Light patterning for two-photon multicell targeting: multispot generation and light induced heating

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The capability to pattern light deep in biological tissue represent a crucial tool for multi-cell optical manipulation and imaging. In particular, in the field of Neuroscience, the advent of novel optogenetic tools, such as light-sensitive channels and reporters, opened new ways to manipulate and monitor neuronal activity by optical means. The full exploitation of their potential requires the development of optical approaches enabling fast, volumetric, in-depth illumination of multiple targets with single-cell resolution. Phase modulation approaches combined with two photon (2P) excitation and temporal focusing are capable of generating 2D illumination patterns well localized laterally and axially on specific cellular structures of interest[1].

Here we describe an optical scheme to extend this approach to three dimension: the temporally focused 2D excitation pattern is multiplexed over multiple positions allowing the generation of several tens of axially confined spots in an extended volume, going up to 500 μm in the z-direction [2].

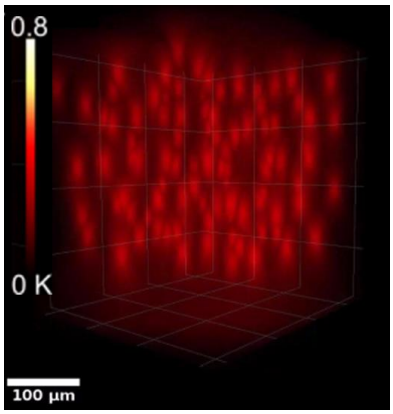

Figure 1: Simulated temperature increase induced by 100 holographic spots in brain tissue.

Additionally, 2P multispot illumination could result in high light powers delivered to the tissue and therefore collateral effects such as heating need to be taken into account. Here we present and experimentally validate a theoretical model that allow to simulate 3D light propagation and heat diffusion in optically scattering samples at high spatial and temporal resolution under different illumination conditions[3]. In particular, we investigate the most commonly used approaches to perform 2P optogenetics photoactivation: single- and multi-spot holographic illumination and spiral laser scanning. The predictions of the model can be useful for the design of optimal illumination conditions in optogenetic or imaging experiments.

References:

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