BRAIN-WIDE MEASUREMENT OF ANTIBODY THERAPEUTIC BIODISTRIBUTION AND TARGET ENGAGEMENT

TRANSLUCENCE BIOSYSTEMS

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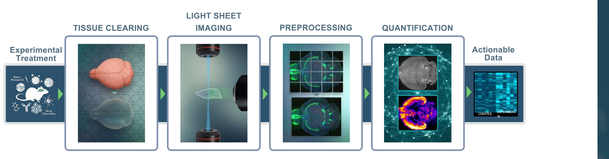
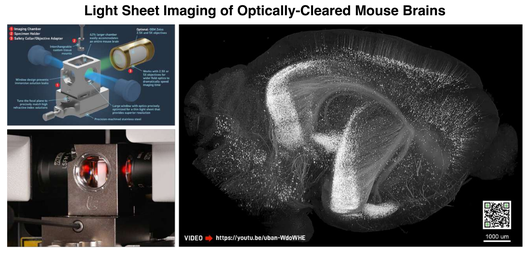
Introduction

- The blood–brain barrier (BBB) heavily restricts large therapeutic antibodies from entering the brain, creating a major challenge for treating CNS diseases like Alzheimer's.
- Conventional 2D histology (thin tissue sections) 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 ML-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.

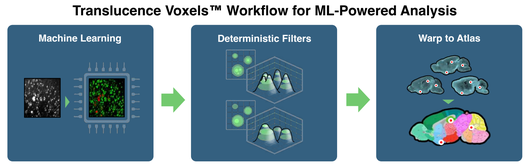
Pipeline For the Generation of 3D Anatomics Data



Light Sheet Imaging of Optically-Cleared Mouse Brains



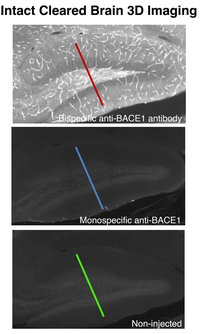
Translucence Voxels™ Workflow for ML-Powered Analysis

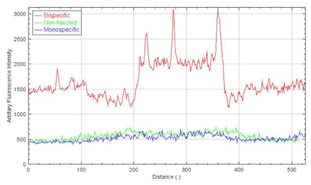


Mice are experimentally manipulated, including treatment with small molecules, antibody therapeutics, cellular therapeutics and gene therapies. Fixed and perfused brains are cleared and immunostained intact and then imaged on the ZEISS Lightsheet Z.1 microscope adapted with our Mesoscale Imaging System™. 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. The Thy1-GFP brain above was imaged in ~25 minutes using this setup. After tiling and stitching in our Stitchy™ software, our machine learning-enabled Voxels™ software produces actionable anatomics data. Voxels 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.

iDISCO Whole-Brain Imaging of IV-dosed anti-BACE1 Antibodies

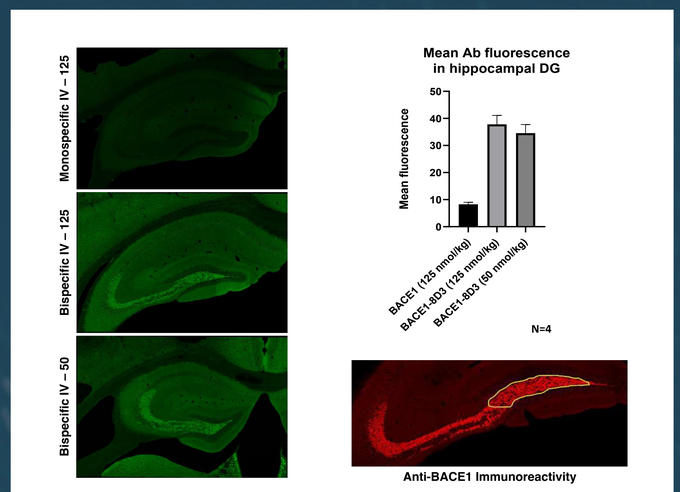
Intact Cleared Brain 3D Imaging





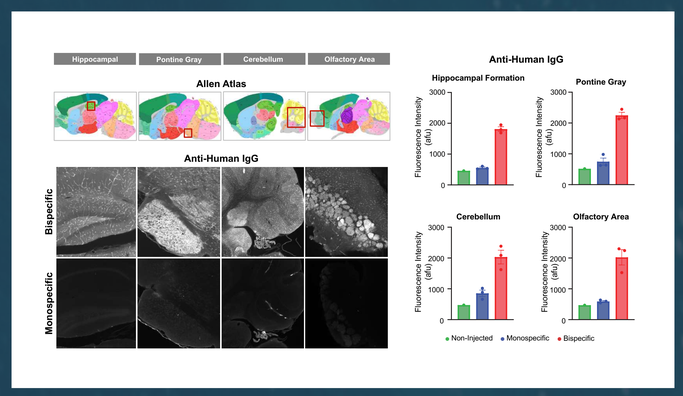
We dosed mice intravenously with various concentrations of a monospecific antibody targeting BACE1 or a bispecific antibody that is similar, but with the addition of a domain binding to the transferrin receptor (TfR1). The transferrin receptor is found in blood vessel endothelial cells where it acts as a carrier to transport iron. Antibodies binding to the transferrin receptor can hijack this mechanism to be transported across the blood-brain barrier. 24 hours after dosing, using our modified iDISCO+ protocol, we cleared and stained perfusion-fixed brains using an anti-human IgG secondary antibody. No immunoreactivity was detected in the brains of mice injected with the monospecific antibody, with background signals identical to brains from non-infected mice. With the bispecific antibody, strong staining was observed in blood vessels, and importantly, in the brain parenchyma.

Validation of BACE1 Targeting of the IV-Dosed Antibodies



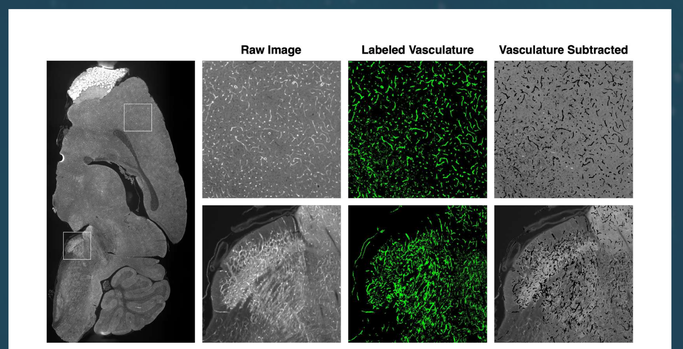
Traditional slice IHC experiments validated that there is enrichment of the IV-dosed bispecific antibody (50 and 125 mmol/kg) vs. the monospecific antibody (125 mmol/kg) in the dentate gyrus (DG) of the hippocampus (green panels and bar graph). This pattern is very similar to that seen in slices from non-dosed mice stained directly with an anti-BACE1 antibody (red staining).

Enrichment of Bispecific Antibody Throughout the Brain



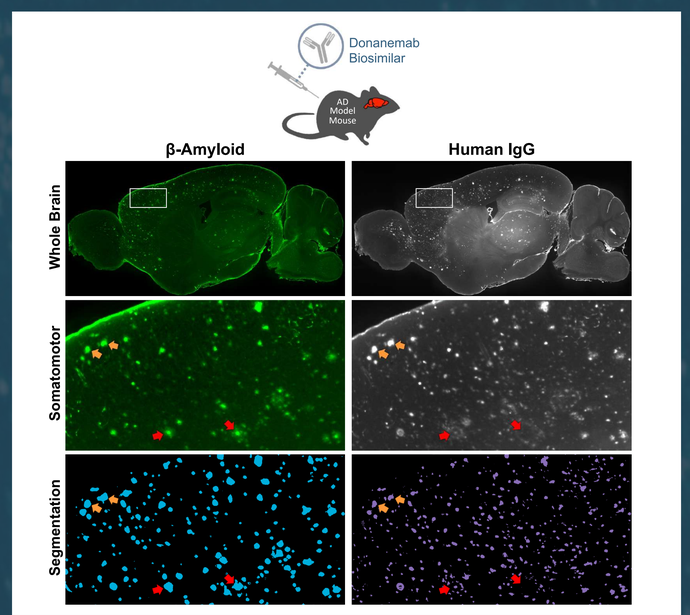
Our modified iDISCO+ protocol allows for imaging of an entire intact tissue giving an unbiased snapshot of immunoreactivity throughout the brain. Consistent with the data from the hippocampus, we discovered that the monospecific antibody has negligible brain penetration, whereas the bispecific antibody is identified in both the vasculature and parenchyma throughout the brain. Automated brain-wide regional quantification measures both the vasculature and parenchymal staining. The bispecific BACE1 antibody is particularly enriched in a number of brain areas including the Pontine Gray, Medulla and Glomeruli in the Olfactory Bulb.

ML-Powered Vasculature Labeling and Subtraction



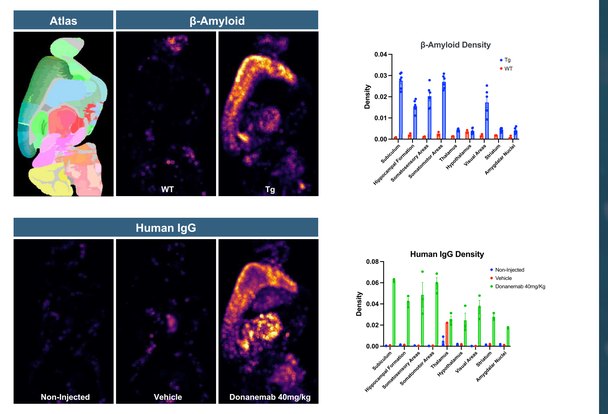
To quantify the pool of human IgG that has crossed the BBB, the staining in the blood vessels must be removed from the calculation. We developed a method that uses machine learning to identify and label vasculature throughout the brain (cortex and pontine gray shown). Based on the vasculature masking, those voxels can be removed from the raw image before performing regional volume quantification.

Automated Identification of β-Amyloid Plaques and Human IgG Distribution



ARTE10 AD model mice were intravenously injected with a biosimilar of Donanemab, a human IgG based antibody therapeutic. Cleared whole brains were cleared, stained, and imaged for β-Amyloid (Left) and Human IgG (Right). The presence of IgG signal colocalizing with β-Amyloid plaques suggests successful brain penetration and plaque engagement by the therapeutic antibody. Whole-brain and zoomed-in somatomotor cortex images show raw signal and segmentation outputs for β-Amyloid and Human IgG. Accurate object detection is observed across both channels. Orange arrows mark plaques where Human IgG colocalizes with β-Amyloid. Red arrows highlight a distinct population of plaques with less localized IgG signal.

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 whole-brains. 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 right 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.

Summary

- Brain-wide distribution of IV-dosed bispecific anti-BACE1/anti-TfR1 antibodies in mice was visualized using a modified iDISCO+ tissue clearing protocol with anti-human IgG detection. The antibodies showed high parenchymal and vascular localization, aligning with traditional IHC patterns.
- To specifically measure the parenchymal levels of Ab therapeutic that have crossed the BBB, we developed a ML-powered analysis pipeline that masks and removes the blood vessels from the regional quantification.
- In ARTE10 AD model mice treated with Donanemab, regional quantification showed a strong correlation between amyloid plaque density and Human 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.