Real-time Caspase 3/7 measurement of suspension and adherent cells using the Celigo image cytometer

Surekha Bonasu[1], Sarah Kessel[1], Leo L Chan[1], Jean Qiu[1], Dmitry Kuksin[1]
[1]*Revvity Health Sciences, Inc., 360 Merrimack St., Suite 200, Lawrence, MA 01843*

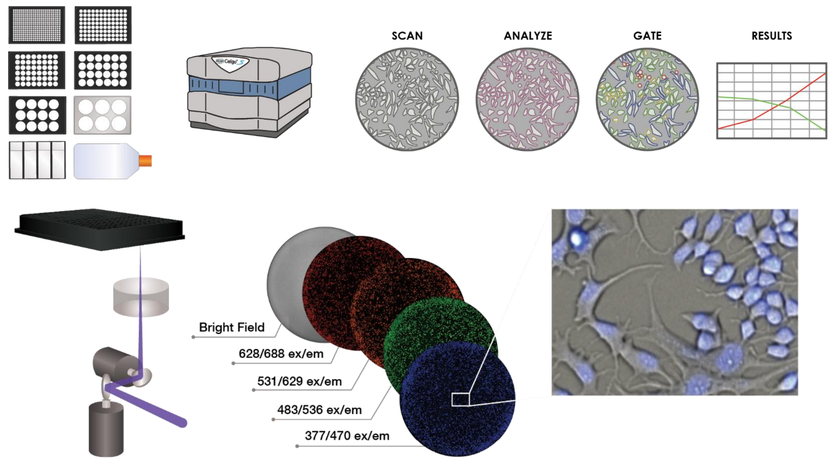
1. ABSTRACT

A novel assay has been developed for real-time staining and detection of active caspases 3 and 7 in apoptotic cells using the Celigo, a micro-well imaging system. Traditionally, this type of apoptosis assay is an end point assay and is performed using fluorescently labeled Annexin-V. However, by coupling this image cytometry system (Celigo) developed by Nexcelom Bioscience LLC (Lawrence, MA) with live-cell dye for caspase 3/7 we are able to image and analyze adherent and suspension cell lines without cell perturbation. Since apoptotic cells are often fragile and can be easily washed off the plate, performing an in-the-plate wash-free assay for the detection of caspase activity is of significant value to the research community. Because the image cytometer is capable of capturing bright-field and fluorescent images as well as perform gating operations based on fluorescence intensity, it is possible to not only carry out live continuous monitoring by detecting a green caspase 3/7 signal but also perform an end point assay by counterstaining the cells with Hoechst and thereby determine a percent nucleated cells that are apoptotic. Here we successfully demonstrate performing real-time continuous monitoring and end-point caspase 3/7 assays using adherent MDA-MB-231 and suspension Jurkat cells stained with Nexcelom ViaStain™ Caspase 3/7 reagent in a 96-well format and imaged on the Nexcelom Celigo image cytometer. This combination of live cell staining and real-time image acquisition provides researchers a unique tool for examining dynamic apoptotic events in a micro-well environment

2. EXPERIMENTAL OUTLINE

1. Seed MDA-MB-231 and Jurkat cells overnight at 10,000 and 20,000 cells per well respectively
2. Add 3 µM Staurosporine and Nexcelom ViaStain™ Caspase 3/7 reagent to MDA-MB-231 and Jurkat cells. Control wells receive only Nexcelom ViaStain™ Caspase 3/7 reagent.
3. Image the same plate over multiple time points (0, 3, 6 hrs) to acquire the kinetic measurement of the number of apoptotic caspase 3/7 positive cells.
4. End point measurement was performed at hour 6 by counter staining the cells with Hoechst

3. DIRECT CELL COUNTING BY CELIGO IMAGING CYTOMETRY

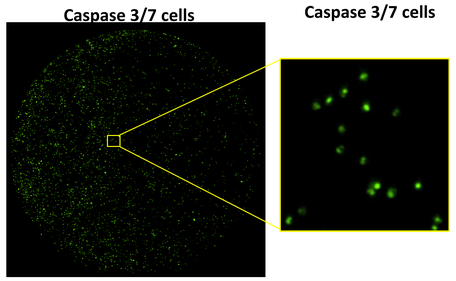


1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplate and captures images using bright-field and 4 fluorescent channels
2. Proprietary optical design enables uniform illumination and consistent edge contrast
3. Accurately quantify cells and colonies with non-invasive method
4. The measured parameters are used to generate cell proliferation kinetic data, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, and ADCC cytotoxicity results
5. System stiches multiple fields of view into a full resolution image

4. CELIGO IMAGE CYTOMETRY PROCEDURE

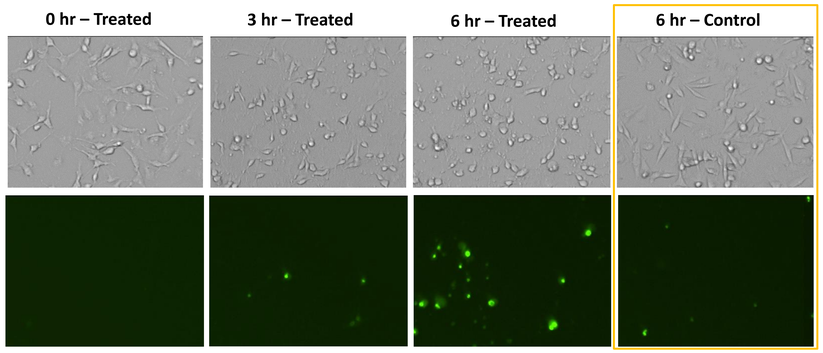
Seed cells in a 96-well plate. Allow cells to grow overnight → Add drug and caspase 3/7 reagent simultaneously → Image and analyze on the Celigo at 0, 3, 6 hrs after adding drug/stain → Stain with Hoechst to obtain total cell number and % of apoptotic cells

5. Hi-RESOLUTION WHOLE WELL IMAGING

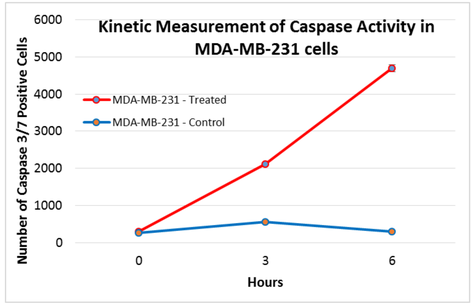


- Hi-res whole well images for any size microwell plate is acquired by the Celigo image cytometer
- Shown on left is a whole well and a zoomed-in image of a 6 hr, drug treated caspase 3/7 positive MDA-MB-231 cells
- Uniform imaging of the entire well allows for fast and accurate direct cell counting

6. IMAGING AND ANALYSIS OF MDA-MB-231 CASPASE 3/7 POSITIVE CELLS

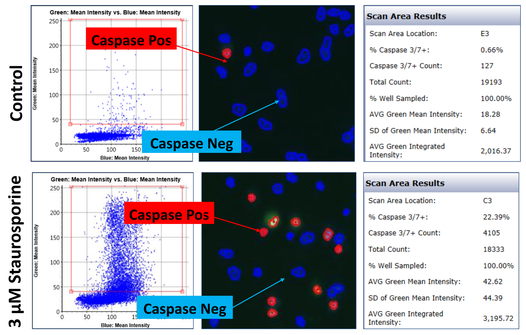


- Whole-well bright field and green fluorescence (caspase 3/7) images were captured on the Celigo. Micrographs above, show representative images for 0, 3, 6 hr time points for both treated and control samples.
- Cells treated with 3 µM staurosporine showed an increase in the number of caspase 3/7 positive cells in a time-dependent manner.

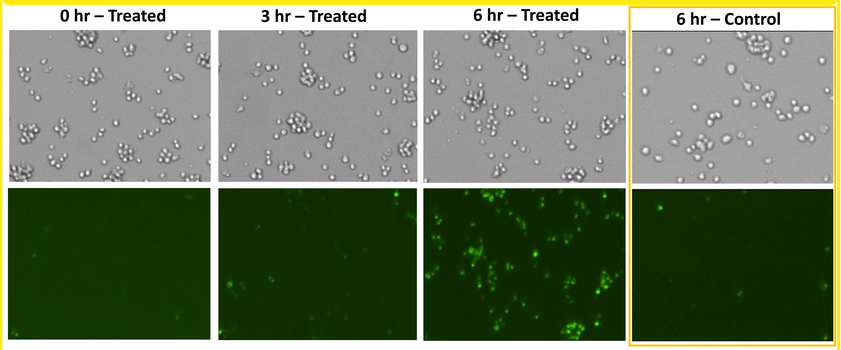


- Kinetic measurement of caspase 3/7 activity in MDA-MB-231 cells was determined by performing direct cell counting
- Green caspase 3/7 positive cells were automatically counted on the Celigo and gathered data was exported to excel as a .csv file
- Cells treated with staurosporine showed a time-dependent caspase 3/7 activity
- For the end-point assay MDA-MB-231 cells are counterstained with Hoechst to determine the total number of nucleated cells
- Nucleated cells with a green caspase 3/7 signal are scored as positive for apoptosis
- On the left, the scatter plot and associated images show blue out lined cells (caspase 3/7 ^{neg}) and red outlined cells are (caspase 3/7 ^{pos})
- The software automatically reports the total number caspase 3/7cells, the total number nucleated cells and the percent of caspase 3/7 cells in the entire population

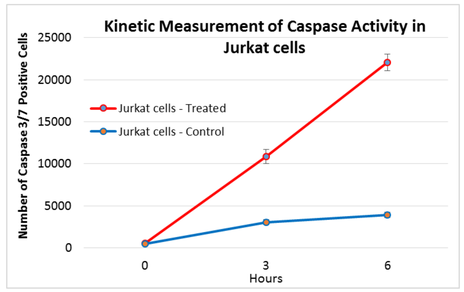
End-point: Caspase 3/7, Hoechst Analysis



7. IMAGING AND ANALYSIS OF JURKAT CASPASE 3/7 POSITIVE CELLS

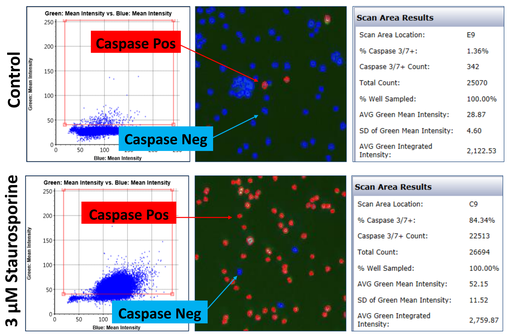


- Micrographs above, show representative bright field and fluorescent images for 0, 3, 6 hr time points for both staurosporine treated and control samples.



- Kinetic measurement of caspase 3/7 activity in Jurkat cells was determined by performing direct cell counting
- Green caspase 3/7 positive cells were automatically counted and acquired data exported to excel as a .csv file
- Cells treated with staurosporine showed a time-dependent caspase 3/7 activity
- For the end-point assay, Jurkat cells are counterstained with Hoechst to determine the total number and percent of caspase 3/7 cells in the entire population
- Hoechst positive cells with an associated green caspase 3/7 signal are scored as positive for caspase 3/7
- On the left, the scatter plot and linked fluorescent images show caspase 3/7 negative cells outlined in blue and caspase 3/7 positive cells outlined in red

End-point: Caspase 3/7, Hoechst Analysis

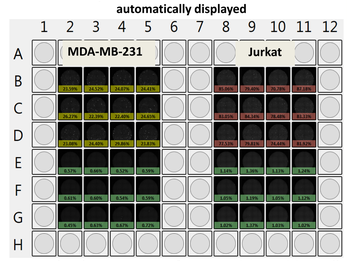


8. CONCLUSION

- Use Celigo image cytometer to perform caspase 3/7 kinetic and end-point assays using adherent and suspension cell lines
- Fast acquisition of high resolution images. Bright field, Caspase 3/7, and Hoechst fluorescent images of an entire 96 well plate took ~ 15 minutes
- Use Celigo built-in gating interface to quickly analyze end-point multi-parameter assays

References:
- Cohen GM. (1997) Caspases: the executioners of apoptosis. *Biochem J*. 326: 1–16.
- Cen H, et al. (2008) Devd-Nucview488: A Novel Class of Enzyme Substrates for Real-Time Detection of Caspase-3 Activity in Live Cells. *FASEB J.* 22(7):2243-2252.
- Fu X, at al. (2014) Overcoming endocrine resistance due to reduced PTEN levels in estrogen receptor-positive breast cancer byco-targeting mammalian target of rapamycin, protein kinase B, or mitogen-activated protein kinase kinase. *Breast Cancer Res*. Sep 11;16(5):430.

Plate map with thumb-nail images and results is automatically displayed



- Results show percent of caspase 3/7 positive cells