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Imaging using 2-photon microscope through GRIN lens

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We use this protocol and it's working

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Abstract

This is the standard protocol for 2-photon imaging with Bruker microscope through GRIN lens used for Rodrigues-Vaz and Athalye et al, 2025.

Materials

- Behavior setups
- 70% ethanol
- 10% ethanol
- 2-photon laser scanning microscope (Ultima In Vivo, Bruker)
- Femtosecond Ti:saphire laser (Chameleon Vision-S, Coherent)
- Low repetition photostimulation laser (Monaco 1035-40, Coherent)
- Lens paper
- Distilled water

Safety warnings

-Wear appropriate PPE as required by your institution.

Ethics statement

This protocol was approved by Columbia University IACUC. Please do not perform any of these procedures unless there is prior approval from the institution's animal ethics committee.

Before start

-Mice were implanted with head plate and GRIN lens and habituated to being head fixed.
-Behavior setup was prepared and placed under microscope for *in vivo* experiments.
-2-photon laser and Pockels cell controller were turned on.
-If doing holographic stimulation, calibration step must have been performed before. See "Holographic calibration" protocol for this.

General preparation

1 Confirm laser is aligned with target at objective output.

1.1 If doing holographic stimulation, confirm that stimulation laser is also aligned

2 Head fix mouse in behavior setup under microscope.

3 Find top of GRIN lens using 4X Nikon objective. If necessary, clean top of the lens with wet cotton tip.

4 Swap objectives – Use 16X water-immersion Nikon objective – and find top of the GRIN lens again.

5 Turn off all lights in the room and switch to using 2-photon microscope for imaging.

6 Find desired field of view (FOV). If matching same FOV across multiple days follow steps below:
a. Load BOT file (brightness over time) that labeled ROIs from previous session – these ROIs were extracted using Suite2p with custom trained classifier to identify medium spiny neurons (MSNs).
b. Find XYZ coordinates that better match last session's FOV
c. To continuously monitor the imaging power, we used a beam pickoff (Thorlabs BSF10-B) and power meter (Thorlabs PDA100A2). Pockels cell bias was adjusted accordingly to maintain imaging power throughout session.

7 Record 1000 frames of functional and structural images – green and red PMTs, respectively. These are used to identify cell types.

8 Turn off red PMTs and red acquisition window.

9 Turn on imaging acquisition for 60000 frames (or for as long as behavior session lasts) followed by all behavior boards.

10 Record session, confirming that all TTLs are being recorded in Prairie's voltage recording as expected by design.

11 At the end of the session, turn off all behavior boards and then the imaging recording.

12 Acquire another 1000 frames of functional and structural images – turn on red PMTs and acquisition window to do so.

13 Collect z-stack 50 um above and below imaged FOV, 2 um between each step.
Each image is an average of at least 16 frames.

14 Turn off PMTs, rise objective and remove mouse from head-fixation.

15 Convert data using "Image Ripping" software.

16 Transfer data from local computer to storage locations.

End of day procedure

17 Clean objectives with wet lens paper.

18 Turn off Pockels cell controller and laser.

19 Turn on lights in the room and take the mice to their holding room.