Cellular Phenotypic Profiling
Combining Live Imaging & Cell Painting Techniques

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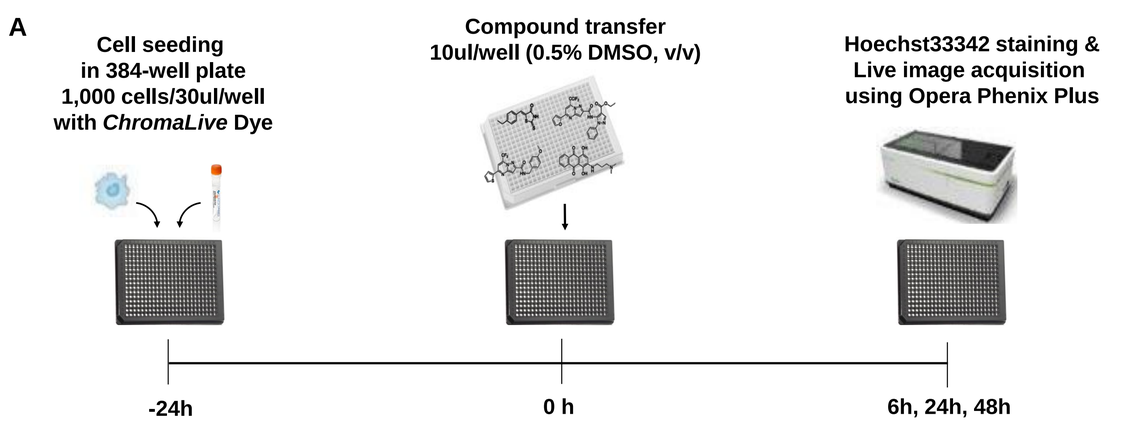
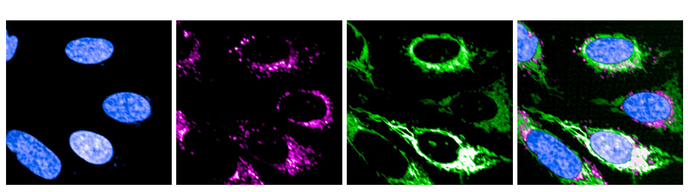
Abstract

High-throughput screening and cell painting techniques that enable phenotypic profiling are becoming increasingly versatile and central to successful drug discovery pipelines; however, these require cell fixation and multiple staining steps. ChromaLive (Saguaro Technologies) Dyes applies cell painting technology in live cells to measure phenotypic changes. The unique staining and spectral properties of the dye can detect various cellular states such as cell survival, death, apoptosis, quiescence, ER stress, and autophagy. We leverage ChromaLive's distinctive properties to profile compounds in live cell kinetic mode. First, we acquired standardized images of stained cells treated with compounds of known function and analyzed them using cell painting techniques to extract thousands of features at a single level population. The obtained profiles can then be used to build and discover dynamic associations between the cellular states over the treatment course of each compound. We finally applied StratoMineR™ (Core Life Analytics), a web-based data analysis platform with built-in modules to seamlessly measure and investigate the phenotypic outcomes from ChromaLive staining. In this study, we demonstrate the feasibility and utility of live cell dyes to profile compounds in an effortless workflow over conventional fixed staining techniques.

Introduction





Channel name	Excitation	Emission
CL 488-Red	488 nm	630-750 nm
CL 561-Yellow	561 nm	570-630 nm
Hoechst33342	375nm	447-460 nm

Fig 1. Overview of Assay. (A) U2OS cells were plated in a 384-well plate with ChromaLive Dye. The cells were treated with various concentrations of compounds. Hoechst staining and images were acquired at 6, 24, and 48 hours on the Opera Phenix Plus High Content Imager (Revvity). (B) Representative live-cell images of U2OS cells stained with ChromaLive (Blue: Hoechst33342, Magenta: CL 488-Red and Green: CL 561-Yellow). (C) Recommended excitation and emission settings for ChromaLive stains.

Results

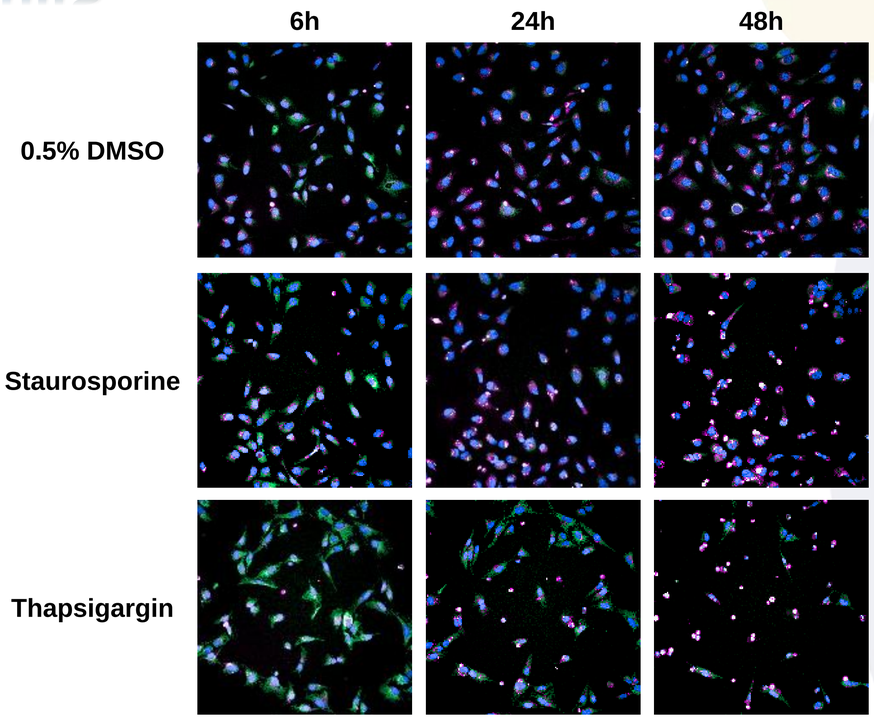


Fig 2. ChromaLive-stained cells treated with compounds at different time points. Staurosporine, known to induce apoptosis. Thapsigargin, a known inducer of ER stress and cell death.

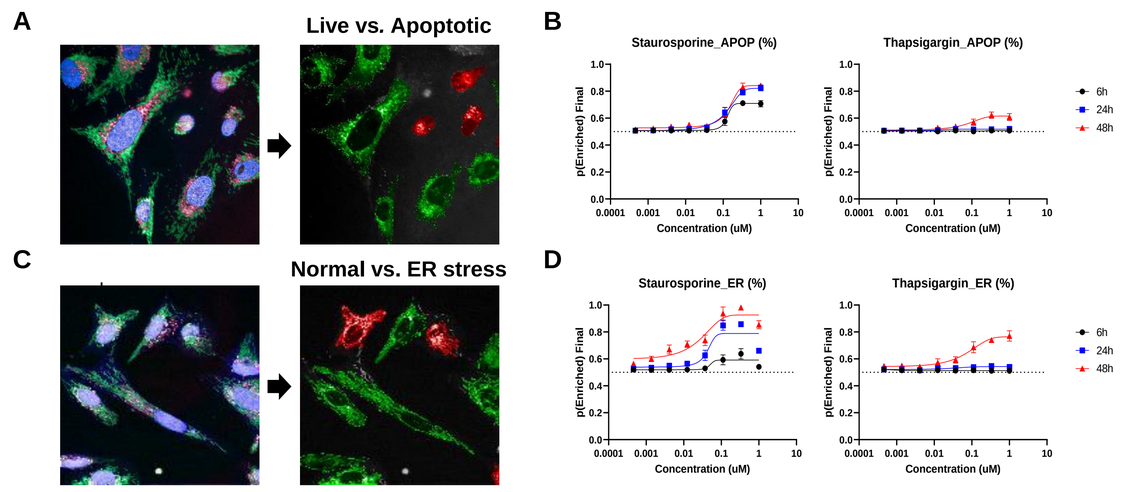
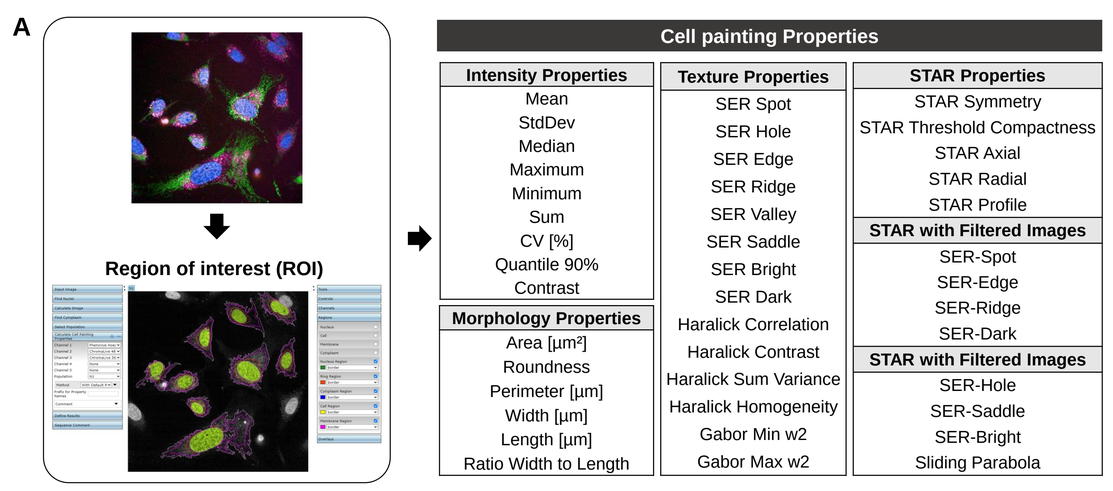


Fig 3. Training Mode of Linear Classifier in Harmony Software. Identify cell phenotypes using PhenoLOGIC machine learning. Train the software to identify different cell types and PhenoLOGIC combines the most meaningful parameters to accurately classify both cell types. (A) Classification Live (green) vs. Apoptotic (red) cell phenotype. (B) Dose response curve of apoptotic phenotype in cells treated with staurosporine or thapsigargin. (C) Classification Normal (green) vs. ER Stress (red) cell phenotype. (D) Dose response curve of ER stressed phenotype in cells treated with staurosporine or thapsigargin.



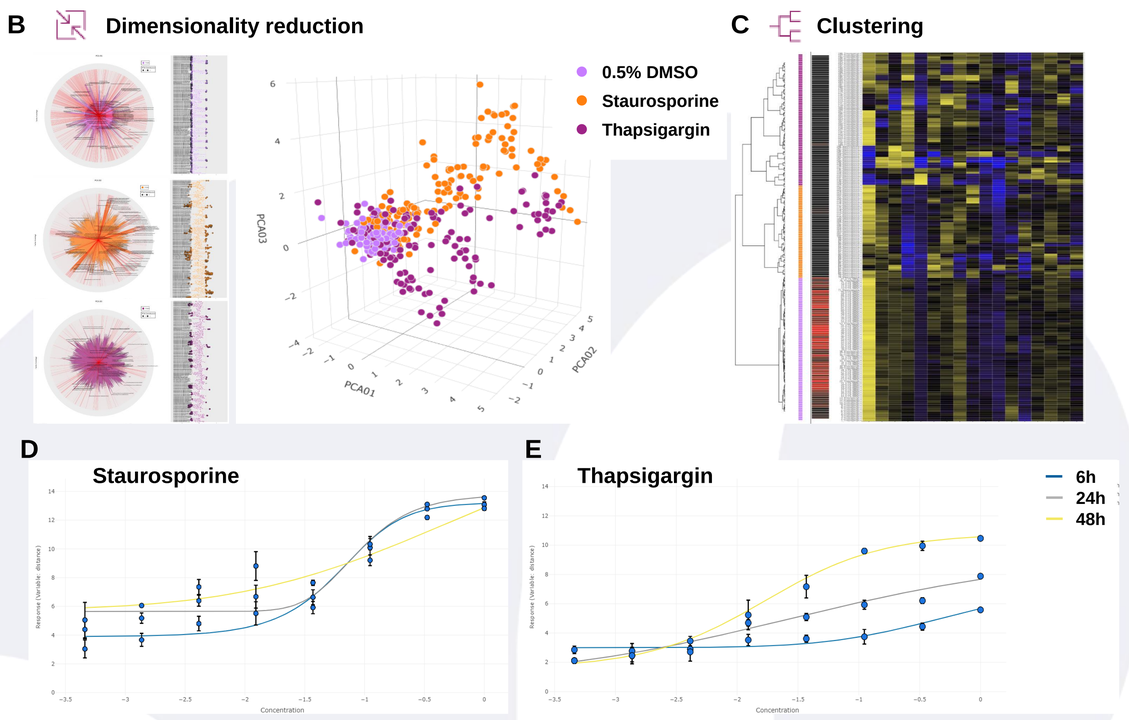


Fig 4. Unsupervised Data Analysis. (A) Calculate Cell Painting Properties in Harmony Software. Calculates a large set of intensity, morphology, and texture properties for a set of regions and channels. Calculate all properties at once instead of using a separate building block for each channel, region of interest (ROI) and feature. (B) Data analysis using StratoMineR™. Dimensionality reduction to reduce the complexity of the data. Principal component analysis (PCA) of cells treated with different concentrations of staurosporine or thapsigargin. (C) Results from the cluster analysis are represented as a hierarchical dendrogram. The dose response curves for staurosporine (D) or thapsigargin (E) using phenotypic distance score ranking at different time points. x-axis: concentration, y-axis: phenotypic distance score.

Summary

- **ChromaLive dyes enable real-time phenotypic profiling in living cells, as well as quantification of more traditional disease-related phenotypes such as apoptosis, autophagy, and ER stress.**
- **The training mode of a linear classifier using Harmony and the analysis of unsupervised feature data using StratoMineR™ enable detection of dynamic phenotypic changes in living cells, with similar results.**
- **Additional image analysis using biologist-friendly analysis tools can help predict the drug's mechanism of action.**

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