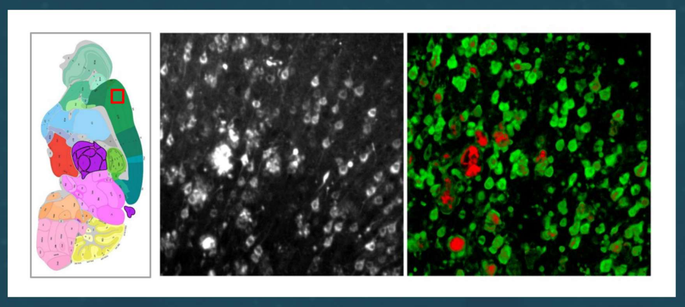
Mice are experimentally manipulated, including treatment with small molecules, antibody therapeutics, cellular therapeutics and gene therapies. Fixed and perfused brains are cleared and immunostained intact and then imaged on the ZEISS Lightsheet Z.1 microscope. After tiling and stitching in our Stitchy software, our AI-powered Voxels software produces actionable anatomics data.

Light Sheet Imaging of Optically-Cleared Mouse Brains



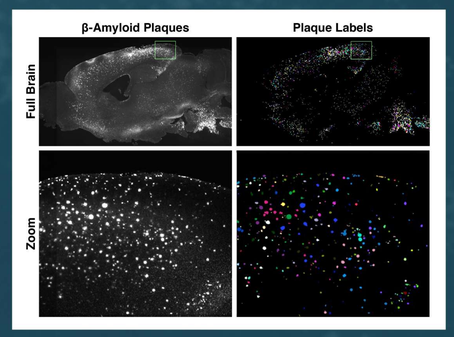
Our Mesoscale Imaging System™ adapts the ZEISS Lightsheet Z.1 and 7 microscopes for imaging large tissues in high refractive index solutions with mesoscale optics. This Thy1-GFP brain was imaged in ~25 minutes.

Using Machine Learning to Distinguish Cell Body and Plaque Amyloid Staining



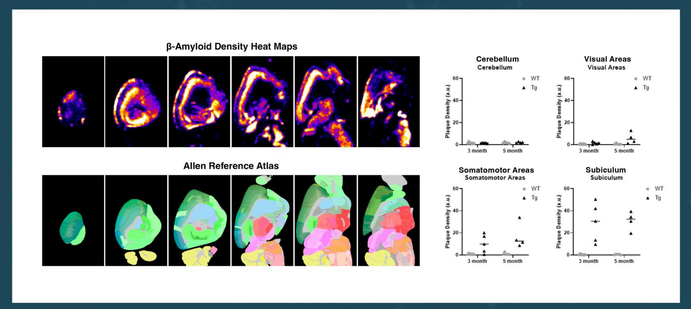
The 6E10 monoclonal anti-Amyloid antibody stains conformers of amyloid found in both plaques and neuronal cell bodies. Machine learning algorithms were trained to determine the probability of individual voxels in the image being part of a plaque or a cell body. The plaque algorithm output is in red and the cell bodies in green.

Labeling of 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. The labels shown are represented in 2-dimensions but all of the labeling and quantification is done in 3-dimensions.

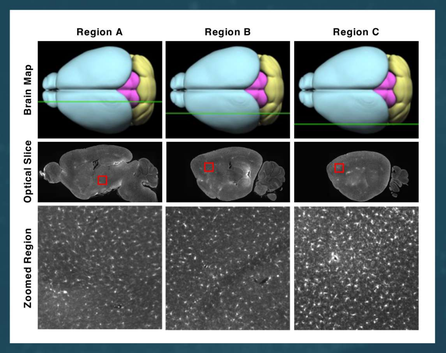
Brain-Wide Regional Quantification of β-Amyloid Plaques



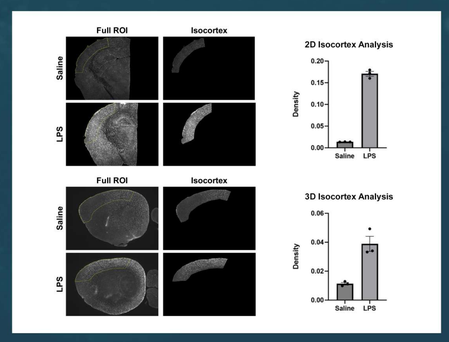
After segmenting amyloid plaques throughout the brain, we register individual brains with the Allen Reference Brain and use a warping function to shift the x,y,z coordinates of individual plaques into a standard coordinate space. We then use the 3D brain map to produce automated regional quantification of amyloid plaque density.

Whole-Brain Immunostaining of Iba1(+) Microglia

Intact mouse brains from experimental animals are removed and processed with 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.

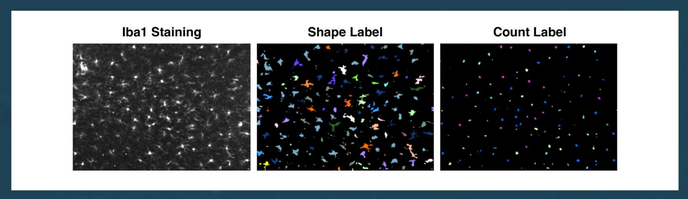


3D Whole Brain Quantification is Comparable to Traditional 2D IHC



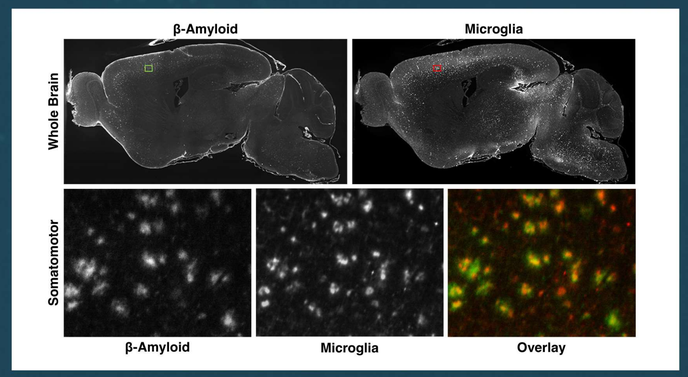
Comparative analysis using traditional 2D and advanced 3D imaging techniques consistently reveals that LPS treatment significantly increases Iba1 marker density in the Isocortex. Both 2D and 3D analyses prove adept at detecting these treatment effects, offering distinct advantages: 2D analysis excels in localized effect detection, making it ideal for targeted screening, while 3D analysis provides a holistic view of brain-wide regional changes, confirming the utility of both methods in neuroinflammatory research. While this analysis indicates a slightly better assay window for 2D IHC, these comparisons were not on identical portions of the Isocortex and a clear gradient in the Iba1 effect is seen throughout the Isocortex. Choosing a different region for the 3D analysis may produce similar results to the 2D analysis.

Machine Learning-Enabled Microglia Shape and Count Workflows



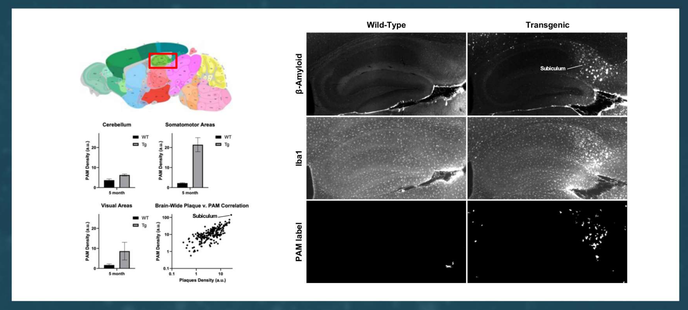
We trained machine learning algorithms to help segment individual microglia. In one case, we predicted the full volume of microglia to make shape determinations and in the other case we identified only the soma to generate counts. The labels shown are represented in 2-dimensions but all of the labeling and quantification is done in 3-dimensions.

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.

Summary

- Our AI-powered workflow can distinguish between β-amyloid in plaques and neuronal cell bodies, and can generate unbiased anatomically precise brain-wide measurements of Amyloid plaques.
- In LPS-treated mice we have quantified the activation of microglia with traditional 2D immunohistochemistry and with 3D tissue clearing, demonstrating that our AI powered quantification provides brain wide insights that are comparable to measurements made with traditional 2D methods.
- We can also identify Iba1(+) microglia throughout the entire brain. In control vs LPS brains, we have developed two machine learning-enabled workflows; one counts the microglia and the other measures microglial shape.
- In 5xFAD Tg mice, microglia colocalize with β-amyloid plaques. Our automated methods can identify plaque-associated microglia (PAM) throughout the brain and their expression correlates with brain-wide measurements of Amyloid plaques.

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