3D holographic optical stimulation in vivo

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In order to study functional neuronal connectivity and neuronal computations in the living brain it is important to be able to artificially generate arbitrary patterns of neuronal activity. The optogenetic toolbox is constantly becoming richer and allows targeting specific neuronal populations. Currently, one of the main bottle necks is the need to deliver light with sufficient spatial and temporal control in order to control subsets of neurons of a genetically defined neuronal population. Various projection systems based on spatial light modulators can be used to generate 2 dimensional patterns for optical stimulation. Digital micromirror devices (DMDs, Texas Instruments) allow ultra-fast switching of 2-dimensional patterns. However neuronal structures and functional modules are inherently 3-dimensional. We are therefore exploring the possibility to generate 3 dimensional patterns in vivo by digital holography using a phase only spatial light modulator (Hamamatsu Photonics). In a first step we compare the holographic stimulation system to a conventional DMD based system. Exploiting a fast sCMOS camera system (Andor Neo) for testing spatio-temporal resolution we find that switching times for holographic patterns allow for stimulation of up to 60 Hz. Local stimulation intensities achieved are more than 2 orders of magnitude higher than with a DMD system and a LED light engine. This substantially reduces the latency of optically evoked action potentials in an in vivo preparation stimulating mouse olfactory bulb mitral cells expressing channelrhodopsin 2 under the Thy1 promoter. In order to obtain 3D optical stimulation we are testing a combination of Fresnel lenses and holographic patterns generated by a modified Gerchberg-Saxton algorithm versus a ‘prism & lenses’ approach to generate holograms corresponding to individual points distributed in the three-dimensional space.