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Simultaneous Multi-plane Imaging of Neural Circuits

Highlights

- Two-photon microscope with an SLM module for fast, flexible beam redirection
- Flexibly controlled, multiple-field-of-view functional imaging
- Constrained nonnegative source separation algorithm to separate signals from multiple regions
- Simultaneous multilayer functional in vivo imaging in the mouse cortex

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In Brief

Yang et al. demonstrate a novel approach for simultaneously imaging multiple layers of the mouse cortex. They combine holographic two-photon microscopy with advanced computational source extraction to create a flexible platform for studying mesoscale neural circuits at multiple depths of the brain with cellular resolution.

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SUMMARY

Recording the activity of large populations of neurons is an important step toward understanding the emergent function of neural circuits. Here we present a simple holographic method to simultaneously perform two-photon calcium imaging of neuronal populations across multiple areas and layers of mouse cortex in vivo. We use prior knowledge of neuronal locations, activity sparsity, and a constrained nonnegative matrix factorization algorithm to extract signals from neurons imaged simultaneously and located in different focal planes or fields of view. Our laser multiplexing approach is simple and fast, and could be used as a general method to image the activity of neural circuits in three dimensions across multiple areas in the brain.

INTRODUCTION

The coherent activity of individual neurons, firing in precise spatiotemporal patterns, is likely to underlie the function of the nervous system, and so methods to record the activity of large neuronal populations appear necessary to identify these emergent patterns in animals and humans (Alivisatos et al., 2012). Calcium imaging can be used to capture the activity of neuronal populations (Yuste and Katz, 1991), and one can use it, for example, to image the firing of nearly the entire brain of the larval zebrafish with single-cell resolution (Ahrens et al., 2013). However, the larval zebrafish is transparent; in scattering tissue, where nonlinear microscopy is necessary (Denk et al., 1990; Williams et al., 2001; Zipfel et al., 2003; Helmchen and Denk, 2005), progress toward imaging large numbers of neurons in three dimensions has been slower. In fact, in nearly all existing two-photon microscopes, a single laser beam is serially scanned in a continuous trajectory across the sample in a raster pattern or with a specified trajectory that intersects targets of interest along the path. To image several focal planes, one needs to change the focus and then reimage. This serial scanning leads to low imaging speeds, which necessarily become slower with increases in the number of neurons or focal planes to be imaged. Since the inception of two-photon microscopy, there have been many efforts to increase the speed and depth of imaging. One approach is to use inertia-free scanning using acousto-optic deflectors (AODs) (Duemani Reddy et al., 2008; Otsu et al., 2008; Grewe et al., 2010; Kirkby et al., 2010; Katona et al., 2012). Another approach is to parallelize the light and use many laser beams instead of a single one. Parallelized multifocal scanning has been developed (Bewersdorf et al., 1998; Carriles et al., 2008; Watson et al., 2009), as well as scanless approaches utilizing spatial light modulators (SLMs), which build holograms that target specific regions of interest (Nikolenko et al., 2008; Ducros et al., 2013; Quirin et al., 2014). As a further innovation of SLM-based imaging, we describe a novel "hybrid" multiplexed approach, combining traditional galvanometers and an SLM to provide a powerful, flexible, and cost-effective platform for 3D two-photon imaging. We demonstrate its performance by simultaneously imaging multiple areas and layers of the mouse cortex in vivo.

In particular, one key challenge in nonlinear microscopies is expanding the spatial extent of imaging while still maintaining high temporal resolution and high sensitivity. This is because of the inverse relationship between the total volume scanned per second and the signal collected per voxel in that time. Our SLM hybrid-multiplexed scanning approach helps overcome this limitation by creating multiple beamlets that scan the sample simultaneously, and leverages advanced computational methods (Pnevmatikakis et al., 2016 [companion paper in this issue of *Neuron*]) to extract the underlying signals reliably.

RESULTS

The basic configuration of our SLM microscope consists of a two-photon microscope, with traditional galvanometers, along with an added SLM module. Figure 1A shows a schematic of the SLM-based multiplexed two-photon microscope. The layout is based on that described in detail in Nikolenko et al. (2008) and Quirin et al., (2014) and is similar to that in Dal Maschio et al. (2010). The SLM module was created by diverting the input path of the microscope, prior to the galvanometer mirrors, with retractable kinematic mirrors onto a compact optical breadboard with the SLM and associated components. The essential features of the SLM module are folding mirrors for redirection, a pre-SLM afocal telescope to resize the incoming beam to match the active area of the SLM, the SLM itself, and a post-SLM afocal telescope to resize the beam again to match the open aperture of the galvanometers and fill the back focal plane of the objective appropriately. The SLM is optically conjugated to the galvanometers and to the back aperture of the microscope objective (see Experimental