15. Playing the piano with the brain: Holographic imaging and manipulation of neural activity

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Status

Optical microscopy is one of the most powerful tools used in biology. The ability to visualize living structures and events over a wide range of scales has led to fundamental discoveries. At the same time, there are limitations which need to be overcome for even more effective interrogation of living tissues. For example, in conventional microscopes, the sample is either illuminated simultaneously across the entire imaging field (wide-field illumination) or sequentially, pixel by pixel (point-scanning illumination). The wide-field approach can image at high speed as it captures a two-dimensional image at once using a camera, but it suffers from pixel crosstalk generated by light scattering. In point-scanning approach, a single pixel detector captures the fluorescent signals and builds an image, pixel by pixel; when using two-photon excitation, it greatly diminishes the crosstalk by light scattering. But, while suited to image deep in scattering tissue, as a point scanning method, two-photon microscopy has slow imaging speed.

Exploring methods in the space between the above two illumination patterns could add extra freedom in microscope design and help overcome some of the fundamental limits of conventional microscopes. One example of such methods are spatial light modulators (SLMs), where arbitrary illumination patterns can be delivered to the sample, making computer-generated holograms (Fig. 1a). SLMs have enabled new functionalities in microscopy, such as fast three-dimensional (3D) imaging. Furthermore, holographic microscopy can be used not only to "read" information from the sample, but also to "write" information, such as through photostimulation, into the sample. While holographic microscopy was initially developed for optical tweezer[1,2] more than twenty years ago, the last ten years have witnessed its growing applications in neuroscience[3-6]. Many designs have been made specifically to image or manipulate neural activity with higher spatiotemporal resolution in 3D, using two-photon excitation.

Holographic imaging of neural activity. Neural activity can be optically reported by activity-dependent fluorescence signals from calcium or voltage indicators in the neurons. For a high signal-to-noise ratio (SNR), two-photon excitation is desirable, particularly for *in vivo* applications. Conventional two-photon microscopes use sequential point illumination and are thus slow. Using an SLM, the laser beam can be divided into multiple beamlets, each of which targets a specific neuron[4,5], and a camera can be used to capture the signal. The hologram essentially multiplexes the laser, significantly increasing imaging speed. Such selective illumination also reduces phototoxicity. A strength of this method further lies on its fast 3D imaging capability. The computer generated hologram can project beamlets over a group of neurons (>100) distributed in 3D. Using a cubic phase plate at the detection path, signals from neurons at different depths can stay in focus on the camera and be simultaneously imaged[7].

Holographic photostimulation of neural activity. Using holographic patterning, neural activity of designated cells or neural processes can be simultaneously activated or inhibited in 3D with high spatial specificity through optochemistry[3,4] or optogenetics[8]. Depending on the dynamics of the actuators, laser power budget and the required spatiotemporal resolution, holograms can be combined with other techniques, such as galvanometer scanning or temporal focusing, to deliver different types of photostimulation patterns to the sample[6]. Using two-photon excitation, holographic photostimulation enables precise mapping of the connectivity or function of neural circuits. In particular,

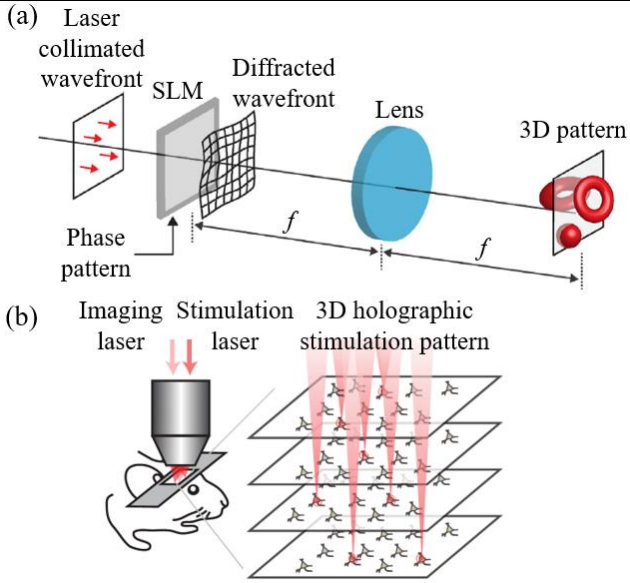

Figure 1. (a) Generation of a 3D image pattern through computer generated holography. *f*, focal length of the lens. (b) "Playing the piano" with neural circuits: simultaneous volumetric calcium imaging and 3D holographic patterned photostimulation in mouse cortex.

when pairing it with two-photon imaging, neural activity can be simultaneously recorded and manipulated with single-cell precision *in vivo*[9,10] (Fig. 1b). Such an all-optical approach, as if one were "playing the piano" with the neurons, provides a powerful tool to study neural circuits *in vivo*.

Current and Future Challenges

For both imaging or photostimulation, the goal is to deliver light to a territory as large and as deep as possible, with a spatiotemporal resolution as high as possible, while maintaining optimal SNR. While the integration of holographic patterning into modern microscopes has largely advanced this goal, there are still many challenges that need to be solved.

Firstly, the essence of holographic imaging is to multiplex the laser to probe many regions of interest simultaneously. The above-mentioned approaches use cameras to detect and spatially demultiplex the signal. But in scattering tissues, pixel crosstalk between different excited sources could strongly degrade the signal. Crosstalk naturally increases with a higher degree of multiplexing. So how to maximize the multiplexing (thus the imaging throughput) while being able to reconstruct the signal with high fidelity poses a challenge.

Secondly, in holographic photostimulation experiments, as the number of simultaneously targeted neurons increases, higher laser power is required, which could impose a heavy heat load on the brain. Furthermore, off-target photostimulation could happen, which effectively reduces spatial resolution.

Finally, one of the goals in neuroscience is to understand how animal behavior is generated from neural activity. All-optical piano experiments, where neural activity can be simultaneously recorded and manipulated, appear ideal for these studies. This requires a closed loop between recording and manipulation of neural activity, and observation of animal behavior. This needs development of both hardware and software that can seamlessly work together at high speed[11]. For all-optical experiments, it is also necessary to increase the co-expression level of activity reporters (e.g. calcium indicators) and actuators (e.g. opsins) in the same neuronal population, and further separate their excitation spectrum to minimize the crosstalk between imaging and photostimulation.

Advances in Science and Technology to Meet Challenges

Joint innovations across multiple disciplines in engineering and statistics could address some of the above challenges, and enhance the performance and capability of holographic illumination microscopy for brain research.

We recently developed a multiplane imaging system where multiple beamlets scan the sample at different depths (or regions), with their signal being summed and collected by a single photomultiplier tube. Using prior structural information and calcium decay dynamics, neural activity from the different scanned planes can be then computationally reconstructed[12] using a constrained nonnegative matrix factorization algorithm[13] (Fig. 2). This approach has a high SNR and greatly increase imaging throughput in scattering tissues. This is an example of the increasing integration of advanced hardware design with statistical algorithms.

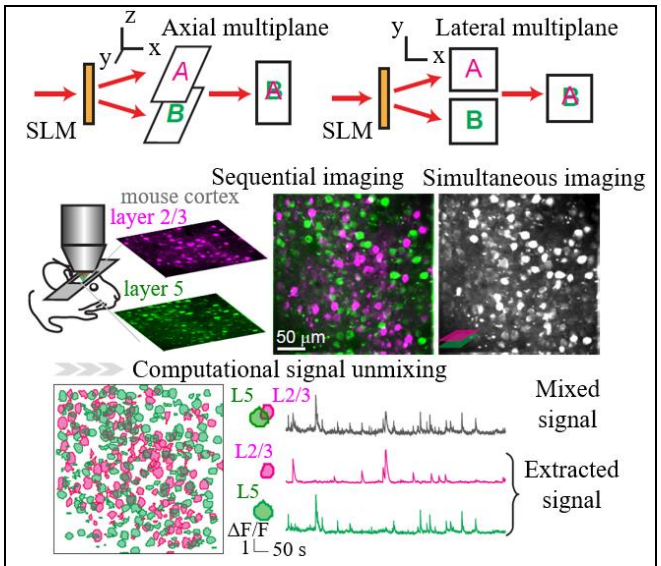

Figure 2. Simultaneous multiplane imaging and computational demixing of signals[12]. Top panel illustrates two types of simultaneous axial/lateral multiplane imaging. Bottom panel shows simultaneous calcium imaging of layer 2/3 and 5 of mouse visual cortex *in vivo*, and signal separation using a constrained nonnegative matrix factorization algorithm[13].

On the photostimulation side, using two-photon holography one can simultaneously photostimulate more than 80 neurons *in vivo* with low laser power[10], using low-repetition-rate lasers. Also, with somatic restricted opsin, off-target photostimulation is greatly suppressed[14]. Research is being devoted to engineering new calcium indicators and opsins, and fusing them together in the same viral vector for better co-expression in cells[15,16].

Holographic imaging and photostimulation will benefit from the development of three-photon excitation and adaptive optics and also help both methods, thanks to the flexibility that SLMs afford for the design of point spread function (PSF). Both techniques aim to penetrate deeper into the scattering tissue. Their integration with holographic illumination will greatly improve the overall optical quality, and enable the interrogation of the neural activity in deeper brain regions with minimal invasiveness.

Finally, technical improvements in SLMs, such as in switching speed, numerical aperture, phase modulation, array size or pixel number could increase the temporal dynamics, optical efficiency and uniformity of the hologram, and thus improve the overall quality of the holographic illumination[12,16]. SLMs with >1,500 pixels in each dimension and >1 kHz switching speed would be very beneficial for current experiments. A switching speed >10 kHz would further open doors to many new experiment designs, for both imaging and photostimulation. While nematic liquid crystals continue to improve, progress in other technologies such as microelectromechanical system devices could enable a next wave of contributions.

Concluding Remarks

Holographic illumination is revolutionizing optical microscopy, enabling novel capabilities for neuroscience, like the ability to read and write neuronal activity in a neural circuit, playing it as if it were a piano. Thanks to these developments, advances in understanding neuronal circuits with cellular resolution have been made. As SLMs can mimic arbitrary transfer functions, they can serve as universal optics in microscopes. With future multidisciplinary approaches, holographic microscope will continue to advance and broaden their biological applications.

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