Mar 10, 2025

DIO-SPOTlight Live 2-Photon Imaging v.250227

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6dj35vqe/v1

Matt Oliver[1,2], Victoria Hall[1,2]

[1]Calakos Lab; [2]Duke University

Matt Oliver
Duke University

DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6dj35vqe/v1

Protocol Citation: Matt Oliver, Victoria Hall 2025. DIO-SPOTlight Live 2-Photon Imaging v.250227 . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6dj35vqe/v1

Protocol status: Working
We use this protocol and it's working

Created: February 27, 2025

Last Modified: March 10, 2025

Protocol Integer ID: 123548

Keywords: ASAPCRN, DIO-SPOTlight 2-photon, photon imaging, live tissue preparation, spotlight, tissue, imaging

Funders Acknowledgements:
ASAP
Grant ID: ASAP-020607

Abstract

This protocol contains the steps taken for live tissue preparation and two-photon imaging.

Prepare acute brain slices

1 Anesthetize mouse and perform intracardial perfusion with ice cold N-methyl-D-glucamine (NMDG)-based artificial cerebrospinal fluid (ACSF).
   a. ACSF (90 mM NMDG, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 35 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 5 mM sodium ascorbate, 2 mM thiourea, 3 mM sodium pyruvate, 10 mM $MgSO_4$ heptahydrate, 0.5 mM $CaCl_2$ dihydrate, and saturated with 95% O_2 and 5% $CO_{2)}$

2 Quickly remove the brain and slice into 250 um sagittal slices using a vibratome.

3 Immediately transfer slices to a recovery chamber containing NMDG ACSF for 10 to 15 min at 32°C.

4 Transfer heat recovered slices to a holding chamber containing ACSF and let rest for approximately 1 hour
   a. ACSF (124 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$,2 mM $CaCl_2$, 26 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 10 mM glucose, saturated with 95% O_2 and 5% CO_2, pH 7.4, 300 mOsm/l)

Two-photon imaging

5 Place the slices into a perfusion chamber and superfuse with oxygenated ACSF.

6 Image slices using a Bruker Investigator Multiphoton Imaging System or equivalent.

7 Set the emitted light wave-length to 920 nm on the Coherent Chameleon I Ti:Sapphire femtosecond laser.
   a. Keep laser power between 35 and 50 mW

8 Set up image stack using galvo-galvo scanning with frame rate of 1 Hz with 3-4 µm depth increments at either 1X or 4X optical zoom.

9 Collect fluorescence signal using a 16X W objective and Quad GaAsP photomultiplier tubes (PMTs).

10 Split the SPOTlight red and green channels using a 565lpxr dichroic mirror followed by a 525/70 bandpass PMT filter.