Rapid dual-views projection microscopy with two-photon glutamate uncaging for neural imaging

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Abstract

Here, we present a rapid dual-view projection imaging method with two-photon glutamate uncaging capability based on an upright Bessel beam light-sheet microscopy setup. Compared with light-sheet imaging, our projection imaging method can significantly improve the volumetric rate for neural imaging. In addition, an independent laser scanning module is designed for 2P uncaging, allowing simultaneous synaptic resolution of stimulation and 100 Hz volumetric imaging of neural activity in deep tissue. Imaging results from mouse brain slices under 2P glutamate uncaging will be presented.

Abstract

The study of neural function requires tools with high spatial and temporal resolution to manipulate and monitor neural activity over large volumes of brain tissue. One of the key goals of neural imaging is to develop fast volumetric imaging techniques with low phototoxicity to brain tissue. Two-photon (2P) light-sheet microscopy has demonstrated its high-speed 2D neural imaging capability in deep tissue. However, its 3D imaging speed is still limited. To improve the volumetric imaging speed for neural imaging, we develop a fast dual-view projection imaging method based on our previously established 2P Bessel beam light-sheet microscope. We also introduce a 2P uncaging module to the system for synaptic resolution neural stimulation.

For dual-view projection imaging, a pencil-shaped two-photon Bessel beam scans the imaged volume at 100 Hz. A camera and a PMT, tightly synchronized with the laser scanners and an electrically tunable lens (ETL), collect two orthogonal view projection images at a rate of 100 Hz. The 2P uncaging module can independently access any selected position with synaptic resolution within the 3D imaging volume. We demonstrate our system by performing projection imaging of glutamate-evoked Ca2+ activity in a neuron at 100 Hz using 2P uncaging. This method provides a solution for rapid 3D volumetric imaging in brain tissue and has the potential to extend the application of existing light-sheet microscopes.

Rapid dual-views projection microscopy with two-photon glutamate uncaging for neural imaging

Research on neuronal functions requires high spatial and temporal resolution tools for manipulation and neural activity monitoring over a large volume of brain tissue. Two-photon (2P) excitation and uncaging are powerful tools for deep-tissue neural imaging and localized subcellular-resolution neural stimulation through photochemical release of caged compounds. Rapid 2P imaging techniques further accelerate the imaging speed for neural imaging. In the previous studies, we developed a rapid 2P Bessel beam light-sheet imaging in the brain tissue [1, 2]. However, its volumetric imaging speed is still limited.

Herein, we introduce a rapid dual-view projection imaging method based on our previously established 2P Bessel beam light-sheet microscope. It greatly improves the volumetric imaging rate to 100 Hz. Our system also adds a 3D two-photon uncaging module. Both the projection imaging and 2p uncaging can conduct fast focus tuning through electrical tunable lenses (ETLs), one for light-sheet imaging focusing and one for 2P uncaging, respectively. The system enables synaptic resolution of stimulation and 3D imaging neural activity in deep tissue.

Design of the rapid dual-views projection microscope

We combine 2P projection imaging with 2P uncaging stimulation for all-optical investigation of neural function in deep tissue. Figure.1 shows the schematic of the system. The optics of the dual-view projection microscope is based on an upright 2P Bessel beam light-sheet microscope by adding a PMT to collect Y-projection image in the excitation arm. For the camera arm, an ETL is placed at the conjugated plane of the detection objective back focal plane and synchronized with the scanning Bessel beam to create a Z-projection image on the camera.

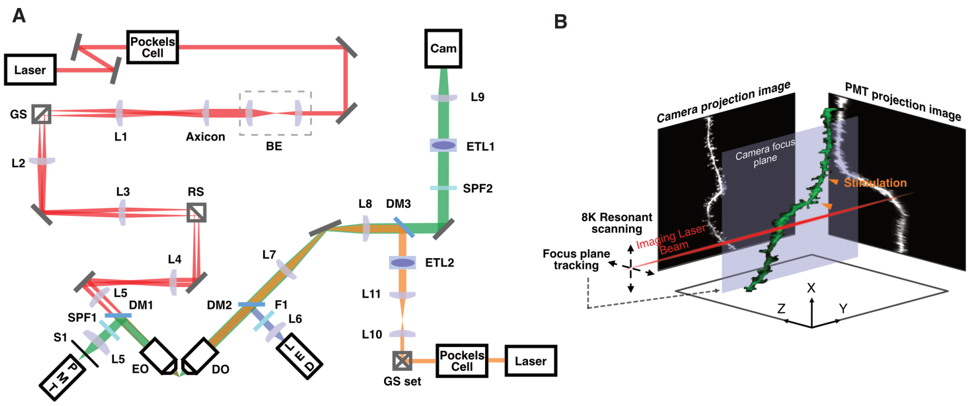

Figure.1: Rapid 2P Bessel beam projection imaging with 2P stimulation ability. (A) System schematic. The microscope is based on an upright configured 2P Bessel beam light-sheet microscope. The generated 2P Bessel beam is scanned across the imaging volume at 100 Hz by the galvo scanner (GS) and the resonate scanner (RS). The extended Bessel beam creates a projection on the PMT through the excitation objective (EO). And with a synchronized ETL, the camera captures another projection image from a different view of the image volume. The 2P uncaging module consists of a second Ti-sapphire laser, a second Pockels Cell, a set of glavo scanners and an additional ETL for independent stimulation control.

(B) The 2P Bessel beam is scanned across the imaged volume. Fluorescence signals are projected onto the PMT in Y-direction and the camera sensor in Z-direction, respectively, generating orthogonal dual-view projection images. The stimulation is also synchronized with the projection imaging process.

For the 2P uncaging, we use another Ti-sapphire laser for uncaging glutamate at 720 nm. The uncaging beam is independently controlled by second sets of Pockels cell, galvo set, and ETL. The projection imaging volume and the optical stimulation space are rigorously co-registered through a fully automatic calibration protocol, allowing simultaneous high-resolution 3D optical stimulation and imaging for the system.

Dual-view projection imaging of 2P uncaging of glutamate-induced neural Ca^{2+} activities

The 2P uncaging module to the microscope allows us to perform two-photon glutamate uncaging on a GCaMP8m-expressed cortical neuronal dendrite. Viruses (flex.GCaMP8m × Cre) were injected into the motor cortex of a B6 mouse brain. 300 μm thick brain slice was obtained and perfused in the oxygenated ACSF with 1 mM DNI-Glutamate. Neuronal dendrites were imaged at 100 Hz volume rate using 920 nm 130 mW excitation power. Figure. 2(A) presents the selective projection images from a 30-second-long time-lapse image series, during which 1 ms × 10 stimulation with 1 ms interval of 720 nm laser pulse were focused to uncage glutamate near a spine head on the dendrite at 5 seconds after the recording start. Figure. 2(B) shows the Ca^{2+} traces from the whole image series, indicating increased intracellular Ca^{2+} after the 2P uncaging event.

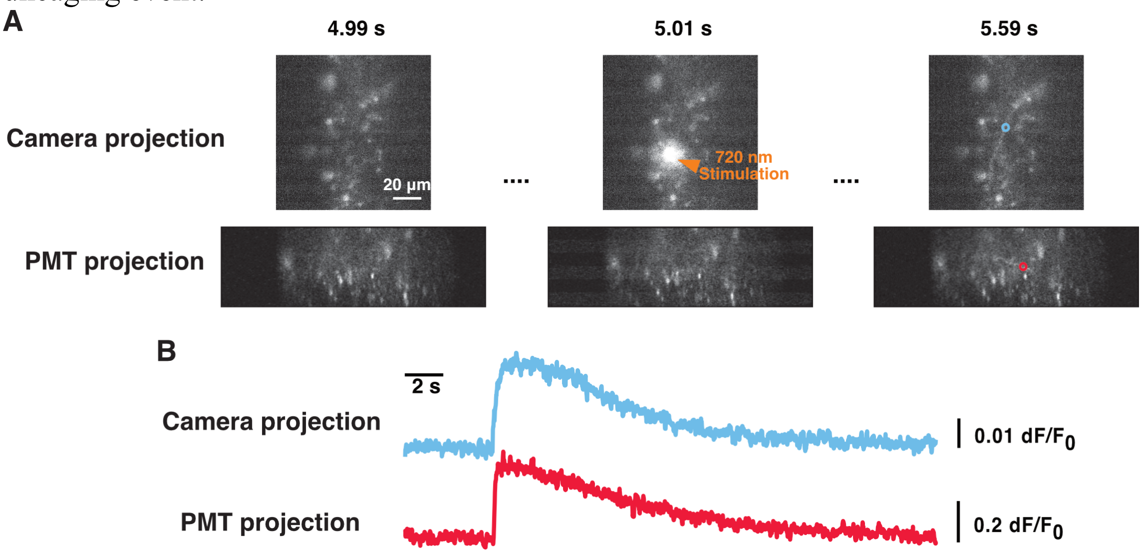

Figure.2: Projection imaging of calcium responses induced by two-photon glutamate uncaging. The volume was imaged at 100 Hz. **(A)** Representative projection images from the 30 sections recording. The images from 4.99 s after the recording start show the status just before the stimulation. The bright spot of the camera projection image at 5.01 s shows the stimulation position. At 5.59 s, both the camera and PMT images shows the active dendrite. (B) Ca2+ traces form the selected regions marked within (A).

References

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2. D. Xu, J. B. Ding, and L. Peng, "Depth random-access two-photon Bessel light-sheet imaging in brain tissue," Opt Express **30**, 26396-26406 (2022).