BRAIN-WIDE QUANTIFICATION OF AMYLOID PLAQUES, MICROGLIA, AND ANTIBODY THERAPEUTICS IN AD MODEL MICE

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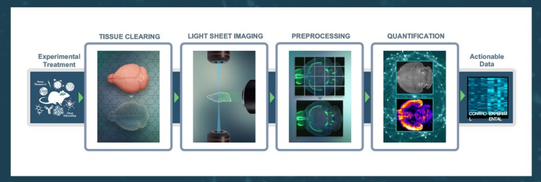
Introduction

- The brain is protected and it is difficult to get large molecule therapeutics into it. Despite this, there have been recent approvals for human IgG based antibody therapeutics like Kisunla™.
- Conventional 2D histology provides only a narrow, biased view of drug distribution, often limited to a few regions of interest and potentially missing the full extent of where a therapeutic penetrates into the brain.
- 3D tissue clearing and light sheet imaging solve these issues by allowing whole-brains to be imaged at cellular resolution, revealing the complete spatial distribution of biomarkers and drugs across 100s of brain regions.
- Combining whole-brain 3D tissue clearing and imaging with AI-powered analysis enables unbiased quantification of Human IgG Antibody therapeutic penetration and target engagement throughout the brain, offering a far more comprehensive assessment than traditional methods.
- In this experiment, we have applied our whole brain 3D tissue clearing and ML-powered quantification techniques to measure a Donanemab (Kisunla™) biosimilar and Amyloid plaques in ARTE10 AD model mice.

Pipeline For the Generation of 3D Anatomics Data

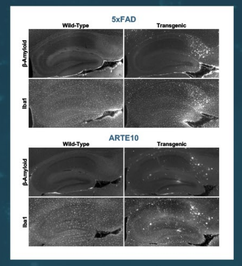


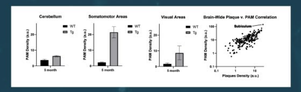
Mice are experimentally manipulated, including treatment with small molecules, antibody therapeutics, cellular therapeutics and gene therapies. Fixed and perfused brains are cleared and immunostained intact and then imaged on the ZEISS Lightsheet Z.1 microscope. After tiling and stitching in our Stitchy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

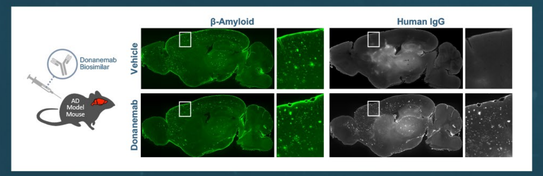
Plaque Associated Microglia Present in Both 5xFAD and ARTE10 AD Model Mice





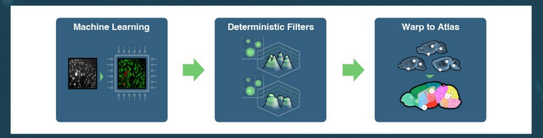
In both 5xFAD and ARTE10 transgenic mouse models, Iba1(+) microglia colocalize with Amyloid plaques and adopt an activated morphology not observed in wild-type brains. Using our machine learning–powered workflows, we selectively detect plaque-associated microglia (PAMs) in 5xFAD mice, distinguishing them from homeostatic Iba1(+) microglia. Regional variation in PAM levels reflects local plaque burden: for example, the cerebellum shows modest plaque and PAM density, while regions like the somatomotor cortex exhibit high plaque load and correspondingly elevated PAM levels. Brain-wide analysis reveals a strong correlation between plaque and PAM density. The ARTE10 AD model was selected for the subsequent Human IgG therapeutic engagement study.

Whole Brain Visualization of β-Amyloid and Donanemab Biosimilar



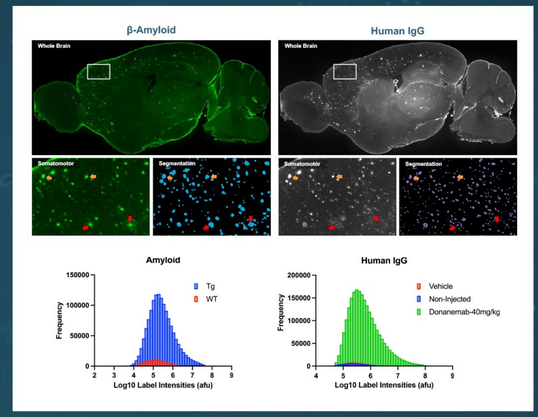
ARTE10 AD model mice were intravenously injected with a biosimilar of Donanemab, a human IgG based antibody therapeutic, or with a Vehicle control. Cleared whole brains were stained and imaged for β-Amyloid (green) and Human IgG (gray). Representative whole-brain optical slices from both treatment groups are shown, with zoomed regions highlighting plaque-rich areas. In Donanemab-treated animals, punctate IgG signal colocalizes with β-Amyloid plaques, indicating successful brain penetration and target engagement. In the vehicle treated animals we did not observe any Human IgG staining.

Translucence Voxels™ Workflow for AI-Enabled Analysis



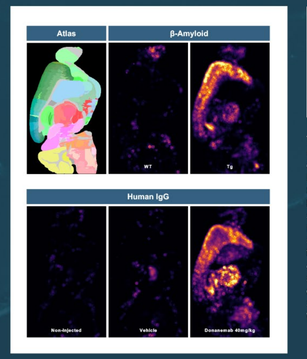
Our Voxels™ Quantification pipeline begins with machine learning–based voxel classification, generating probability maps of objects of interest across whole-brain images. These probability maps are then refined using deterministic filters to produce segmented objects. In parallel, each brain is aligned 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.

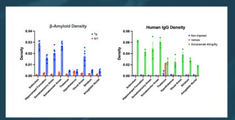
Automated Identification of β-Amyloid Plaques and Human IgG Distribution



Whole-brain and somatomotor cortex images show raw signal and segmentation outputs for β-Amyloid (left) and Human IgG (right). Accurate object detection is observed across both channels. Red arrows mark plaques where Human IgG colocalizes with β-Amyloid. Orange arrows highlight a distinct population of plaques with less localized IgG signal. Histograms show log-transformed intensity distributions of segmented objects, enabling quantitative comparison across genotypes (Amyloid) and treatment groups (Human IgG).

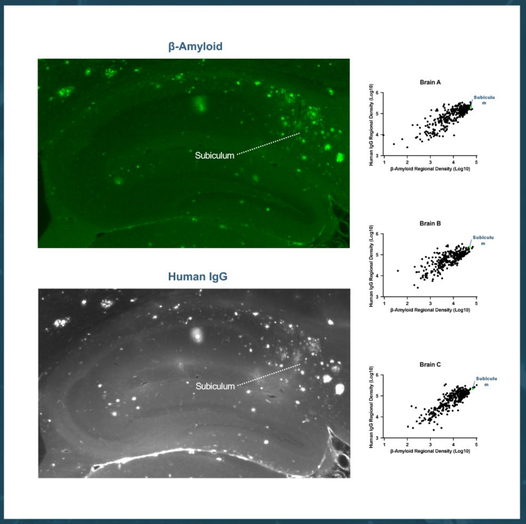
Distribution and Regional Quantification of β-Amyloid Plaques and Human IgG





The panel on the left displays averaged heatmaps representing the spatial distribution of segmented objects in atlas-aligned brain regions. The first image is the reference atlas plane, followed by β-Amyloid (top row) and Human IgG (bottom row) channels. Each heatmap reflects group-level averages across all samples, except for the Vehicle group, which includes only one sample. The graphs on the above show regional quantification grouped by treatment. In the β-Amyloid channel, Tg animals exhibit high object density in the Subiculum and widespread signal across the isocortex. In the Human IgG channel, Donanemab-treated (40mg/kg) animals show strong signal in the Subiculum and Isocortex, with additional signal in the striatum and hindbrain.

Correlation of Segmented Objects ion Tg Animals Dosed with Donanemab (40mg/kg) Across Regions



Raw images of β-Amyloid and Human IgG (left column) demonstrate strong colocalization of plaques and IgG signal in the Subiculum of ARTE10 mice treated with Donanemab biosimilar. Correlation plots (right column) reveal quantitative association between β-Amyloid and Human IgG objects across Tg animals treated with Donanemab. The correlation analysis was restricted to regions with a volume greater than 10,000 voxels. After filtering, 374 regions were included in the analysis.

Summary

- Plaque-associated microglia levels correlate with regional plaque burden in both ARTE10 and 5xFAD mice. These findings informed our use of the ARTE10 model for therapeutic studies.
- Whole-brain 3D imaging of cleared AD model mice revealed widespread distribution of β-Amyloid plaques and human IgG therapeutic (Donanemab) throughout the brain following intravenous drug treatment.
- Automated analysis with our AI-powered Voxels™ software confirmed substantial accumulation of human IgG in β-Amyloid–rich regions of Donanemab-treated transgenic mice, with treated animals showing significantly higher brain IgG levels than untreated controls.
- Regional quantification showed a strong correlation between amyloid plaque density and IgG levels, indicating that the antibody preferentially localizes to areas containing amyloid and is engaging its target (amyloid plaques) across the brain.
- These findings validate our tissue clearing and AI quantification workflow as an effective platform for measuring brain biodistribution and target engagement of CNS antibody therapeutics, demonstrating a powerful new tool for unbiased, brain-wide evaluation of therapeutic efficacy.

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