ALLEN INSTITUTE for NEURAL DYNAMICS

RECORDING NEURONS' SYNAPTIC INPUT PATTERNS WITH SCANNED LINE PROJECTION MICROSCOPY

PSTR 241.30

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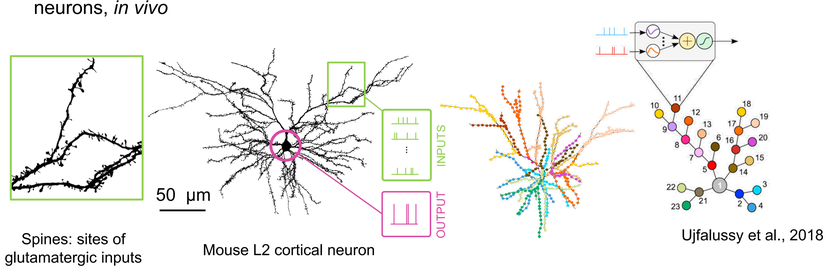
MOTIVATION

Measuring input-output transformations of neurons in behaving mice

- Input-output operations in neurons are the fundamental unit of computation in both biological and artificial neural networks
- We want to generate mechanistic explanations of computations in the brain in terms of how signals are transformed by neurons
- Systems neuroscience has no methods to measure complex input patterns to mammalian neurons, neither in circuits nor in single neurons
- We are creating methods to simultaneously record many inputs and outputs of individual neurons, *in vivo*



Spines: sites of glutamatergic inputs

Mouse L2 cortical neuron

Ujfalussy et al., 2018

Record many synaptic inputs (glutamate) and somatic firing output (calcium) in single neurons → Fit models of input-output transformations, plasticity, circuit computations, etc

REQUIREMENTS

Imaging input patterns requires fast, sensitive measurements

TINY: Synapses have a volume of ~1 femtoliter each

MANY: Neurons each have many synapses (sometimes >10,000)

INTACT: We study computations of intact circuits in behaving animals

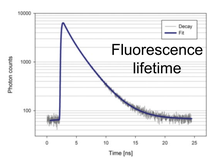
FAST: Input integration occurs over ~10 ms, requiring >100Hz recordings

We need:

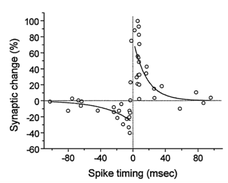
NEUROTRANSMITTER INDICATORS with extremely high brightness, sensitivity, and photostability, to record high-SNR signals from many synapses at high speed.

IN VIVO MICROSCOPES with fast and flexible scanning capabilities

- Imaging femtoliter volumes in scattering brain tissue requires multiphoton imaging
- Multiphoton imaging records pixels sequentially, with pixel rates limited by fluorescence lifetime (~200 MHz)
- Dense raster scanning is far too slow to record entire neurons at >100Hz



Imaging requires ~5-10 ns per pixel

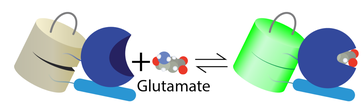


Neurons integrate signals with with <10 ms precision

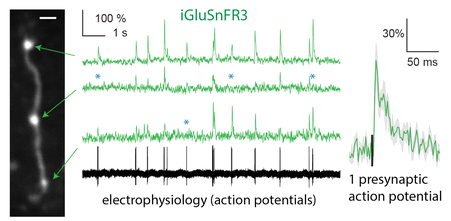
Bi et al 2001

NEUROTRANSMITTER INDICATORS

iGluSnFR3



Aggarwal et al. 2023:
'**Glutamate indicators with improved activation kinetics and localization for imaging synaptic transmission**'



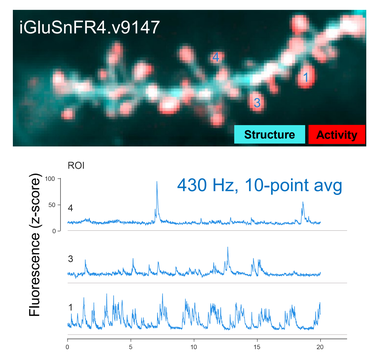
iGluSnFR4 Development

- **/w GENIE Project Team**
- **Full combinatorial screen** of 11 sites from iGluSnFR3 dev.
- ~10,000 combo variants imaged /w field stimulation in neurons
- 70 variants screened for optical minis (single vesicle release)
- 10 variants selected for in vivo testing

See PSTR507.23 (Janelia GENIE Project Team) for in vitro screen info

- In vivo screen in progress: 4 variants tested
- AAV2/1, 2.5e12 titer, Mouse Visual Cortex (V1)
- 40 mW, ~100-300 um below surface, 1030m, NA 1.0
- 430 Hz frame rate 2D imaging
- Full-field visual stimulation @ 0.5 Hz

Variants show >50 SNR at single synapses *in vivo*

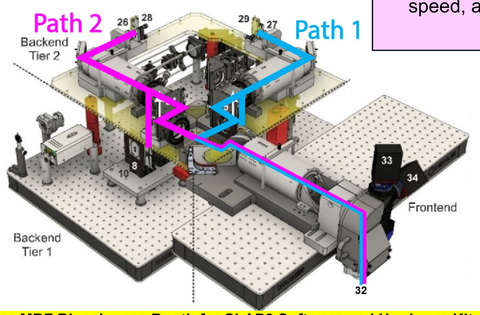


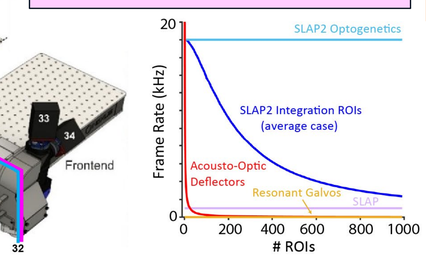
SLAP2 MICROSCOPE

Two-photon microscope based on a novel scan system optimized for random access imaging of many targets:

- A line focus is scanned across a DMD, illuminating 1 column at a time
- The DMD selects a small fraction of pixels in each column to relay to the sample. The selected pixels are excited simultaneously.
- After each line scan, the pattern on the DMD is updated.
- A remote focus system rapidly steers the beam in depth at the sample.

SLAP2 has two simultaneously-recorded paths that are scanned interleaved:





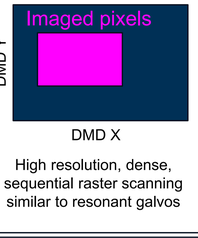
SLAP2's unique scan system allows it to:

- Efficiently image many targets with **no per-target access time cost**, unlike AOD-based random access microscopes
- Flexibly trade off resolution versus speed, allowing extremely fast imaging

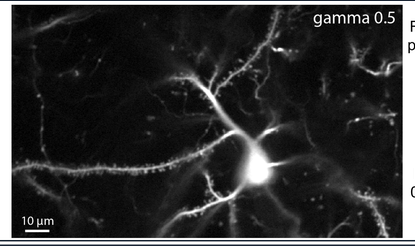
See MBF Biosciences Booth for SLAP2 Software and Hardware Kits

IMAGING MODES

Simple Raster



High resolution, dense, sequential raster scanning similar to resonant galvos



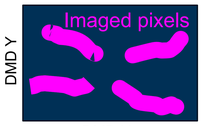
Raster scans are performed by setting 1 row of pixels 'ON' at a time, in sequence. All other light is discarded, creating a point.

Field of view 220 x 300 microns per DMD

450 microns of axial remote focus

Diffraction-limited two-photon excitation at NA 0.8 (<2 um axial, 0.7 um lateral FWHM) across >80% of imaging volume

Raster ROIs

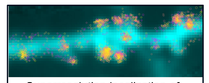


High resolution sequential raster scanning of arbitrary sets of pixels.

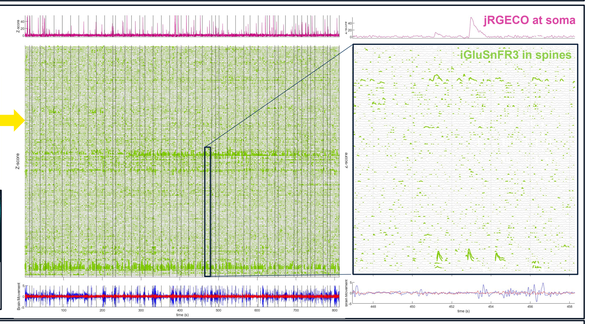
Frame rate in each column is proportional to 1/(# imaged pixels in column).

Arbitrary pixels in field of view can be selected.

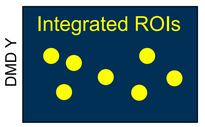
Enables recording of >200 spines at >100 Hz, with high resolution and SNR, in behaving mice.



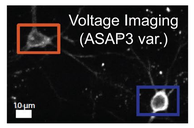
Superresolution localization of release events (zoom from recording of >200 synapses)



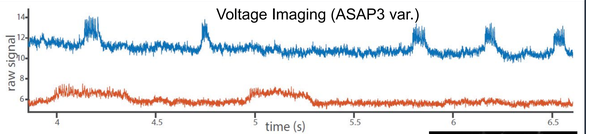
Integration



The pixels in each column of each ROI are merged into one measurement. Frame rate in each column is proportional to 1/(# ROIs in column).

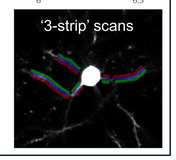


Vertical resolution within each integration ROI is lost, trading resolution against speed.

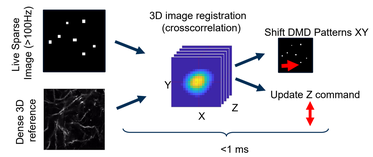


Enables >10kHz voltage imaging of multiple neurons at once

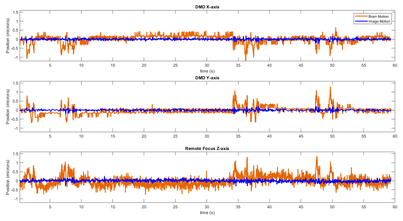
Enables high-speed 'strip scans' of dendrites: 3.6 kHz/plane (3 strips) >100 Hz volume

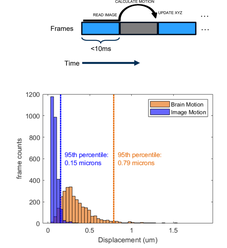


SLAP2 Online Motion Correction



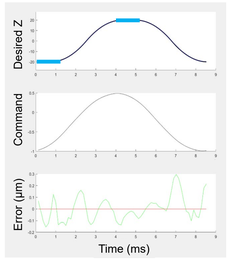
- Full-image based motion correction
- >100 Hz update rate (limited by imaging rate/2)
- <150 nm residual brain motion (95th %ile) when imaging at 200 Hz

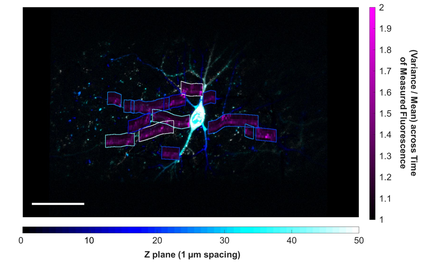




3D imaging

- Closed loop, model-based control of remote focus piezos (>100 Hz volume rate)
- Strip scans of >60 planes possible at 100 Hz using both fields of view
- In progress: Data processing, online motion correction, speed optimization for 3D scans





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