

Celigo[®] provides an alternative method to Cell Titer-Glo[®] for proliferation studies in suspension cells

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Introduction

Traditional cell proliferation assays involve multiple steps, expensive reagents, and are fixed end-point assays, prohibiting the same cells from being repeatedly assessed over a time course. Ignyta was searching for a new, reagent-free proliferation detection method to determine the number of suspension cells per well. This new method should generate results comparable to their current one. Here, Ignyta[®] and Nexcelom Bioscience carried out a reagent-free cell proliferation assay employing the Celigo[®] Image Cytometer¹ using suspension cells and proprietary compounds. The same experiment was performed using the Cell Titer-Glo[®] Assay², and the proliferation results were compared directly to Celigo.

Materials and Methods

Four suspension cell types, including Ba/F3 parental cell line (Cell A), Ba/F3 expressing an oncogenic gene (Cell B), an oncogenic gene mutant A or B (Cell C and D respectively) were plated at a concentration of 5,000 cells/well and mixed in the presence of various concentrations of four drugs (1-4) at Day 0. On Day 3, the Celigo was used to image and analyze cell proliferation using the bright-field application, where whole-well images of each well were captured and analyzed in less than 5 minutes per plate. No reagent or additional incubation periods were required.

The same plate imaged and analyzed by Celigo was then used to evaluate cell proliferation following the standard Cell Titer-Glo protocol, meaning the same wells were analyzed by both methods for a true comparison. Dose-response curves and IC₅₀ values were calculated and compared between the two proliferation assessments.

Conclusions

Cell proliferation results between the two methods of Celigo and Cell Titer-Glo were comparable and the generated data values were highly correlated ($r^2=0.998$)

- Celigo provides a simple, rapid, reagent-free way to determine cell proliferation in suspension cells
- Celigo provides images for visual verification of all results
- Celigo is not an end-point assay, meaning that the cells remain alive during the proliferation analysis, so cell proliferation can be assessed repeatedly over multiple time points throughout an experiment rather than single, fixed end points, thereby reducing time, supplies, and cost

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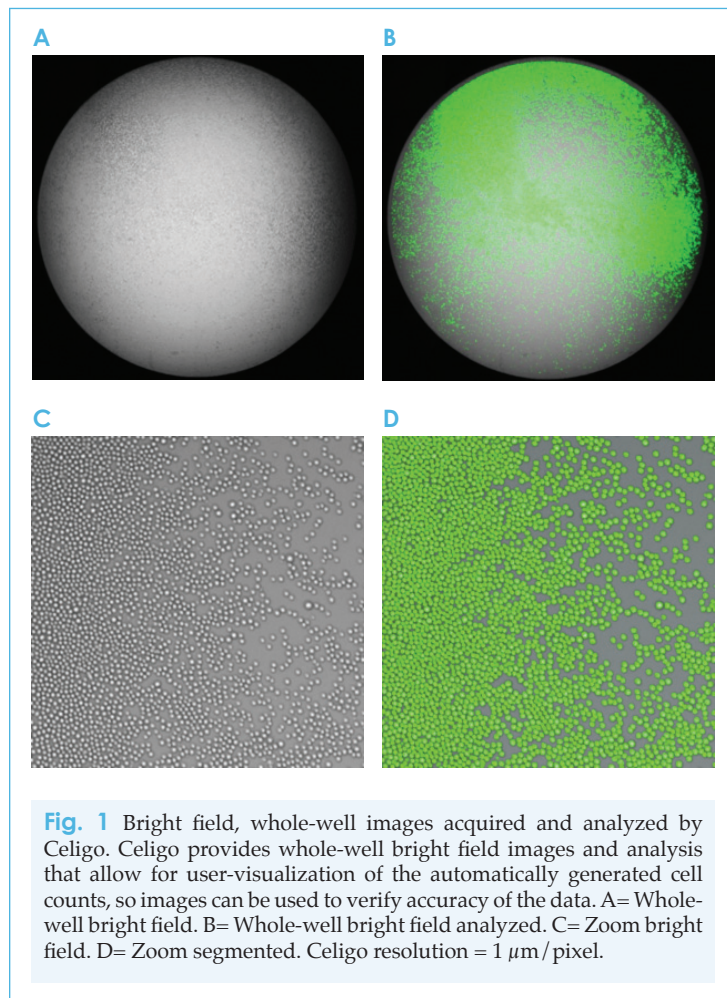
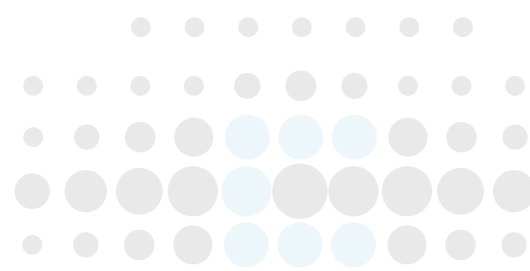


Fig. 1 Bright field, whole-well images acquired and analyzed by Celigo. Celigo provides whole-well bright field images and analysis that allow for user-visualization of the automatically generated cell counts, so images can be used to verify accuracy of the data. A= Whole-well bright field. B= Whole-well bright field analyzed. C= Zoom bright field. D= Zoom segmented. Celigo resolution = 1 μm/pixel.

| | Celigo Cell A | CTG Cell A | Celigo Cell B | CTG Cell B | Celigo Cell C | CTG Cell C | Celigo Cell D | CTG Cell D |
|--|---------------|------------|---------------|------------|---------------|------------|---------------|------------|
| Drug 1 | 2.695 | 2.533 | 3.294 | 8.720 | 4.034 | 5.510 | 848.7 | 1099 |
| Drug 2 | 1.515 | 1.697 | 1.493 | 3.300 | 1.863 | 2.234 | 62.99 | 91.35 |
| Drug 3 | 11.88 | 10.85 | 51.60 | 45.32 | 14.39 | 14.27 | 66.32 | 133.3 |
| Drug 4 | 1.796 | 2.003 | ~4.067 | ~4.239 | 2.391 | 3.269 | 3.925 | 6.508 |
| Correlation across all cell types and drugs tested: $r^2=0.9987$ | | | | | | | | |

Table 1 Side-by-side comparison of IC₅₀ values (nM) for cell types A-D with drugs 1-4 between the Celigo (dark gray columns) and Cell Titer-Glo (light gray columns). CTG= Cell Titer-Glo



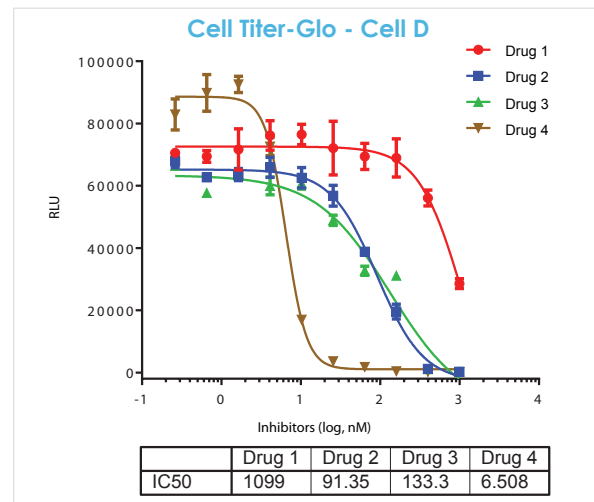
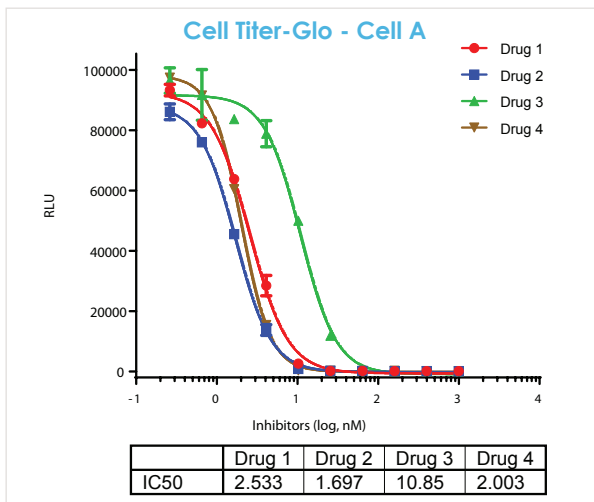
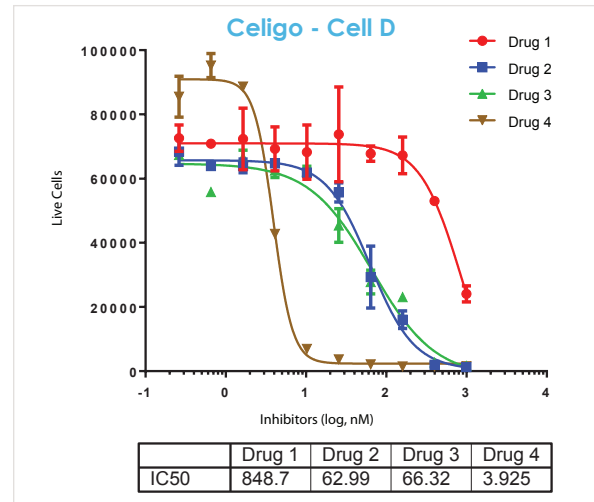
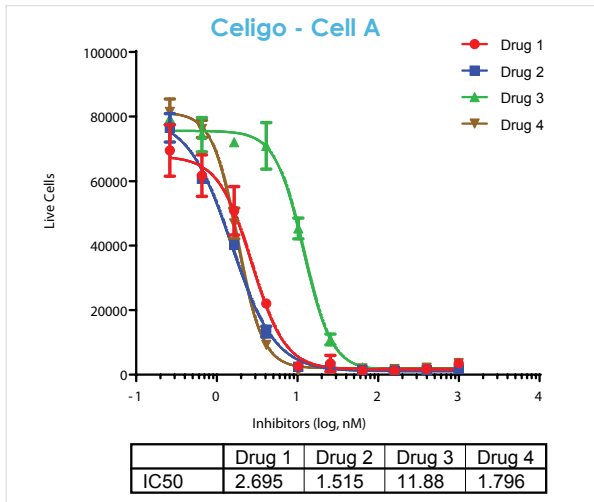


Fig. 2 Side-by-side comparison of IC₅₀ curves for cell types A (left) and D (right) treated with Drugs 1-4 between the Celigo (top row) and Cell Titer-Glo (bottom row). All concentrations in nM.

References

1. Wang, YC et al. (2011) Different mechanisms for resistance to trastuzumab versus lapatinib in HER2-positive breast cancers--role of estrogen receptor and HER2 reactivation. *Breast Cancer Res.* 13(6):R121
2. Crouch, SP et al. (1993) The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *J. Immunol. Methods* 160, 81-8.