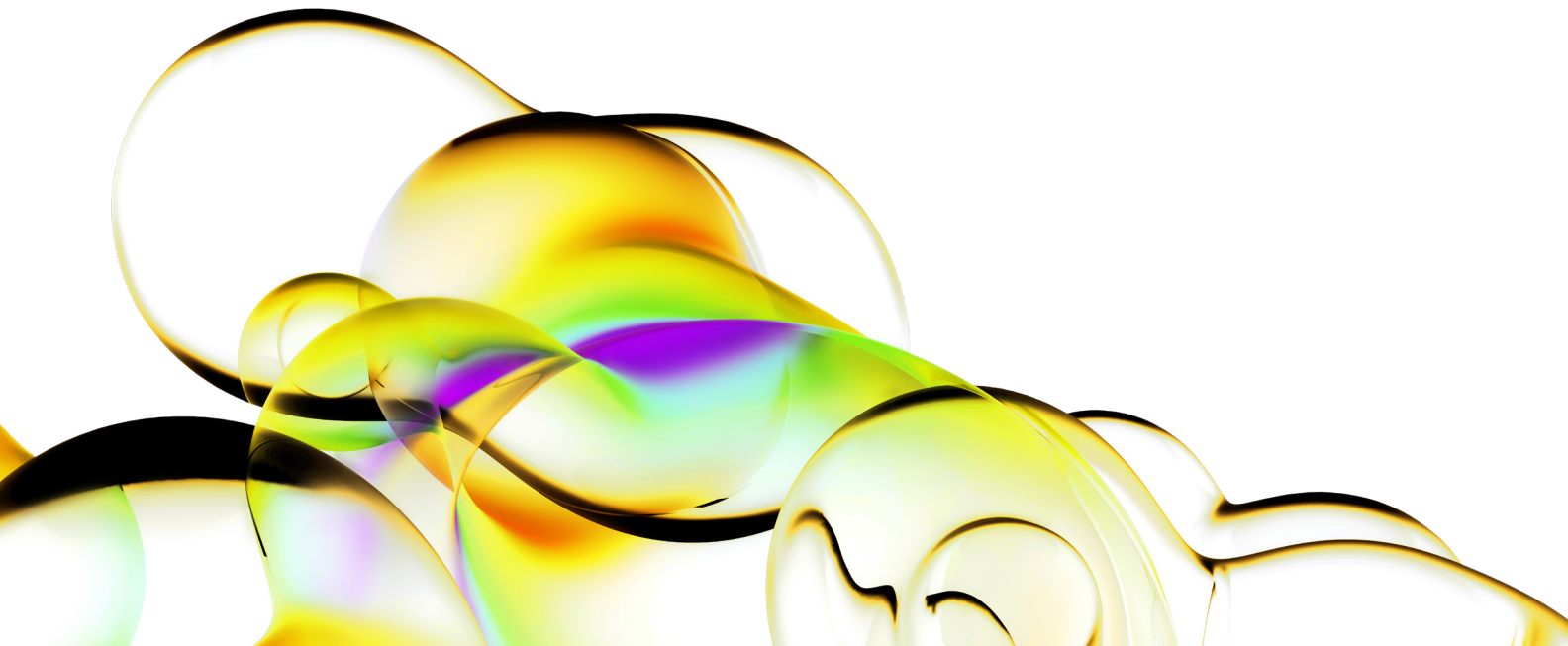
APPLICATION NOTE

Automated cell growth tracking for cytotoxicity, proliferation.

Label-free, non-destructive, no need to trypsinize adherence cells

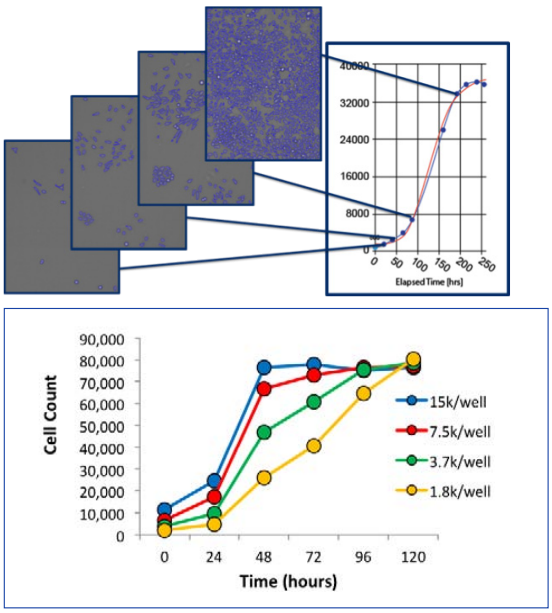
The Growth Tracking application automatically integrates label-free cell counts of the same well/flask from different time points to provide direct measurement of growth rates and doubling times, which is also a good overall assessment of cell health.



Two methods to produce a label-free proliferation curve

Method 1

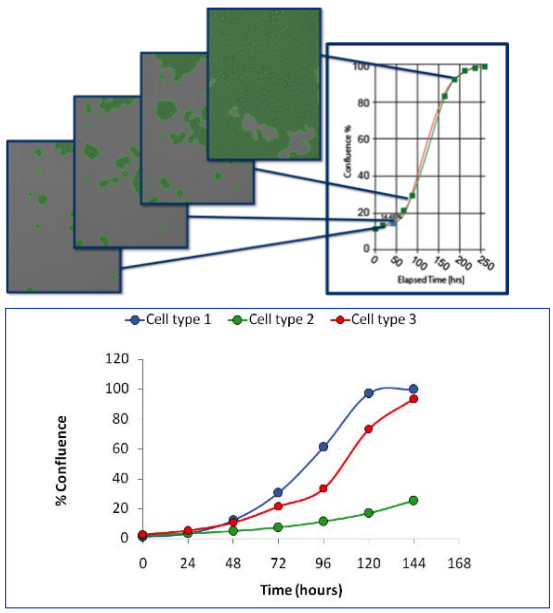
The first method is to use cell segmentation, where each cell is identified by the image analysis software to produce cell count. This method requires customization of the analysis parameters for each cell type.



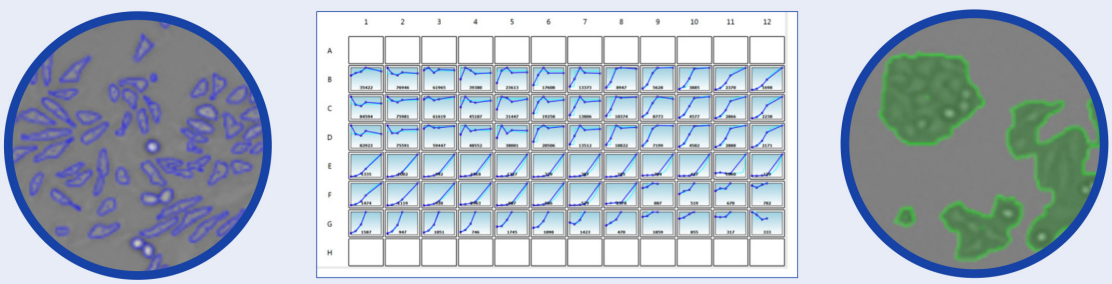
Cell growth tracking using Celigo. Growth curves for CHO (DUXB11) cells grown in 96 well plates over 120 hours. Cells were imaged and counted using the Celigo Cell Counting application.

Method 2

The second method is to measure confluence of the cell colonies. This method is most universal and applicable to the majority of cell types without customization.



Streamlined cell culture maintenance using Celigo. Example of workflow and output for monitoring adherent cell cultures in 96 well plates. Cells can be imaged and counted in culture vessels without staining or harvesting. Celigo updates and returns growth curves automatically.

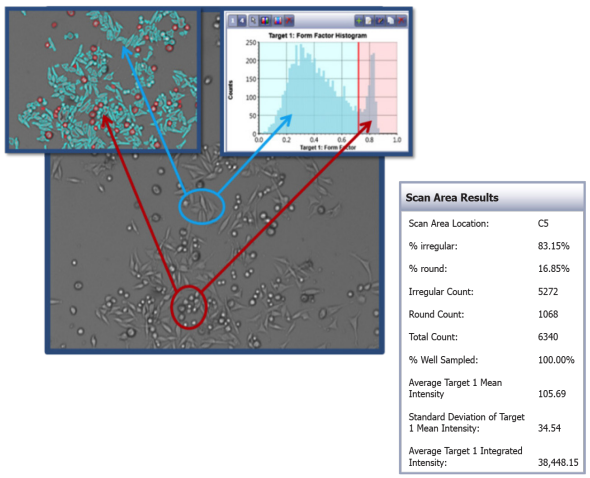


Cell growth curves are displayed in 96-well plate.

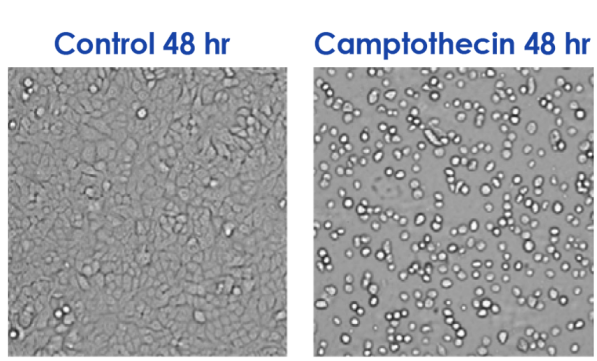
Morphology screen of toxic compounds

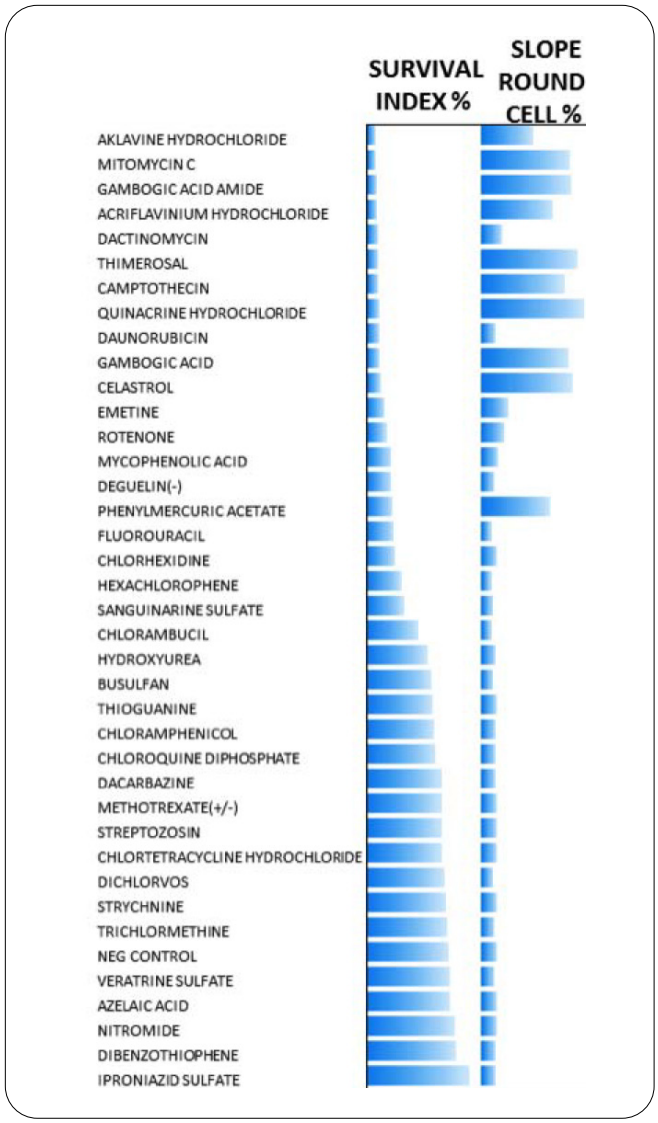
A mixture of adherent and suspension cells were seeded into a 96-well plate. Using the Celigo® gating interphase we can identify two distinct populations using morphology only. Designed to produce the enhanced bright field cell image, in combination with the advance image analysis software, Celigo has been used to identify, characterize and monitor specific cell sub-populations based on morphological features.

Celigo imaging cytometer records cell images, displays gated cell images, calculates cell morphological parameters and plots the data for population gating.

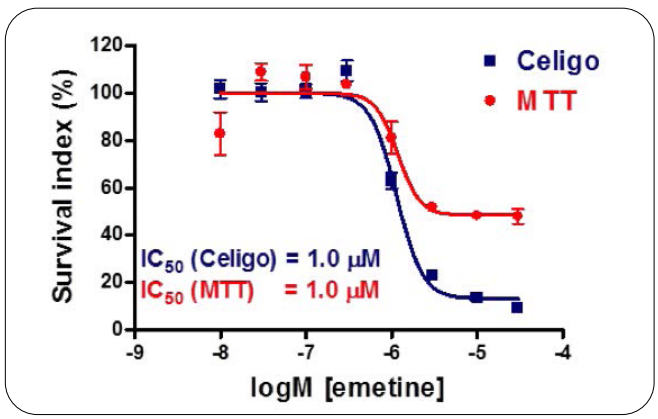


Alteration in cellular morphology was used as an indicator of compound toxicity. Above we can observe an example of the rounding up of an adherent cell after 48 hrs treatment with Camptothecin. Using this alteration in morphology in a label-free assay, we screened a number of compounds. Below you can observe the % survival index calculated from this assay.





Cytotoxicity assay for drug screening

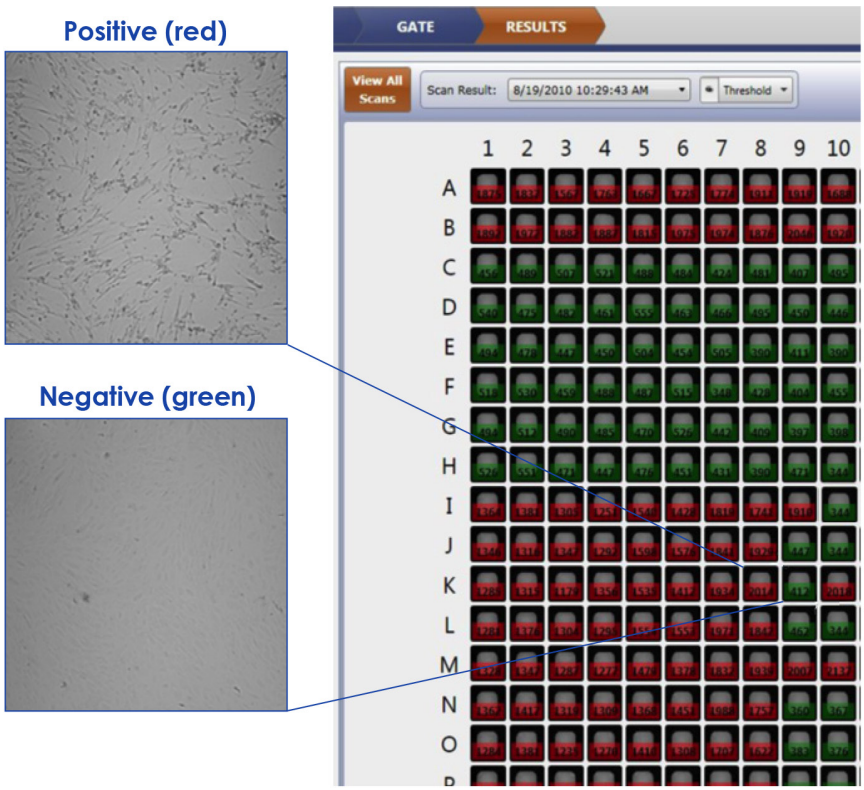


Dose-responses measured with the Celigo Cytometer versus MTT assay. HL-60 and A549 cells were treated with compounds from 1 nM to 30 µM.

Automated Cytopathic Effect (CPE) assay

Celigo imaging cytometer has been applied to provide automated, rapid assessment of viral infectivity in a range of microtiter plate formats. Using f-theta optics, Celigo provides high quality, whole well images using brightfield illumination. Automated segmentation and analysis provides quantitative output of CPE based on characteristic changes to the host cell monolayer.

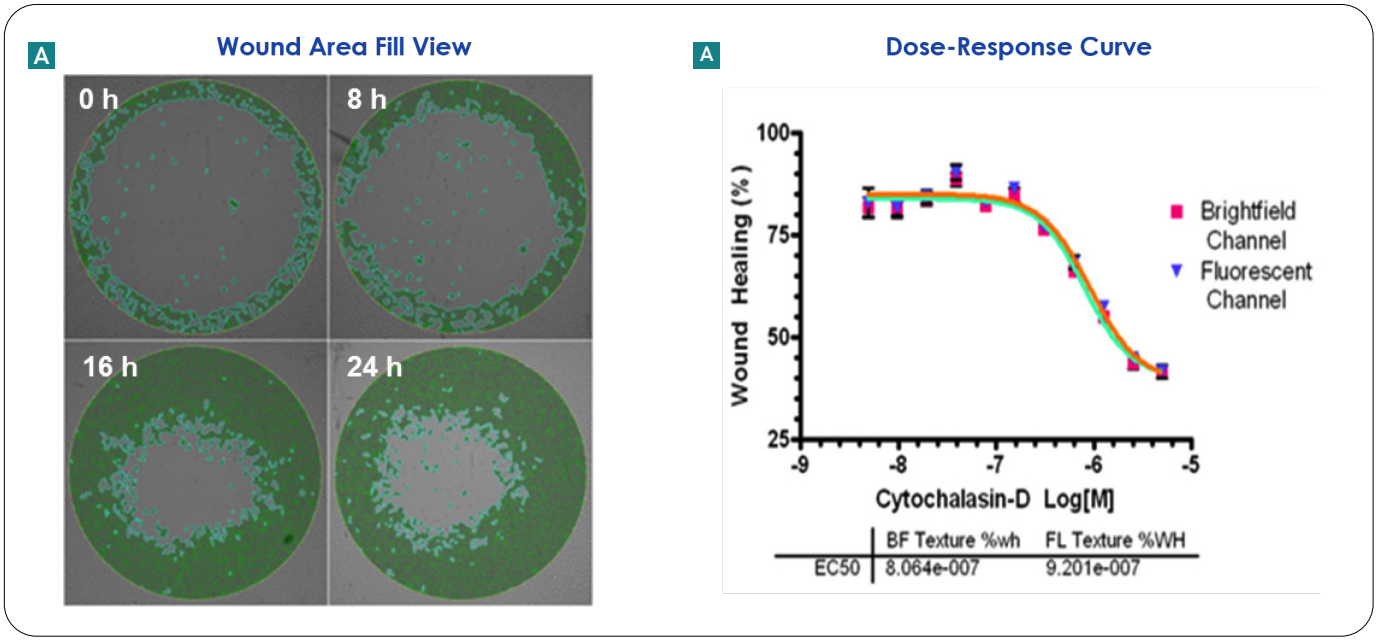
- Objective segmentation and quantitative output of magnitude of infection
- Automated sample analysis reduces time, labor and variability
- High throughput and scaleable – less than 10 min for 96- or 384-well plate
- Capture high resolution, whole well images for documentation, audit trail or manual assessment of CPE
- Supports related fluorescent based functional assays relevant to infectivity (e.g. expression analysis, apoptosis)



Bovine Viral Diarrhea (BVD) infection of Bovine Turbinate cells (BT2). Viral inoculum was serially diluted across plate. Threshold function indicates red wells (infected) vs. green wells (uninfected). Celigo results demonstrate 100% correlation with manual CPE assessment via light microscopy

Wound healing assay

Celigo imaging cytometer has been applied to provide automated, rapid assessment of wound healing using the Oris Platypus plate technology. Automated segmentation of cells or confluency in both bright field and fluorescence is able to provide a quantitative output of wound healing / migration.



Dose-response of % wound healing measured with the Celigo Cytometer using brightfield and fluorescence.
(A) Wound area fill view at 0, 8, 16 and 24 hours. Confluence detection of the area covered within the wound is followed over time and a pseudo colour green fill mask is added to aid visualization.
(B) HT1080 cells were plated in a 96-well Oris plate and allowed to grow to confluency. Upon removal of the plug the cells were treated with a dose response of Cytochalsin D and the confluence readout was read in brightfield or in fluorescence after staining with Cell Tracker Green.

For research use only. Not approved for diagnostic or therapeutic use.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
(800) 762-4000
www.revvity.com