

# All-optical electrophysiology in iPSC-derived neurons with synthetic NIR voltage reporter

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**Abstract:** We demonstrate a robust and reproducible protocol to achieve all-optical electrophysiology in iPSC-derived and primary cell cultures using synthetic voltage sensors and genetically encoded optogenetic actuators while minimizing cell death due to viral transfection. © 2020 The Author(s)

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## 1. Introduction

Traditionally, neuronal electrical properties have been evaluated using intracellular electrophysiology, which is low-throughput and labor intensive. Recent advances in optical microscopy and optogenetics offer a new experimental paradigm – “all-optical electrophysiology” – where optical voltage sensors and optogenetic (OG) actuators are combined for all optical stimulation and readout [1]. Applied to human-induced pluripotent stem cells (hiPSCs) [2], all-optical electrophysiology opens unprecedented opportunities for high-throughput phenotyping of neurons and neuronal networks possessing unique genetic background of individual patients. In prior studies, the voltage sensor and OG actuator were co-expressed using viral transfection [1]. However, for hiPSCs, transfection with large or multiple plasmids can often result in cell death. This can in turn bias the composition of the remaining neuronal networks towards cell types that are resistant against the transfection procedure. Moreover, this bias may differ across patient and control genotypes undermining the use of hiPSC-based models for biomedical research. Thus, we sought to establish an alternative protocol that uses synthetic voltage sensors, which can be easily and safely delivered to cell cultures mitigating the cell death problem. All-optical electrophysiology requires that the voltage sensor and OG actuator are spectrally orthogonal in order to minimize the crosstalk, i.e., avoiding excitation of the voltage sensor by light that controls the OG actuator and vice versa. Therefore, we chose BeRST (Berkeley Red Sensor of Transmembrane potential) [3] as the most red shifted among the available synthetic voltage indicators. BeRST requires no genetic manipulation, can be easily delivered to all cells and have high sensitivity for detection of single spikes [3]. We optimized our protocols of cell growth and imaging to enable robust, sensitive and reproducible voltage imaging. We further combined BeRST imaging with OG stimulation using an OG actuator CheRiff controlled by blue light, to enable all-optical electrophysiology. Further, we co-labeled our cells with a synthetic calcium indicator Oregon Green BAPTA-1 (OGB1) that facilitated diagnostics and screening for active cells prior to conducting all-optical electrophysiology.

## 2. Methods

*Cell cultures:* Human neurons were derived from hiPSC cultures as described in [4] and replated on Polyhornitine/Laminin-coated MatTek plates. Imaging was performed after 8 weeks of differentiation. Expression of OG actuator was carried out via lentiviral transfection of CheRiff-EGFP plasmid. Primary hippocampal cultures were prepared from postnatal day 0 rat pup brains as described in [5] and plated on Laminin coated MatTek plates. *Optical setup and recordings:* Cells were imaged on a custom-built epifluorescence inverted microscope (Fig. 1A) under constant perfusion with Tyrode’s buffer (pH=7.4) at 28°C. Excitation of BeRST was achieved using a 500mW 635-nm CW laser (Opto Engine LLC) with an intensity at the sample of 25 W/cm<sup>2</sup>. CheRiff was controlled using a 100-mW 473-nm CW laser (Cobolt 06-MLD) with fast analogue modulation (2 MHz). The same 473-nm laser was used for imaging of OGB1. Images were collected with a 40X oil objective

