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We use this protocol and it's working

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High-Throughput Multiplex Live-Cell Imaging of iPSC-derived neurons

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ABSTRACT

Live-cell high-throughput imaging of multiplexed dyes to reveal organelle dynamics (nucleus, mitochondria and lysosomes) in live iPSC-derived neurons.

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Keywords: Live-cell imaging, high-throughput, multiplex, iPSC, neurons

1 Live-cell multiplexed imaging was performed in 96-well plates (PerkinElmer). Cortical neurons were plated at a density of 20,000 - 40,000 cells/well after at least 50 days of neural induction. Neurons were then maintained until imaging (>70 days) with twice weekly feeding.

2 Cells were then washed with Hank's balanced salt solution (HBSS; Invitrogen)

3 Cells were then incubated with 10 µM Hoechst (62249, ThermoFisher Scientific), 25 nM TMRM (T668, Thermo Fisher Scientific) and 250 nM LysoTracker Deep Red (L23492, Thermo Fisher Scientific); 500 nM SYTOX green (S7020, Thermo Fisher Scientific) in HBSS for 30 minutes at room temperature.

4 Live-cell images were acquired using an Opera Phenix High-Content Screening System (PerkinElmer). Hoechst was imaged using the 405 nm laser, SYTOX green labelling was imaged using the 488 nm laser, TMRM was imaged using the 516 nm laser, and LysoTracker was imaged using the 647 nm laser. Live-cell imaging was performed in HBSS. ~20 fields of view were taken per well with each field taken at 5 focal planes in the *z*-axis.