A high-throughput direct adherent cell analysis method for cell cycle, apoptosis, and viability using Celigo image cytometer

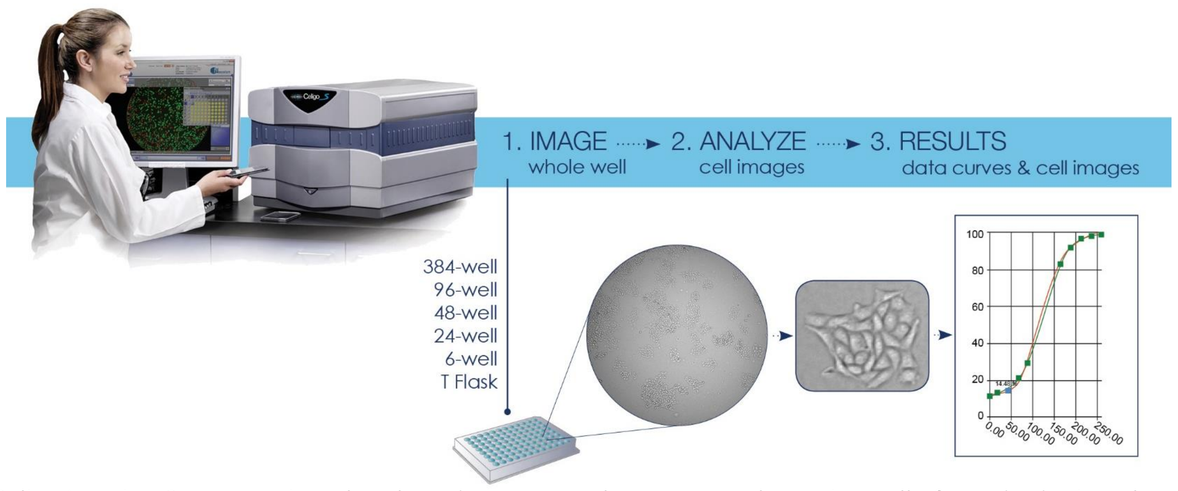
Leo L. Chan[1], Scott Cribbes[1], Sarah Kessel[1], Olivier Déry[1], Dmitry Kuksin[1], and Jean Qiu[1]

[1]Revvity Health Sciences, Inc., 360 Merrimack St., Suite 200, Lawrence, MA 01843

1. ABSTRACT

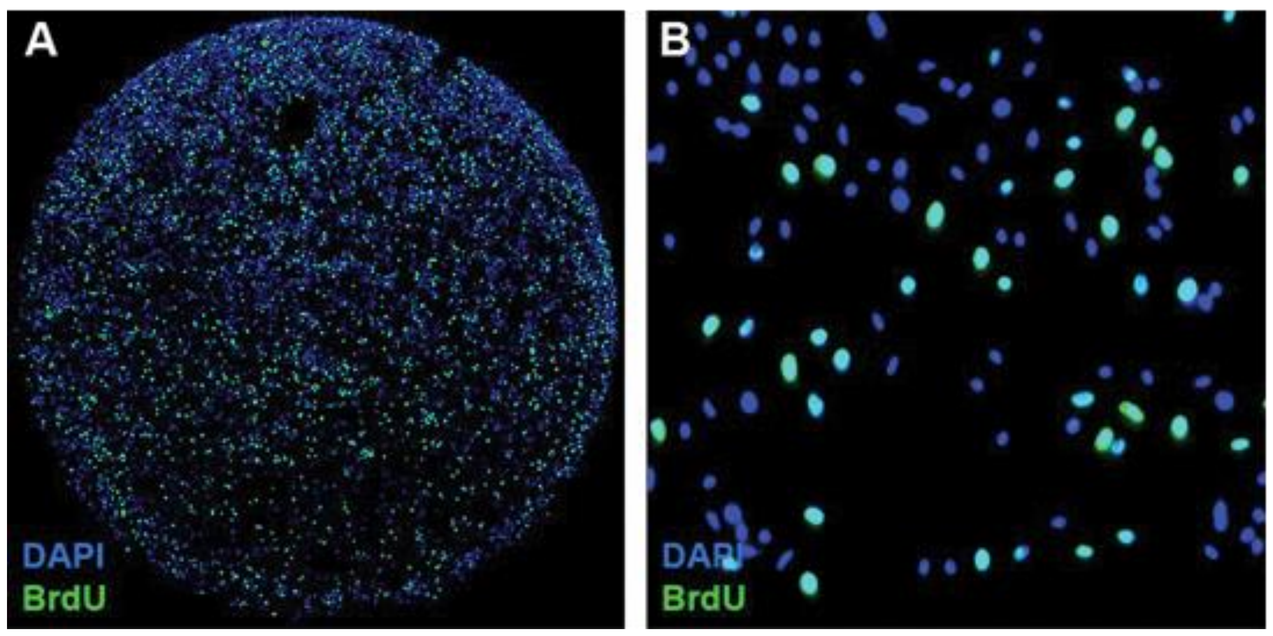
Apoptosis and cell cycle play an important role in various aspects of preclinical pharmaceutical drug discovery and validation. The ability to quickly determine the cytotoxic effect of chemical compounds on cancer cells allows researchers to efficiently identify potential drug candidates for further development in the pharmaceutical discovery pipeline. Recently, a plate-based imaging cytometry system, Celigo Imaging Cytometer, has been used to for high-throughput fluorescence cell cycle and apoptosis analysis. In this study, we demonstrate the use of Celigo imaging cytometry for apoptosis and cell cycle detection by studying the dose response effect of nocodazole on cell cycle and staurosporine on apoptosis. For cell cycle analysis, the cells are labeled with propidium iodide and BrdU. For apoptosis analysis, the cells are labeled with Annvexin V-PE and Hoechst 33342. The experimental results were evaluated to validate the imaging cytometric capabilities of the Imaging Cytometry system. The plate-based imaging cytometer utilizes bright-field and three fluorescence channels (Blue, Green, and Red) for multi-channel analysis. By utilizing the F theta lens technology, uniform bright-field image is captured for more accurate cell counting and analysis of the entire well. In addition, Celigo analysis software is used to report numerous parameters allowing detailed fluorescence-based cell population characterization. The ability of Celigo to rapidly and cost-effectively perform plate-based fluorescent assays has the potential of improving research efficiency, especially for adherent cells where plate-based cytometer does not require trypsinization for cell population analysis.

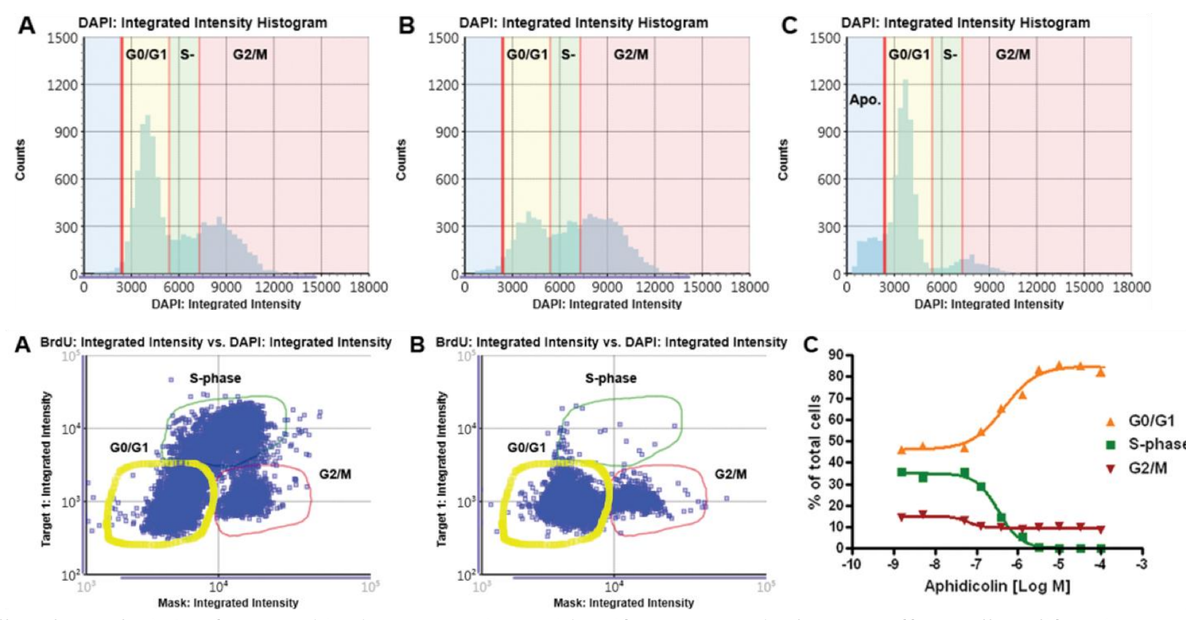
2. CELIGO IMAGING CYTOMETRY FOR APOPTOSIS AND CELL CYCLE ANALYSIS



1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and captures bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. 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

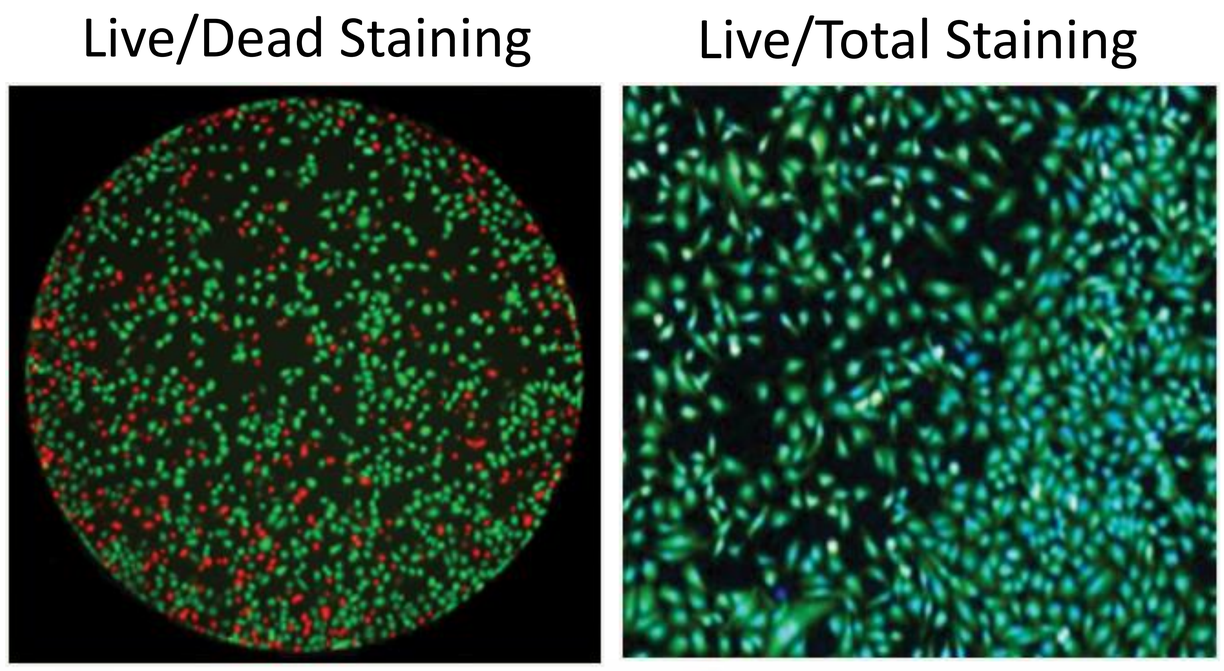
3. CELL CYCLE ANALYSIS USING CELIGO IMAGE CYTOMETRY





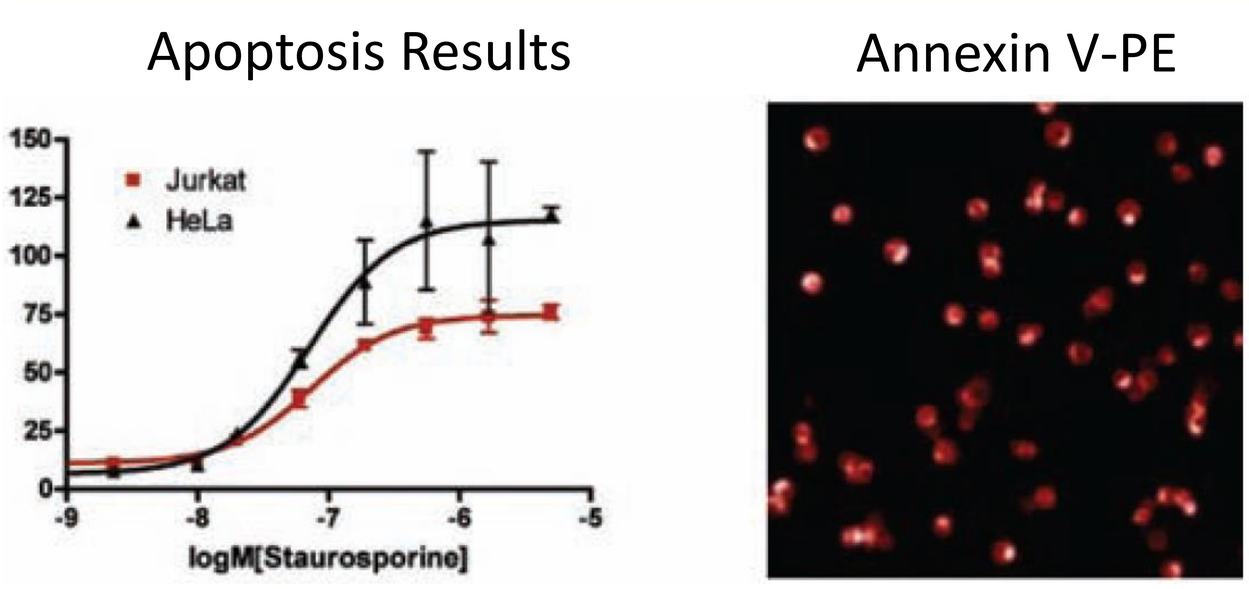
- Cell cycle analysis Is often used in drug screening to identify compounds that can affect cell proliferation and growth
- Celigo image cytometer can be used to measure the G0/G1, S, and G2 phase in a cell population to determine the effect of treatment
- In this work, A549 cells are stained with DAPI, a fluorescent nuclear dye, and BrdU, a thymidine analog, to incorporate into cells for detection of DNA content and replication
- BrdU uses a primary anti mah monoclonal antibody and anti Gam AF-488 secondary antibody
- A549 cells are treated with nocodazole for 18 hours, at 150 nM (middle) and 50 µM (right)
  - The results showed reduction in G0/G1 phase at 150 nM, and increase in apoptotic cells at 50 µM
- A549 cells are treated with aphidicolin fro 18 hours, from 1 nM (left) to 100 µM (middle)
  - The results showed large S phase population, while at high dosage, that population is completely eliminated
- The aphidicolin EC50 for G0/G1 and S phases, are 463 and 335 nM, respectively

4. CELL VIABILITY ANALYSIS USING CELIGO IMAGE CYTOMETRY



- Cell viability assay is used to investigate cellular cytotoxicity induced by chemical compounds, antibodies, mechanical force, etc.
- Fluorescent staining methods such as Calcein AM, propidium iodide, and Hoechst 33342 can be used to identify and count live, dead, and total cells using Celigo image cytometer
- In this work, Hydrogen Peroxide is used to induce cell death in A549 and Jurkat cells
- The cells are treated with H2O2 from 0.1 to 12 µM for 4 hours
- After 4 hours, the samples are scanned and analyzed with Celigo image cytometer to measure viability
- Jurkat cells showed IC50 and EC50 at 2.2 and 3.2 mM, respectively
- A549 cells showed IC50 and EC50 at 2.2 mM

5. APOPTOSIS ANALYSIS USING CELIGO IMAGE CYTOMETRY



- Annexin V-based measurement is commonly used for the detection of early to late stage apoptosis
- The Annexin V labels the change in position of phosphatidylserine (PS) on the cell membrane
  - In normal healthy cells, the PS molecules are localized on the inner layer of the cell membrane
  - In apoptotic cells, the PS redistribute to the outer layer of the membrane that becomes exposed to the extracellular environment
  - PS translocation occurs during early stage apoptosis, and the PS remain on the outer until the membrane break apart
  - Therefore, by using Annexin V and propidium iodide, early and late stage apoptosis can be determined
- In this experiment, Jurkat and HeLa cells are treated with Staurosporine for 18 hours from 0.5 to 5000 nM, which is a drug that can induce apoptosis
- Jurkat cells are then stained with Annexin V-PE and Hoechst 33342 to determine apoptotic cell percentages using Celigo image cytometer
- The EC50 values are 73 and 72 nM for HeLa and Jurkat cells, respectively

6. SUMMARY AND CONCLUSION

In conclusion, the Celigo image cytometer has been demonstrated to perform cell viability, cell cycle, and apoptosis analysis, which are three critical cell-based assays for immunological, oncological, and toxicological research. By utilizing an automated image cytometry system, drug screen results can be quickly obtained using high throughput plate format, and significantly increase the productivity of research capabilities.

References

- Sasaki et al. Flow cytometric estimation of cell cycle parameters using a monoclonal antibody to bromodeoxyuridine. Cytometry. 1986; 7(4):391-5.