

## HOLOGRAPHIC-BASED ALL-OPTICAL DISSECTION OF NEURAL CIRCUITS

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The development of genetically encoded photosensitive actuators and sensors able to trigger and detect the neuronal activity (optogenetics) has revolutionised neuroscience, promising to increase our capacity to dissect how neuronal circuits function. Reaching this goal requires the developments of optical methods enabling that light is properly delivered into the tissue to target and manipulate neurons in spatiotemporal patterns that mimic physiological neuronal network activity, while optically monitoring their responses.

The light delivery strategy needs to be robust to scattering, spanning multiple spatial scales (cell bodies, dendritic branches or spines from one to multiple cells) and featuring high spatial and temporal resolution. Two-Photon Computer Generated Holography coupled with Temporal Focusing (TF-CGH) allows shaping the illumination to match the structures or circuits of interest, enabling 3D targeting of neurons either individually or in groups with micrometers range resolution, resilience to scattering, and controlling neuronal firing with –ms resolution. The combination of TF-CGH illumination with two-photon scanning imaging has thus the potential to achieve the spatial and temporal sophistication necessary for an “all-optical” dissection of neural circuits. Here, we describe a holographic-based all-optical strategy with the necessary flexibility and precision to enable investigations of neuronal circuits in the retina and *in-vivo* in the visual cortex. In the visual cortex we showed *in-vivo* optical control of neuronal firing with –ms temporal resolution and sub-ms temporal precision in L2/3 of anesthetized mouse V1. In the mouse retina, we used this approach to dissect how rod bipolar cells contribute to shape direction selectivity in retinal ganglion cells.