

BRAIN-WIDE CELLULAR RESOLUTION MEASUREMENT OF NEURONAL ACTIVITY, MICROGLIAL ACTIVATION AND β -AMYLOID DEPOSITION



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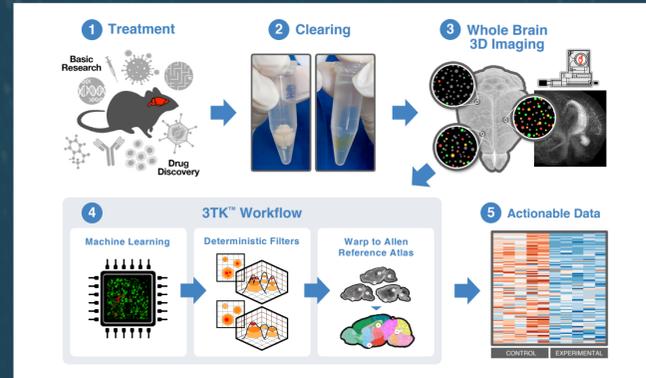


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Introduction

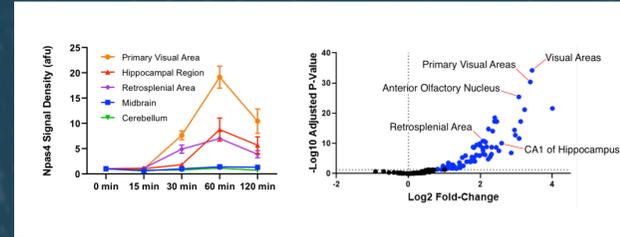
- Recent advances in optical clearing and light sheet imaging have opened an exciting new avenue for brain-wide, cellular resolution immunostaining at the forefront of a dimensional shift from 2D to 3D histology.
- With our optimized iDISCO-based clearing methods and our Mesoscale Imaging System™ for the ZEISS Lightsheet Z.1 microscope, we can image entire mouse brains in ~10 min. Further, our machine learning-enabled 3TK™ software identifies individual cells or protein aggregates throughout the brain and aligns them to the Allen Reference Atlas to produce a regionalized read-out of patterns across 100's of brain areas.
- We first demonstrate this technology with a cellular-resolution brain-wide assay of neuronal activity. Traditional immediate-early gene (IEG) products such as cFos are turned on by neuronal activity, but also by cAMP and various paracrine factors. Npas4 is unique among IEGs in that it is neuron-specific and exquisitely tuned to Ca²⁺ signaling. With our recombinant rabbit monoclonal anti-NPAS4 antibody (www.npas4.com) we can measure recent changes in neuronal activity throughout the brain.
- Next, we apply this technology to measure microglial responses during neuroinflammation and β -Amyloid plaques in Alzheimer's Disease model 5xFAD mice. Our automated analysis of microglia and β -Amyloid co-staining allows for brain-wide quantification of plaque-associated microglia.
- These studies demonstrate the utility of tissue clearing, light sheet imaging and our machine learning-enabled 3TK software for the brain-wide measurement of neuronal activity and Alzheimer's disease-related pathology.

Pipeline For the Generation of 3D Anatomic Data



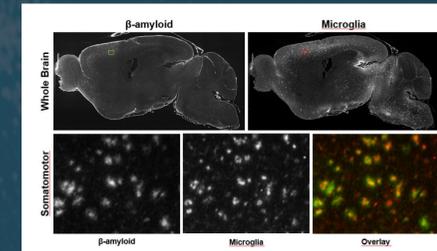
Mice are experimentally manipulated, including treatment with small molecules, antibody therapeutics, cellular therapeutics and gene therapy. Fixed and perfused brains are cleared and immunostained intact and then imaged on the ZEISS Lightsheet Z.1 microscope. After tiling and stitching in our Stitchy software, our machine learning-enabled 3TK software produce actionable anatomics data.

Quantification of Neuronal Activity Across 100's of Brain Regions



We identified many areas throughout the brain with increased activity after light exposure. Blue dots in the volcano plot represent regions with significantly up-regulated Npas4 as identified with a modified implementation of the DE-Seq2 statistical package typically used with RNA-Seq data.

Co-Localization of Microglia and β -Amyloid in 5xFAD Mice



Brains from 5 month old 5xFAD mice were immunostained for microglia (anti-Iba1) and β -Amyloid (6E10). Optical slices from intact brains demonstrate the brain-wide nature of the staining. Zooming in reveals that rather than having the typical spacing seen in control and LPS-treated brains, activated microglia are intimately associated with β -Amyloid plaques – plaque-associated microglia.

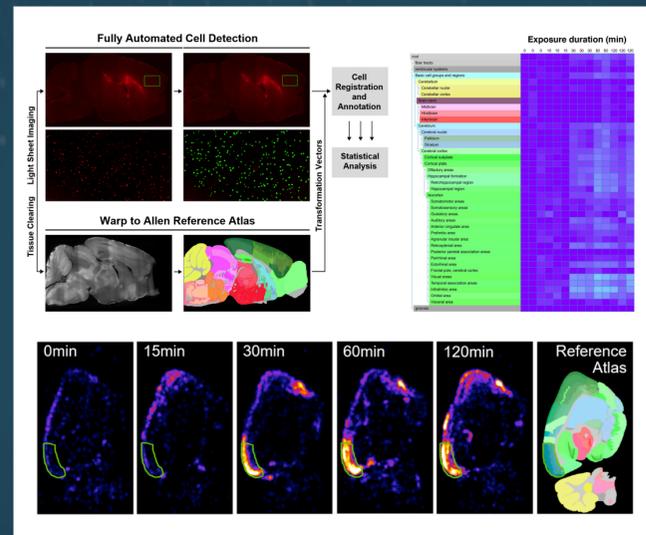
Light Sheet Imaging of Optically-Cleared Mouse Brains



VIDEO <https://youtu.be/uban-WdoWHE>

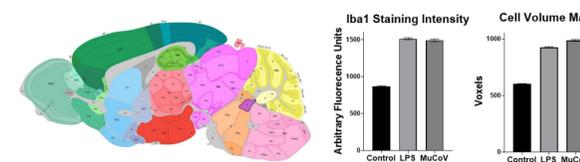
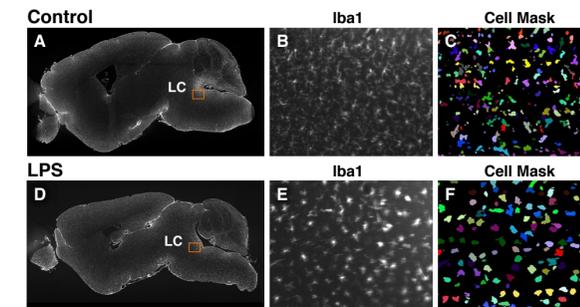
Our Mesoscale Imaging System adapts the ZEISS Lightsheet Z.1 and 7 microscopes for imaging large tissues in high refractive index solutions with mesoscale optics. This Thy1-GFP brain was imaged in ~25 minutes.

Brain-Wide Monitoring of Npas4 Expression After Light Exposure



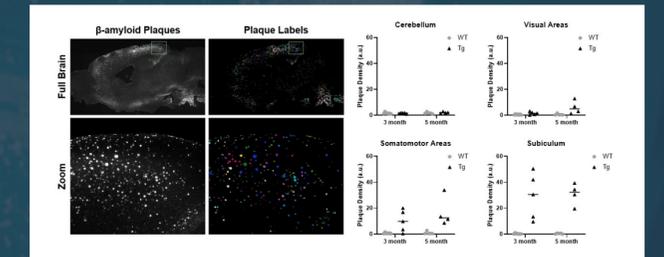
3TK identifies Npas4-expressing neurons, maps them to the Allen Reference Atlas and quantifies Npas4 expression across 100's of brain regions. Light exposure after 24 h in the dark drives strong Npas4 expression within 30 min in various regions throughout the brain.

Brain-Wide Measurement of Microglial Responses to Neuroinflammation



Brains from mice treated for 7 days with Lipopolysaccharides (LPS) were cleared and stained with an anti-Iba1 antibody. A and D show optical slices through the intact brains. Zoomed images of the Locus Coeruleus demonstrate the prototypical condensing of activated microglia (B,E), which is also seen in the brain-wide cellular masks produced by 3TK (C,F). Quantification across the brain demonstrates increased Iba1 staining intensity and object size in LPS-treated brains and in brains from mice infected with a murine coronavirus.

Brain-Wide Regional Quantification of β -Amyloid Plaques



Plaques in brains from 3 month and 5 month old WT and 5xFAD mice were immunostained and imaged with a light sheet microscope. Individual plaques throughout the brain were identified with 3TK. After warping imaged brains onto the Allen Reference Atlas, plaque density measurements were produced across 100's of brain regions. Data from 4 exemplar regions are shown. Each symbol represents one brain.

Summary

- Our iDISCO-related tissue clearing methods and machine learning-enabled 3TK software produce automated cellular-resolution quantification across 100's of anatomical regions.
- With our anti-Npas4 recombinant rabbit monoclonal antibody, we can identify recently active neurons throughout the mouse brain, generating brain-wide signatures of neuronal activity.
- In transgenic 5xFAD Alzheimer's disease model mice, we used anti-Iba1 antibodies and the 6E10 monoclonal antibody to produce brain-wide measurements of microglia and β -Amyloid. Strong colocalization in the Tg mice demonstrates the ability to measure plaque-associated microglia.
- These new methods for whole-brain, next generation 3D immunohistochemistry and anatomics are ideally suited to pre-clinical studies for unbiased, complete and anatomically precise mapping of the efficacy of CNS therapeutics.

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