BRAIN-WIDE QUANTIFICATION OF A BBB-CROSSING ANTIBODY THERAPEUTIC TARGETING AMYLOID PLAQUES

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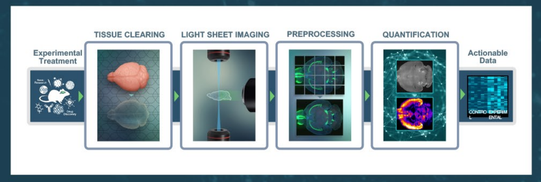
Introduction

- The blood–brain barrier (BBB) presents a significant hurdle for antibody therapeutics, often limiting their ability to penetrate the brain and engage pathological targets such as β-Amyloid plaques.
- Traditional 2D histology is constrained to thin sections and select regions, offering only a partial and potentially biased assessment of antibody biodistribution.
- 3D tissue clearing combined with light sheet imaging enables whole-brain visualization at cellular resolution, capturing the complete spatial distribution of both pathology and therapeutic antibodies.
- Combining whole-brain 3D tissue clearing and imaging with AI-powered analysis enables unbiased measurement of antibody penetration, plaque colocalization, and regional differences across hundreds of brain regions.
- Here, in an AD mouse model we apply the pipeline to evaluate a Brainshuttle™-enabled bispecific antibody designed to bind to TfR1 to transport across the BBB and target amyloid plaques.

Pipeline For the Generation of 3D Anatomics Data

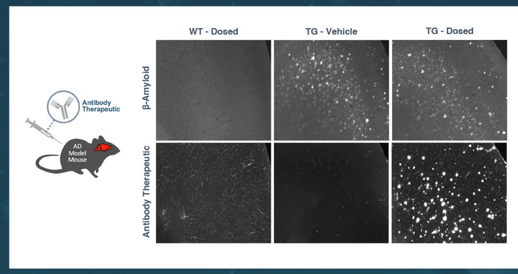


Mice are experimentally manipulated, including treatment with small molecules, antibody therapeutics, cellular therapeutics and gene therapies. Fixed and perfused brains are cleared and immunostained intact and then imaged on the ZEISS Lightsheet Z.1 microscope. After tiling and stitching in our Stitchy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

Light Sheet Imaging of Optically-Cleared Mouse Brains

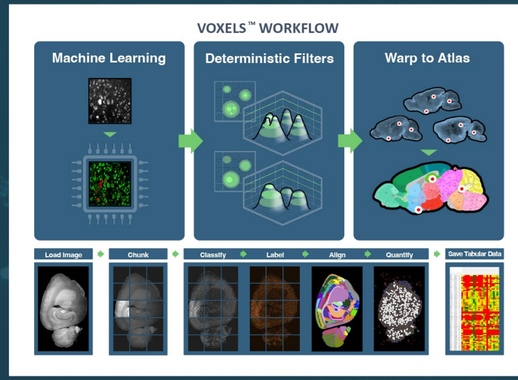
Our Mesoscale Imaging System™ adapts the ZEISS Lightsheet Z.1 and 7 microscopes for imaging large tissues in high refractive index solutions with mesoscale optics. This Thy1-GFP brain was imaged in ~25 minutes.

Visualization of β-Amyloid Plaques and Human IgG Distribution in AD Model Mice



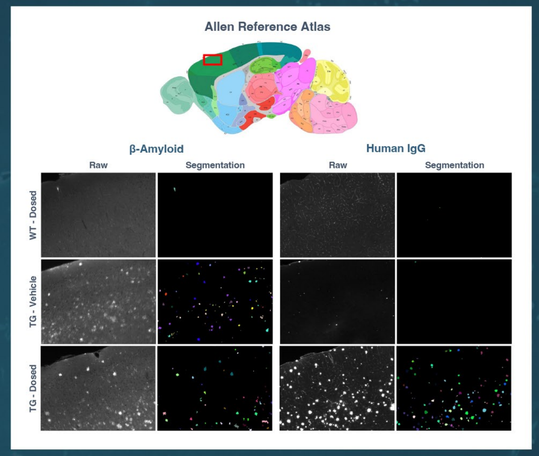
Raw fluorescent optical slice images of β-Amyloid plaques (top row) and human IgG therapeutic distribution (bottom row) in wild-type (WT) and transgenic (TG) Alzheimer's disease model mice. In WT animals dosed with the Brainshuttle™-enabled antibody therapeutic antibody, hIgG can be detected in the vasculature. In TG-vehicle mice, extensive β-Amyloid deposits are present but there was no human IgG signal, as expected. In contrast, TG mice dosed with the antibody therapeutic demonstrated widespread human IgG accumulation colocalizing with β-Amyloid plaques. These results confirm successful blood–brain barrier penetration and target engagement.

Translucence Voxels Workflow for AI-Enabled Analysis



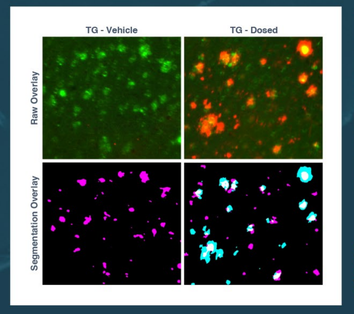
Our Voxels™ Quantification pipeline begins with machine learning–based voxel classification, generating probability maps of objects of interest across whole-brain images. These probability maps are then refined using deterministic filters to produce segmented objects. In parallel, each brain is aligned to a common reference atlas using linear and nonlinear transformations. Segmented objects are then warped into this common atlas space, enabling regional quantification across hundreds of brain regions. Metrics are extracted hierarchically, supporting both region-specific and whole-brain analysis. The Voxels™ quantification pipeline is a fully automated workflow that provides reproducible, high-throughput quantification across diverse imaging datasets, helping scientists turn terabyte-scale datasets into actionable insights

Automated Whole-Brain Segmentation of β-Amyloid and Human IgG Signals



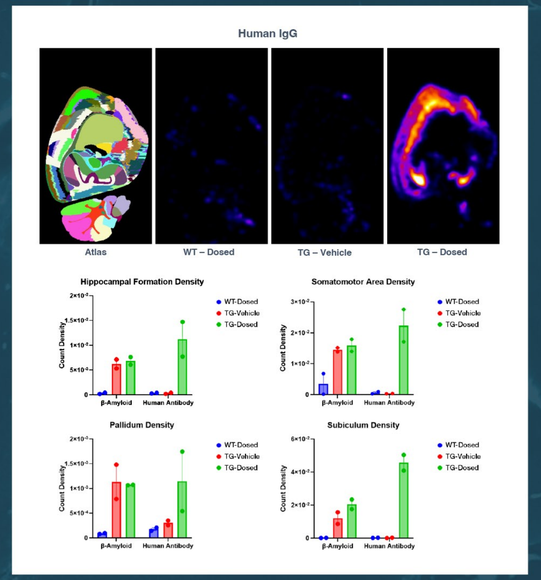
Representative raw and segmented images of β-Amyloid (left) and human IgG (right) staining are shown for wild-type (WT) and transgenic (TG) AD-model mice. All of the cropped images are pulled from the motor cortex, indicated on the Allen Reference Atlas. Raw fluorescence images display expected signal distributions across treatment groups, while segmented overlays demonstrate accurate object detection and signal isolation using the Voxels™ segmentation workflow. These results highlight the robustness of the accuracy of the segmentation pipelines in capturing biological patterns while minimizing background and preserving morphological detail across conditions.

β-Amyloid and Human IgG Signal and Segmentation Colocalization



Representative overlay images show raw fluorescence (top) and corresponding segmentation outputs (bottom) for β-Amyloid and antibody therapeutic signals in vehicle and dosed AD-model mice. The raw images show no human IgG signal in vehicle animals and colocalization β-Amyloid and human IgG signals in dosed animals. Segmentation results, generated using our Voxels™ platform, accurately delineate discrete β-Amyloid plaques and antibody-labeled regions. In TG-dosed animals, the segmentation shows clear overlay of the segmented objects, similar to the overlay seen in the raw overlays

Distribution of Segmented Objects in β-Amyloid and Human IgG Channels



Human IgG heatmaps and corresponding regional quantification highlight antibody biodistribution across multiple anatomically defined brain regions in wild-type (WT) and transgenic (TG) AD-model mice. Brains were aligned to the Allen Reference Atlas for the generation of anatomical data. TG mice dosed with the Brainshuttle™-enabled antibody therapeutic exhibited strong antibody accumulation within the hippocampal formation, somatomotor cortex, pallidum, and subiculum, aligning with regions of high β-Amyloid plaque burden. In contrast, WT and TG vehicle-treated animals displayed minimal antibody signal. These findings confirm robust brain penetration and target engagement of the the Brainshuttle™-enabled antibody therapeutic.

Summary

- In wild-type mice dosed with the Amyloid-largeting Brainshuttle antibody, binding to TfR1 resulted in hIgG staining in blood-vessels throughout the brain with minimal exposure in the brain parenchyma.
- In contrast, transgenic brains dosed with the Brainshuttle antibody showed intense human IgG staining that colocalized with amyloid plaques.
- Using our Voxels™ software, we segmented and quantified the human IgG signal and β-amyloid plaques across hundreds of brain regions. Regions with high amyloid plaque burden showed correspondingly elevated levels of the human antibody therapeutic.
- These findings demonstrate the utility of our integrated tissue clearing and analysis platform for monitoring BBB penetration, biodistribution, and target engagement across the entire brain.