Microsecond Timescale Selective Access Two-photon Targeting for Functional Measurements in Tissue

Vicente J. Parot[1,2,3*], Shane Nichols[3*], Guilherme Testa-Silva[3,4], & Adam E. Cohen[3,4,5]
*[1]Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, 40 Blossom St, Boston MA 02114, USA; [2]Division of Health Sciences and Technology, Massachusetts Institute of Technology, 77 Mass Ave, Cambridge MA 02139, USA; [3]Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford St, Cambridge MA 02138; [4]Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase MD 20815, USA; [5]Department of Physics, Harvard University, 17 Oxford St, Cambridge MA 02138. *These authors contributed equally.*
vparot@mgh.harvard.edu; cohen@chemistry.harvard.edu

Abstract: Dense sampling of voxels limits time resolution in two-photon microscopy. We developed a system to scan sequentially through 512 excitation spots at 2 microseconds between spots, enabling targeted recording of neurotransmitter release at millisecond time resolution in intact tissue.

1. Microsecond Selective Access Scanner

Two-photon (2P) scanning microscopy can report Ca^{2+} transients of up to ~10^4 neurons deep in the living mouse brain, with time resolution of several hundred milliseconds. Recent advances in all-optical electrophysiology enable manipulation and recording of much faster electrical spikes of neurons and neurotransmitter release events [1-3], which require ~1 ms time resolution. Current high-speed optical systems are limited to <100 cells due to spatiotemporal resolution limitations. Raster scanning 2P microscopes suffer from a trade-off in spatial and temporal resolution. Efforts to increase speed by splitting the power among N targets lead to an N-fold decrease in 2P excitation efficiency. Random access scanning using acousto-optic devices has bandwidth limited to ~50 kHz point-targeting rate, limited scan angles, and low efficiency [4].

To enable millisecond time resolution 2P excitation of ~500 neuronal targets, we designed a hybrid scanning system combining high speed galvanometric scanning in one dimension (1D) with space division multiplexing of a static holographic mask. A collimated femtosecond laser was focused by a cylindrical lens onto a diffraction-limited line, reflected on a galvo mirror, and reimaged onto a liquid crystal reflective spatial light modulator (SLM, Meadowlark ODP512). The focused line covered one row of pixels in the SLM, and was translated transversally by the galvo scanner so that each laser shot landed on a successive row of SLM pixels. The holographic reflection from the SLM was focused in 1D by another cylindrical lens, and imaged to the sample plane through a 25x objective (Olympus XLPLN25XWMP2). The sample plane was scanned periodically at 400 Hz in one dimension by galvo motion, and holographically targeted in the second dimension, with a different 1D hologram imparted by each row of the SLM. This strategy enabled sequential addressing of selected target locations over a large area (0.5 mm x 0.5 mm) in the sample plane. A schematic representation of the scan system is shown in Fig. 1A.

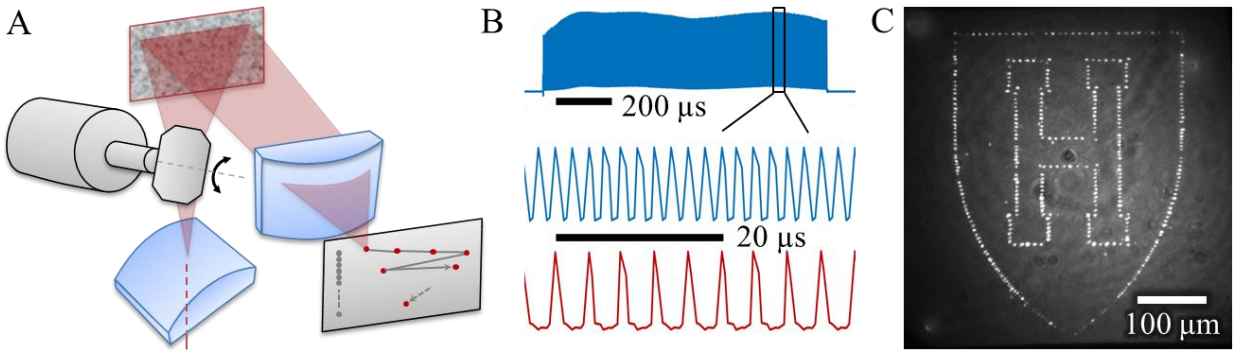

Fig 1. High-speed selective access 2P scanning. (A). Schematic of the optical system. A combination of periodic scanning and static holography multiplexing achieves fast 2D selective target addressing. A circular lens between galvo and SLM is not depicted. (B). Two-photon fluorescence waveform recorded from a fluorescein volume sample for timing calibration purposes. Top: Full single scan of 512 SLM rows in 1 ms. Middle: zoom-in of the detection of every laser pulse at 500 kHz, after diffraction off the SLM. Bottom: Similar scan while diffracting every other SLM row out of the field of view. (C). Fluorescent image of ~500 photoconverted targets after sequential scanning through all targets in 1 ms.

To allow efficient 2P excitation, we synchronized the galvo control waveform to the laser oscillator (Amplitude Satsuma, 1030 nm), and calibrated the galvo trajectory so each optical pulse landed on a successive SLM row. This arrangement can direct the full available laser power to selective locations sequentially at microsecond time intervals. To confirm this capability, we configured all rows of the SLM to direct light to the sample, and recorded 2P-excited fluorescence from a dye sample using a PMT (Hamamatsu H11526-20-NF). Fig. 1B shows the envelope of a full scan of the SLM over 1 ms (top), zooming in on the detection of each pulse at 500 kHz repetition rate (middle). Configuring every other row of the SLM to direct light outside the sample demonstrated holographic manipulation of individual laser pulses (bottom). To document the addressing flexibility and large area covered by the scanning system, we recorded a camera image (Fig. 1C) of ~500 targets in a thin solid fluorescent layer that were photoconverted by sequential scanning of the 2P excitation through them in 1 ms. This fast scan was repeated 8 times targeting the same locations.

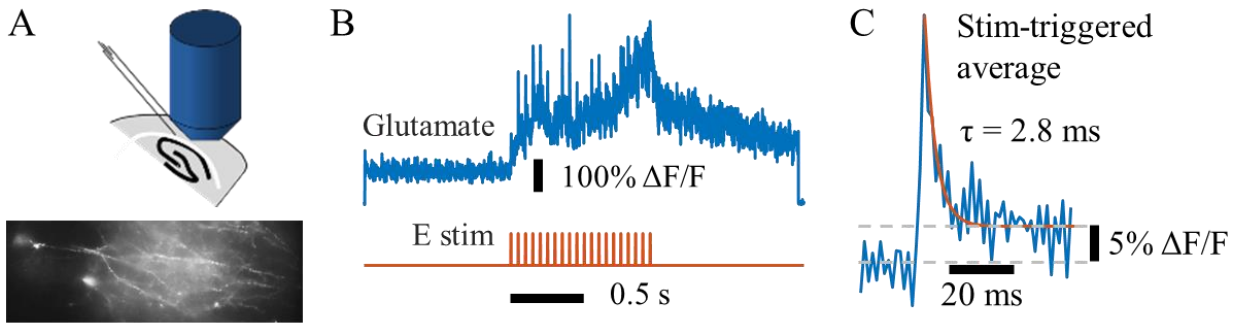

Fig 2. High-speed measurements of glutamate release. (A) Top: schematic of acute slice optical recording with electrical stimulation. Bottom: static fluorescence image of a neuron expressing yGluSnFr in an acute brain slice. (B) Top: fast rises in the extracellular Glutamate concentration were detected upon synaptic release elicited by electrical pulses. Accumulation of glutamate upon repeated stimulation indicates synaptic facilitation. (C) Stimulation-triggered average quantified fast decay and facilitation.

2. Synaptic Release of Glutamate

We applied this instrument to measure extracellular glutamate concentration reported by virally transduced yGluSnFr(A184S) [5] in acute brain slices. Fig. 2A shows a schematic of the experimental preparation and an example wide-field fluorescence image of the expression pattern. A bipolar electrode in CA3 delivered 2 ms pulses at 20 Hz for 1 s while 12 mW of 1030 nm excitation was targeted to a yGluSnFr-expressing cell in CA2. Fluorescence was recorded by a PMT. A fluorescence trace at 800 Hz, shown in Fig. 2B (top), showed fast rises in response to extracellular stimulation (Fig 2B, bottom) and cumulative increase in amplitude, consistent with electrically induced synaptic release and short-term synaptic facilitation. On average (Fig. 2C), elicited transients decayed with a time constant of 2.77 ms, and sustained a 5% increase in fluorescence after decay.

These results are consistent with known hippocampal physiology [6] and demonstrate measurement of activity recorded at 800 Hz indicating synaptic neurotransmitter release and facilitation, achieved with a system capable of efficient selective 2P excitation of 512 sequential targets at 2 microsecond intervals. The number of targets was limited by SLM rows (512), and the interval between targets was set by the laser repetition rate (500 kHz). The scan period was fixed by twice the product of rows and interpulse interval, plus turnaround time. Further work can readily increase these parameters to achieve >1000 targets using an SLM with more rows, and faster addressing and scan rate using higher laser repetition rates. These tools have the potential to enable efficient large-scale, kHz-resolved targeted recording and possibly stimulation *ex vivo* and *in vivo*.

3. References

[1] Lin, Michael Z., and Mark J. Schnitzer. "Genetically encoded indicators of neuronal activity." Nature neuroscience 19.9 (2016): 1142.

[2] Chen, I-Wen, et al. "In Vivo Submillisecond Two-Photon Optogenetics with Temporally Focused Patterned Light." Journal of Neuroscience 39.18 (2019): 3484-3497.

[3] Adam, Yoav, et al. "Voltage imaging and optogenetics reveal behaviour-dependent changes in hippocampal dynamics." Nature 569.7756 (2019): 413.

[4] Weisenburger, Siegfried, and Alipasha Vaziri. "A guide to emerging technologies for large-scale and whole-brain optical imaging of neuronal activity." Annual review of neuroscience 41 (2018): 431-452.

[5] Marvin, Jonathan S., et al. "Stability, affinity, and chromatic variants of the glutamate sensor iGluSnFR." Nat. Methods 15 (2018): 936-939.

[6] Debanne, Dominique, et al. "Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation affects subsequent release." The Journal of physiology 491.1 (1996): 163-176.