Fast Kinetic Imaging and Analysis of Intracellular Dynamics in Human iPSC- derived Neurons

Stefan Letzsch, Alexander Schreiner, Angelika Foitzik, Marcel Waschow, Norbert Garbow, Karin Boettcher

1 Overview / Key Features

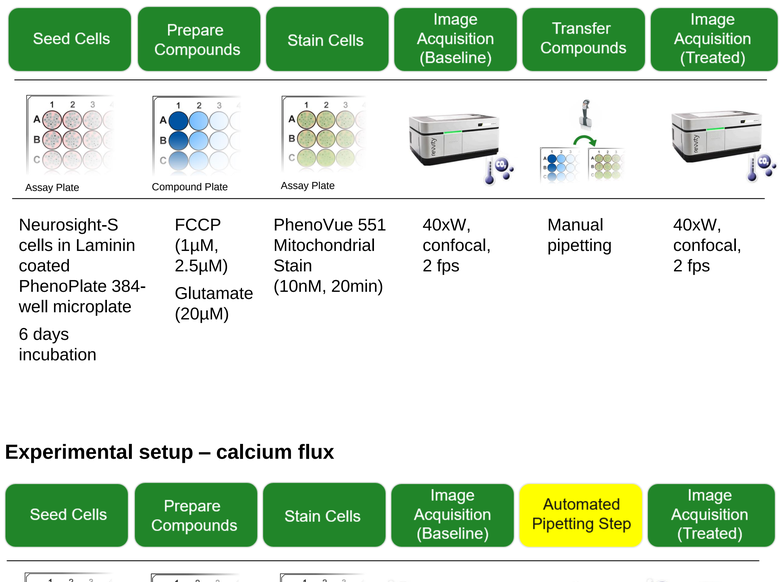
- Fast frame rate imaging to accurately capture rapid cellular responses
- Reliable quantification of mitochondrial dynamics in neurons
- Reliable quantification of calcium activity in single neurons
- Measure immediate compound responses using onboard liquid handling
- Flexible kinetic acquisition settings for each channel avoid unnecessary data acquisition

2 Introduction

- Study of intracellular dynamic processes is fundamental for unraveling the mechanisms of a broad spectrum of diseases and to develop effective drugs and therapies
- Particularly in the field of neuroscience and neurodegenerative diseases two intracellular events are of critical importance: mitochondrial movements and calcium signaling in neurons
- By capturing and analyzing kinetic image sequences, we gain valuable insights into the dynamics of mitochondria in live cells to shed light on their role in neuronal health and dysfunction
- Furthermore, our approach enables the precise assessment of calcium flux in single neurons, crucial for understanding the fundamental processes of neuronal communication and potential disruptions associated with neurodegenerative disease or neurotoxic insults
- Here we apply fast kinetic imaging using our high-content imaging and analysis systems to visualize and evaluate these dynamic events in human iPSC-derived neurons with high temporal resolution

3 Methods

Experimental setup – mitochondrial dynamics



Experimental setup – calcium flux

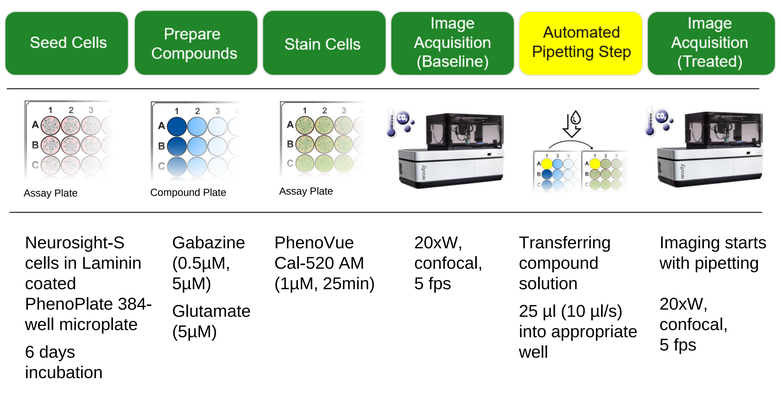


Figure 1: Experimental setup of the mitochondrial dynamics assay and calcium flux assay.

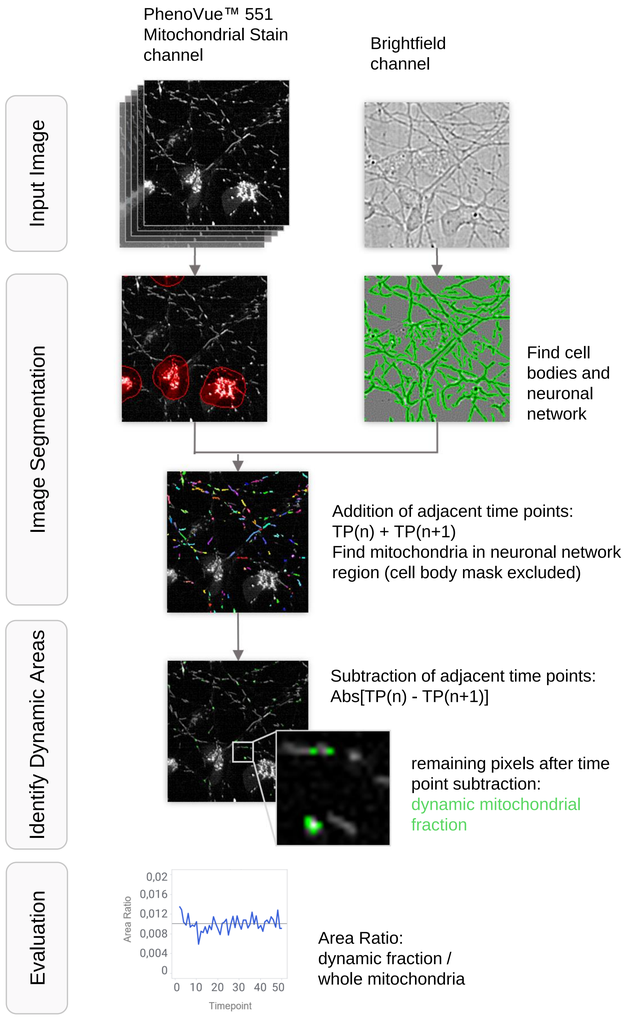


Figure 2. Image analysis sequence in the Harmony software for the evaluation of mitochondrial dynamics over time.

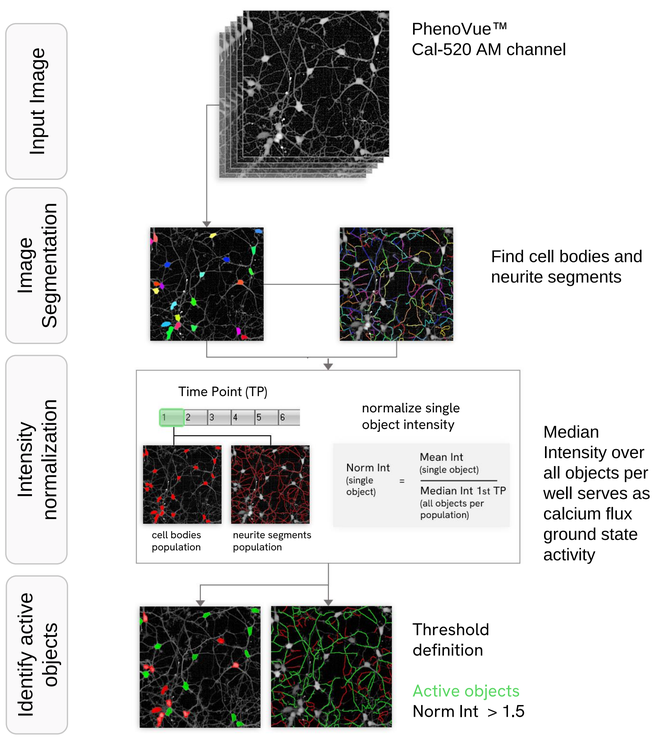


Figure 3. Image analysis strategy in the Harmony software for the evaluation of calcium flux in neurons..

4 Results

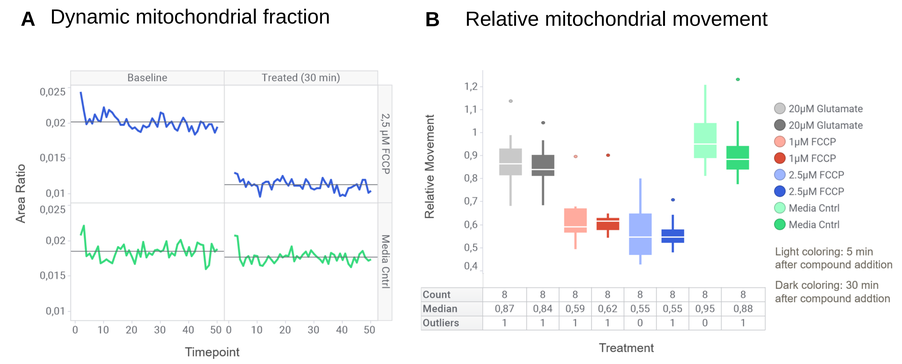


Figure 4: Evaluation of mitochondrial dynamics. (A) The ratio of dynamic mitochondrial fraction over time is shown for two representative wells before and after treatment: untreated medium control in green and FCCP-treated sample in blue. (B) Box plot of the relative mitochondrial movement compared to baseline levels calculated from the mean values of the area ratio over time. Besides untreated medium control (green), cells were treated with 20 µM Glutamate (gray), 1 µM FCCP (red) or 2.5 µM FCCP (blue). Values from two measurements are shown: 5 minutes after compound addition (light coloring) and 30 minutes after compound addition (dark coloring).

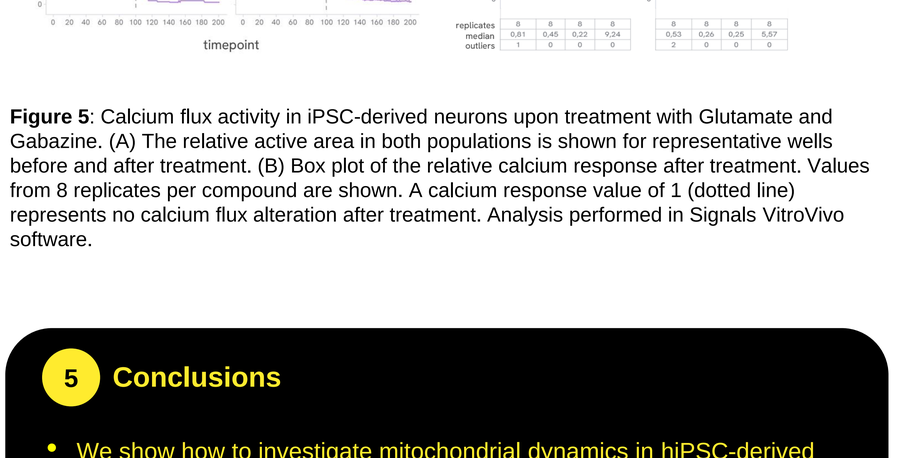


Figure 5: Calcium flux activity in iPSC-derived neurons upon treatment with Glutamate and Gabazine. (A) The relative active area in both populations is shown for representative wells before and after treatment. (B) Box plot of the relative calcium response after treatment. Values from 8 replicates per compound are shown. A calcium response value of 1 (dotted line) represents no calcium flux alteration after treatment. Analysis performed in Signals VitroVivo software.

5 Conclusions

- We show how to investigate mitochondrial dynamics in hiPSC-derived neurons using the Operetta CLS high-content analysis system
- Utilizing the PhenoVue 551 Mitochondrial stain combined with gentle, high-resolution imaging enables the reliable detection of mitochondrial dynamics without compromising mitochondrial function
- we show how to investigate compound effects on spontaneous calcium signaling events in human iPSC-derived neurons using the Opera Phenix Plus high-content screening system
- Combining the PhenoVue Cal-520 AM stain with gentle, fast kinetic high-resolution imaging enables the reliable detection of calcium flux without compromising neuronal function
- In summary, fast kinetic live cell imaging combined with high-content screening represents a promising strategy to analyze calcium response activity in real time and at large scale

For more information about products used in this experiment

Microplates (PhenoPlates) | **CRISPR Cas-9 Gene editing** | **PhenoVue Cellular Reagents** | **HCS Instruments** | **Signals VitroVivo** | **Cellular Imaging Solutions**

Revvity Inc., 940 Winter Street, Waltham, MA USA (800) 762-4000 or (+1) 203 925-4602 www.revvity.com