New Non-Toxic Fluorescent Stain to Probe Cell Physiology in High Content Screening

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Overview

High-content screening, in combination with the use of perturbing agents such as small molecules or gene-editing approaches, has already shown great potential for identifying new therapeutic treatments [1].

Being confined to a finite number of time points or cellular states per assay limits what precious information we can get from these cells.

Here we present a novel non-toxic and mix-and-read fluorescent stain that provides unparalleled insights into cellular physiology through indefinite live-cell assays and the simultaneous detection of multiple cellular phenotypes. Data that validate these unique properties and applications for drug discovery in the context of laboratory automation are hereby presented.

Introduction

ChromaLive is a new type of non-toxic and mix-and-read fluorescent stain that generates a unique signature for every cellular state. This work aims to validate these unique properties, and present use-cases for drug discovery applications, in the hope of helping generate new insights into the performance of new therapeutic treatments.

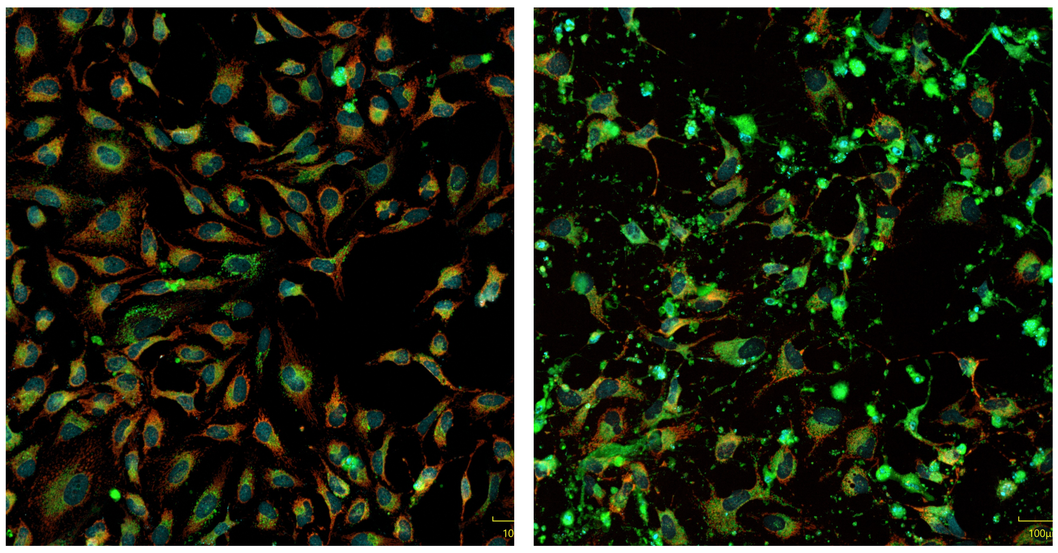

Figure 1. Here, *ChromaLive* is combined with Hoechst 33342 in Staurosporine-treated cells. *ChromaLive* is a small organic molecule that incroporates into lipid bi-layers and stains cell structures indiscriminately. It is multi-chromatic and therefore has 3 excitation and emission pairs: 488/600nm, 488/700nm, and 561/600nm. The first pair is omitted in this image, the second appears green, and the thrid appears yellow and red. Left: at 0h. Right: at 25h.

Quantification of Cellular Phenotypes

The changes highlighted by *ChromaLive* in cells can be leveraged to quantify different cellular phenotypes such as autophagy, ER stress, apoptosis, quiescence, live, and dead.

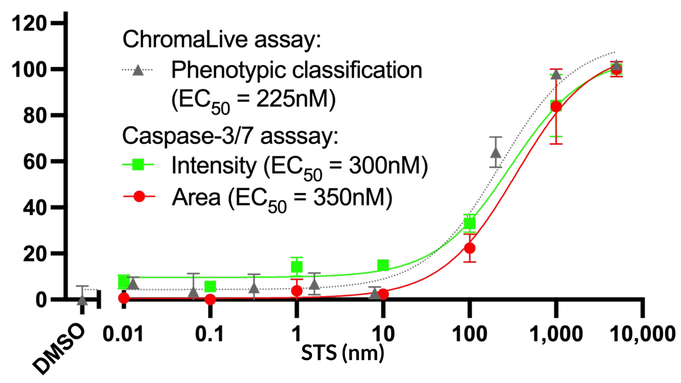

Figure 2. Apoptosis – 24h. Phenotypic dose-responses can be generated for different celllular phenotypes. Here, *ChromaLive* is compared ag inst CellEvent™ Caspase kit. The CellProfilerAnalyst classification tool was used

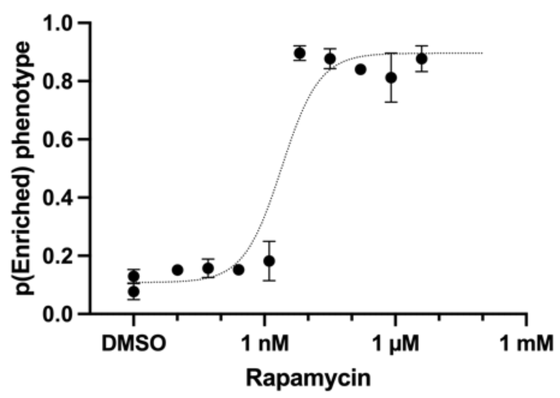

Figure 3. Autophagy– 72h. Here, *ChromaLive* is used to quantify rapamycin-induced autophagy, which is detected below 10nM

To quantify cellular phenotypes using *ChromaLive*, images are acquired in three different channels, allowing for data collection of three intensity profiles and of distinct cell features, which together can be used to classify cell types and cell status. Importantly, *ChromaLive* spectral changes, such as increased intensity in apoptotic cells, can be used to distinguish live and dead states (see figure 1).

Validation of (Non-)Cytotoxicity

ChromaLive dye has shown little to no effect on cell transcriptome, and on cultures of pluripotent stem cells, primary cells, and cell lines. Absence of cytotoxic effects on cell health and great photostability were also observed in 40-day cultures.

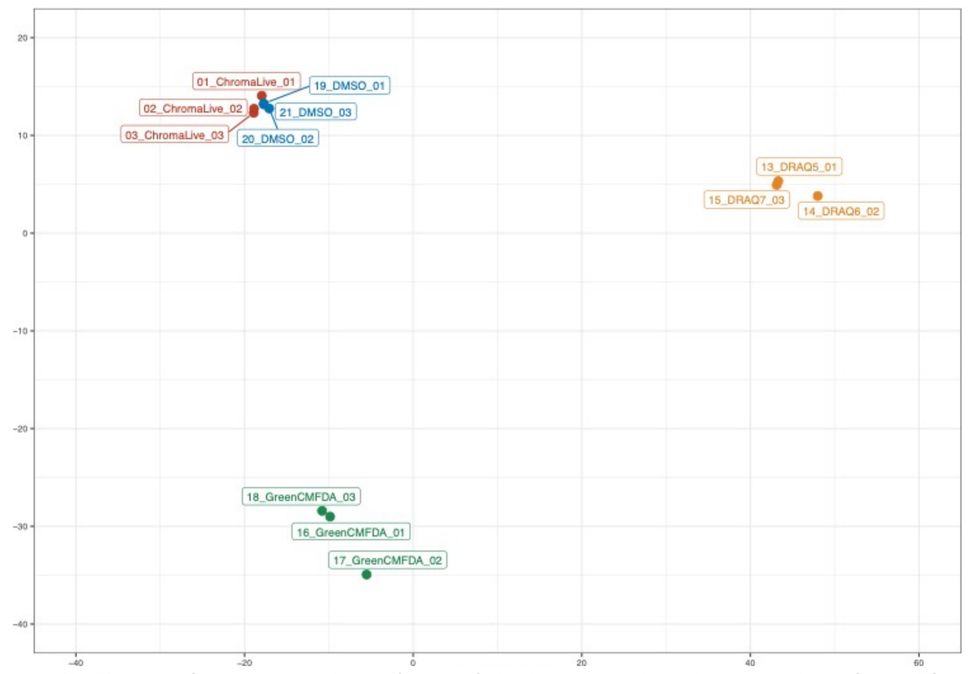

Figure 3. Principal component analysis plot depicting the transcriptional profile of *ChromaLive* (red) relative to a DMSO control (blue) and other commercial dyes: CellTracker™ green (green) and DRAQ5 (yellow).

"Live Cell Painting"

With multi-parametric analysis of images of cells stained with *ChromaLive*, it is possible to perform morphological profiling assays, which serve as an orthogonal modality for drug discovery.

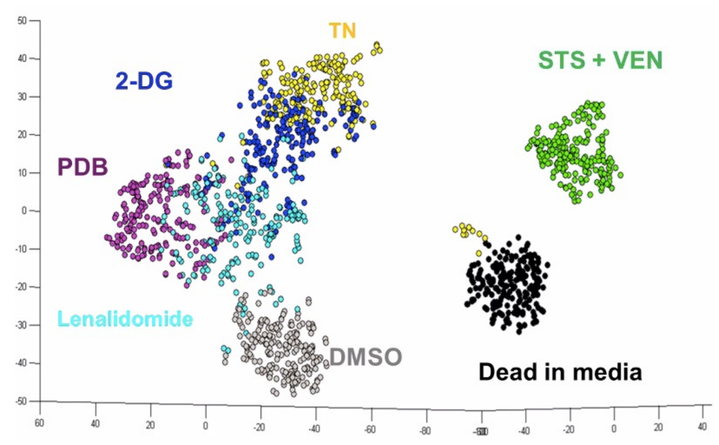

Figure 4. CLL cells from 40 patients stained with *ChromaLive* and imaged by confocal microscopy. A t-SNE plot of image features shows good separation of drug responses.

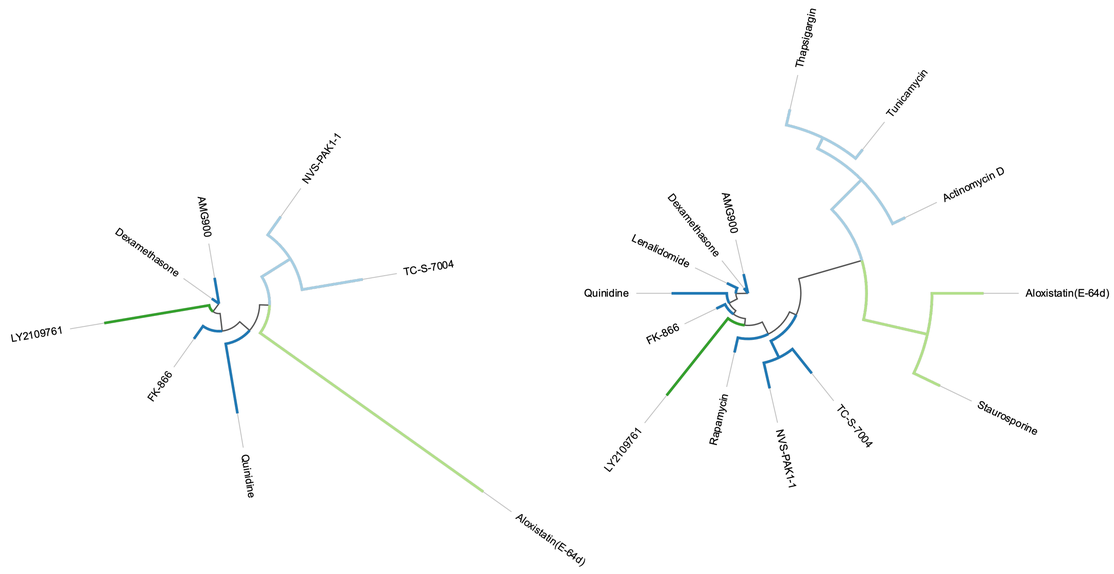

Figure 5. Dendrogram visualizations from cells stained with *ChromaLive* show good phenotypic separation of the control compounds of the JUMP-CP consortium: on their own (right), and together with five compounds of known phenotypes (left). Analysis using ViQi's AutoHCS software.

Concluding Remarks

Cells can reveal a lot about the performance of new therapeutic treatments, but we are still limited by the inability of current probes to be used with live cells or to provide more complete information on cellular physiology.

The validation of *ChromaLive*'s unique properties and usability in different applications shows its potential for extracting the precious information contained in cells. Hence, *ChromaLive* dye contitutes a new insightful tool for drug discovery research.

References

1. Oppermann, S., Ylanko, J., Shi, Y., Hariharan, S., Oakes, C. C., Brauer, P. M., ... & Andrews, D. W. (2016). High-content screening identifies kinase inhibitors that overcome venetoclax resistance in activated CLL cells. Blood, The Journal of the American Society of Hematology, 128(7), 934-947.