Effects of reducing axial resolution in two-photon calcium imaging on retrieving functional neuronal activity

Ha Yun Anna Yoon[1], Genesis M. Ferrer Imbert[1], Ryan G. Natan[1], Adam S. Charles[2], and Na Ji[1]
[1]University of California, Berkeley, Berkeley, CA 94720, [2]Johns Hopkins University, Baltimore, MD 21025

POSTR574.05

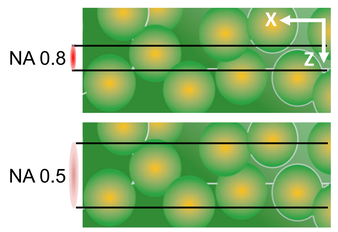
National Institutes of Health The BRAIN Initiative
U01 NS118300, R01 NS109553

Abstract

Two-photon calcium imaging is widely used in neuroscience to record population activity of neurons in vivo. In recent development of two-photon microscopy methods, optical resolution is sometimes sacrificed in pursuit of recording activity from ever larger numbers of neurons. We investigated how reducing resolution, especially along the axial direction, impacts the quality of calcium imaging data. With both NAOMi simulation and recordings from the same neurons in the mouseprimary visual cortex in vivo using two-photon excitation foci with axial full widths at half maximum (FWHM) ranging from 3.6 μm to 21 μm, we observed increasing neuropil contamination with the decrease of axial resolution, which can severely reduce the accuracy of functional characterization of properties such as orientation selectivity. With ongoing work incluing testing the ability of popular calcium analysis pipelines in extracting accurate activity information, we aim to provide benchmarks and guidelines for calcium data analysis and future microscopy development efforts.

Neuropil contamination

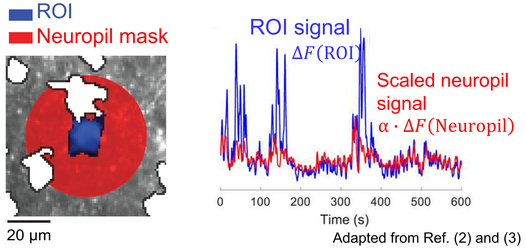
- **Axial elongation of excitation focus causes contamination of soma signal by fluorescence from the neuropil(1)**



Axial PSFs of 0.8 and 0.5 NA excitation focus compared with 10-μm-diameter somata

Adapted from Ref. (1)

- **Neuropil subtraction for removing neuropil contamination**



Adapted from Ref. (2) and (3)

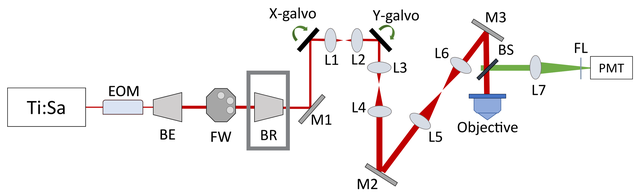
$\alpha = 0.7$[4] is a common choice.

- **Calcium analysis pipelines (Suite2P[3], CNMF[5,6], and EXTRACT[7]) develop strategies for removing neuropil contamination**

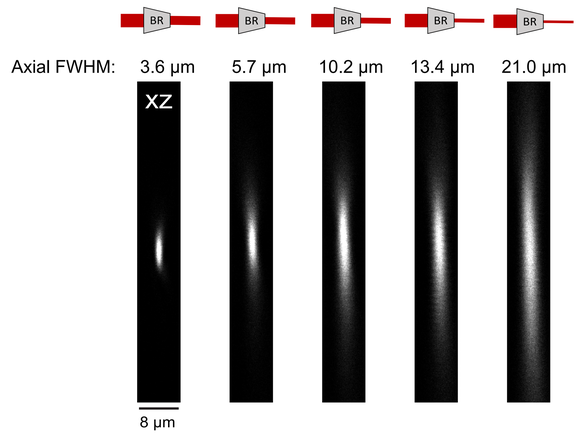
Problem statement

- **How does decreasing axial resolution affect neuropil contamination?**
- **Are common approaches for neuropil subtraction sufficient?**

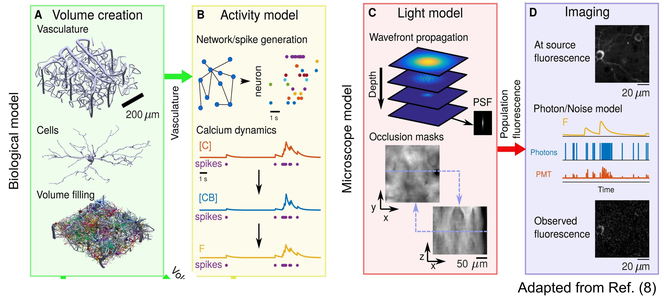
Controlling axial resolution



- **Controlling axial resolution with a motorized beam reducer (BR)**
- **Underfill microscope objective to increase axial FWHM**



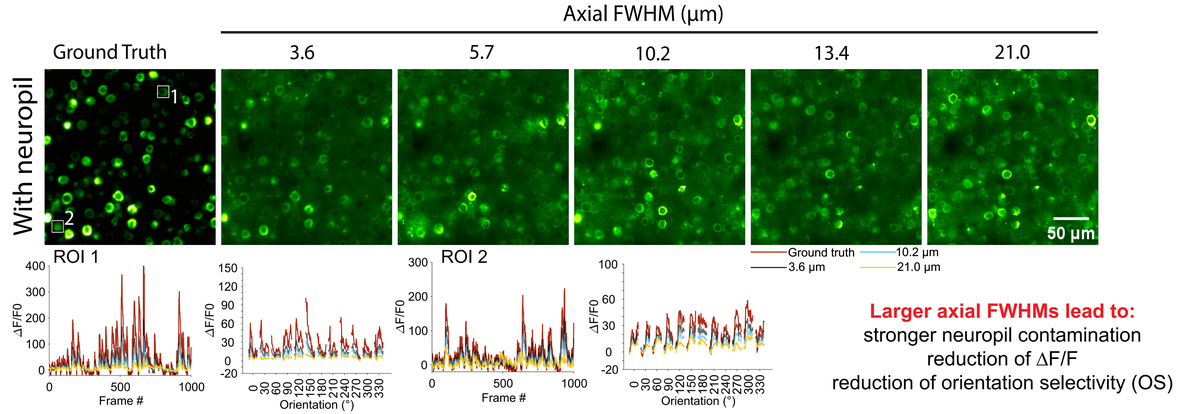
NAOMi simulation



Adapted from Ref. (8)

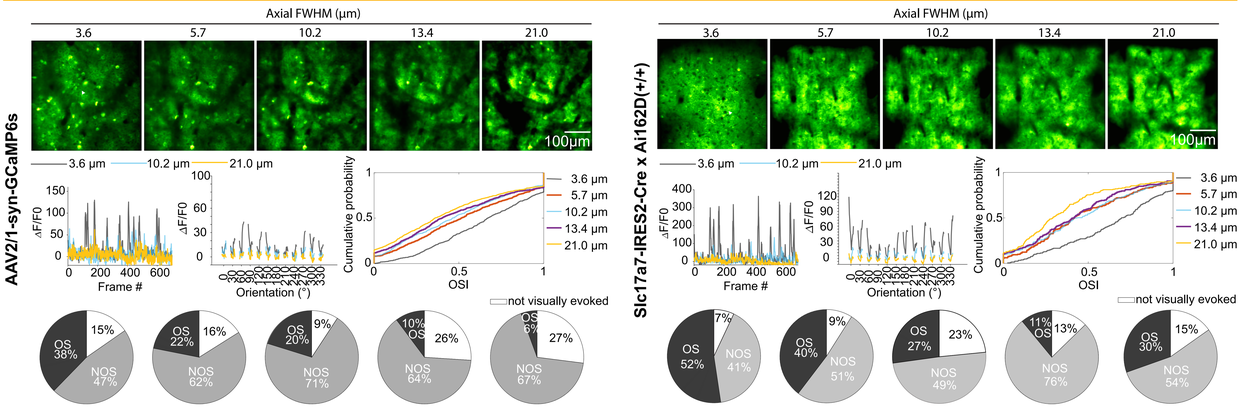
- Simulate 3D cell volume
- Assign an experimentally acquired calcium imaging trace to each neuron
- Calculate resulting images with different axial FWHMs
- Consider both fully cytosolic expression and soma-targeted expression

NAOMi simulation for 3D volume with neuropil expression

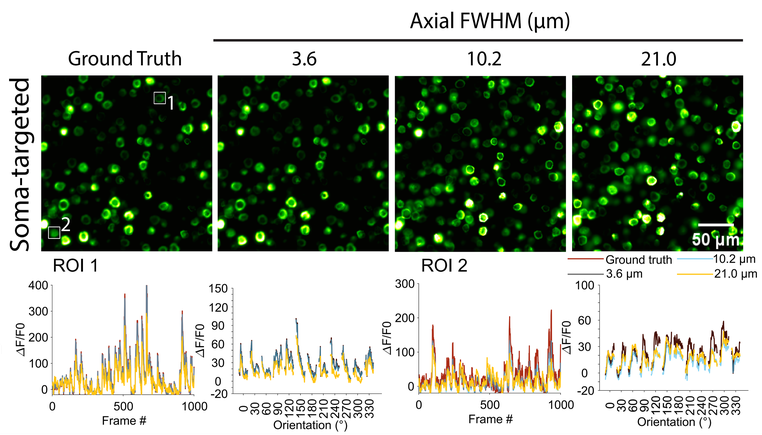


Larger axial FWHMs lead to:
stronger neuropil contamination
reduction of ΔF/F
reduction of orientation selectivity (OS)

In vivo imaging of V1 neurons with different axial FWHMs



NAOMi for soma-targeted expression



Conclusion & Future work

1. **Reducing axial resolution worsens neuropil contamination in both simulation and experiment.**
2. **Standard subtraction method does not fully remove neuropil contamination, leading to errors in functional characterization of (e.g., OS) neurons.**
3. **High axial resolution leads to more accurate measurement.**
4. **Soma-targeting may be an experimental solution.**

Future work:

1. **Test soma-targeted indicator in vivo.**
2. **Test other neuropil contamination removal strategies including CNMF(E) and EXTRACT.**

References: (1) Göbel, W. & Helmchen, F. Physiology 22, 358–365 (2007). (2) Dipoppa, M. et al. Neuron 98, 602–615.e8 (2018). (3)Pachitariu, M. bioRxiv 061507 (2017). (4) Chen, T.-W. et al. Neuron 94, 866–879.e4 (2017). (5) Pnevmatikakis, E. A. et al. Neuron 89, 285–299 (2016). (6) Zhou, P. et al. Elife 7, e28728.(2018). (7) Inan, H. et al. bioRxiv 436279 (2021). (8) Song, A. et al. Journal of Neuroscience Methods 358:109173, (2021)