Synaptic resolution two-photon stimulation and imaging of neural activity with Bessel beam light-sheet microscopy

Dongli Xu[1, 2], Jun B. Ding[2*] and Leilei Peng[1]**

[1]College of Optical Science, The University of Arizona, Tucson, AZ 85721, USA

[2]Department of Neurosurgery, Department of Neurology and Neurological Sciences, Wu-Tsai Neuroscience Institute, Stanford University, Stanford, CA 94305, USA

** dingjun@stanford.edu*

*** lpeng@optics.arizona.edu*

Abstract: We report combining two-photon Bessel beam light-sheet microscopy with two-photon uncaging of glutamate to perform all-optical neural stimulation and imaging in deep-tissue at synaptic resolution. Imaging results from mouse brain slice under 2P uncaging of glutamate will be presented .© 2022 Dongli Xu, Jun B. Ding and Leilei Peng

1. Introduction

Neurons communicate with each other with various neurotransmitters crossing synapses. Two-photon (2P) uncaging of glutamate provided neuroscientists with a direct optical tool to manipulate neural activity in brain tissue through uncaging chemical messengers at synaptic resolution. By incorporating the 2P uncaging into two-photon high-speed light-sheet volumetric imaging, we conduct high-resolution functional mapping of neural circuit deep in the brain tissue. Our system integrates a fast two-photon Bessel beam light-sheet microscope with a 3D two-photon uncage module. Both are capable of 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.

2. Methods

Two-photon(2P) excitation is a powerful tool for both deep tissue imaging of brain and localized subcellular-resolution neural stimulation through photochemical release of caged compounds. Light-sheet 2P imaging techniques further accelerates 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]. Here, we combine 2P uncaging stimulation with 2P Bessel beam light-sheet microscopy for all optical investigation of neural tissue. Fig.1 shows the schematic of our system. For 2P light-sheet imaging, an ETL is used to conduct rapid z-refocusing for rapid 3D scanning. For 2P uncaging, the stimulation light is independently controlled by a set of galvo scanners and a second ETL. The light-sheet imaging field-of-view and the optical stimulation position are co-registered through a rigorous and fully automatic calibration protocol. This new system allows simultaneous high-resolution 3D optical stimulation and imaging.

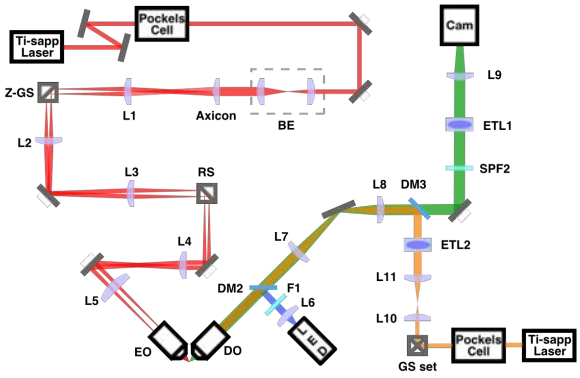

Fig. 1. Schematic of Two-photon Bessel beam light-sheet imaging system. We used two Ti-sapphire lasers for light-sheet imaging at 920 nm and two-photon uncaging of glutamate at 720 nm, respectively. The excitation Bessel beam is created by an axicon lens and scanned by a resonant scanner (RS) to create a two-photon excitation light-sheet. The fluorescence signals are imaged onto a camera through an electrical tunable lens (ETL1) synchronized with a z galvo scanner (Z-GS). The uncaging laser is independently controlled by a second set of galvo scanners (GS set) and ETL (ETL2).

3. Results

3.1. Thy1-YFP mouse brain slice 3D 2P light-sheet imaging

The 2P Bessel light sheet microscope is capable of perform 3D synaptic resolution imaging of neural tissue at 100 FPS. Fig. 2 shows rendering of a typical 3D image stack, taking from a Thy1-YFP mouse brain slice. The 3D image contains over 86 light-sheet optical sections with 1 μm depth intervals, scanned by incrementally changing imaging ETL-driven current and corresponding z-galvo scanner voltage. The imaging frame rate was 100 Hz, resulting in a volumetric rate of 1.2 Hz. Neuronal dendrites and spines can be clearly visualized in the rendering images. The FWHM of the cross-section over a spine neck proves the system has a 0.5 μm lateral resolution, which meets the resolution predicted by the optical design.

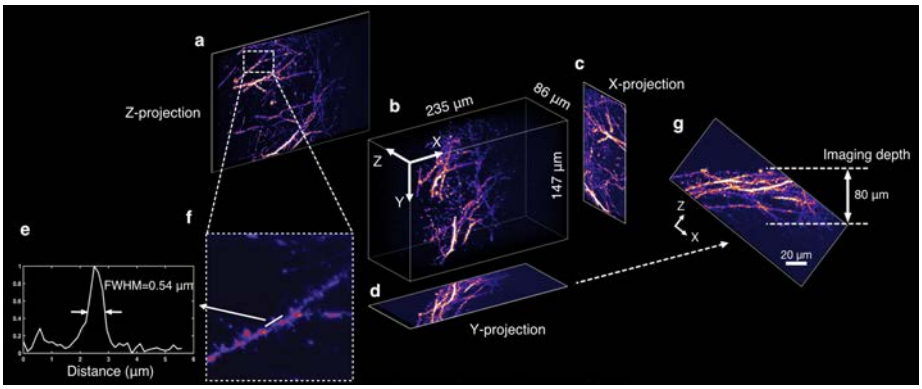

Fig. 2. Volumetric imaging of a neural structure in a Thy1-YFP mouse slice at 100 frames per second. The volume size is 235 × 147 × 86 μm^3. **(a-d)** A 3D rendered image and maximum intensity projections of the whole imaged volume. **(e)** The cross-section profile of a spine neck marked in (f) shows the spatial resolution of the light-sheet microscope is 0.54 μm. **(f)** An enlarged view of a dendrite marked in (a). **(g)** shows that the microscope can acquire high-quality light-sheet imaging data at a depth of 80 μm.

3.2. Two-photon uncaging of glutamate in mouse brain slice

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) was 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 20 Hz framerate using 920 nm 130 mW excitation power. Fig 3. presents a 25-second-long time-lapse image series, during which a 1 ms pulse of 720 nm stimulation laser light was focused to uncage glutamate near a spine head on the dendrite at 1.85 seconds after the record start. Fig. 3a-e. shows the increase of green fluorescence from the GCaMP8m, indicating increased intracellular Ca^{2+}. Fig. 3f plots a typical Ca^{2+} time-trace at subcellular locations.

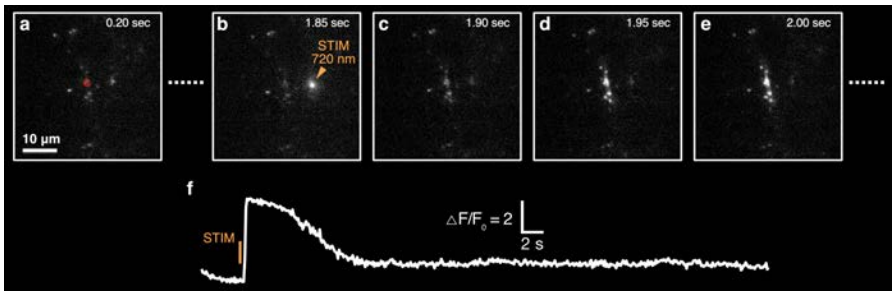

Fig. 3. Representative 2p Bessel light sheet time-lapse images of calcium responses induced by two-photon glutamate uncaging. **(a)** GCaMP8m intensity image before the two-photon uncaging event. **(b)** A 1-ms 720 nm stimulation laser pulse (orange arrow) was focused near a spine to uncage glutamate. **(c-e)** Successive light-sheet images exhibit Ca^{2+} signal changes. **(f)** The $\Delta f/f_0$ trace of total GCaMP8m signals in the area marked with a red circle in (a).

References

[1] M. Zhao, H. Zhang, Y. Li, A. Ashok, R. Liang, W. Zhou, and L. Peng, "Cellular imaging of deep organ using two-photon Bessel light-sheet nonlinear structured illumination microscopy," Biomed. Opt. Express **5**, 1296-1308 (2014).

[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).