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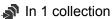
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Freely moving imaging



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ABSTRACT

We have developed a new micro-fiber array approach capable of chronically measuring and optogenetically manipulating local dynamics across over 100 targeted locations simultaneously in head-fixed and freely moving mice, enabling investigation of cell-type and neurotransmitter-specific signals over arbitrary 3-D volumes . This protocol includes the steps for imaging in freely-moving mice. Please contact us (mwhowe@bu.edu) if you are interested in using this technique.

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- 1 For visualizing fibers in behaving mice custom-built miniscopes (Liberti et al., 2016; Liberti et al., 2017) were positioned above the fiber bundle, focused with an XYZ manipulator, and cemented in place with Metabond (Parkell) under brief isoflurane anesthesia.
 - **1.1** Note: Permanent implantation was used for initial experiments in this study, but baseplate attachment is equally viable.
- 2 Mice were allowed to recover for 24-48 hrs after attachment, after which imaging was performed in a custom built acrylic arena as mice foraged for Froot Loops (Kellogg's).
- The miniscope GRIN objective had an N.A. of 0.45 for high-efficiency light collection and the imaging area was approximately $800 \times 600 \mu m$, allowing visualization of a substantial fraction of the fiber bundle.
- 4 Both behavioral video and fiber signals were acquired at 30Hz using custom MATLAB acquisition software.
- Neural imaging data were acquired from DAQ and CMOS PCBs from the UCLA Miniscope project (Pnevmatikakis and Giovannucci, 2017).

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- Illumination intensity was delivered by a 470 nm LED (Luxeon Rebel, Lumileds) at an intensity of 0.1-0.25 mW/mm2, controlled by a D-A interface (National Instruments USB-6009) and BuckPuck LED controller (LEDdynamics, Inc).
 Imaging data were motion-corrected with NormCorre73, corrected for minor background light leak using spatial high-pass filtering, and converted to ΔF/F.
- 8 Intensity timeseries for each fiber were extracted using manually selected circular ROIs.
- 9 Locomotor activity was quantified using manual tracking in ImageJ.