Advancing neurophotonics using 3D optical-field patterns

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A novel system advances neurophotonics beyond its physical limitations using tools previously employed for multibeam optical manipulation.

Recent advances in photonics, chemistry, and genetics jointly provide powerful tools for analysis of the mammalian brain, particularly of its circuitry and ability to process information. In the 1990s, the development of two-photon 3D microscopy had a major impact on neuroscience. The technique is now widely used in combination with electrophysiology and photochemical reagents for imaging neuronal networks. Using tailored optical fields for analysis of neuronal circuitry is foreseen as the next major advance that will transcend the physical limitations of conventional electrophysiology for probing neuronal activity at multiple sites in all three dimensions.

Splitting a single laser into multiple beams has been used for simultaneous transfer of linear momentum, a technique commonly referred to as multiple-beam optical tweezers. Development of devices capable of dynamic spatial phase encoding has further pushed the field of multibeam micromanipulation to its limits. In neuroscience, holography has been demonstrated for single- and two-photon photolysis of caged neurotransmitters. When uncaged by light, neurotransmitters are liberated and bind to receptors at the dendrites. They can thus mimic synaptic transmission from neighboring neurons. Neurons receive some 10,000 of these synaptic contacts every second. Neuronal firing is typically the result of activation of small subsets of these contacts in distributed dendritic locations.

Using single-photon activation for neurotransmitter uncaging, however, results in a poorly localized response along the optical axis, rendering attribution to a specific uncaging site on the dendritic tree impossible. Highly localized responses can be obtained with two-photon uncaging. However, thus far projections of spot patterns have been restricted to a single plane. While such systems incorporate a lens function to move the planar light pattern along the optical axis, simultaneous stimulation at arbitrary sites in 3D cannot be achieved. For realistic analyses of how dendritic inputs determine neuronal activity in circuits, the system will, therefore, require both localized excitation in 3D and arbitrary 3D spot positioning.

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Figure 1. Image sections selected from various axial depths and (bottom right) a 2D projection of the entire neuron. Points for photostimulation at arbitrary 3D dendritic locations are shown as red dots.
We have constructed a fully dynamic system that can project multiple focal spots at arbitrary positions in 3D and is capable of delivering various forms of light-matter interaction. The same system can also be used for optical manipulation of arrays of particles through transfer of linear and orbital angular momentum, demonstrating optimal use of the available laser power while maintaining diffraction-limited optical resolution. Our system is, therefore, the realization of a fully dynamic and multifunctional microscope where multibeam optical tweezers, 3D two-photon microscopy, volume-holographic storage, nonlinear microfabrication, nanosurgery, and complex neurophotonics can be performed.

We use the holographic projection method to split up an incident femtosecond-pulsed titanium:sapphire laser into multiple focal spots. Each spot can be encoded with helical wavefronts to produce multiple optical vortices. To form rapidly changeable focal-spot and vortex patterns, we derive an analytic expression of the input-phase hologram using superposition and a combination of prism, lens, and vortex functions. The calculated phase holograms are encoded onto a programmable spatial-light modulator and consequently project multiple focal spots at the sample. The system also functions as a two-photon microscope, with two acousto-optic modulators as high-speed beam scanners for acquiring 3D images. Rendering the 3D morphology of the whole neuron, especially its dendrites, enables accurate positioning of the stimulation sites. We use conventional whole-cell recording techniques (micromanipulators, micro-electrode, and signal amplifiers) to study neuronal activity and record the electrical responses of the neuron after photostimulation.

To demonstrate photostimulation in 3D using two-photon excitation, we filled a neuron (from a rat-brain slice) using a patch pipette with fluorescein (10mM), which then diffused throughout the cell. This isolates imaging of a single neuron from the other neurons in the brain slice. We obtained a stack of 60 optical sections by moving the sample stage over subsequent 1μm distances. Figure 1 shows image sections and a 2D projection (integrated image) of the neuron. The depth (z) positions are indicated at the bottom of each frame with respect to an arbitrary position (z = 0mm) of the nominal focal plane of the objective lens. To perform photolysis, the caged compound can either be applied through a microinjector (picospritzer) or bath-applied together with the artificial cerebrospinal fluid that maintains the slice. Specific regions of interest from different dendritic locations can be used to investigate how different parts of the dendrite contribute to firing the neuron. The projected 2D image shows multiple excitation points where localized photostimulation of caged neurotransmitters can be achieved. Depending on the strength of the stimulus and synapses, the neuron depolarizes or may even generate action potentials. Experiments that study such integrative properties of dendrites are among the major challenges that can be efficiently approached with this system.

In addition to neurophotonics, the system can also be used for micromanipulation of mesoscopic particles. Both linear and orbital angular momentum can be transferred to optically trapped particles, thereby setting up a multifunctional microscope capable of manipulating particle arrays, 3D two-photon imaging, multisite and highly localized two-photon fluorescence excitation, and nonlinear photostimulation. Implementation of the analytic expression of the phase hologram reduces the computational complexity, and the system can be used for dynamic micromanipulation, as well as instantaneous positioning of photostimulation sites within biological tissues (e.g., neurons). Our system enables investigation of fundamental questions with regard to the mechanisms of information processing in the human brain. We continue to pursue research along these lines.

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Vincent Daria is jointly affiliated with the John Curtin School of Medical Research and the Research School of Physics and Engineering. His experience in using 3D optical fields for multibeam optical tweezers and two-photon microscopy led him to develop biophotonics tools for analysis of neuronal circuitry.

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Christian Stricker is an associate professor of systems physiology at the Medical School with research laboratories at the John Curtin School of Medical Research. His research focuses on information exchange within small networks in the neocortex, which requires development of new optical tools to stimulate and record at many sites simultaneously.

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References