

## Introduction

The use of cell-based assays continues to grow in basic and applied sciences research as the importance of assessing cellular processes in biologically meaningful systems increases. Quality control considerations for assay optimization include appropriate growth media, optimal cell densities, suitable growth phases, and other factors which likely influence cell-based assay outcomes. Current cell culture practices often rely on manual methods (e.g., manual observation of plates or flasks, hemacytometers) to address these considerations, but manual approaches can be subjective and inaccurate, and are not truly scalable or repeatable. These techniques, and some of their automated alternatives (e.g., automated microscopy, flow cytometry), require invasive harvesting and/or staining of cultures, and measure only a small representative sample of the total population. An ideal solution to these issues would provide a non-invasive, non-destructive manner to assess cell populations within their normal growth environment.

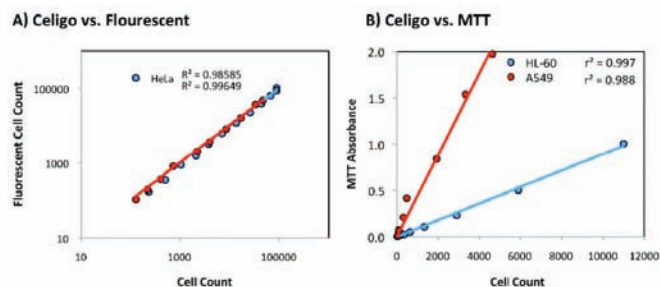
The Celigo™ cytometer is a bench-top, brightfield and fluorescent imaging system capable of identifying and counting adherent and non-adherent cells within standard culture vessels. Celigo's unique optical system enables rapid and accurate imaging of live cells within entire wells of multi-well plates. The Celigo Label-Free Cell Counting application determines cell counts or confluence by imaging cells in culture flasks and microplates without disrupting or removing them. Direct counts are obtained for every cell in every well, eliminating the need for calibration curves and cell number estimation. For laboratories maintaining many cell lines or large numbers of individual cultures, the Celigo Label-Free Cell Counting application provides rapid, accurate, and non-invasive monitoring of cell growth with minimal culture disturbance.

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. This application note demonstrates the cell counting capability of Celigo and presents relevant case studies.

## Approach and Results

### Cell Counting Validation

Label-free cell counting accuracy was validated by comparing counts from the Celigo cytometer to fluorescent counts of the same cultures. HeLa and Jurkat cells were plated in serial dilution in 96 or 384 well microplates. Cells were labeled with a fluorescent nucleic acid stain (Hoechst) and imaged in brightfield and fluorescence on the Celigo cytometer. A strong linear relationship between data obtained from the brightfield and fluorescent counts was observed ( $r > 0.98$ ), demonstrating that the Celigo cytometer counted cells accurately in label-free mode (Fig. 1A). Selected counts were also verified by manual counting.

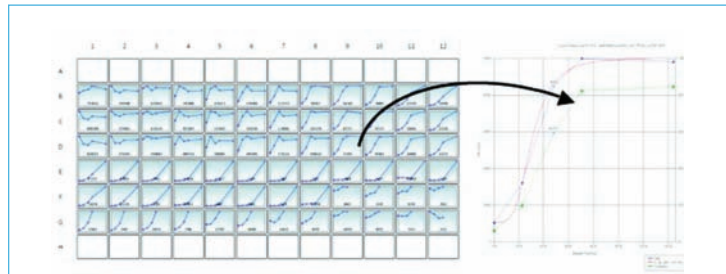


**Fig. 1** Correlation of the Celigo cytometer's label-free mode and traditional methods. (A) HeLa and Jurkat cells were plated in a dilution series in 96 well plates and counted in brightfield and fluorescence on Celigo with resulting  $r^2/\Lambda$  values of  $> 0.98$ . (B) HL-60 and A549 cells were plated in a dilution series in 384 well plates and counted in brightfield only 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. When monitoring different cell types, separate calibration curves were essential with MTT assays whereas Celigo label-free cell counting provided direct cell number data.

The dynamic range of the Celigo cytometer's cell counting application was also evaluated in 384-well plates as compared with an MTT assay, a metabolic assay commonly used for monitoring relative cell numbers. The Celigo cytometer demonstrated a strong linear relationship to MTT over a broad range of cell densities tested (Fig. 1B). These data demonstrate that the Celigo cytometer accurately monitored changes in cell numbers over a range of plating densities from 100 to  $> 10,000$  cells in wells of a 384-well plate.

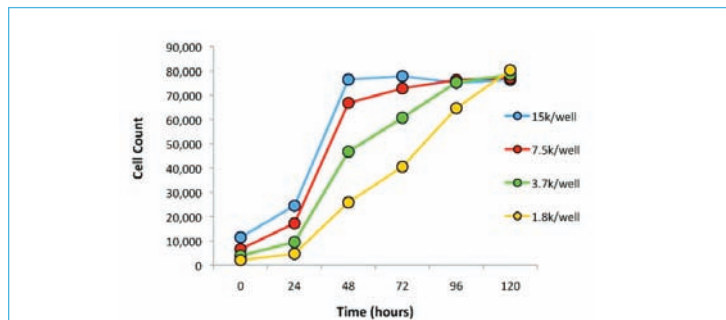
## Growth Tracking

The Celigo provides clear and direct information regarding cell growth rates in every well of a plate (Fig. 2A), as well as more detailed view of a given well (Fig. 2B).

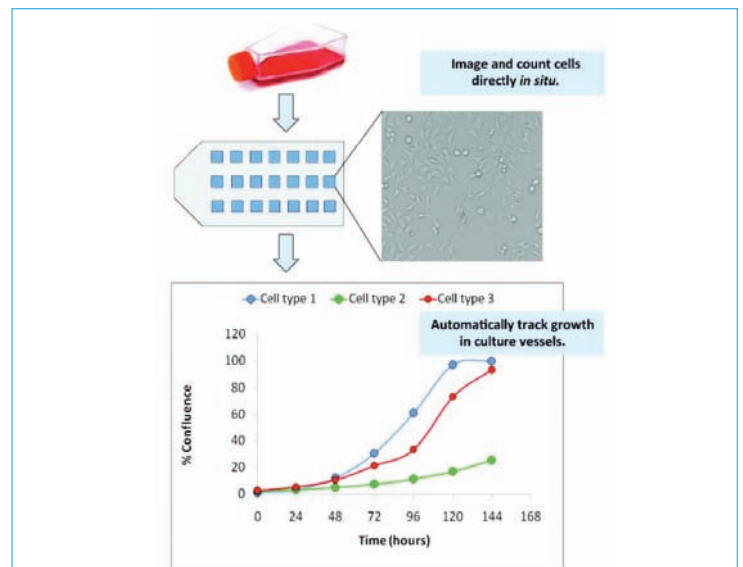


**Fig. 2** *In situ* growth tracking using Celigo. Growth curves for (A) entire plate of CHO cells grown in 96W plate over 150 hr, and (B) from a given selected well from Fig 2A allowing detailed inspection of the raw count data, curve fit and confluency. The same wells were imaged and counted on multiple days using the Celigo Label-Free Cell Counting application. Double clicking on a given well provides detailed view of data.

Celigo growth tracking was also demonstrated using DUXB11 CHO cells grown in 96 well plates and with HeLa cells grown in T-25 flasks. CHO cells were seeded at four densities (15,000, 7,500, 3,700, and 1,800 per well) and counted at six time points over 120 hr. Growth curves were plotted using total cell counts per well. These data demonstrate the utility of Celigo in monitoring cell growth *in situ* over various time frames.



**Fig. 3** *In situ* 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.



**Fig. 4** Streamlined cell culture maintenance using Celigo. Example of workflow and output for monitoring adherent cell cultures in T-25 flasks. Cells can be imaged and counted in culture vessels without staining or harvesting. Celigo updates and returns growth curves automatically.

## Conclusions

The data presented here establish the use of the Celigo Cell Counting application for general cell culture monitoring. The accuracy of Celigo label-free cell counting was verified in adherent and non-adherent cells by comparison to established methods (fluorescent cell counting and MTT assay). Because Celigo counts cells without harvesting or other perturbations, it can be used to track growth of cultures in various flasks and well plates, as demonstrated above. The Celigo Cell Counting application simplifies the tedious tasks of maintaining cell cultures and optimizing growth conditions necessary in many life sciences laboratories today. Cells can be monitored at any time point without the need for staining or harvesting. Because entire wells are imaged *in situ*, total cell counts of adherent as well as non-adherent cells are possible, and risk of culture contamination is substantially reduced. Celigo enables laboratories of any size to streamline their cell culture workflow, increasing the resources that can be devoted to cell based research rather than simple cell maintenance.

## References

1. Mosmann, T, J. of Immunol. Methods. 1983 Dec 16;65(1-2):55-63.