

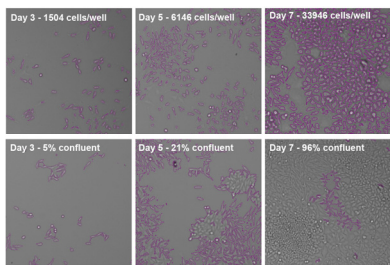
Brightfield and Fluorescent Image Analysis for Screening Applications using the Celigo Adherent Cell Cytometer

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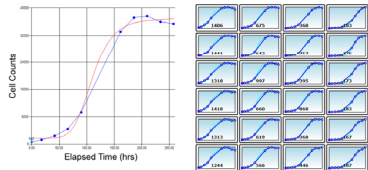
Abstract

The Celigo adherent cell cytometer is a multi-channel imager well suited for all image-based screening applications. The flexibility and ease-of-use of the Celigo facilitates the optimization of cell-based assays and the subsequent evaluation of assay performance. Using large field-of-view optics and a proprietary high speed imaging system, the Celigo rapidly images multiple fields-of-view without moving the plate. Nearly all microplate formats (from 1536-well to 6-well plates) can be fully- or partially-imaged in brightfield and 3 fluorescent channels. Additionally, unique brightfield optics allow accurate segmentation of cells right to the edge of the well and a rich flow cytometry-like gating interface accommodates a wide variety of cell-based assays. We present here whole-well imaging and label-free brightfield cell counting applications that allows the Celigo user to generate growth curves over time and monitor cell counts and confluence at the individual well level. These brightfield applications permit the screening of drugs altering cell proliferation and growth rate. We also highlight the capacity of the Celigo to image large objects making it suitable for analysis of large structures such as embryoid bodies, tumor spheres or small organisms. Finally, the analysis of complex samples by imaging is a challenge for high-content screening applications. The Celigo software provides multi-channel analysis of brightfield and fluorescent images and means of identification of cells based on intensity and morphology parameters. Moreover, a powerful yet easy-to-use gating interface allows the analysis of cell populations similarly to flow cytometry. Data also shows a variety of fluorescent assays for assessment of cell health, DNA synthesis, cell cycle or expression of cell surface markers. Overall the brightfield and fluorescent imaging capability of the Celigo combined with powerful analysis features and a potential integration with automation make it suitable for high-throughput screening applications.

Brightfield Cell Counting and Growth Tracking Analysis



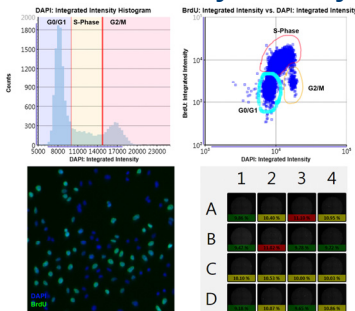
The Celigo allows monitoring label-free cell growth. HeLa cells were plated at low density in a 96-well plate. The same well can be imaged over time and growth characteristics can be determined. The top panels show brightfield cell counting of the well over time (from left to right). The lower panels show confluency segmentation over time (from left to right).



Cell growth characteristics measured on Celigo. The Celigo direct cell counting application reports label-free growth curves for individual wells and multi-well plates (left and right panel respectively). The application also reports doubling time and doubling rate for each well of a plate (not shown).

- Rapid scanning and analysis of multi-well plates.
- Easy-to-use interface to image and identify a wide variety of cells in brightfield.
- Determine growth characteristics of cells in situ, directly where they are grown.
- Reports growth curves, cell counts, confluency, doubling time, and doubling rate for each well.
- Analyze cells growing in T-flasks.

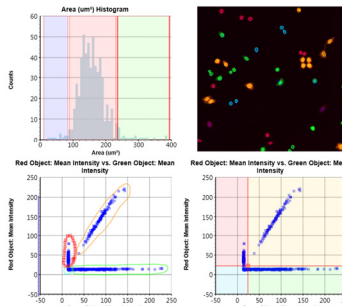
Fluorescent Cell Cycle Analysis



Cell cycle analysis on Celigo. A549 cells were analyzed for cell cycle phase determination using the Expression Analysis application of the Celigo software. A histogram representation of DNA content similar to flow cytometry is shown on the upper left panel. The typical "Horse Shoe" visualization of the cell cycle is achieved by plotting the integrated intensity of DNA synthesis stain vs. DNA content stain (upper right panel). The lower left panel shows a 2-color image of the BrdU and DAPI stains. The lower right panel shows a heat map of the plate level data results for a specific output such as percentage of cells in S-phase.

- Celigo permits a histogram representation of DNA content similar to flow cytometry.
- A typical "Horse Shoe" scatter plot representation is used for determination of each phase of the cell cycle.

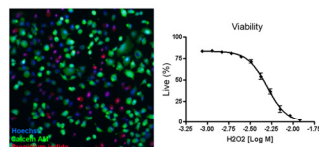
Fluorescent Marker Analysis



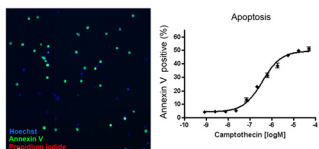
Celigo gating interface. HeLa cells expressing either GFP or RFP were mixed with CHO-K1 cells expressing YFP. Hoechst was used to stain the cells prior to imaging and allowed identification of the nuclear mask of each cell. Green and red fluorescence intensities were measured within the nuclear mask. The Celigo software features histogram (upper left) and scatter plot (lower plots) representations of the segmentation data. Cell populations expressing the green, yellow and red fluorescent proteins were identified and cells were classified using a color overlay (upper right).

- Celigo software provides a rapid solution for multi-channel analysis of brightfield and fluorescent images.
- A powerful, yet easy-to-use, gating interface allows the analysis of cell populations similarly to flow cytometry.

Fluorescent Cell Health Assays



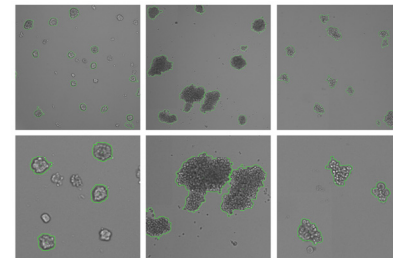
Cell viability application on Celigo. Representative images and concentration response plot for cell viability application. The left image is a 3-colors overlay of HeLa cells stained with calcein AM, propidium iodide and hoechst pseudo-colored in green, red and blue respectively. The right graph is a concentration response curve reporting the percentage of live HeLa cells treated with hydrogen peroxide.



Phosphatidylserine externalization application on Celigo. Representative image and concentration response plot for PS externalization application. The left image is a 3-colors overlay of Jurkat cells stained with annexin V, propidium iodide and hoechst pseudo-colored in green, red and blue respectively. The right graph is a concentration response curve reporting the percentage of apoptotic Jurkat cells treated with camptothecin.

- Quick assessment of cell health can be performed using the Celigo.
- Simple and rapid analysis of cell viability and apoptosis in many diverse cell types.

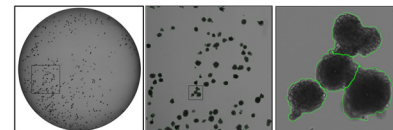
Tumor Sphere Analysis



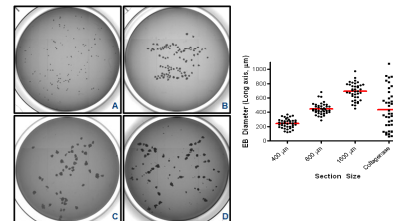
Analysis of mammospheres on Celigo. MCF7, MDA-MB-436 and SKBR3 (from left to right, respectively) cells were cultured in ultra-low attachment 96-well plates for 10 days. Cells were imaged on the Celigo using the Colony Counting: Embryoid Body application. Size, shape and counts of mammospheres are analyzed and reported. The bottom panel shows zoomed images.

- Size, shape, and counts of tumor spheres are reported.
- Application is suitable for studying effect of drugs on tumor spheres.

Embryoid Body Analysis



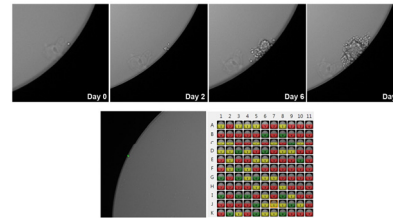
Analysis of embryoid bodies on Celigo. Embryoid bodies (EBs) were generated using the LEAP instrument from Cyntellect and the EB number and size were analyzed on the Celigo using the Colony Counting: Embryoid Body application. The whole-well surface of a 96-well plate was acquired using the brightfield channel of the instrument (left panel). The center and right panels shows zoomed images of the EB segmentation.



Analysis of embryoid bodies size on Celigo. EBs of various sizes were generated on the LEAP instrument and their size was measured on the Celigo. The left panel shows the whole-well view of small, medium and large size EBs (panel A, B, C, respectively) and random size EBs generated using collagenase (panel D). The right panels shows a graphic representation of EB diameter for each well.

- Celigo permits whole-well imaging of suspension EBs.
- Automated morphometric analysis of EBs greatly reduces the time needed to characterize cultures prior to investing in long-term differentiation protocols.

Single Clone Validation



Confirmation of clonality on Celigo. CHO-S cells were plated at a concentration of 1 cell/well in a low volume 384-well plate. Brightfield images were acquired at various time after plating and colonies were visualized at the location where a single cell was detected in the well. The top panel shows the development of a colony over a 8-day period. The lower panel shows the segmentation of a single cell at the edge of the well and a heatmap view reporting cell counts for each well.

- With superior brightfield image quality, the Celigo can reliably visualize individual cells in a well, even if the cell is adjacent to the well wall, and allows for verification that a clone originates from a single cell.

Conclusion

The Celigo adherent cell cytometer is a bench top imager suitable for a variety of screening applications. We have showcased the whole-well brightfield and fluorescent imaging capability of the instrument to analyze single cells or multi-cellular structures. Moreover, the diversity of easy-to-use applications combined with powerful analysis features make the Celigo a platform of choice for drug screening.