A REMOTE FOCUSING SYSTEM FOR 3D HOLOGRAPHIC STIMULATION

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The use of light as an active tool to control neuronal activity has recently become the technique of choice in studies such as the probing of neuronal circuits or mapping of synaptic connectivity. An optimized approach requires the ability to address light to the preparation in flexible 2D and 3D patterns that mimic physiological excitation. We have recently proposed one-photon and two-photon Digital Holography (DH) [1-3] as an efficient way of shaping a laser beam to generate 2D multiple diffraction limited spots or, alternatively, excitation domains that accurately cover sub-cellular structures. Although the possibility of generating 3D holographic excitation patterns has already been demonstrated, its actual implementation in biological experiments requires simultaneous positioning of excitation spots in 3D and imaging of the sample at different axial planes. This would allow access to the 3D organization of neuronal circuits as well as whole arborizations of individual neurons, which are rarely confined to a single axial plane.

Here we propose to use the remote focusing system developed by Botcherby et al. [4] combined with one-photon 3D-DH as a simple method to decouple the position of the 3D excitation pattern from the imaging plane. This method can be easily implemented in combined electrophysiological and imaging set-ups. The starting point of the remote focusing technique is to recreate a stigmatic, remote image of the sample. The constraints for a perfect imaging system are given by Maxwell’s theorem and require the magnification in all spatial directions to be equal to the ratio of the refractive indices in the object (sample) and in the image (remote) space. As in Botcherby’s system, two objectives are employed sequentially to magnify and demagnify the image of the sample: magnifications are chosen to match Maxwell’s criterion, so that spherical aberrations are negligible on a large z-range (∓ 150 µm in our system). The remote image of the sample is reflected back through the second objective by using a small mirror and imaged through a beam-splitter on a CCD camera. In this way, imaging of the sample in multiple planes is achieved remotely through the second objective and is decoupled from the holographic excitation pathway, for which laser light passes only through the first objective.

REFERENCES: