Optica Biophotonics Congress: Biomedical Optics 2024
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Publishing Group 2024

Time-Multiplexed Miniaturized Two-Photon Microscopy

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Abstract: We propose a time-multiplexed miniaturized two-photon microscope (TM-MINI2P), enabling a two-fold increase in imaging speed while maintaining a high spatial resolution. Using TM-MINI2P, we conducted high-speed in-vivo calcium imaging in mouse cortex.

1. Introduction

Miniature multiphoton microscope [1] is a powerful tool to investigate the neuronal basis of complex and ethologically relevant behavior, as it could record brain activity (e.g. through calcium indicators [2]) when the animal freely behaves. As the image is built pixel by pixel by raster scanning the focal spot across the sample, there is a general tradeoff among the imaging frame rate, the resolution of the spatial sampling grid and the field of view (FOV). We recently demonstrated a two-photon miniscope where we engineered the point-spread-function (PSF) into an elliptical beam [3]. A larger area of the sample was thus imaged in one measurement, leading to a higher imaging speed. While suitable for cellular resolution, its resolution is lower than the conventional point-by-point scanning approach. Here, we introduce a time-multiplexing technique [4] in the miniaturized two-photon microscope [1] (TM-MINI2P) which maintains a high spatial resolution while increases the imaging speed. This approach utilizes a pair of spatially and temporally distinct beams to image two regions of the FOV, where the signal of individual region could be distinguished by the detection time of their fluorescence. This enables high-resolution imaging with 2x of the imaging speed compared to the conventional approach. We demonstrated the capabilities of the TM-MINI2P in recording neural activity in mouse cortex *in vivo*, over a ~400×375 μm^2 FOV at 1.62 fps (galvo-galvo mode) and a ~160×375 μm^2 FOV at 17.17 fps (galvo-resonant-scanner mode).

2. Methods and Results

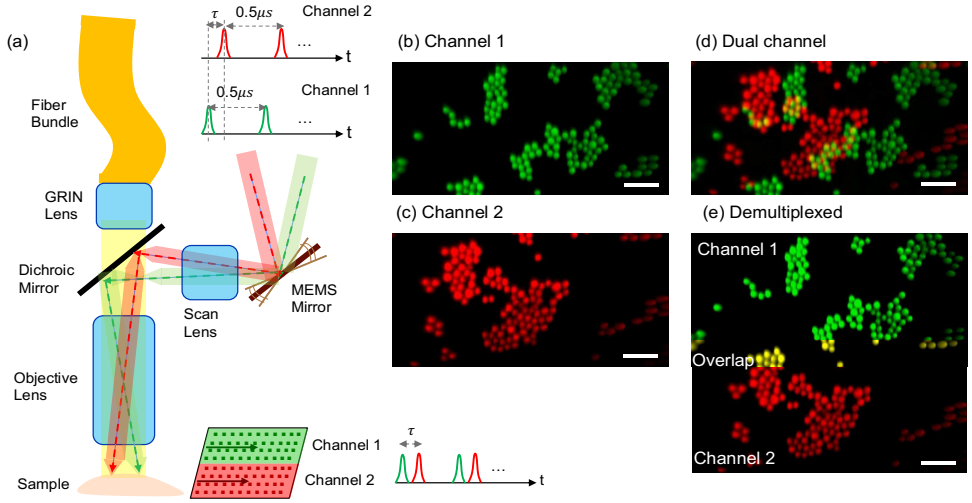

Fig. 1. (a) Layout of the TM-MINI2P. Two laser pulse trains with 2 MHz repetition rate are temporally separated by time τ and directed towards two distinct regions on the sample. (b-d) Fluorescence images of 12-μm fluorescent beads sample with (b) channel 1 excitation (green) only, (c) channel 2 excitation (red) only, and (d) dual channel excitation. (e) Temporal demultiplexed signal from (d). The overlapped imaging region from the two channels is colored as yellow. Scale bar: 50 μm. GRIN lens, graded-index lens.

The two excitation channels in the TM-MINI2P (Fig. 1a) were generated by splitting a 920-nm femtosecond laser pulse train (2 MHz repetition rate) into two paths and delaying one from the other through free space optical path. The temporal delay of the two paths is $\tau = 20\ ns$, which well exceeds the fluorescence lifetime of GCaMP (~6-7 ns) and thus minimizes crosstalk between the two channels. The two beams were then coupled to two hollow-core photonic crystal (HC-PhC) fibers, reached the two-photon miniaturized microscope [1], and focuses on two different regions on the sample. Through the micro-electromechanical-system (MEMS) scanner, each beam scanned and imaged half of the entire FOV. The emitted fluorescence passed through a dichroic mirror and was coupled to a fiber bundle through a gradient index lens, before being detected by a photomultiplier tube. To demultiplex the signal from the two channels, we set two temporally separated window to gate and integrate the fluorescence signal from each excitation

pulse at the digitizer. We validated our TM-MINI2P through a phantom sample made of randomly distributed 12-μm fluorescent beads (Fig. 1b-e). In the dual-channel recording (Fig. 1d), the signal of each channel matched well with the single-channel recording when one of the channel was manually blocked (Fig. 1b-c), confirming the minimum crosstalk between the two channels. Based on the calibrated separation distance of the two beams, the entire FOV (Fig. 1e) could be constructed. Here, a ~400×375 μm^2 FOV was recorded by using half of the MEMS' scanning angle range on the slow axis, thus doubling the imaging speed, compared to the conventional single-beam approach.

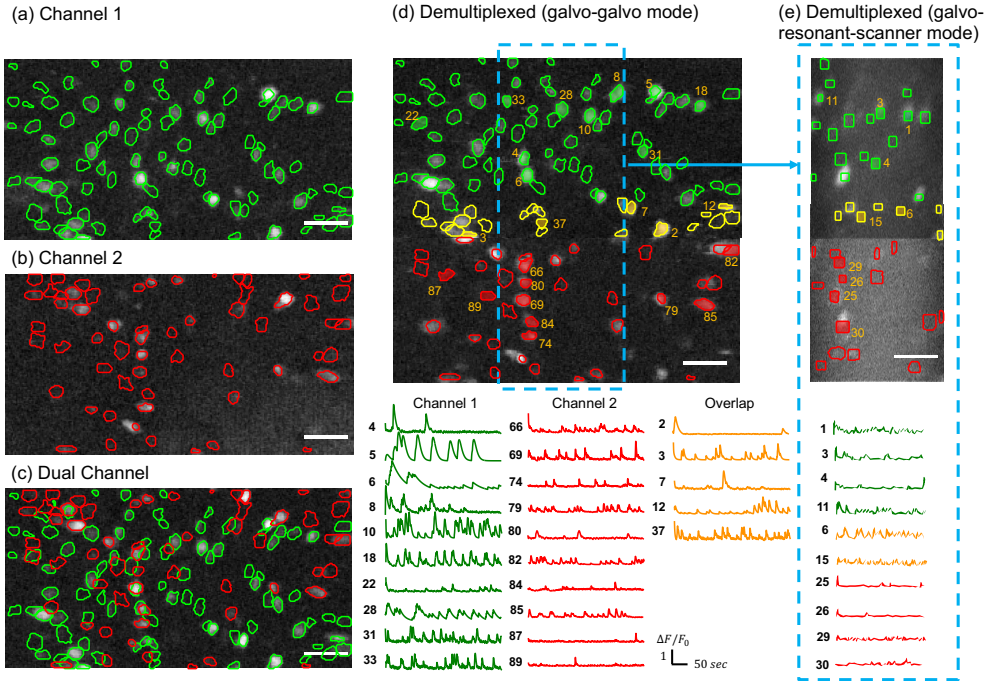

Fig. 2. Calcium imaging of mouse cortex *in vivo* through TM-MINI2P. (a-c) Max-correlation projection of the recording of (a) channel 1 and (b) channel 2 by blocking one excitation path, and (c) dual channel, with the CNMF-extracted neuronal cell body contours overlaid. (d) Demultiplexed dual channel recording [from (c)] with the CNMF-extracted neuronal activity traces of selected cells for each channel (green/red). The spatial footprint and temporal activity trace of the neurons in the overlapped regions of the two channels were labeled in yellow. Galvo-galvo scanning mode were used, resulting in a frame rate of 1.62 fps. (e) Same as (d), but with galvo-resonant-scanner mode, at a frame rate of 17.17 fps. Scale bar: 50 μm.

We demonstrated the capability of TM-MINI2P for in-vivo calcium imaging of mouse cortex (Fig. 2), in both galvo-galvo (GG) mode (1.62 fps) and galvo-resonant-scanner (GS) mode (17.17 fps). At GG mode (Fig. 2d), a ~400×375 μm^2 FOV (256 × 256 pixels) was reconstructed by interleaving the two channels in time. At GS mode (Fig. 2e), we set the scanning frequency of the MEMS mirror to be 2.06 kHz at the fast axis, and imaged at 400 pixels per line, where one pixel was excited by one pulse. As the operation frequency was well beyond the resonant frequency of the MEMS mirror (1.25 kHz), the FOV shrank to ~160×375 μm^2. A constrained nonnegative matrix factorization (CNMF) algorithm [5, 6] was used to extract the spatial footprint and temporal activity traces of the neurons in the FOV.

3. Conclusion

We reported the TM-MINI2P which enabled a two-fold increase in imaging speed while maintaining a high spatial resolution in 2P miniaturized microscope. Using TM-MINI2P, we demonstrated calcium imaging in mouse cortex *in vivo*. Ongoing work includes optimizing the optical design and MEMS scanning system to increase the imaging speed and FOV, and incorporating an electrically tunable lens to performs high-speed volumetric calcium imaging on freely-behaving mice.

4. Acknowledgment

We acknowledge support from National Institute of Neurological Disorders and Stroke and National Eye Institute (R01NS118289), National Science Foundation (CAREER 1847141) and Burroughs Wellcome Fund (Career Award at the Scientific Interface 1015761).

5. References

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