

SIMULTANEOUS 3D TWO-PHOTON IMAGING AND PHOTOSTIMULATION FOR ARTIFICIAL PERCEPTION

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1. SIMULTANEOUS 3D TWO-PHOTON IMAGING AND PHOTOSTIMULATION

Understanding brain function requires technologies that can measure, as well as control the activity of large neuronal populations with high fidelity in space and time. We developed a multiphoton approach based on acousto-optics (AO) to simultaneously measure and optically stimulate (activate or suppress) the activity of cortical neurons with millisecond precision and subcellular spatial resolution. We demonstrate the ability to perform simultaneous photostimulation of up to 250 neurons distributed in 3D in a $600 \times 600 \times 500\text{-}\mu\text{m}^3$ volume of brain tissue. Using AO for two-photon stimulation has multiple advantages compared to other methods. First, compared to holographic stimulation, optical resolution is preserved even with the growing number of stimulation locations, allowing the precise activation of individual neurons with virtually no crosstalk between neighboring neurons. Second, interlaced stimulation can be used by exploiting the fast switching time of AO imaging. During the stimulation period, we performed stimulation at every odd frame and imaged Ca^{2+} activity at every even frame. This allowed us to follow Ca^{2+} activity during the imaging period (Fig. 1.).

2. PHOTOSTIMULATION DURING BEHAVIOR

Despite the astonishing advances in the field, it is still not clear how various percepts arise in the cortex. Combined imaging and photostimulation can be a great tool for answering these questions: it can help us understand, on the basic cellular level, how behaviorally consequential percepts are initiated. We designed a robust behavioral protocol, where the underlying neural activity can be measured with high precision. Our custom analysis code can motion-correct the imaging data, detect activity and co-activated ensembles in real-time. Contextual correlation between the detected ensemble and behavior could be tested with photostimulation.

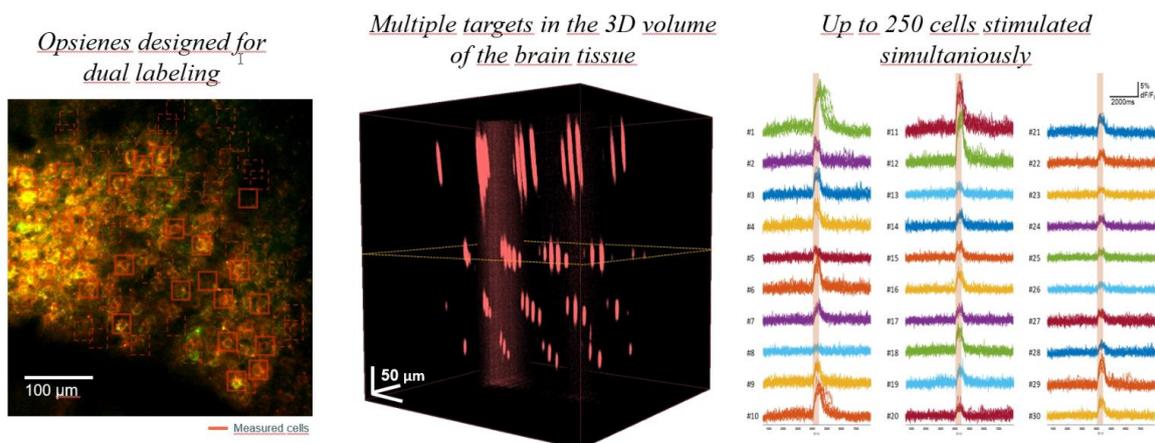


Figure 1: (Left) Dual AAV labeling, (Middle) Multiple 3D stimulating points with reserved optical resolution, (Right) Exemplified stimulated cells, overlaid curves represent repetitions of the same stimulation pattern, note the stability and reliability of the stimulation.