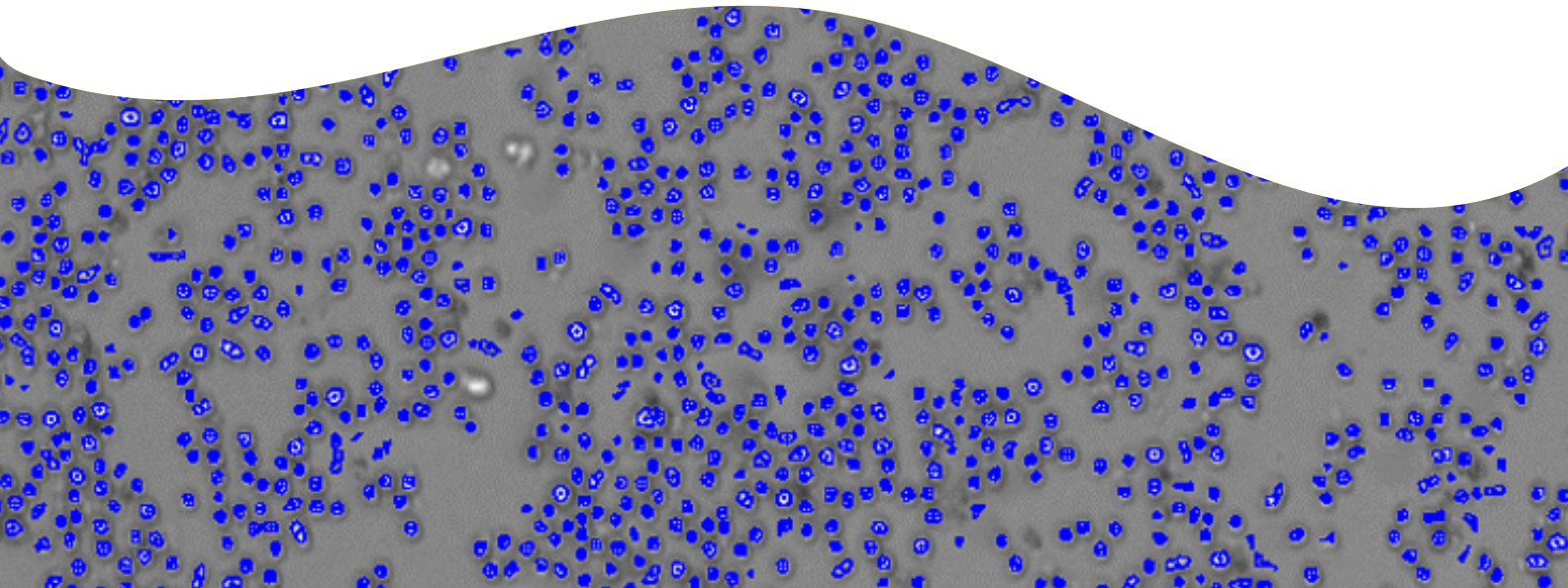
APPLICATION NOTE

A rapid and label-free *in situ* assay method for cell proliferation and drug toxicity using Celigo imaging cytometer.

Introduction

Inhibition of cell proliferation is a sensitive marker of cytotoxicity. Many proliferation assays use indirect measurements or require harvesting and/or staining of cells, analyzing only a subset of the treated cells. These assays are destructive and do not allow for kinetic measurements. The Celigo® Adherent Cell Cytometer is a novel, cell imaging instrument that combines whole well, in situ imaging with automated software for label-free brightfield image analysis [1-4].

In this study, Celigo was used to screen a compound library for effects on cell proliferation in adherent and non-adherent cell lines. Human lung carcinoma (A549) and promyelocytic leukemia (HL-60) cells were treated with a panel of compounds to inhibit proliferation. Finally, the Celigo system used image-based analysis to measure changes in cell morphology upon compound treatment. These data indicate that certain anti-proliferative compounds can have secondary effects on cell health or physiology, which manifest changes in cell morphology.



Materials and methods

Whole well imaging and analysis

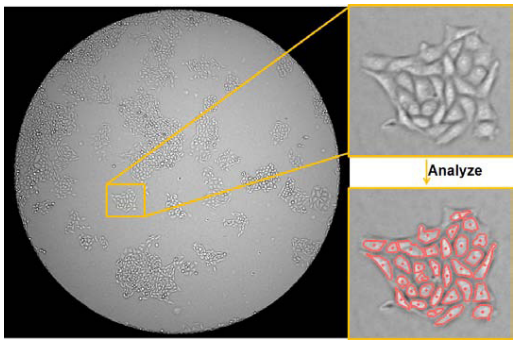


Figure 1. The Celigo Cytometer acquires high-quality whole-well images of a 384 well microplate without mechanical stage movements. The proprietary analysis algorithm identifies the individual cells (orange outlines) and provides quantitative outputs. This capability enables critical assessment of all cells within a well, even at the edges, eliminating the need for averaging or cell count estimation.

Brightfield cell counting validation

- Brightfield label-free cell counting accurately counts adherent and non-adherent cells in microplates.
- Direct cell counting results are cell-type independent.

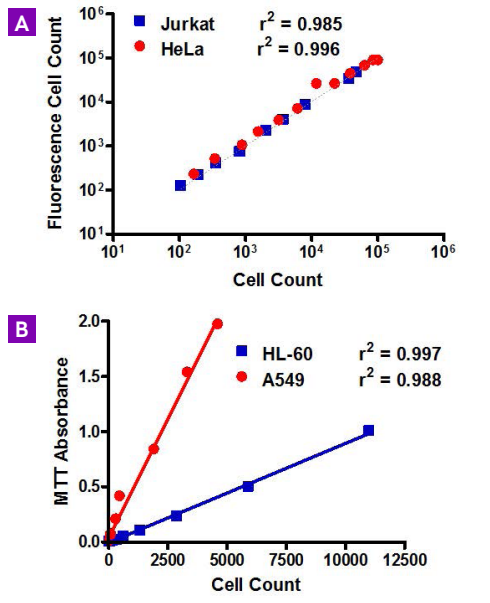


Figure 2A and B. (A) HeLa and Jurkat cells were plated in a dilution series in 96 well plates and counted in brightfield and fluorescence on Celigo with resulting r^2 values of >0.98. (B) HL-60 and A549 cells were plated in a dilution series in 384-well plates and counted in brightfield using the Celigo Cytometer. Replicate plates were processed using the MTT assay. Cell counts correlated well with MTT absorbance over a broad range of cell densities.

Celigo cytometer

- Analyzes cell cultures *in situ* with minimal disturbance.
- Eliminates enzymatic disruption of sample during preparation procedures.
- Images cells in brightfield and fluorescence on the same platform.
- Analyzes every cell in every well in brightfield mode with no “edge effect”.

Results

Label free monitoring of effects on proliferation

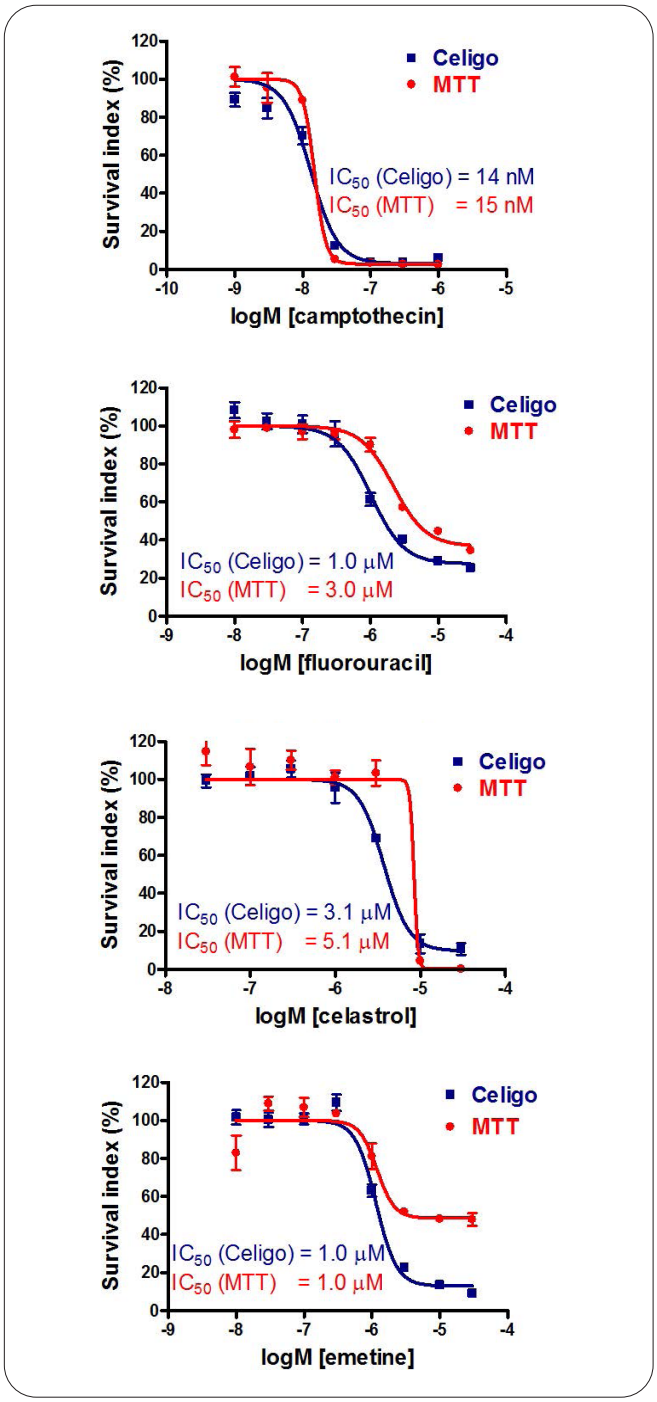


Figure 3. Brightfield label-free mode can quantify effects of compounds on cell proliferation in human cells. HL-60 and A549 cells were treated with compounds from 1 nM to 30 µM (depending on compound) for 48 hr in 384-well microplates. The Survival Index (% remaining cells or MTT signal compared to negative control, DMSO) was calculated for each treatment well and IC_{50} values were determined for each compound. Good correlation of label-free direct cell counting assay results with MTT metabolic endpoint assay.

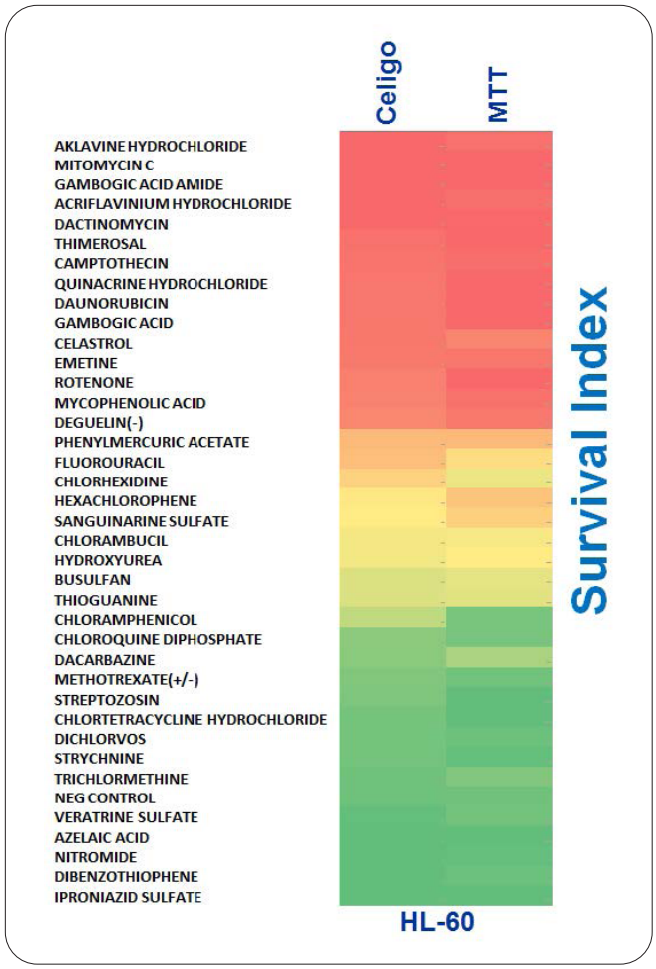


Figure 4. Good correlation of label-free direct cell counting assay results with MTT metabolic endpoint assay. Heat map of Survival Index (SI%) for compounds tested at a single concentration (10 µM) in the HL-60 cell line and assayed using the Celigo Cytomer's label-free cell counting application or MTT assay. The average of two independent experiments is presented. Survival Index is represented by colors ranging from Red to Green. Red = low survival, yellow = medium survival, green = high survival. Values were ranked by percentile, with the 10th percentile survival being the lowest, the 50th percentile the medium, and the 90th percentile the highest survival.

In Situ growth tracking

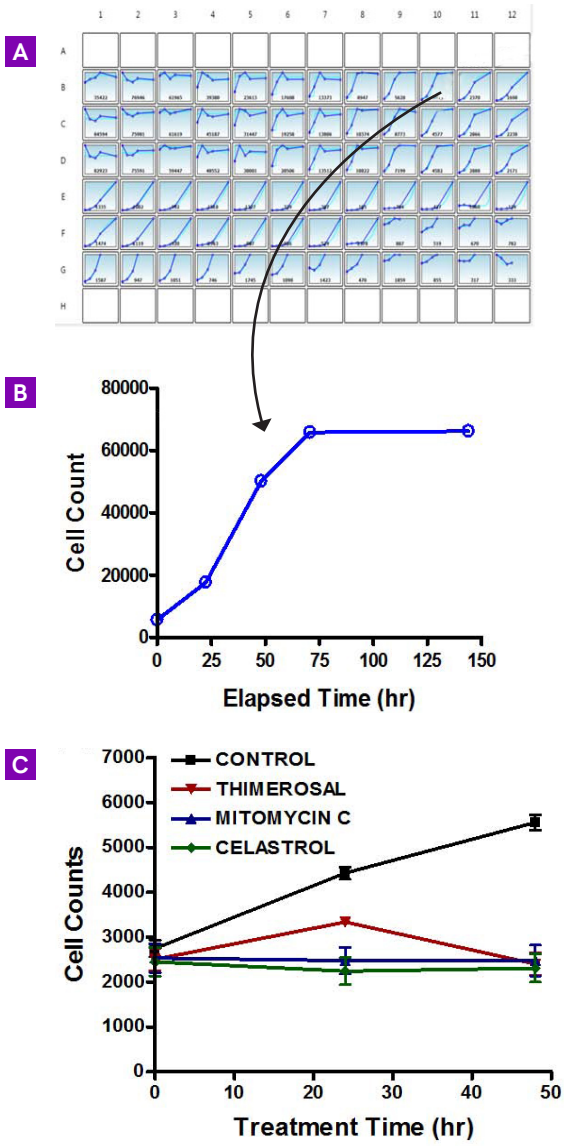


Figure 5A-C. Kinetic growth tracking detects early growth inhibition. Growth curves for (A) entire plate of CHO cells grown in 96W plate over 150 hr, and (B) from a given selected wee same wells were imaged and counted on multiple days using the Celigo Label- Free Cell Counting application. (C) A549 cells were treated with 10 µM compound, and proliferation was monitored on the Celigo Cytometer at 0, 24, and 48 hr.

Kinetic, label-free morphology tracking

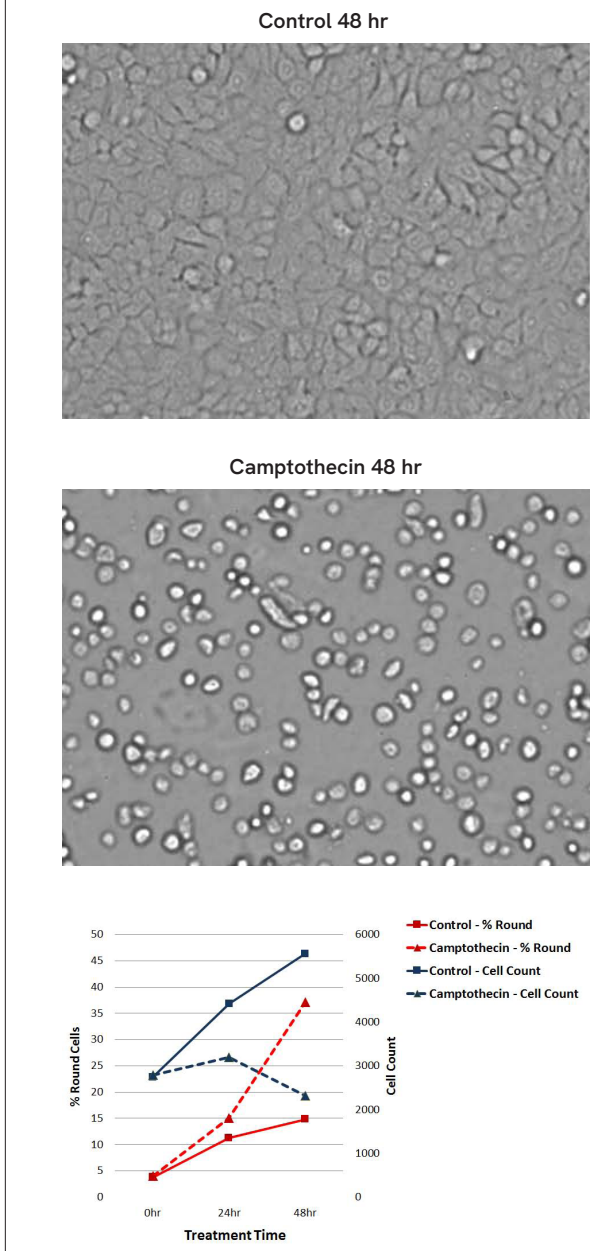


Figure 6. Brightfield label free mode can kinetically monitor cell morphology and growth. Here images showing A549 cells treated with 10 µM camptothecin or DMSO (negative control) for 48 hr are shown. The increase in round cell morphology of treated cells was more than twice that for untreated cells (red lines) while there was the opposite trend in cell proliferation (blue lines).

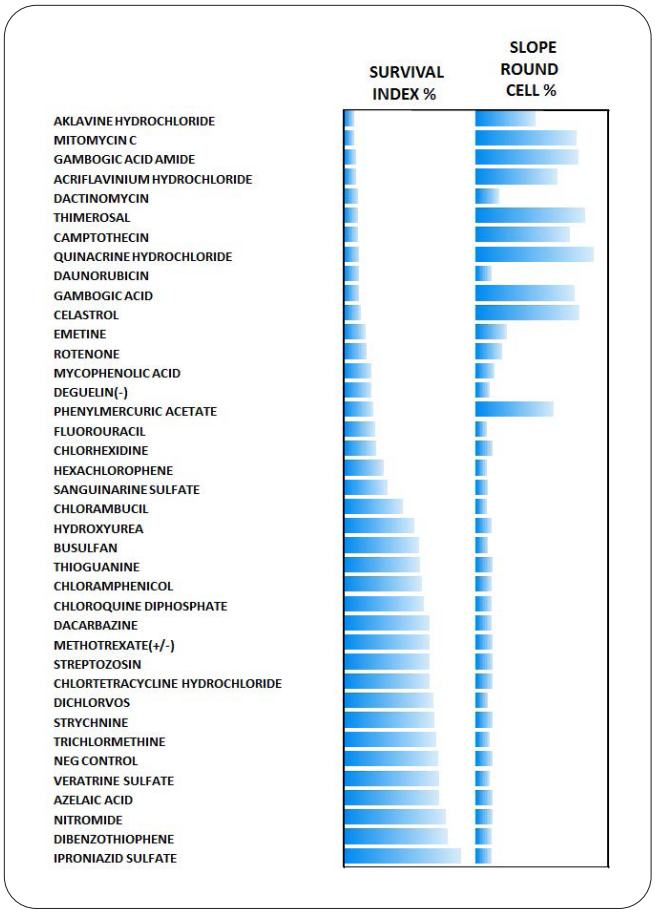


Figure 7. Morphological analysis identified growth-inhibiting compounds that demonstrated differential effects on morphology suggesting different mechanisms of action. Survival Index and Slope of "round" cell percentage for A549 cells treated with 36 compounds. The length of the blue bar corresponds to the magnitude of the value. A general trend of increasing round cell morphology with decreasing survival is evident from the iSCIP readout.

Conclusions

These data demonstrate the use of label-free, brightfield cell counting to identify compounds that inhibit growth of human cells in culture. The Celigo adherent cell cytometer accurately counts cells *in situ*, and direct cell counting is independent of cell type. Growth inhibition results are comparable to a common, endpoint MTT assay. In addition, label-free growth tracking identifies early growth inhibition, which potentially reduces the assay time. Finally, direct detection of cells in brightfield mode enables morphological assessment in parallel to growth inhibition. The Celigo cytometer provides a rapid, lower cost, and sensitive method for assessing compound effects on cell proliferation and cell toxicity.

References

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2. Bian, S., et al., P2X7 integrates PI3K/AKT and AMPK-PRAS40-mTOR signaling pathways to mediate tumor cell death. *PLoS One*, 2013. **8**(4): p. e60184.
3. Al-Kasspooles, M.F., et al., Preclinical antitumor activity of a nanoparticulate SN38. *Invest New Drugs*, 2013. **31**(4): p. 871-80.
4. Hsieh, J.L., et al., Acquisition of an enhanced aggressive phenotype in human lung cancer cells selected by suboptimal doses of cisplatin following cell deattachment and reattachment. *Cancer Lett*, 2012. **321**(1): p. 36-44.

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