Scanned Line Angular Projection Two Photon Laser Scanning (SLAP2) Microscopy for Real-Time (Kilohertz Rates) Volumetric in Vivo Imaging at Subcellular Resolution

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Abstract

Calcium imaging has been the gold standard for functional in-vivo imaging of neural activity. However, calcium imaging lacks the temporal resolution to capture action potentials in neurons. Although new fluorescent voltage and neurotransmitter indicators, such as Voltron, have been developed to overcome this limitation, the emerging labeling mechanisms require new imaging methods to capture neuronal activity at kilohertz rates. SLAP2 microscopy uses a novel and unique scan technology capable of imaging in vivo mouse brains and in vitro brain slices at subcellular spatial resolution at kilohertz scan rates.

Preliminary raster scanning images of glutamate signals acquired in-vivo from excitatory neurons in the mouse visual cortex in the laboratory of Dr. Podgorski have validated that the SLAP2 technology can detect transience in synaptic activity. Going forward we plan to extend this concept and validate the system's ability to image with kilohertz scanning rates by employing fast ROI scanning methods. With a temporal resolution of milliseconds (i.e., 1000 Hz and higher), we believe that SLAP2 will enable many experimental paradigms focused on imaging living neuronal circuits. The fast, large-scale microscopic imaging advancement will also expand our ability to accurately characterize physiological activity in three-dimensional culture and organoid models.

Methods

The SLAP2 microscope is a 2-photon laser scanning microscope with three imaging modes for direct and fast sampling of voltage and neurotransmitter signals. The type of imaging mode is determined by the sequence of illumination patterns presented on the Digital Micromirror Device (DMD, Fig. 3F). To create the illumination patterns, the DMD switches individual pixels in a FOV "on" and "off" at full scan rates, allowing random-access imaging of ROIs with minimal mechanical movement overhead, increasing imaging speeds by roughly 30x that of a resonance scanning 2-photon microscope.

Imaging Mode	Use Case	DMD Sequence
Raster Scanning	Structural Imaging	DMD Pixels for a single row are switched on
Fast Region of Interest Scanning	Fast measurement of change in fluorscent singal	Sequence of DMD illumination masks
Tomographic Scanning	Structural imaging and fast measurement of change in fluorscent singal	All DMD pixels switched on

For fast ROI scanning, a scheduling algorithm generates a sequence of DMD illumination masks to sequentially scan all selected ROIs. In fast ROI scanning, an entire ROI is illuminated within one line sweep, and the fluorescent signal is measured and integrated for the ROI. This imaging method does not generate raster images but directly measures the change of fluorescence signal of brain regions at fast rates.

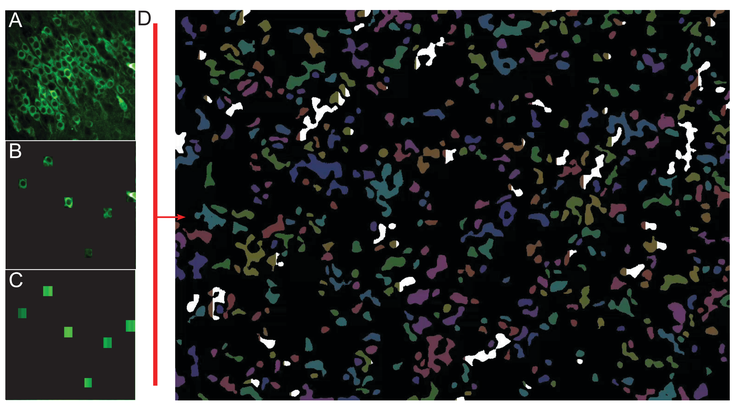


Figure 1. (A) a raster scanned image for a single timepoint of neurons with a GCaMP6 calcium indicator. (B) 6 cell bodies from the raster scan image are selected for activity monitoring using fast ROI scanning. An imaging mask is defined using the structural information from image (A). (C) For fast region of interest scanning, a laser line is swept across the digital mirror device (DMD). The DMD pixels act as an illumination mask (black), and relay light to the sample only for active regions of interest (ROIs). The integrated intensity from the entire line scan is obtained for each pixel column in the active ROIs. (D) The DMD can change the illumination mask at the end of each line sweep to collect data from a new set of ROIs. Within one sweep, only ROIs that do not overlap in the direction perpendicular to the line sweep can be sampled. A scheduling algorithm groups regions of interest into line sweeps to ensure even and efficient sampling of all ROIs.

+ Match the Pace of Neural Communication

A vertical laser line is horizontally swept across the DMD 10,800 times per second. Capturing the activity of multiple cell body-sized volumetric ROIs scanned at kilohertz rates, SLAP2 can directly sense voltage and Ca++ signals.

+ Increase Information Throughput

Simultaneously image multiple fields of view, optical z levels, or orientations with the addition of surfboard modules.

+ Minimize Mechanical Overhead

Piezo-Mounted Mirrors increase speeds when imaging in 3 dimensions by focusing optically. Bi-stable DMD mirrors tilt +/- 12 degrees to quickly image random access ROIs.

SLAP2 Microscope Optical Path

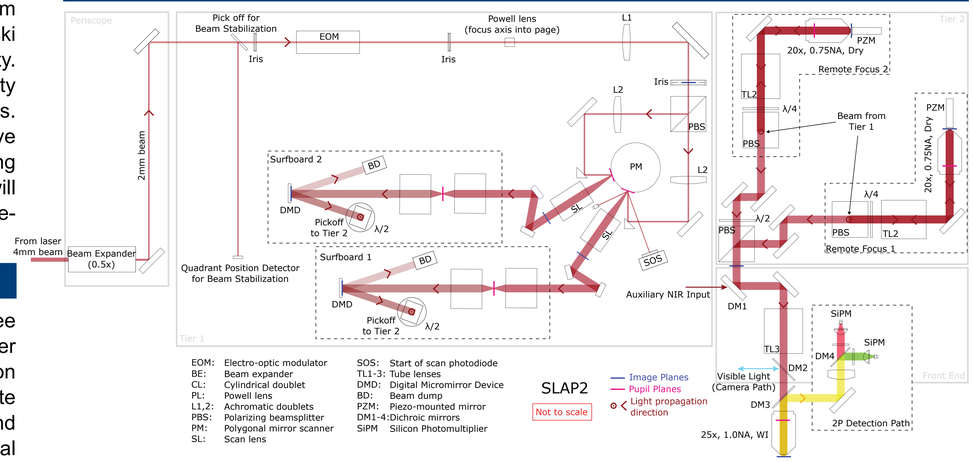


Figure 2. A femtosecond laser for 2-photon excitation (1030nm, 60W) is coupled into the microscope via a periscope. The Gaussian beam profile is transformed into a line focus by a Powell lens. The laser line is scanned across a Digital Micromirror Device (DMD) (1280x800 pixel array) by a Polygonal Scanner (28 faces, 21,500rpm -> 10.8kHz line rate). The DMD is conjugated to the image plane and acts as an illumination mask to selectively illuminate regions of interest in the sample. A remote focusing system positions the focus axially within the sample (600µm range). Two Multipixel Photon Counters (MPPCs) convert the fluorescent emission from the sample into voltage signals. The SLAP2 microscope is equipped with two independent scan arms, each equipped with a DMD and a remote focus system. The field of view of each scan path can be positioned independently within the sample. The DMD pixel array yields a field of view of 320µm x 200µm in the sample.

SLAP2 Microscope Layout

Figure 3

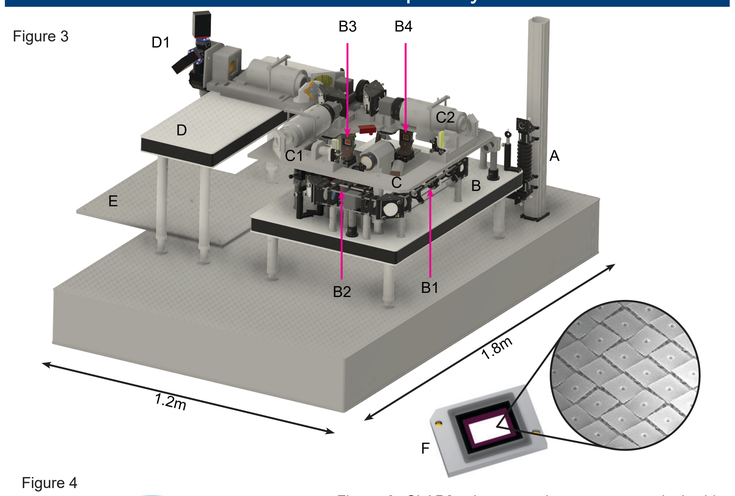


Figure 4

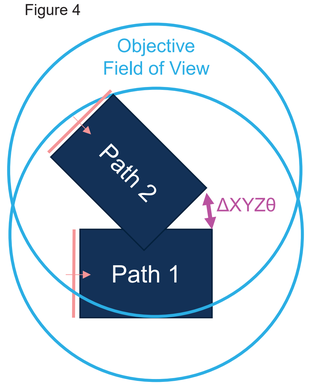


Figure 3. SLAP2 microscope layout on an optical table. (A) Periscope. (B) Tier 1. (B1) Powell Lens. (B2) Polygonal Line Scanner.Figure 3. SLAP2 microscope layout on an optical table. (A) Periscope. (B) Tier 1. (B1) Powell Lens. (B2) Polygonal Line Scanner. (B3) Surfboard 1. (B4) Surfboard 2. (C) Tier 2. (C1) Remote Focus 1. (C2) Remote Focus 2. (D) Front End. (D1) Objective lens and 2P Detection Path. (E) Platform for Sample. (F) Digital Micromirror Device, 1280x800 pixel array, pixel pitch 10.8µm (Image credit: Douglass M., Texas Instruments)

Figure 4. The SLAP2 microscope has two independent optical paths that can be arbitrarily positioned laterally within the objective field of view. Each path is equipped with a dedicated DMD and a remote focusing device. The two paths can simultaneously and independently scan different regions of the sample at different axial locations. Because of the layout of the microscope's optical path, the fields of view of path 1 and path 2 are rotated 45° relative to each other.

Results

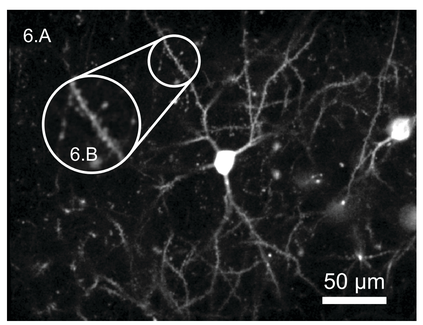
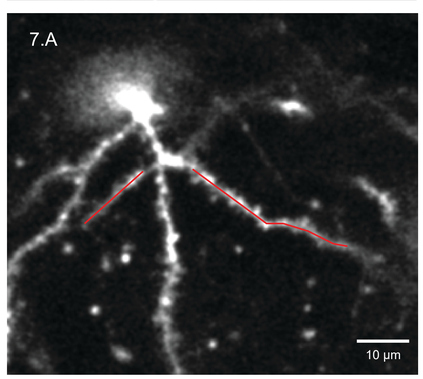


Figure 6.A. Full field raster scan of mouse excitatory cortical neurons expressing iGluSnFR3.NGR and jRGECO1a. The full width half maximum of the point spread function (PSF) is 0.7µm laterally, and 2.6µm axially. The microscope is diffraction limited at a numerical aperture of 0.7. The SLAP2 microscope resolves dendritic spines (detail 6.B). The frame rate of a full field raster scan of 320µm x 200µm is 12.5Hz.



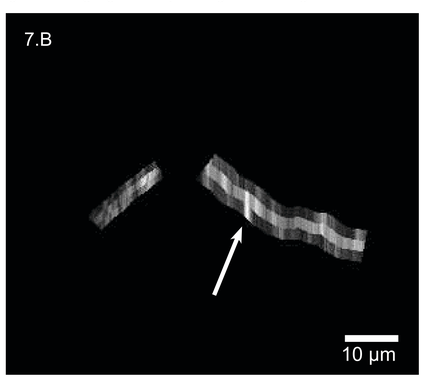


Figure 7. Multi strip integration scan of a mouse excitatory cortical neuron. First a anatomical raster scan is performed (7.A), and two dendrites are selected for a strip integration scan (highlighted in red). The selected dendrites are each scanned with three integration strips: The center strip captures the spines above and below the dendrite; the side strips capture the spines on each side of the dendrite separately (7.B). The frame rate for the strip integration scan is up to 3.6kHz (10.8kHz line rate / 3 strips). The arrow in figure 7.B highlights a captured glutamate signal.

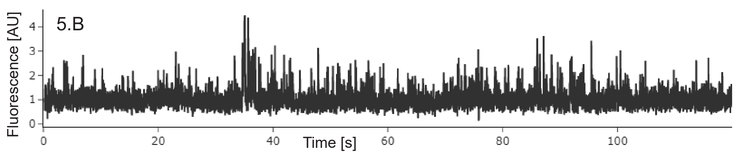


Figure 5.A Functional strip raster scan of mouse cortical excitatory neurons expressing a glutamate indicator (iGluSnFR3). The activity trace of the spine in the white circle is shown in figure 5.B as a function of time. The frame rate of the raster strip recording is 260Hz.

Future Directions

We intend to validate that the fast ROI imaging method employed by the SLAP2 microscope is comparable to recording results obtained with traditional 2-photon microscopes. Through similar studies, we will demonstrate the enhanced temporal resolution achievable with Fast ROI imaging in comparison to the raster scanning mode. Improvements to the SLAP2 prototype through hardware refinements and software developments will help increase reliability and performance. Through optimizations like the implementation of automatic hardware calibration and imaging configuration workflows, complex acquisitions comprised of both structural and functional activity imaging of multiple neurons will be possible. Enhancements like motion correction functionality will help improve imaging results. Automated cell segmentation and ROI definition features will be employed to streamline multi-mode acquires.

Acknowledgements and References

- Kazemipour A et al. Kilohertz frame-rate two-photon tomography. Nat Methods. 2019 Aug
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