Quantifying Apoptosis with Time-Lapse Images of Live Cells using a New Nontoxic Dye and Automated AI Analysis

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Overview

Dysregulation of apoptosis is associated with numerous diseases, such as cancer, neurodegenerative, and autoimmune conditions, making it a critical target for drug discovery. Traditionally, apoptosis quantification methods have relied on fixed cells and stains that negatively impact cell viability, which limit the ability to capture dynamic apoptotic events in live cells [1].

In this study, we present a novel approach that combines a new non-toxic dye with automated AI image analysis for real-time visualization and quantification of apoptosis in live cells, without compromising on cell viability.

Our work addresses a critical need in the field of apoptosis research by providing a non-invasive, high-throughput approach for quantifying apoptotic events in live cells.

Introduction

We present a method for staining cells using ChromaLive nontoxic dye, setting acquisition parameters on the CellVoyager CQ1 confocal high-content system, and generating dose-response analyses with the AutoHCS tool.

Using HeLa cervical cancer cells treated with staurosporine, a known inducer of apoptosis [2], we performed two time-lapse imaging assays to successfully quantify apoptosis: a first time-lapse of 24h with 10-min intervals and 4 doses, and a second of 6h with 30-min intervals and 10 doses.

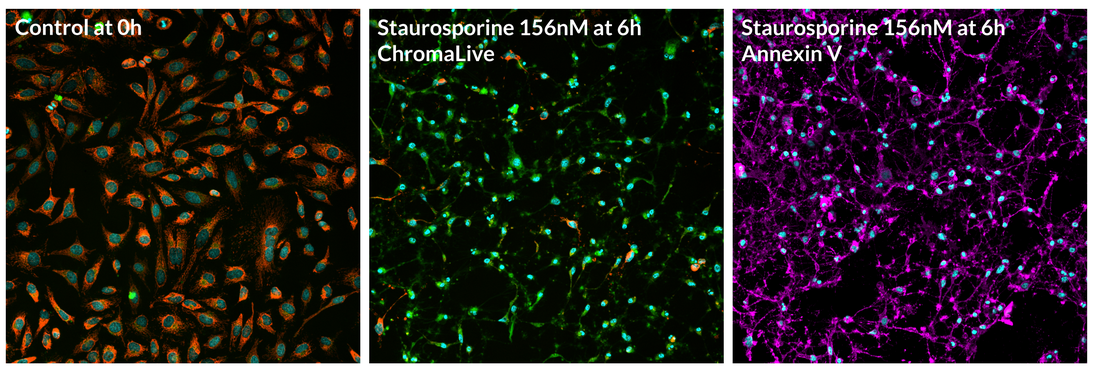


Figure 1. Images of HeLa cells stained with ChromaLive or Annexin V. Healthy cells in DMSO control at 0h (left) and apoptotic cells treated with 156nM staurosporine at 6h (middle and right). Hoechst33342, ChromaLive, ChromaLive, Annexin V.

Method - Staining to Results in 3 Simple Steps

First, the fluorescence of ChromaLive dye only upon incorporation into cell membranes allowed for its addition at the cell-seeding step. Hence, HeLa cells were seeded with medium containing ChromaLive and incubated overnight. Hoechst 33342 was added at low concentration for minimal cell perturbation and to allow sufficient staining for cell segmentation during analysis.

Second, staurosporine treatments were added the next day, before performing a 6-hour time-lapse imaging assay. The environment-controlled chamber and low phototoxicity of the CellVoyager CQ1 imager made it ideal for imaging every 30 minutes. After 6 hours, Annexin V staining was performed in order to confirm apoptosis in cells (Figure 1).

Third, images were uploaded to ViQi Inc.'s AutoHCS web-based AI analysis tool. Images were tiled (4x4) and features were calculated using custom software and run through a feature classifier. AIs were then trained to predict either dose (Dose AI) or a target phenotype from the control condition (Target Phenotype). Individual AIs were trained as outlined in 'Quantifying Apoptosis in Live Cells'.

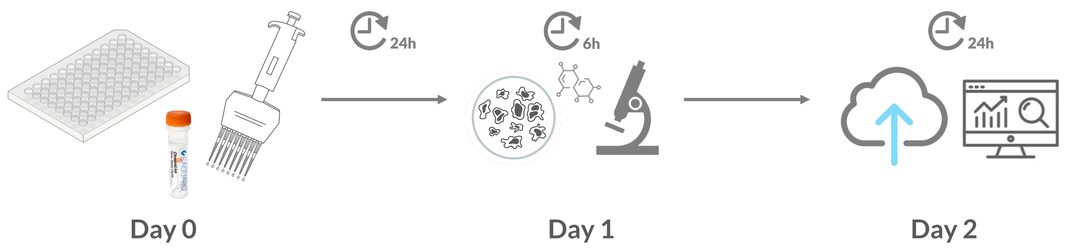


Figure 2. Workflow for staining, imaging and analyzing apoptosis in live cells.
First, cells were stained with ChromaLive and plated in a 96-well plate. Then, imaging was performed the next day. Images were acquired at 20x magnification with 4 channels. MIP (Max Intensity Projection) images generated from 3 z-slices were used for further quantification. Lastly, images are uploaded to ViQi Inc. servers, and a full report is generated within 24 hours.

References

1. Gelles JD, Chipuk JE. Robust high-throughput kinetic analysis of apoptosis with real-time high-content live-cell imaging [published correction appears in Cell Death Dis. 2017 May 4;8(5):e2758]. Cell Death Dis. 2016;7(12):e2493.
2. Nicolier M, Decrion-Barthod AZ, Launay S, Prétet JL, Mougin C. Spatiotemporal activation of caspase-dependent and -independent pathways in staurosporine-induced apoptosis of p53wt and p53mt human cervical carcinoma cells. Biol Cell. 2009;101(8):455-467.
3. ViQi AutoHCS: https://viqiai.com/autohcs

Staining and Imaging of Live Cells

The staining of cells with ChromaLive dye was performed at the cell-seeding step, which is typically recommended for this non-toxic dye. ChromaLive is better suited for phenotypic profiling and multi-parametric analysis, but increased intensity in one of its channels (Figure 3) indicates cell death, and presents a great visual cue for the imaging scientist. Hence, with increased apoptosis, more signal is typically observed in ChromaLive-stained cells.

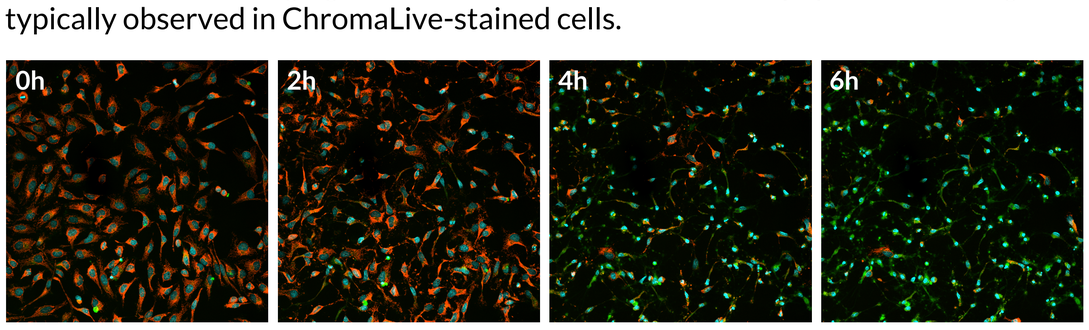


Figure 3. ChromaLive-stained cells treated with Staurosporine at different timepoints. Cells treated with 78.1nM staurosporine. Hoechst33342, ChromaLive, ChromaLive.

Time-lapse imaging follows a simple process with the CellVoyager CQ1 instrument. Its highly-sealed chamber stage can keep the environment at 37°C and 5% CO_2 with humidity, and although ChromaLive has a long stokes shift, lasers and emission filters can easily be adjusted. In addition, particular attention must be taken when setting an imager's fluorescent channels with ChromaLive dye (Figure 4).

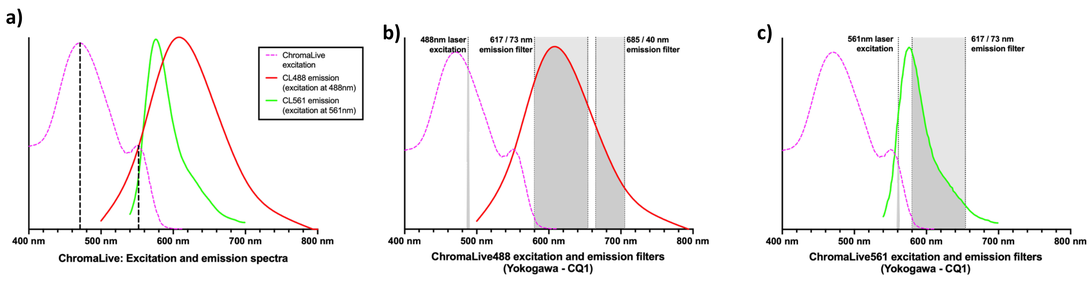


Figure 4. Setting up lasers and emission filters used for ChromaLive on the CQ1 imager
Fluorescence spectrum of ChromaLive (a) and selected filters (gray) on the CQ1 (b and c).

Quantifying Apoptosis in Live Cells

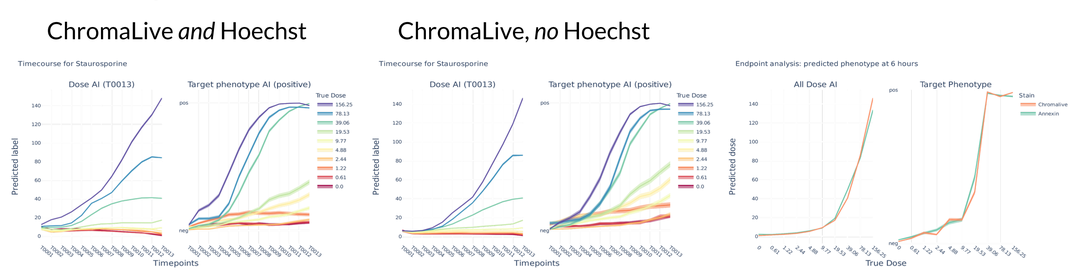
ViQi Inc.'s Automated High-Content Screen (AutoHCS) is a robust and flexible web-based analysis tool that uses AI to evaluate high-content screens. Here, AutoHCS is used to evaluate the dose-response of staurosporine. By strategically dropping channels from the analysis, we demonstrate that the nuclear stain Hoechst does not play a significant role in distinguishing different doses of staurosporine. Further, we show that ChromaLive dye matches the efficacy of Annexin V in determining the extent of apoptosis. Finally, in a second, longer screen, we demonstrate the significant effect of time on cell phenotype, which can be corrected for by training an AI per timepoint.

a) Channel-specific effects

ChromaLive *and* Hoechst

ChromaLive, *no* Hoechst

b) Endpoint assay



c) Correcting for time

AI trained at final timepoint

AI trained per timepoint

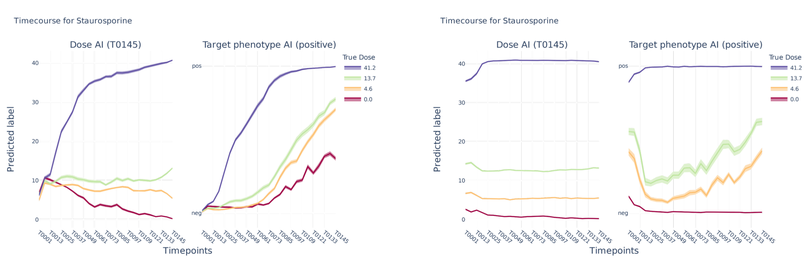


Figure 5. Line represents mean value of predictions and shaded region is the standard error of the mean for all predictions at that timepoint/dose. **a)** 6-hour time course with 10 doses of staurosporine. Each panel displays a 'Dose AI' trained on dose at the final timepoint and a 'Target phenotype' trained on dose 0.0, first timepoint vs. dose 39.06, last timepoint. The individual plots depict AI prediction across time for each dose in the screen. Left) AI prediction when trained on all dyes including nuclear stain Hoechst. Right) AI predictions when trained without Hoechst. **b)** True dose against predicted label for both Dose AI and Target Phenotype AI when trained solely on Annexin V channel and solely on ChromaLive channels. **c)** 24 hour timecourse with 4 doses of staurosporine. Left) Full timecourse predicted using an AI trained as in (a). Right) Each point of timecourse predicted using individual AI trained only on that timepoint to correct for time.

Conclusion

Our study introduces a new method of quantifying dynamic apoptotic events by using a novel non-toxic dye, state-of-the-art imaging and accessible AI analysis.

The presented approach provides researchers with a seamless and powerful way of circumventing the limitations of traditional fixed-cell methods, and gaining a more nuanced understanding of apoptosis and disease mechanisms.