Three-dimensional imaging and photostimulation by remote-focusing and holographic light patterning

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Access to three-dimensional (3D) structures in the brain is fundamental to probe signal processing at multiple levels, from integration of synaptic inputs to network activity mapping. This can be achieved with optical techniques, since both imaging and photostimulation have been performed in 3D. However, to reach a full optical control of 3D structures, these approaches need to be combined into a unique optical system. One of the main challenges, in this respect, is to axially decouple the imaging and stimulation planes when both optical pathways are combined (as it is often the case) into the same microscope objective.

In this contribution, we present an optical method for independent three-dimensional photoactivation and imaging by combination of digital holography with remote-focusing [1]. One-photon digital holography provides an efficient way of shaping a laser beam to generate 3D multiple diffraction limited spots at the vicinity of a microscope objective focal plane [2]. This is obtained by modulating the spatial phase of a laser beam in a plane conjugated with the objective back focal plane. Remote-focusing was originally proposed Botcherby et al. [3] as a method for rapid 3D scanning. Here, we perform a full characterization of the remote focusing system and demonstrate compensation of spherical aberration for out-of-focus imaging in a range of at least 300 μm. In addition, we demonstrate for the first time that remote focusing can be adapted to perform scanless epifluorescence and functional imaging along arbitrary tilted planes. An example is shown on Fig 1. Images of the apical dendrite of a CA1 pyramidal neuron were obtained before and after tilting the imaging plane by 10°. Tilting the image plane allowed following the whole length of the apical dendrite inside the field of view (200 × 150 μm), whereas only a small portion of dendrite was in-focus with an imaging plane perpendicular to the optical axis (dashed red line).

We apply our method to perform functional imaging along tilted dendrites of hippocampal pyramidal neurons in brain slices, after photostimulation by multiple spots glutamate uncaging. An example is shown in Fig. 2, where we performed glutamate uncaging on a secondary oblique dendrite, while recording Ca\(^{2+}\) signals from the principal one. A z-scan (Fig. 2 b-c) was performed with the remote focusing unit, to measure the inclination of the principal dendrite and to place the uncaging spots (red arrows) along a secondary dendrite. The imaging plane was then tilted by 11° to follow the inclination of the principal dendrite (Fig. 2d), leaving the secondary dendrite (and the uncaging spots) out-of-focus. Ca\(^{2+}\) responses were recorded following light stimulation: the ΔF images are shown in Fig. 2e. A small out-of-focus signal was observed in the region corresponding to the excited secondary branch, which spreads in the full visible portion of the in-focus principal dendrite. The corresponding ΔF/F traces (single trial, Fig. 4f) were calculated for regions of interest (ROIs) chosen along the apical dendrite (Fig. 4d, cyan).

Finally, by bringing extended portions of tilted dendrites simultaneously in-focus, we could monitor the spatial extent of dendritic calcium signals, and were able to show a shift from a widespread to a spatially confined response upon blockage of voltage-gated Na+ channels [1].

To conclude, we present an innovative combination of digital holography and remote-focusing, allowing for independent 3D imaging and photoactivation. This system provides a simple optical method to stimulate neuronal dendrites in 3D and record evoked responses in arbitrary planes,
while preserving spatial information on signal distribution with high resolution (<μm). The combination of tilted imaging with digital holography permits a precise stimulation of specific points distributed in 3D throughout the dendritic tree, and makes our setup particularly useful to study phenomena of dendritic integration and compartmentalization, but also activity-dependent dendritic plasticity.

References


Figure 1. CA1 pyramidal neuron filled with OGB-1, imaged before (Top) and after (Bottom) tilting the imaging plane to compensate for the inclination of the apical dendrite to the surface of the slice (10°). Scale bar: 10 μm.

Figure 2. Independent 3D photostimulation and Ca²⁺ imaging along a tilted plane in CA1 pyramidal neurons filled with OGB-1. (a) Schematic of the uncaging configuration: MNI-glutamate photolysis is performed at three locations on a thin branch off the apical dendrite (red arrows; z=18, 22.5, 34.5 μm). (b) Fluorescence image of the neuron schematized in (a), at the focal plane (z=0). (c) Maximum intensity projection obtained from a z-stack of the neuron (40 frames; Δz=1.5μm), indicating the position of the uncaging spots, which are axially displaced with respect to the tilted imaging plane. (d) Image of the neuron after tilting the imaging plane by 11°. (e) Ca²⁺ response (ΔF) measured 100 ms after photolysis. (f) Ca²⁺ responses (ΔF/F) measured from three ROIs along the apical dendrite (d, 1–3 cyan lines) at 10Hz. Scale bars: 10 μm.