Two-photon optogenetics with millisecond temporal precision and single cell resolution

V. Emiliani

Neurophotonics Laboratory
Wave front engineering microscopy group
CNRS UMR8250, University Paris Descartes
45, rue des Saints Pères
75270 Paris Cedex 06, France

Optogenetics has revolutionized neuroscience by enabling remote activation or inhibition of specific populations of neurons in intact brain preparations through genetically targeted light sensitive channels and pump. Nevertheless, studying the role of individual neurons within neuronal circuits is still a challenge and requires joint progresses in opsin engineering and light sculpting methods. Here we show that computer generated holography using an amplified pulse laser combined with high light sensitive opsins [1,2] enable precise control of neuronal firing with millisecond temporal precision, single cell resolution and unprecedented low illumination level using cortical brain slices.

We also show a new optical system enabling remote axial displacement of temporally focused holographic patterns, as well as generation of multiple temporally focused holographic targets occupying separate axial planes. We demonstrate the capabilities of the system in two different experimental paradigms. In a first one we use the system to photoconvert, with cellular resolution, tens of Kaede protein expressing neurons occupying separate axial planes in live zebrafish larvae [3]. In a second one the set up is used to probe the functional connectivity between the bipolar cell layer and the ganglion cell layer in the mice retina**.**

1. E. Ronzitti, R. Conti, V. Zampini, N.C. Klapoetke, D. Tanese, E. Papagiakoumou, E.S. Boyden, V. Emiliani,
Sub-millisecond optogenetic control of neuronal firing by two-photon holographic photoactivation of Chronos,
BioRxiv. Doi 10.1101/062182. (2016).
2. E. Chaigneau, R. Ronzitti, M. Gajowa, G. Soler-Llavina, D. Tanese, A. Brureau, E. Papagiakoumou, H. Zeng, V. Emiliani,
Two-photon holographic stimulation of ReaChR,
Front. Cell. Neurosci. (2016).
3. O. Hernandez, E. Papagiakoumou, D. Tanesee, K. Felin, C. Wyart and V. Emiliani,
Three-dimensional spatiotemporal focusing of holographic patterns
Nature Communication, 7, 11928 (2016).