Bright field and Fluorescent Image Analysis for Screening Applications using the Celigo Imaging Cytometer

WHITE PAPER
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**Introduction**

The Celigo adherent cell cytometer is a multi-channel imager suited for all image-based screening applications. Celigo’s flexibility and ease-of-use facilitates the optimization of cell-based assays and the subsequent evaluation of assay performance [1-5]. 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 bright field and 3-fluorescent channels. Additionally, unique bright field optics allow for accurate segmentation of cells at the edge of the well and a rich, flow cytometry-like gating interface accommodates a wide variety of cell-based assays.

Here, we present whole-well imaging and label-free bright field cell counting applications that allow the Celigo user to generate growth curves over time and monitor cell counts and confluence at the individual well level. These bright field 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 bright field and fluorescent images and can identify cells based on intensity and morphology parameters. Moreover, a powerful yet easy-to-use gating interface permits the analysis of cell populations in a way that is similar to flow cytometry. A variety of fluorescent assays for assessment of cell health, DNA synthesis, cell cycle or expression of cell surface markers is available. Overall, the bright field 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.
Results

Bright Field Cell Counting and Growth Tracking Analysis

- Rapid scanning and analysis of multi-well plates.
- Easy-to-use interface to image and identify a wide variety of cells in bright field.
- 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.

Figure 1A and B.

(A) The Celigo enables the monitoring of label-free cell growth. HeLa cells were plated at low density in a 96-well plate. The same well were imaged over time and growth characteristics were determined. The top panels show bright field cell counting of the well over time (from left to right). The lower panels show confluency segmentation over time (from left to right). (B) Cell growth characteristics measured on Celigo. The Celigo direct cell-counting application reported label-free growth curves for individual wells and multi-well plates (left and right panel, respectively). The application also reported doubling time and doubling rate for each well of a plate (not shown).
**Fluorescent Cell Cycle Analysis**

- Celigo permits a histogram representation of DNA content similar to flow cytometry.
- A typical “horse shoe” scatter plot representation is used to determine each phase of the cell cycle.

**Figure 2.**

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.
**Fluorescent Marker Analysis**

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

**Figure 3.**
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) representing 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).
Fluorescent Cell Health Assays

- 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.

**Figure 4A and B.**

(A) Cell viability application on Celigo. Representative images and concentration response plot for cell viability application. The left image is a 3-color overlay of HeLa cells stained with calcein AM, propidium iodide and Hoechst pseudo-colored green, red and blue respectively. The right graph is a dose-response curve reporting the percentage of live HeLa cells treated with hydrogen peroxide. (B) Phosphatidylserine (PS) externalization application on Celigo. Representative image and concentration response plot for PS externalization application. The left image is a 3-color overlay of Jurkat cells stained with annexin V, propidium iodide and Hoechst pseudo-colored green, red and blue, respectively. The right graph is a dose-response curve reporting the percentage of apoptotic Jurkat cells treated with camptothecin.
**Tumor Sphere Analysis**
- Size, shape, and counts of tumor spheres are reported.
- Application is suitable for studying effect of drugs on tumor spheres.

**Figure 5.**
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 were analyzed and reported. The bottom panel shows zoomed in images.
**Embryoid Body Analysis**

- 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.

**Figure 6A-D.**

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 show a graphic representation of EB diameter for each well.
**Single Clone Validation**

- With superior bright field 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.

**Figure 7.**
Confirmation of clonality on Celigo. CHO-S cells were plated at a concentration of 1 cell/well in a low volume 384-well plate. Bright field images were acquired at various times 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 an 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.

**Conclusion**

The Celigo adherent cell cytometer is a bench top imager, suitable for a variety of screening applications. We have showcased the whole-well bright field and fluorescent imaging capabilities 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.
References


