

# MEASUREMENT OF NPAS4 AND CFOS FOR BRAIN-WIDE SNAPSHOTS OF NEURONAL ACTIVITY



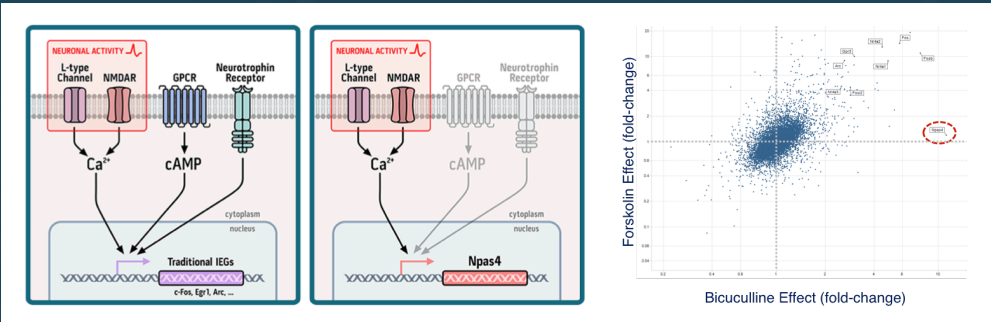
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## Introduction

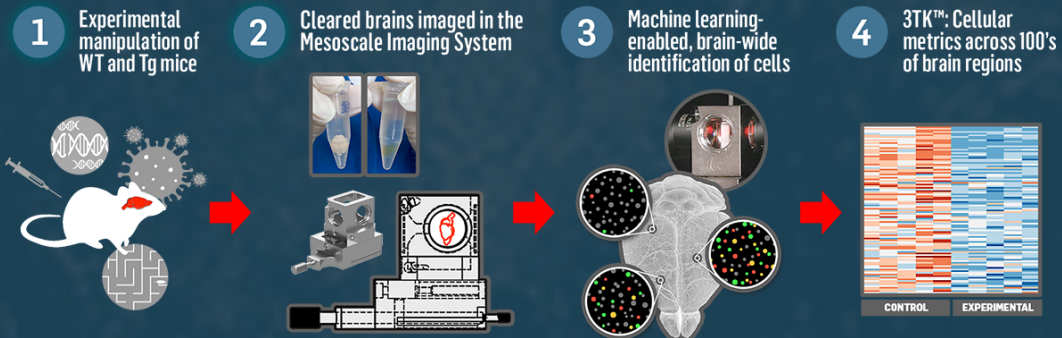
- Recent advances in optical clearing and light sheet imaging have opened an exciting new avenue for brain-wide, cellular resolution immunostaining at the forefront of a dimensional shift from 2D to 3D histology.
- Translucence Biosystems has developed the Mesoscale Imaging System™ which adapts the ZEISS Lightsheet Z.1 microscope, designed for imaging small live organisms, to image large intact tissues with unprecedented speed and resolution.
- 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 Ca<sup>2+</sup> signaling. We developed a recombinant rabbit monoclonal anti-NPAS4 antibody (Activity Signaling: [www.npas4.com](http://www.npas4.com)) that we are using to generate cellular-resolution brain-wide signatures of neuronal activity.
- Using a modified version of iDISCO, we are optically clearing and immunostaining intact brains to mark neurons expressing cFos and Npas4. Our machine-learning enabled software, 3TK, surveys 3D-rendered whole brains to identify individual stained cells, map them to the Allen Brain Reference Atlas and quantify their expression across 100's regions.
- Our data show that cFos is expressed in a large number of neurons in control brains, whereas Npas4 expression at baseline is much lower. Behavioral manipulations that increase neuronal activity drive the expression of both cFos and Npas4, but the lower baseline expression of Npas4 provides for a higher fidelity readout of neuronal activity.

## Npas4 Expression is Exquisitely Tuned to Neuronal Activity



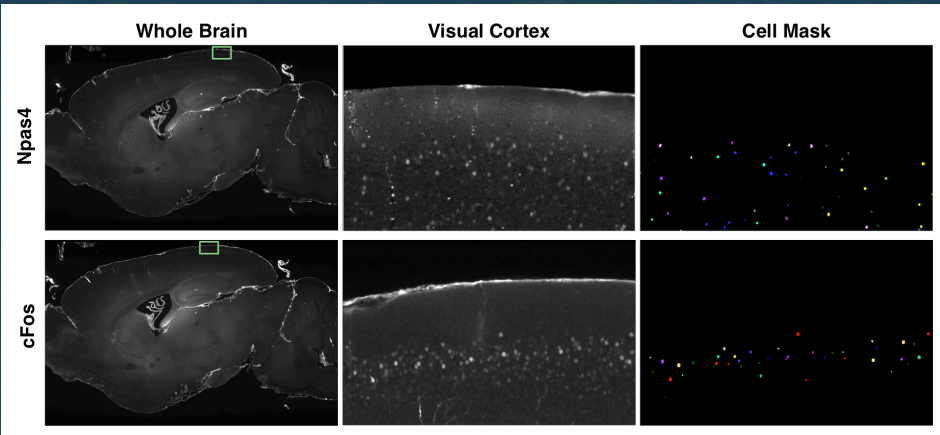
Neuronal activity, cAMP elevations and other signaling pathways drive the expression of traditionally-used IEGs such as cFos. Npas4, however, is unique among IEGs in that expression is tuned to Ca<sup>2+</sup>-mediated signaling pathways. In primary-cultured neurons *npas4* is the only mRNA transcript up-regulated by synaptic activity, but not by cAMP elevation.

## The Translucence Pipeline for Brain-Wide Measurement on Neuronal Activity



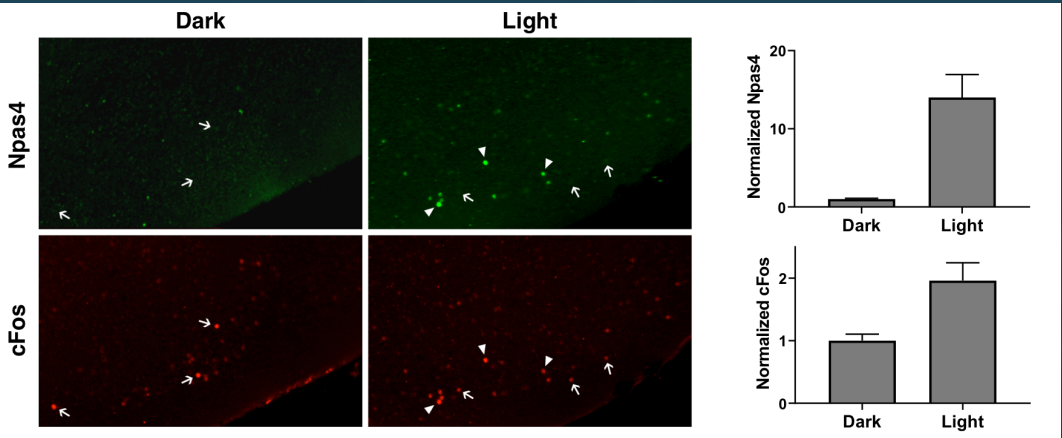
Mice are experimentally manipulated and perfusion-fixed brains are optically-cleared and stained for Npas4 and cFos with an iDISCO-related protocol and imaged with a ZEISS Lightsheet Z.1 microscope equipped with the Mesoscale Imaging System. Our 3TK software quantifies cFos and Npas4 expression across 100's of brain regions.

## Identification of Npas4- and cFos-Expressing Neurons



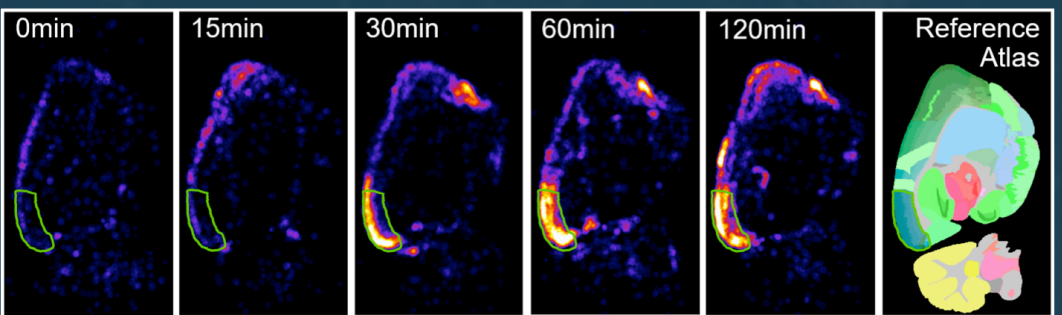
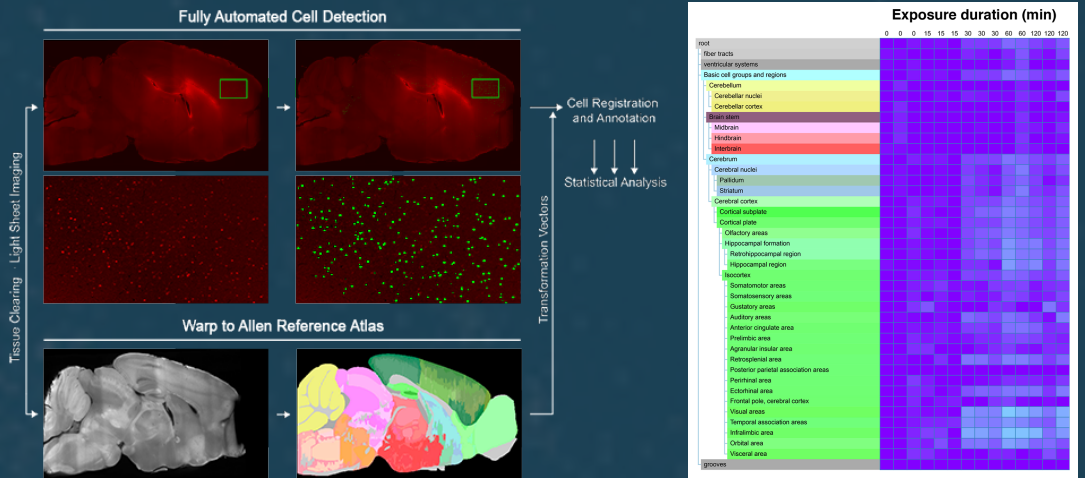
Optical slices from an intact mouse brain showing Npas4 and cFos immunoreactivity. 3TK uses machine learning and a series of deterministic filters to label marked neurons, as shown in the right-hand panels.

## Npas4 Baseline Expression is Lower than cFos



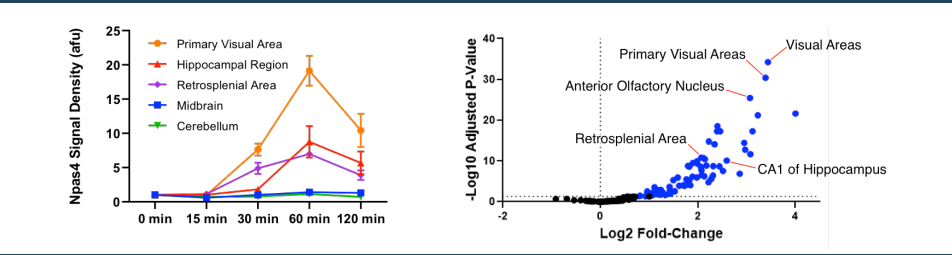
In brains of mice kept in the dark for 24 h, many neurons in the visual cortex express cFos, yet they do not express Npas4. Exposure to light drives the expression of both IEGs in many neurons, but the baseline cFos expression reduces the fidelity of the activity-evoked signal.

## Brain-Wide Monitoring of Npas4 Expression After Light Exposure



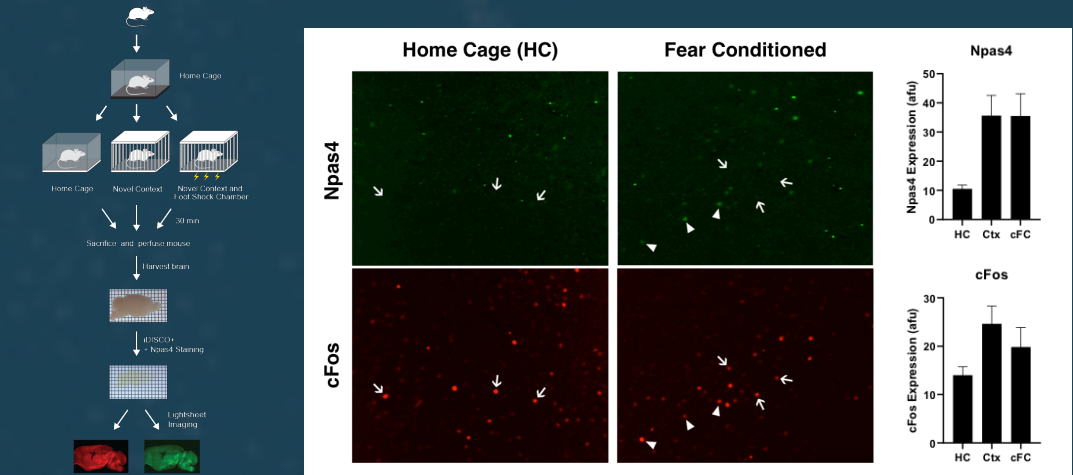
3TK identifies Npas4-expressing neurons, maps them to the Allen Reference Atlas and quantifies Npas4 expression across 100's of brain regions. Light exposure after 24 h in the dark drives strong Npas4 expression within 30 min in various regions throughout the brain.

## 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.

## Brain-Wide Mapping of NPAS4 After Fear Conditioning



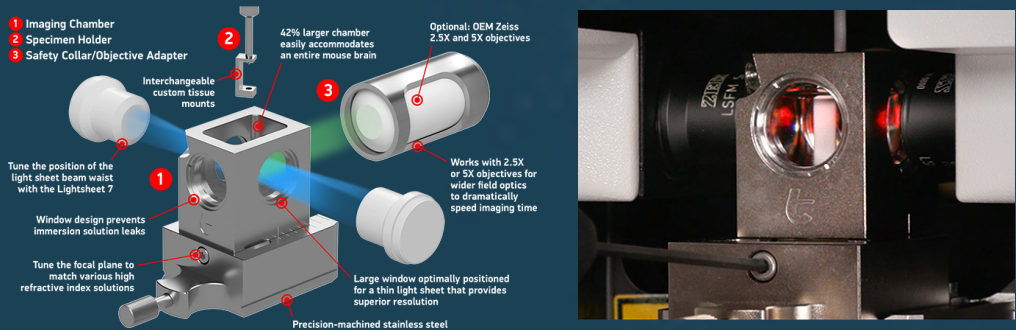
Mice were left in their home cage, subjected to shock in a cFC task or exposed to the shock box but not shocked (novel context). Npas4 had lower baseline expression than cFos in the basolateral amygdala (BLA), where many cells express cFos but not Npas4 (thin shaft arrows). cFC drives the expression of Npas4 and cFos (arrowheads). Both context exposure and cFC increased cFos and Npas4 in the BLA, but the fidelity of the Npas4 signal is better due to the lower baseline expression.

## Summary

- Npas4* is a unique immediate-early gene in that it is exquisitely tuned to recent neuronal activity and unlike traditional IEG's such as *fos*, *npas4* does not respond to other extrinsic cues.
- Using our anti-Npas4 recombinant monoclonal antibody with a commercially available anti-cFos antibody, we have optimized an iDISCO-based method for brain-wide marking of cFos(+) and Npas4(+) neurons.
- In two experimental paradigms, light exposure after housing in the dark and contextual fear conditioning, we have found that Npas4 expression is substantially lower than cFos at baseline allowing for a higher-fidelity readout of neuronal activity after environmental stimuli.
- With our 3TK software pipeline, we use machine learning-based tools to identify individual cFos- and Npas4-expressing neurons, mapping them to the Allen Brain Reference Atlas to generate whole-brain signatures of recent neuronal activity.
- The elevated expression of cFos over Npas4 at baseline is likely due to factors other than neuronal activity *per se*. While Npas4 provides a more pure readout of neuronal activity, identification and quantification of cFos(+) neurons that do not express Npas4 will help stratify distinct populations of neurons across the brain and provide a deeper understanding of brain-wide responses to environmental stimuli.

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## 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.