

Simultaneous Dual-region Functional Imaging in Miniaturized Two-photon Microscopy

Zixiao Zhang¹, Shing-Jiuan Liu¹, Ben Mattison^{2,3}, Weijian Yang^{1,3,*}

¹Department of Electrical and Computer Engineering, University of California, Davis, Davis, CA 95616, USA

²Department of Biomedical Engineering, University of California, Davis, Davis, CA 95616, USA

³Biomedical Engineering Graduate Group, University of California, Davis, Davis, CA 95616, USA

*weijiang@ucdavis.edu

Abstract: We demonstrate simultaneous dual-region in-vivo imaging of brain activity in mouse cortex through a miniaturized spatial-multiplexed two-photon microscope platform, which doubles the imaging speed. Neuronal signals from the two regions are computationally demixed and extracted. © 2024 The Author(s)

1. Introduction

Miniaturized two-photon (2P) microscopes are emerging transformative tools for studying neuronal activities in-vivo in freely-moving mice [1-4]. These devices have a distinct advantage over conventional benchtop microscopes by enabling the recording of mice brain activity in more natural settings. This advancement opens new avenues for novel scientific explorations, such as investigating the neural mechanisms of ethologically pertinent behaviors including social interactions [5] and navigation [1]. However, a major limitation of 2P miniscopes lies in their relatively low imaging speed, a consequence of the point-by-point excitation beam raster scanning mechanism, which restricts the optical recording throughput that can be achieved. Here, we present a novel spatial-multiplexed 2P miniscope capable of recording neuronal activity simultaneously across two fields of views (FOVs). Leveraging the prior knowledge of neuronal locations and the spatiotemporal sparsity of neuronal activity, we used a constrained nonnegative matrix factorization (CNMF) algorithm [6,7] to demultiplex and extract neuronal signals from the two FOVs imaged concurrently. Our method effectively increases the imaging speed by two times.

2. Spatial multiplexing in a miniaturized 2P microscope platform

We built our spatial-multiplexed miniaturized 2P microscope (Fig. 1a) based on an open-source 2P miniscope [1]. The laser beam from a femtosecond laser first passed through a dispersion compensation module to pre-compensate the group delay dispersion. This compensator was fine-tuned to ensure the pulse width at the sample plane was minimized (~ 200 fs). After the compensator, a 50:50 beam splitter divided the beam into two separate paths. Each individual path was then coupled into a hollow-core photonic crystal (HC-PhC) fiber, each equipped with a half-wave plate at the entrance to prevent double-pulse effects due to the HC-PhC fiber [1]. The beamlets, transmitted through the HC-PhC fibers, reached the 2P miniaturized microscope (indicated by the dashed section in Fig. 1a) and excited two laterally offset foci on the mouse brain (Fig. 1a-b). These two foci were raster-scanned by the micro-electromechanical-system (MEMS) scanner, enabling simultaneous imaging of two FOVs.

In our demonstration, the two FOVs were laterally spaced by $170\ \mu\text{m}$ and covered $400\ \mu\text{m} \times 200\ \mu\text{m}$ each (Fig. 1c). Neuronal signals from both FOVs were concurrently recorded during the dual-FOV imaging, and subsequently demixed and extracted by CNMF algorithm. Compared with the conventional approach where a single beamlet was scanned across the entire FOV, the imaging throughput in our system could increase by a factor of two.

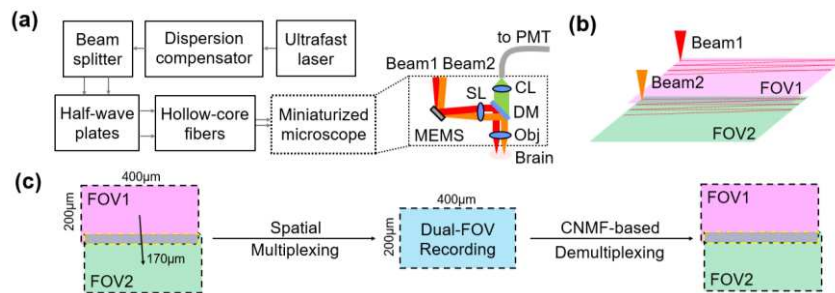


Fig. 1. (a) System setup of the spatial-multiplexed 2P miniscope. (b) Simultaneous dual-FOV imaging via MEMS scanning. (c) Dual-FOV spatial multiplexing and CNMF-based computational demixing. SL: Scan lens. CL: Collection lens. DM: Dichroic mirror. Obj: Objective lens.

3. Simultaneous dual-FOV in-vivo calcium imaging in mice primary visual cortex (V1)

Using our spatial-multiplexed 2P miniaturized microscope platform, we conducted in-vivo calcium imaging of two FOVs simultaneously in layer 2/3 of mouse V1, which was transfected to express the GCaMP6f calcium indicator [8]. The spatial multiplexing and CNMF-based demultiplexing procedures were performed as follows. Initially, individual single-FOV recordings were obtained by blocking each of the two optical paths. The time-series

recordings acquired from each FOV were then analyzed using the CNMF algorithm. This step was essential to identify the spatial footprint and temporal calcium transient characteristics of the active neurons and estimate the background and noise levels within each FOV. The extracted data was stored as prior information for the subsequent demultiplexing. Single-path beam blockers were then removed to enable simultaneous imaging of the two FOVs. For demultiplexing, the dual-FOV recording was analyzed by the CNMF algorithm with the previously stored prior information as initial estimates. The spatial footprint of the neurons was not re-optimized. A similar procedure applied in the single-FOV analysis was used to solve the convex optimization problem for the dual-FOV data to extract the demixed temporal traces of individual neurons and re-estimate the background and noise levels.

The imaging results of the individual FOVs (separately recorded, FOV1 and FOV2, with lateral offset shown in Fig. 1c) and the simultaneously recorded dual-FOV were presented in Fig. 2a, 2b, and 2d respectively. Specifically, Fig. 2a displays the max-correlation projection of a six-minute single-FOV (FOV1) recording, with the magenta contours representing the neuronal footprints extracted by CNMF. The CNMF-extracted temporal activity traces of the neurons were presented in Fig. 2e. Similarly, Fig. 2b and 2f present the single-FOV imaging results of FOV2 with the CNMF-extracted neuronal footprints outlined in green contours, and the representative activity traces respectively. We synthesized a dual-FOV image (Fig. 2c) by arithmetically summing individual FOVs' recordings (i.e. Fig. 2a-b). The synthesized image matched well with the simultaneous dual-FOV imaging result (Fig. 2d). With the neuronal footprints from both FOVs (Fig. 2g) as prior, CNMF successfully extracted the activity traces of the neurons found in FOV1 and FOV2 in the dual-FOV recording (Fig. 2h-i). As a control experiment, we designed the spatial offset of the two FOVs so there was a small overlap (Fig. 1c), indicated by the dashed yellow boxes in Fig. 2a-b. The neurons in the overlapping region (e.g. the two neurons highlighted in yellow and labeled as 1 and 2), were imaged twice and appeared twice in the dual-FOV recording (Fig. 2d). Indeed, correlation of the activity traces of these repeatedly imaged neurons were high (>0.97 for the two highlighted neurons). We note that some active neurons in the dual-FOV imaging (Fig. 2d) were not extracted, likely due to their inactiveness during the single-FOV recordings. Extending the recording duration at single-FOV imaging stage could resolve this issue. Compared to the single-beam imaging scheme in conventional 2P miniscopes, this simultaneous dual-FOV imaging doubles the imaging speed while preserving the spatial resolution. Our method increases the optical throughput and the number of neurons that could be recorded in unit time. It may be further combined with a tunable lens for high-speed volumetric calcium imaging.

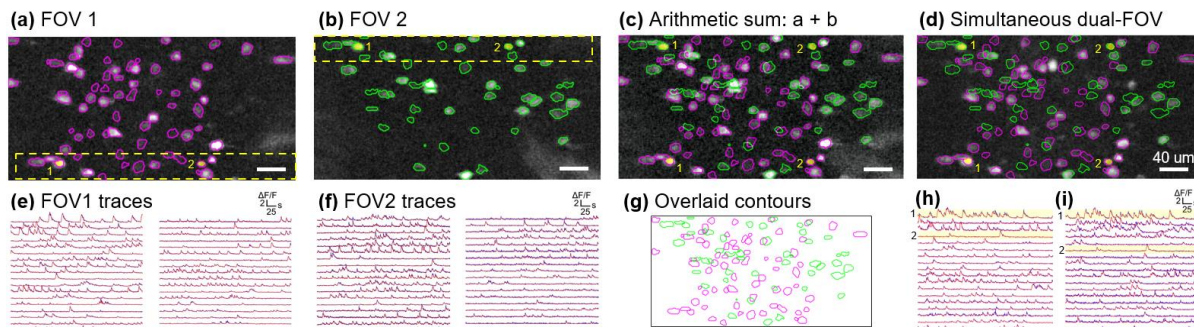


Fig. 2. In-vivo calcium imaging of mice cortex. FOV1 and FOV2 were imaged individually and then together. (a-b) The max-correlation projection of (a) FOV1 and (b) FOV2 overlaid with CNMF-extracted neuronal footprint. (c) Arithmetic sum of (a) and (b). (d) Simultaneous dual-FOV imaging of FOV1 and FOV2. (e) and (f) show the representative CNMF-extracted neuronal activity traces of (a) and (b), respectively. (g) Overlaid spatial component contours from the two FOVs. (h) and (i) show the representative extracted temporal traces of the two FOVs from (d) with CNMF-based demultiplexing. The activity traces of the two labeled neurons in the overlapping regions are highlighted in yellow.

4. Acknowledgement

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5. References

- [1] Zong, Weijian, et al. "Large-scale two-photon calcium imaging in freely moving mice." *Cell* 185.7 (2022): 1240-1256.
- [2] Guan, Honghua, et al. "Deep-learning two-photon fiberscopy for video-rate brain imaging in freely-behaving mice." *Nature communications* 13.1 (2022): 1534.
- [3] Zhao, Chunzhu, et al. "Millimeter field-of-view miniature two-photon microscopy for brain imaging in freely moving mice." *Optics Express* 31.20 (2023): 32925-32934.
- [4] Accanto, Nicolò, et al. "A flexible two-photon fiberscope for fast activity imaging and precise optogenetic photostimulation of neurons in freely moving mice." *Neuron* 111.2 (2023): 176-189.
- [5] Zhang, Chaoyi, et al. "Dynamics of a disinhibitory prefrontal microcircuit in controlling social competition." *Neuron* 110.3 (2022): 516-531.
- [6] Yang, Weijian, et al. "Simultaneous multi-plane imaging of neural circuits." *Neuron* 89.2 (2016): 269-284.
- [7] Giovannucci, Andrea, et al. "CalMAn: an open source tool for scalable calcium imaging data analysis." *elife* 8 (2019): e38173.
- [8] Chen, Tsai-Wen, et al. "Ultrasensitive fluorescent proteins for imaging neuronal activity." *Nature* 499.7458 (2013): 295-300.