A 2-photon acousto-optic lens (AOL) microscope for high-speed 3D imaging of neural activity

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Two-photon microscopy is widely used to study brain function at the synaptic, neuronal and network level. However, present designs are limited in their temporal resolution and in the fact that microscopes are typically designed to image in a single plane. This is problematic for studying neural signaling which occurs on the 1-100ms timescale and is distributed in 3D space. Here, we describe a 3D 2-photon microscope base on a novel acousto-optic lens (AOL) design that overcomes these limitations. We present the design details of the AOL microscope and test its utility by measuring fast neuronal signaling in 3D, both *in vitro* and *in vivo*. We used fluorescent neuronal tracers and calcium indicators to measure AP-evoked [Ca^{2+}] transients in spines, dendritic shafts and somata of neurons from mouse sensory cortex.

To do this we patch-loaded single neurons in layer 2/3 of somatosensory cortex of actue slices with the fluorescent calcium indicator Fluo-4 and the red dye Alexa 594. The red signal was used to form a 3D structural image of the volume by sequentially imaging, point by point, xy planes at different depths that were focused with the AOL (remote focusing). We show that the AOLM can image over a 130μm x 130μm x 150μm (40x 0.8NA) cuboid while spatially resolving spines (~1μm diameter) over the entire focal range. We then arbitrarily selected points of interest (POI) within this volume, and used the AOL to rapidly focus the laser beam to these POIs, thus making functional measurements from many locations in 3D space at kHz acquisition rates.

We then used the microscope to track the activity of neural networks *in vivo*. Fluo-4-AM and the cell tracer Calcein-red-orange were bolus loaded in a population of neurons in layer 2/3 of visual cortex of an anesthetized mouse. A reference image of the 3D field of view spanning 150 microns in depth (170 – 320 microns below pia) was used to select POIs in the visualized neurons. We recorded the activity of up to 100 neurons (~350Hz) while presenting drifting bars of different orientations.

Our results demonstrate that our AOL microscope can make high speed (kilohertz), near-simultaneous measurements of neural activity from individual spines, dendrites and neuronal somata sparsely distributed within a three-dimensional field of view. Moreover, the high spatial resolution of our custom-designed AOL is well suited to spatially resolve fine subcelluar elements, while covering a large depth range.

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