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Multi-plane 3D optical voltage imaging using high-speed multi-Z confocal microscopy

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1. INTRODUCTION

Fluorescent genetically encoded voltage indicators can be combined with optical imaging to provide high-throughput electrophysiologic recordings with single-spike temporal resolution and subthreshold sensitivity.[1] This method, collectively referred to as voltage imaging, promises to revolutionize understanding of neural circuit dynamics.

Compared with other functional imaging methods (e.g. calcium imaging) signal requirements for voltage imaging are extremely demanding; hence, a majority of voltage imaging experiments have been conducted using widefield single-photon excitation, in part to maximize signal collection. This strategy is adequate for sparely labeled preparations, however issues arise when imaging within densely labeled, scattering tissues, which are more representative of the preparations used in circuit mapping. In such scenarios, excess shot noise from out-of-focus and/or scattered background fluorescence degrades the signal to noise ratio (SNR) (plotted in Fig. 1, solid line). Moreover, if the background is itself time-varying, it can introduce artifacts which are difficult to distinguish from real subthreshold voltage transients. A technique which effectively suppresses background and its associated noise will enable more densely labeled in vitro and in vivo imaging experiments, which are critical for the detailed mapping of neural circuits.

To remove unwanted background, the classic solution is to employ optical sectioning techniques, such as confocal microscopy (Fig. 1, dashed line). In this work, we describe the development of a multi-plane confocal microscope that has been specifically designed for voltage imaging.

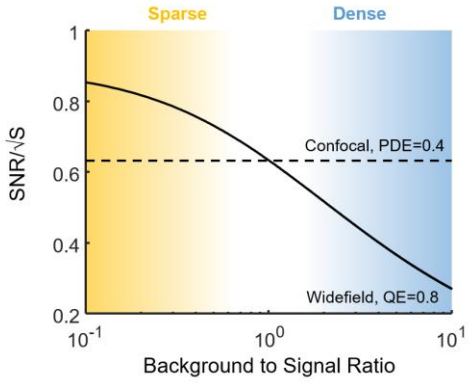

Figure 1. Normalized signal to noise ratio (SNR/√S) vs. background to signal ratio. Widefield imaging (solid line) is superior to confocal imaging (dashed line) in sparse labeling scenarios with low background due to the high quantum efficiency (QE) of modern scientific cameras. Despite the lower photon detection efficiency (PDE, similar to QE) of confocal detectors, background rejection maintains SNR, resulting in better performance relative to widefield in situations with high background.

2. MULTI-Z CONFOCAL SYSTEM

The system is based on Multi-Z confocal microscopy[2], in which a low-NA excitation beam, generated by underfilling the objective back aperture, excites an axially extended (several 10's of μm) range. Fluorescence is collected using the entire NA of the objective, ensuring high collection efficiency. Rather than using a single pinhole, as in conventional confocal microscopy, Multi-Z confocal microscopy uses a series of axially distributed reflective pinholes. As shown in Fig. 1(a), each pinhole collects from a single focal plane while reflecting the rest of the beam on to the next pinhole.

To reach the sampling rate needed to capture fast voltage transients, we equipped the system with a high-speed polygonal scanner, which provided a line scan rate of 117 kHz. With a 16X/0.8 NA water-immersion objective lens, we can image four 150 x 150 μm^2 planes, spanning 50 μm in depth, at 916 Hz (Fig. 2(b)). We used Voltron, a chemigenetic voltage

indicator[3] coupled with a red synthetic dye (JF585) for voltage imaging and Channelrhodopsin-2 (ChR2) for full field blue light optogenetic stimulation.

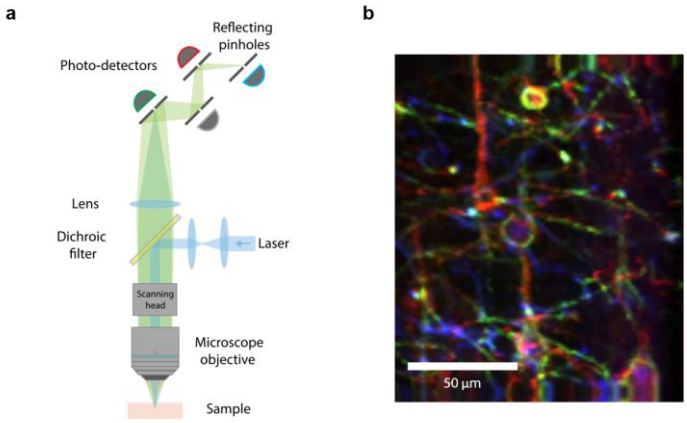

Figure 2. (a) Multi-Z confocal microscopy diagram. Excitation light (blue) underfills the objective creating an axially extended beam of illumination. Fluorescence is collected by a series of axially distributed pinholes which map to different planes in the sample. For clarity, lights paths from only two depths are shown in light and dark green. (b) Triple-plane RGB example image.

3. SLICE IMAGING

Some preliminary results from an acute slice stimulation and imaging experiment are shown in Fig. 3. Additionally, one neuron (indicated in Fig. 3(a)) was simultaneously recorded with patch clamp electrophysiology for validation. Fig 3(b) shows the single trial response under different ChR2 stimulation intensities.

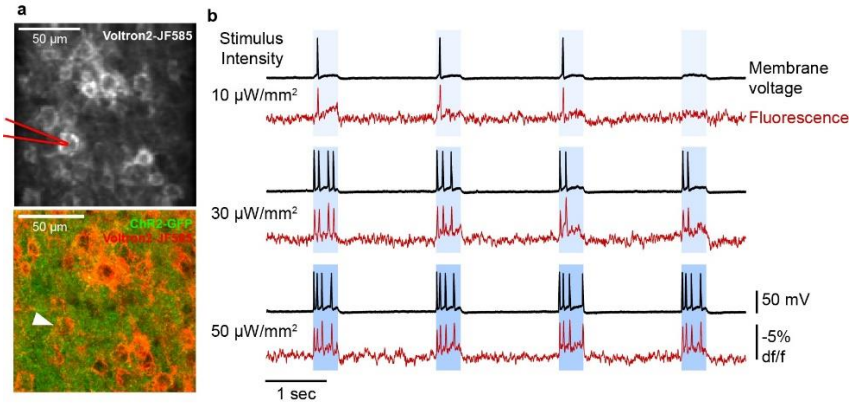

Figure 3. (a) Projection of 4 Multi-Z voltage imaging planes (above) and corresponding post-experiment histology confirming dense Channelrhodopsin-2 (ChR2) and Voltron expression (below). (b) Single-trial, simultaneous electrophysiology and voltage imaging traces from the cell indicated in (a) under different ChR2 stimulation intensities.

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