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Voltage Imaging In Vivo Using Periodic Structured Illumination with Pseudo-HiLo Reconstruction

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Abstract: We utilize pseudo-HiLo (pHiLo) for voltage imaging in awake mice expressing Voltron2$_{552}$ in parvalbumin (PV) interneurons in the somatosensory cortex. We demonstrate increased signal-to-background ratio using pHiLo compared to traditional widefield neural recording. © 2025 The Author(s)

1. Introduction

Genetically encoded voltage indicators (GEVIs) enable the detection of spiking activity and subthreshold oscillations in optical recordings of neuronal activity in vivo. Because short exposure times (0.5-2.5ms) are required for action potentials to be identified using GEVIs, voltage recordings are inherently limited by Poisson noise. The change in recorded fluorescence evoked by a single action potential is a small percentage of the overall signal and must be distinguishable from fluctuations caused by shot noise and out-of-focus background components. The use of structured illumination enables the separation of in-focus and out-of-focus information by exciting the sample using a high frequency grating pattern [1-4]. Using optical sectioning structured illumination microscopy (OS-SIM), the grating is shifted 120 degrees between each frame and in-focus information is retrieved via square-law detection after three consecutive frames [1]. To reduce temporal loss, this process may be performed on an interleaved basis [2]. However, the optical sectioning capability of OS-SIM comes at the cost of decreased signal-to-noise ratio and susceptibility to artefacts caused by dynamic sample changes such as motion [3-4]. Alternatively, HiLo microscopy enables the removal of out-of-focus information by combining a single structured image with a uniform illumination image [5]. High frequency information is extracted from the uniform image as it is inherently in-focus [5]. To obtain the in-focus low frequency information, the spatially varying modulation contrast imparted by structured illumination is assessed in a difference image (obtained by subtracting the uniform image from the structured image) and used to scale the low frequency information of the uniform image [5]. The HiLo reconstruction is then obtained by combining low and high frequency information after scaling for continuity of spatial frequency [5]. In previous work, we used a pseudo-HiLo (pHiLo) reconstruction for high-speed GCaMP8f recording using only periodic structured illumination in the CA1 hippocampal region of an awake mouse [4]. Using the pHiLo technique, an interleaved pseudo-widefield (pWF) stack is first obtained by averaging each structured illumination frame with the preceding (-120 degrees) and succeeding (+120 degrees) frame. The pWF stack is then used in place of the uniform illumination stack and traditional HiLo reconstruction is performed with the structured illumination image at each time point [4]. In this work, we utilize pHiLo for voltage recording from Voltron2$_{552}$ labelled parvalbumin interneurons in the somatosensory cortex of an awake mouse at 870Hz. A reduction of out-of-focus background contamination is indicated by a 2.26x increase in spike ΔF/F for pHiLo compared to a widefield recording from the same neuron.

2. Experimental details and results

A digital micromirror device (Texas Instruments, DLP6500/DLPC900) patterns light emitted from a fiber-coupled 560nm LED (Mightex, 0560-200) after it is collimated using a 10x/.4NA objective (Olympus, UPLXAPO 10x) and filtered at a center wavelength of 535nm (Thorlabs, MF535-22). The beam is focused onto the sample with 35mW/mm^2 of intensity using a 25x/1.1NA objective (Olympus, XLPLN25XWMP2) and emitted fluorescence is separated using a shortpass 567nm dichroic mirror (Thorlabs, DMSP567L). Separated fluorescence passes through a long pass emission filter (Semrock, BLP01-568R-25) and 0.35x magnification coupling (Olympus, U-TV0.35XC) before it is collected by a back-illuminated SCMOS (Hamamatsu Orca-Fusion BT, C15440-20UP). A CamK2a-Cre mouse was injected with 650 nl of the pGP-AAV-syn-FLEX-Volton2-ST-WPRE virus into the somatosensory cortex [6]. After one month, a retro-orbital injection of JF552 was delivered and four hours later the mouse was head-fixed beneath the objective. Data was first recorded using widefield illumination with 35mW/mm^2 intensity at 896 Hz and all DMD mirrors turned on. Structured illumination data was recorded with 20mW/mm^2 at 870 Hz, immediately after the widefield recording, using a grating period of 14 microns at the sample. A pWF image stack was created from the

structured illumination data by pixelwise averaging of each frame with the preceding and subsequent frame. The pHiLo reconstruction was generated in FIJI using the Mertz Lab HiLo plugin with a scaling factor of 1.0 [7]. Figure 1 shows results from the widefield (top) and pHiLo (bottom) image stacks. Figure 1a shows average intensity projections (AIPs) of each recording. Regions of information (ROIs) were segmented using VolPy and are indicated by the red dashed lines in each AIP [8]. The ΔF/F time course for each ROI is shown in Figure 1b. Spike templates for each recording were extracted using VolPy and are shown in Figure 1c [8]. An increase in signal-to-background ratio is indicated by the increase in AIP contrast and for the pHiLo reconstruction as well as a 2.26x increase in spike ΔF/F. A loss of temporal resolution, caused by the three-frame averaging used for pWF stack generation prior to pHiLo reconstruction, is indicated by a ~3x increase in spike template FWHM. Signal-to-noise ratio (SNR), identified using VolPy [8], was 6.05 for the widefield recording and 5.17 for pHiLo.

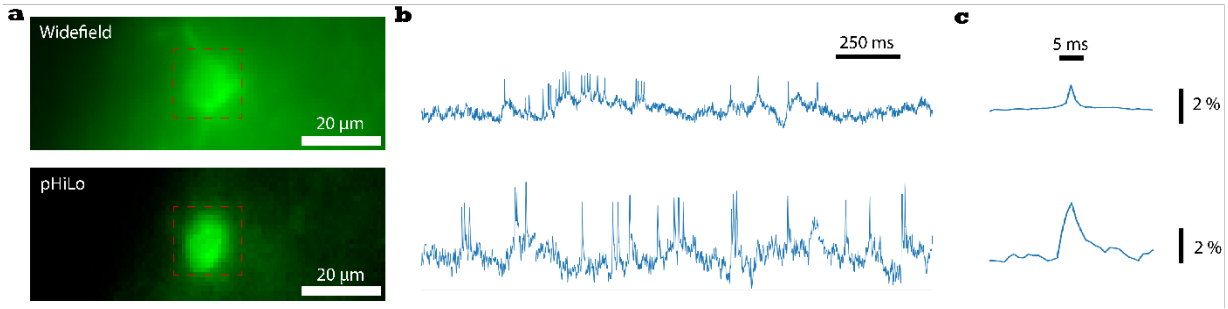

Figure 1. Comparison of widefield (top) and pHiLo (bottom) recordings taken from parvalbumin interneurons expressing Voltron2$_{552}$ in the somatosensory cortex. A. Average intensity projections of each recording. **B**. ΔF/F time courses extracted from the outlined ROIs in (A). **C.** VolPy extracted spike templates for each ROI time course. Data was recorded using 35mW/mm^2 intensity at 896Hz (for the widefield recording) and 20mW/mm^2 intensity at 870Hz (for the pHiLo recording).

3. Conclusion

We utilized structured illumination recording with pHiLo reconstruction to record voltage activity from parvalbumin interneurons expressing Voltron2$_{552}$ in the somatosensory cortex. We compared pHiLo reconstruction with a traditional widefield recording from the same cell. An increase in signal-to-background ratio was indicated by the increase in contrast in the pHiLo AIP and a 2.26x increase in spike ΔF/F. A loss of temporal resolution was indicated by a nearly 3x increase in the FWHM of the extracted spike template for the pHiLo reconstruction compared to widefield.

4. References

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