

Feb 27, 2025

DIO-SPOTlight Confocal Imaging Acquisition v.250227

DOI

<https://dx.doi.org/10.17504/protocols.io.dm6gp97odvzp/v1>

Matt Oliver^{1,2}

¹Calakos Lab; ²Duke University



Matt Oliver

Duke University

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Protocol Citation: Matt Oliver 2025. DIO-SPOTlight Confocal Imaging Acquisition v.250227. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.dm6gp97odvzp/v1>

Manuscript citation:

Oliver, M. L., Caffall, Z. F., Eatman, C. B., Faw, T. D., & Calakos, N. (2024). DIO-SPOTlight Transgenic Mouse to Functionally Monitor Protein Synthesis Regulated by the Integrated Stress Response. *BioRxiv*, 2024.10.14.618312. <https://doi.org/10.1101/2024.10.14.618312>

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Protocol status: Working

We use this protocol and it's working

Created: February 27, 2025

Last Modified: February 27, 2025

Protocol Integer ID: 123537

Keywords: ASAPCRN, DIO-SPOTlight imaging, spotlight immunohistochemistry, details for confocal image acquisition, confocal image acquisition, spotlight image processing, immunofluorescent labeling protocol, thick brain slice, immunofluorescent, spotlight, image processing, brain slice, imaging

Funders Acknowledgements:

ASAP

Grant ID: ASAP-020607

Abstract

Abstract

This protocol contains details for confocal image acquisition of immunofluorescent labeled 40-um thick brain slices and follows the immunofluorescent labeling protocol.

Partner protocols: [DIO-SPOTlight Immunohistochemistry v.250227](#) & [DIO-SPOTlight Image Processing and Analysis v.250227](#)

Guidelines

For comparative analysis of SPOTlight RFP and GFP signal laser power and exposure time should be constant across these acquisition channels. The RFP signal will in general be much lower than GFP.



- 1 **Confocal Imaging Acquisition**
- 2 Power on the Andor Dragonfly 202 spinning disk confocal unit.
- 3 Open Fusion acquisition software
- 4 Secure microscope slide to slide holder and center the object over the slice of interest
- 5 Set up imaging protocol in "Protocol Manager" tab
- 5.1
 - a. Protocol type: Z scan
 - i. Select "Montage Enabled" set mode to "Edge"
 - ii. For 10x objective lens plus 1.5x camera zoom set z-step to appropriate size to capture needed planes of the full brain.

Note: No quantification performed, use discretion to avoid large file size. Z-step will depend on how flat the tissue is.
 - iii. For 40x/1.3 oil immersion objective lens image montage set z-step to 2-3 um

Note: magnification used for single imaging plane fluorescence quantification
 - b. Set up acquisition channels in order of decreasing wavelengths
 - i. Anti-ChAT: 637 nm laser line – 698 nm filter cube
 - ii. Anti-RFP: 561 nm laser line – 594 nm filter cube
 - iii. Anti-GFP: 488 nm laser line – 521 nm filter cube
 - iv. Hoechst/DAPI: 405 nm laser line – 445 nm filter cube
- 6 Add immersion oil and select 40x/1.3 oil immersion objective lens
- 7 Set acquisition settings to yield desired dynamic range of acquired signal and avoid pixel saturation. Once appropriate settings are determined, maintain settings for all images for the given experiment.

Note: For comparative analysis of SPOTlight RFP and GFP signal laser power and exposure time should be constant across these acquisition channels. The RFP signal will in general be much lower than GFP.



- 8 For brain-wide imaging of cholinergic nuclei use anti-ChAT acquisition channels to determine regions of interest. Once identified set XY and Z bounds and acquire.
- 9 For the integrated stress response activation experiment (e.g. vehicle vs tunicamycin) the hippocampus was imaged, not regions containing cholinergic neurons. Set XY and Z bounds and acquire.