Light Beads Microscopy: Rapid Volumetric Brain Imaging by Spatiotemporal Axial Beam Multiplexing

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Summary

Current two-photon scanning microscopy techniques for brain imaging often face challenges in balancing image resolution, acquisition speed, and signal-to-noise ratio (SNR). These challenges become particularly pronounced during volumetric imaging due to the light-scattering properties of brain tissue.

Light Beads Microscopy (LBM) offers a solution by optimizing spatiotemporal signal acquisition to record data at rates limited by the fluorescence lifetime of neural calcium indicators, such as GCaMP. LBM leverages high-energy femtosecond laser pulses at a relatively low repetition rate (~ 4.5 MHz to 5 MHz) to enable the highly temporally multiplexed acquisition of up to 30 axial planes within the same timeframe required for traditional two-photon microscopy to capture a single plane. Additionally, LBM enhances SNR by utilizing one excitation pulse per voxel.

In recent studies, LBM, used in conjunction with a mesoscope [1], has demonstrated the ability to record the calcium dynamics of approximately one million neurons at 2 Hz within a volume of approximately $5.1 \times 6 \times 0.5$ mm in the mouse brain cortex. This breakthrough promises new avenues for exploration in mammalian brain research.

Here, we describe a new system that optimizes and streamlines the LBM implementation by improving its stability, while reducing its footprint and alignment complexity. We also demonstrate integrating LBM into a standard open-source two-photon scanning microscope. This effort aims to broaden accessibility to LBM technology within the neuroscience community, facilitating further innovation and discovery in the field.

Principle & Setup

Axial Beam Multiplexing

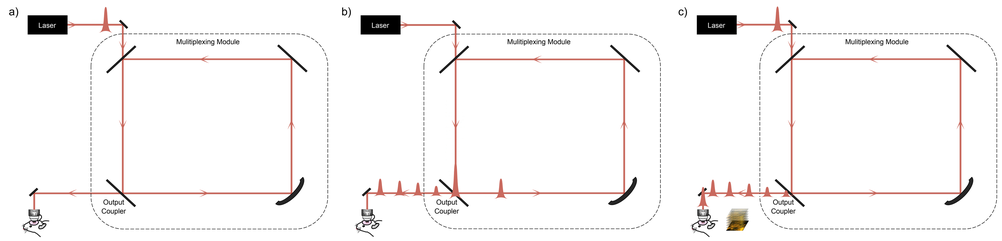

Figure 1: Axial Beam Multiplexing. a) A femtosecond laser pulse enters the multiplexing cavity. b) After several passes inside the cavity, the beam is duplicated, producing up to 32 light beads. With each pass, the newly generated light bead has less pulse energy than the previous one. c) The light beads are directed towards the sample, with each bead focusing at a different axial plane.

Optical Setup

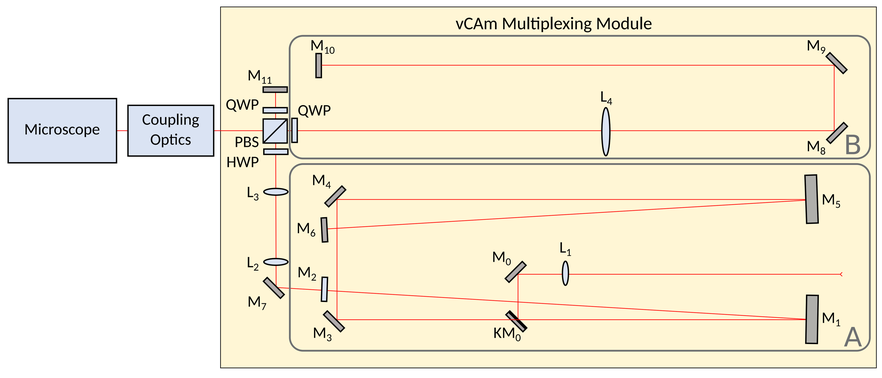

Figure 2: vCAm Multiplexing Module Layout. Our innovative vCAm (Volumetric Calcium Imaging Activity Microscope) multiplexing module duplicates the excitation laser beam up to 32 times. The duplicated beams, referred to as light beads, are precisely focused at different positions along the optical axis. The light beads are evenly spaced and timed to arrive at the sample at predetermined intervals, determined by the cavity length of the vCAm module. (PBS: polarizing beam splitter. QWP: quarter wave plate. HWP: half wave plate. KM: knife-edge mirror. M: mirrors. M_1 and M_5: concave mirrors. M_2: 92/8 beam splitter.)

Signal Processing

Signal Demultiplexing

The vCAm multiplexing module produces two sets of light beads. A first instance, cavity A creates up to 16 light beads while the second instance, cavity B, duplicates the initial 16 beads and acts as a delay line. As a result, cavity A light beads (1 to 16) are temporally interleaved with those from cavity B (17 to 32). This interleaving is taking into account for the temporal demultiplexing of the emission signal and the final volume reconstruction.

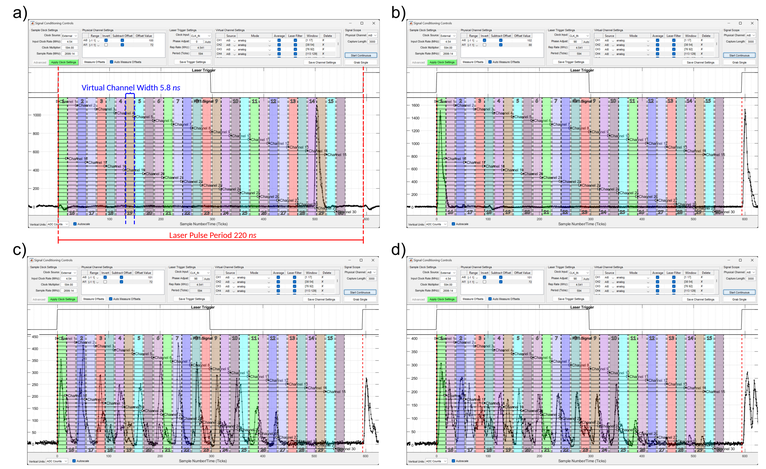

Figure 3: PMT Signal Demultiplexing. Fluorescence signal collected from a wide uniform fluorescent slide. Only 30 imaging planes were possible due to the laser frequency (4.54 MHz corresponding to a 220 ns pulse period). Demultiplexing channels 1 to 15 correspond to excitation from cavity A beads, while channels 16 to 30 correspond to excitation from cavity B beads. a) First bead temporal position before synchronization with the laser trigger. b) Synchronization between the first light bead and the laser trigger. c) All light beads from cavity A reaching the sample are matched to their corresponding demultiplexing channels. d) Light beads from cavity B are allowed to also illuminate the sample.

vCAm Module

Coupling to a microscope

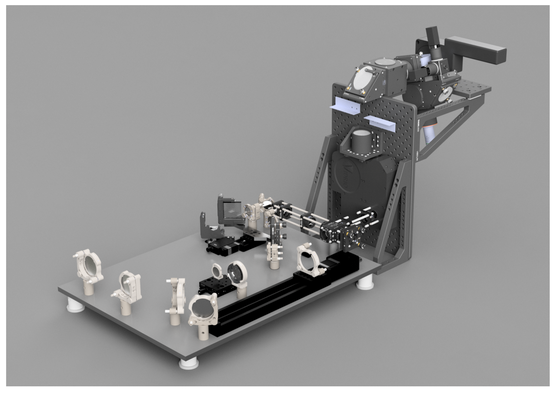

Figure 4: vCAm Multiplexing Module. The vCAm multiplexing module is integrated into an open-source microscope assembled from commercially available parts. The cavity is coupled using a set of relay optics, which depend solely on the characteristics of the microscope.

Proof of Concept

Imaging

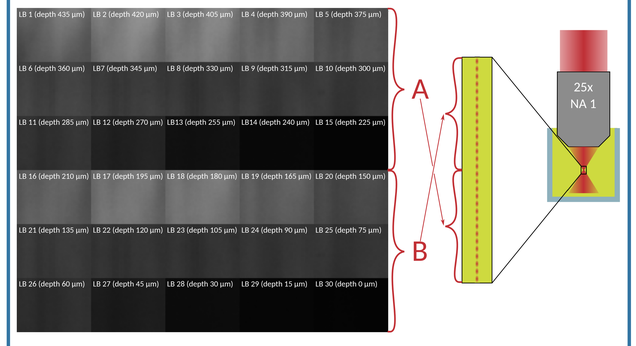

Figure 5: Simultaneous imaging of 30 planes. The vCAm cavity was coupled into the ancillary path of a SLAP2 microscope, with the objective immersed in a solution of fluorescein. The first light bead is focused deep into the sample, approximately 435 µm below the 30th light bead. Since the first light bead penetrates deeper, it has superior pulse energy compared to the other light beads. Conversely, the 30th light bead, being the shallowest, has the lowest pulse energy. (LB: light bead.)

References

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Contact Information

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