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

Axial Beam Multiplexing

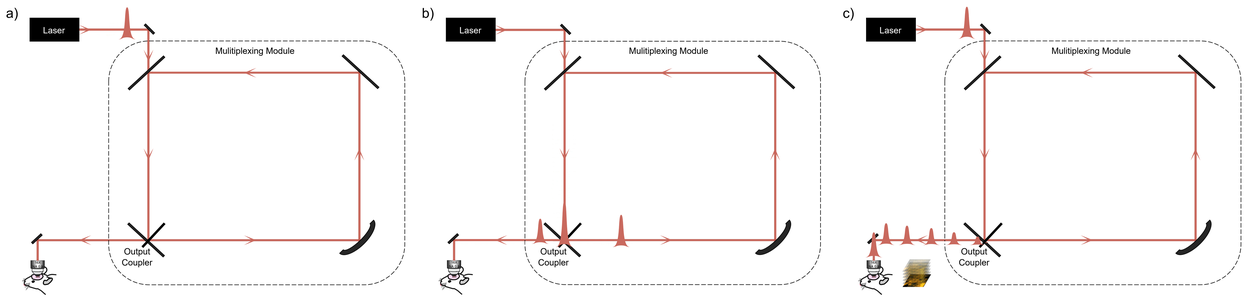

Figure 1: Axial Beam Multiplexing.

Signal Processing

Signal Demultiplexing

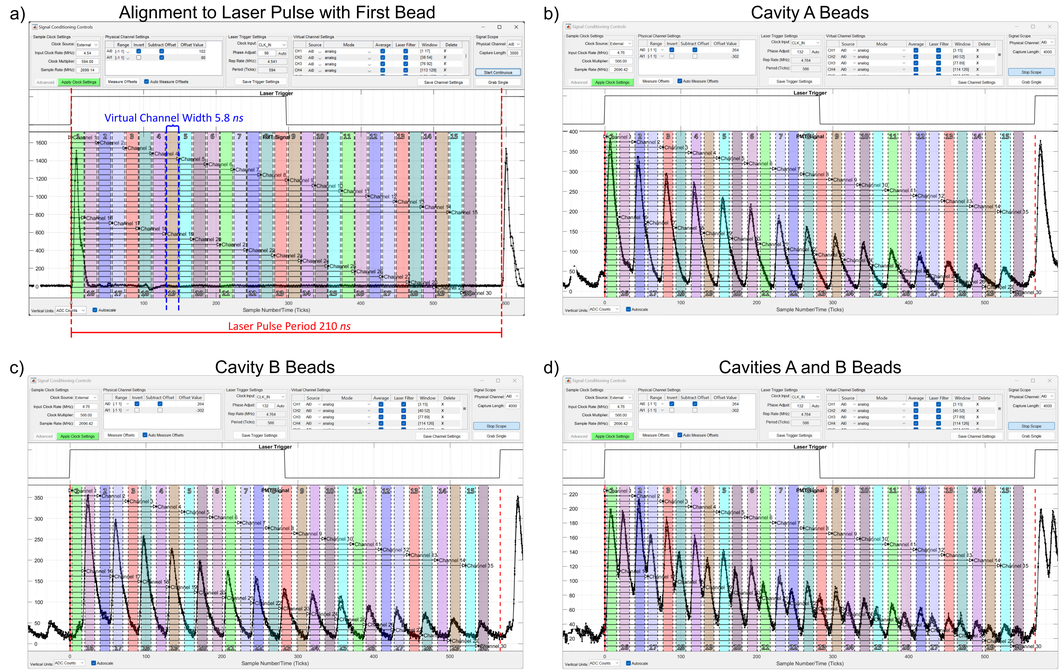

Figure 4: PMT Signal Demultiplexing.

Optical Layout

Optical Setup

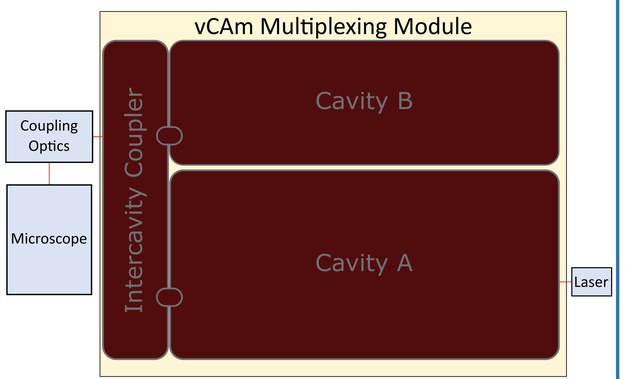

Figure 3: vCAm Multiplexing Module Block Diagram.

vCAm Module

Coupling Light Beads to a DIY Microscope

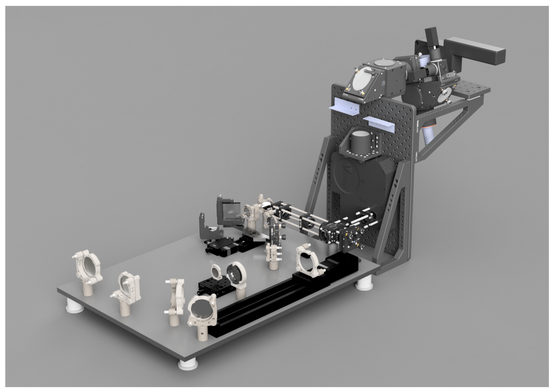

Figure 2: vCAm Multiplexing Module Coupled to a Microscope.

Proof of Concept

Light Beads Imaging in a SLAP2 Microscope

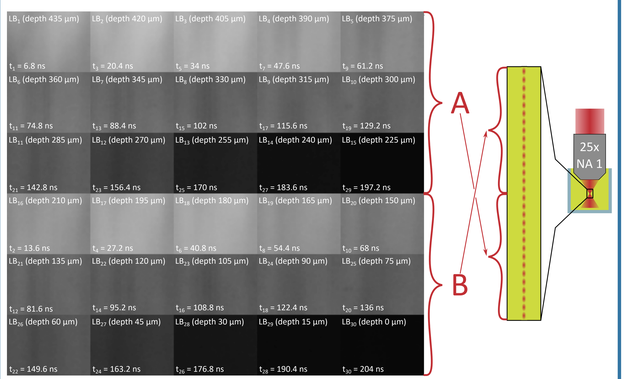

Figure 5: Simultaneous imaging of 30 planes.

Live Brain Imaging

Light Beads Imaging in a 2p-RAM Mesoscope

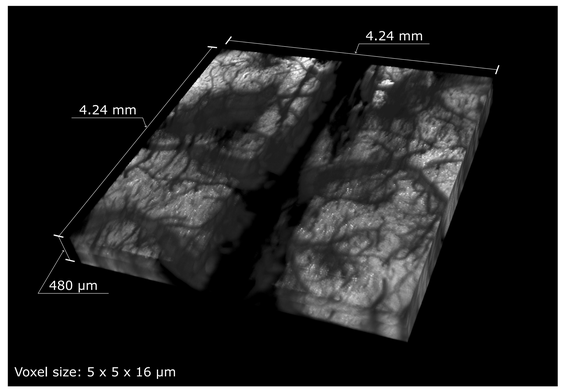

Figure 6: Mouse Dorsal Cortex (Left and Right Hemispheres) [1, 2].

References

[1]J. Demas, J. Manley, F. Tejera, K. Barber, H. Kim, F. M. Traub, B. Chen, and A. Vaziri, "High-speed, cortex-wide volumetric recording of neuroactivity at cellular resolution using light beads microscopy", Nature Methods **18**, 1103–1111 (2021).

[2]J. Manley, S. Lu, K. Barber, J. Demas, H. Kim, D. Meyer, F. M. Traub, and A. Vaziri, "Simultaneous, cortex-wide dynamics of up to 1 million neurons reveal unbounded scaling of dimensionality with neuron number.", eng, Neuron **112**, 1694–1709.e5 (2024).

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Acknowledgment

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Contact Information

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