Abberration Correction in 2P holographic optogenetics in scattering media

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Optical methods to manipulate neural activity with cellular resolution strive for understanding how information is processed in the brain and how the neural circuit structure is related to its specific function. Several microscopy techniques, based on optogenetic tools, have been developed to simultaneously target and record the light from neurons. Here, the stimulation is achieved through two-photon (2P) absorption, which offers a high optical sectioning and an increase of penetration depth (*A. Vaziri, E. Emiliani. Current Opinion in Neurobiology, 2012, Vol 22 (1)*). To rapidly stimulate multiple region of interest the 2P stimulation is combined with digital holography (DH) (*P. Pozzi, D. Gandol, M.Tognolina, G. Chirico, J. Mapelli, E. D'Angelo. Neurophotonics, 2015, Vol 2 (1)*). DH relies on a spatial light modulator (SLM) which patterns the light matching the ROIs we want to stimulate. This approach is still challenging to implement in vivo where optical aberrations, introduced by high scattering media, lead to separation between the imaging and the stimulation plane.

In order to correct for sample-induced aberrations we propose a custom wide field microscope based on 2P holographic stimulation and direct wavefront sensing (*K. Wang, W. Sun, C.T. Richie, B.K. Harvey, E. Betzig, N. Ji. Nature communications, 2015*). A phase pattern, calculated through an iterative algorithm based on non-convex optimization of a custom cost function (*J. Zhang, N. Pegard, J. Zhong, H. Adesnik, L. Waller. Optica, 2017, Vol 4 (10)*), is imposed onto the laser line by a SLM. Simultaneously, a Shack-Hartmann sensor measures the aberrations of the excitation. A correction for the distortions is given by a suitable phase pattern addressed to the same SLM. This technique allows precise and simultaneous excitation of arbitrary patterns in a fluorescent film. Moreover, in order to increase the number of spots in the arbitrary pattern and to minimize the tissue damage, we are testing a cavity dumped Ti:Sapphire system which provides a higher pulse energy with variable repetition rate.

Ultimately, we will benchmark this setup to manipulate synapses in living zebrafish embryos. In particular we will investigate the refinement of the retinal ganglion cell (RGC) circuit to elucidate how plasticity affects large-scale circuit organization in a developing sensory system.