Rapid, label-free, direct cell counting in measurement of cell proliferation for compound screening

Introduction

Cell proliferation is one of the most widely used assay types across a broad range of biological disciplines. Due to their sensitive nature, cell proliferation assays are commonly used to measure cytotoxicity and/or efficacy of drug candidates in oncology, immunology, and stem cells, among other fields.

Despite their widespread use, many traditional proliferation assays are severely limiting because they are invasive and destructive to the cell samples, and rely on indirect methods for determining cell proliferation. For example, many assays only estimate cell number based on measurements of metabolic activity (e.g., MTT, Alamar Blue), ATP content, total protein concentration (SRB), or incorporation of dNTPs (3H-thymidine, BrdU uptake). Metabolic function assays (e.g., MTT) only provide relative cell counts, leading to misinterpretation of results when compounds impact metabolism directly. Assays that rely on flow cytometers, hemacytometers, or microscopes are generally destructive in nature due to harvesting and/or staining of cells, and generally analyze only a sampled proportion of treated cells. These constraints preclude using the cells being analyzed for any subsequent purpose, and also prevent kinetic monitoring of the same cell population over time.

The Celigo™ cytometer provides a label-free assay for direct *in situ* cell counting of live cells, by imaging every cell within all areas of standard multi-well plates and culture flasks (Fig. 1), eliminating the need for cell number estimation. The Celigo cytometer's brightfield imaging mode is non-invasive and non-destructive to the samples, enabling measurement of live cells without the need for forcing cells into suspension or other sample manipulation. Thus, researchers can obtain cell proliferation data at various time points on the same samples, and additional information (e.g., kinetic growth data) as compared with traditional endpoint proliferation assays.

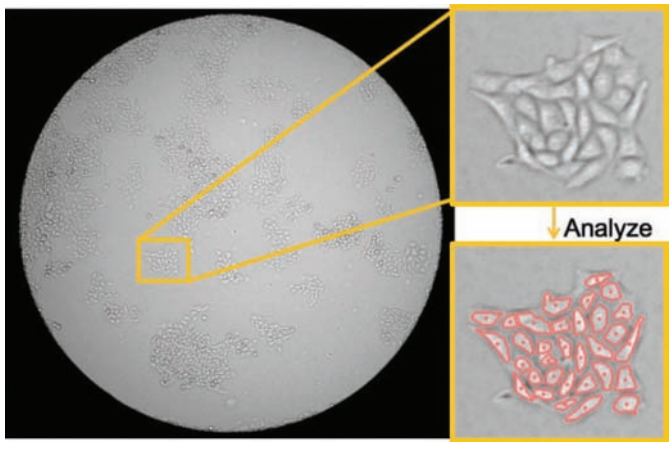

Fig. 1 Whole well image. The Celigo cytometer is unique in its ability to acquire high-quality whole-well images without requiring large numbers of mechanical stage movements. This capability enables critical assessment of all cells within a well, even at the edges, eliminating the need for averaging or cell count estimation.

While the Celigo cytometer works on any cell type, its combined set of features makes it ideal for adherent cells.

In this application note, we demonstrate utility of the Celigo cytometer for measuring cell proliferation in a typical compound screening assay.

Approach and Results

Compound Profiling and Screening

To demonstrate the use of the Celigo cytometer for label-free cell proliferation assays in a drug screening application, data were compared to a conventional MTT assay. Label-free cell counting accuracy and dynamic range was validated previously (See Application Note: Automated, Label-Free Growth Tracking...). The Survival Index (% remaining cells or MTT signal compared to negative control, DMSO) was calculated for each treatment well. With the Celigo cytometer, the Survival Index was calculated for cell counts normalized to pre-treatment counts from the same well. Because cells must be sacrificed with the MTT assay, the Survival Index was calculated for the final day of treatment only. HL-60 and A549 cells were treated in a dose response from 1 nM to 30 μM with compounds selected from a compound library. Both the Celigo cytometer label-free cell counting and MTT absorbance detected a dose-dependent inhibition of growth (decrease in Survival Index), and calculated IC50 values were comparable between the two methods (Fig. 2) and were similar to reported values from the NCI database in HL-60 cells. Overall, the Celigo cytometer and MTT assays produced very similar dose-response curves that were reproducible across experiments.

The Celigo cytometer was used to screen compounds in non-adherent and adherent cells. A total of 36 anti-proliferative compounds were assessed for effects on cell growth using the Celigo cytometer's label-free cell counting application. HL-60 and A549 cells were treated and monitored after 48 hours using label-free cell counting and the MTT assay. Effects on cell proliferation at a single (10 μM) concentration showed general agreement between the label-free and MTT methods (Fig. 3).

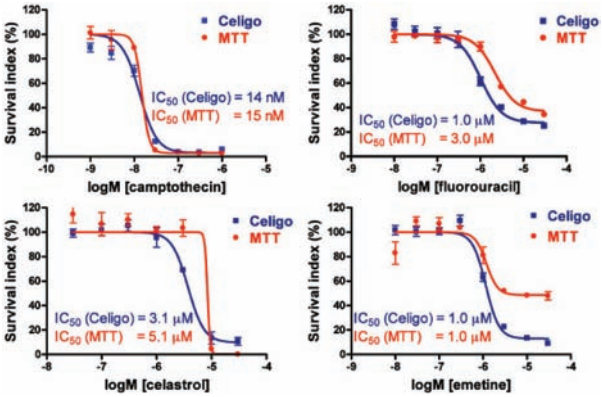

Fig. 2 Dose-responses measured with the Celigo cytometer versus MTT assay. HL-60 (top row) and A549 (bottom row) cells were treated with compounds from 1 nM to 30 µM.

Fig. 3 Comparison of treatment effects measured with the Celigo cytometer versus MTT assay. Heat map of Survival Index (SI%) for compounds tested at a single concentration (10 µM) and assayed using the Celigo Cytomer's label-free cell counting application or MTT assay. 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.

The data for A549 cells illustrate detailed differences between the two assays. Some compounds appear to differentially affect survival versus metabolism. Not surprisingly, compounds with an impact on metabolic function can be misinterpreted using the MTT assay. Overall similarities in the Celigo cytometer and MTT data demonstrate that the cytometer provides reliable data to assess cell proliferation in screening experiments.

The Celigo cytometer's ability to measure proliferation on the same population of cells at multiple time points was demonstrated by growth curves plotted for selected compounds. Differences in growth patterns among compounds were observed with the Celigo cytometer that would not have been observed using an endpoint assay (Fig. 4). Kinetic growth data allows optimization of the treatment time for specific screening assays resulting in earlier detection. For example, assay time could be reduced by 50% for compounds such as celastrol and mitomycin c with the same outcome.Alternatively, adding a second assay read point without needing additional samples reduces overall effort and results in higher quality compound profile data. In addition, imaging the same wells over time reduces the number of experimental replicates needed.

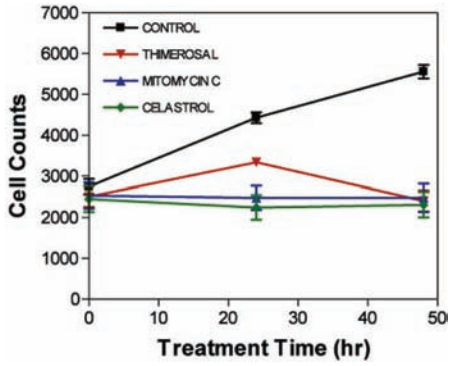

Fig. 4 Determining cell proliferation kinetics using the Celigo cytometer. A549 cells were treated with 10 µM compound, and proliferation was monitored on the Celigo cytometer at 0, 24, and 48 hr.

Conclusions

This application note demonstrates the use of the Celigo cytometer in a label-free, cell-based screening assay to directly monitor cell proliferation. The Celigo cytometer produced similar results to MTT assays, but with several advantages. First, cells do not need to be stained or harvested with the Celigo cytometer, so counts can be made at any time point and the samples can be used for subsequent or alternative assays. Second, direct counts from different cell types can be obtained without requiring calibration curves. Third, high quality, full-well images of cells can be archived and retrieved for review when following up screening hits. Fourth, because the Celigo cytometer measures cells in situ, it is ideal for all cell types including adherent cells, requiring virtually no invasive sample manipulation. Finally, because the Celigo cytometer can assay the same cells over time, effects on kinetic growth patterns in response to compound treatment can be monitored. This additional information will be valuable in optimizing conditions for specific assays, saving time and lowering costs. The Celigo cytometer provides a tool to perform cell proliferation studies in a quick, simple, and low cost manner, while providing the additional advantages of image archiving and kinetic growth data.

References

1. National Cancer Institute (NCI) database: http://dtp.nci.nih.gov/branches/btb/ivclsp.html.

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