Manipulation of neurons with precisely controlled illumination in space and time

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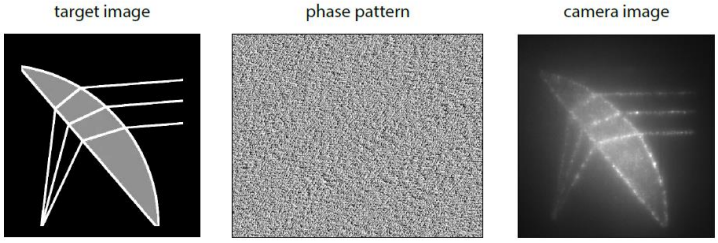
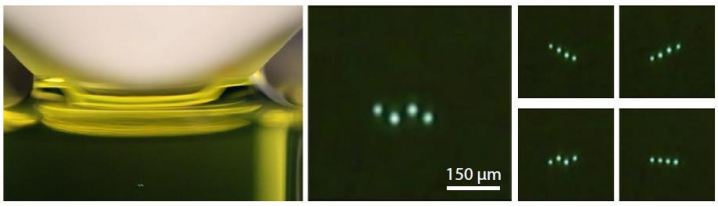
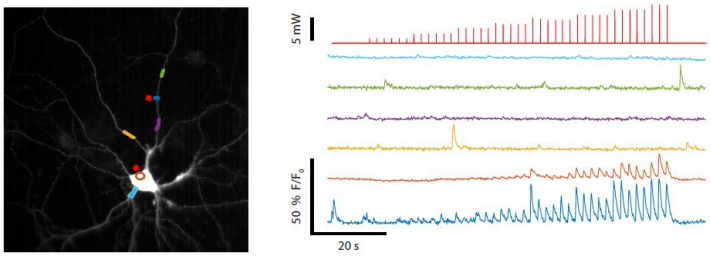
ABSTRACT: Manipulation of cells with light has become a powerful and widely used tool in neuroscience. For this approach to reach its full potential, two conditions need to be met: the precise spatial and temporal control of light delivery inside the sample, and the availability of light-controllable molecules that can modify crucial cellular and neuronal functions. We present a novel holographic illumination system that can be coupled to existing microscopes (e.g. two-photon microscopes) which provides high resolution control for photomanipulation experiments. The system is optimized for two-photon excitation to improve axial confinement of the excitation patterns as well as tissue penetration. 4d light patterns (x,y,z,t) can be defined by the user based on fluorescence images of the sample. In addition to a technical characterization of the system we present biological applications of the illumination module using various types of light-sensitive molecules, including caged compounds and optogenetics molecules.



Projection of a 2D pattern into a homogeneous fluorescent sample, imaged with a camera.



Projection of various 3D patterns into a fluorescein solution, photographed from the side (optical axis is vertical).



Release of caged glutamate in the vicinty of a cultured neurons elicits Ca^{2+}-transients (GCaMP6) selectively close to the illumination sites (red circles) but not in other parts of the cell.