Quantitative confocal microscopy for grouping of dose–response data: Deciphering calcium sequestration and subsequent cell death in presence of excess norepinephrine

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Abstract

Live imaging based testing of ligands and identification of toxic dose range during in vitro studies is a time-intensive process due to the inherent heterogeneity present in cell responses. In this context, high magnification imaging and large-scale data visualization remains challenging for analysis of toxicity during the drug screening as well as selection of dose-range. To address this challenge, we propose the measurement of cytosolic calcium ion (Ca2+) using spinning disk confocal microscopy at a higher resolution for generation of imaging data that can be visualized using uniform manifold and projection (UMAP). First, we performed large scale experiments and showed norepinephrine induced increase in Ca2+ flux in HeLa cells for a large range of doses. Secondly, the time-series dataset was mapped in 2D plane using UMAP. We also show that the proposed framework can be used to depict the relative distribution of various responses corresponding to a range of drug doses. To the best of our knowledge, this is the first attempt for UMAP visualization of time-series dose-response and identification of Ca2+ signature in the toxic dose-range. Such quantitative microscopy can be used for prediction of toxic drug dose range, and identification of drugs that lead to lytic cell death.

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