

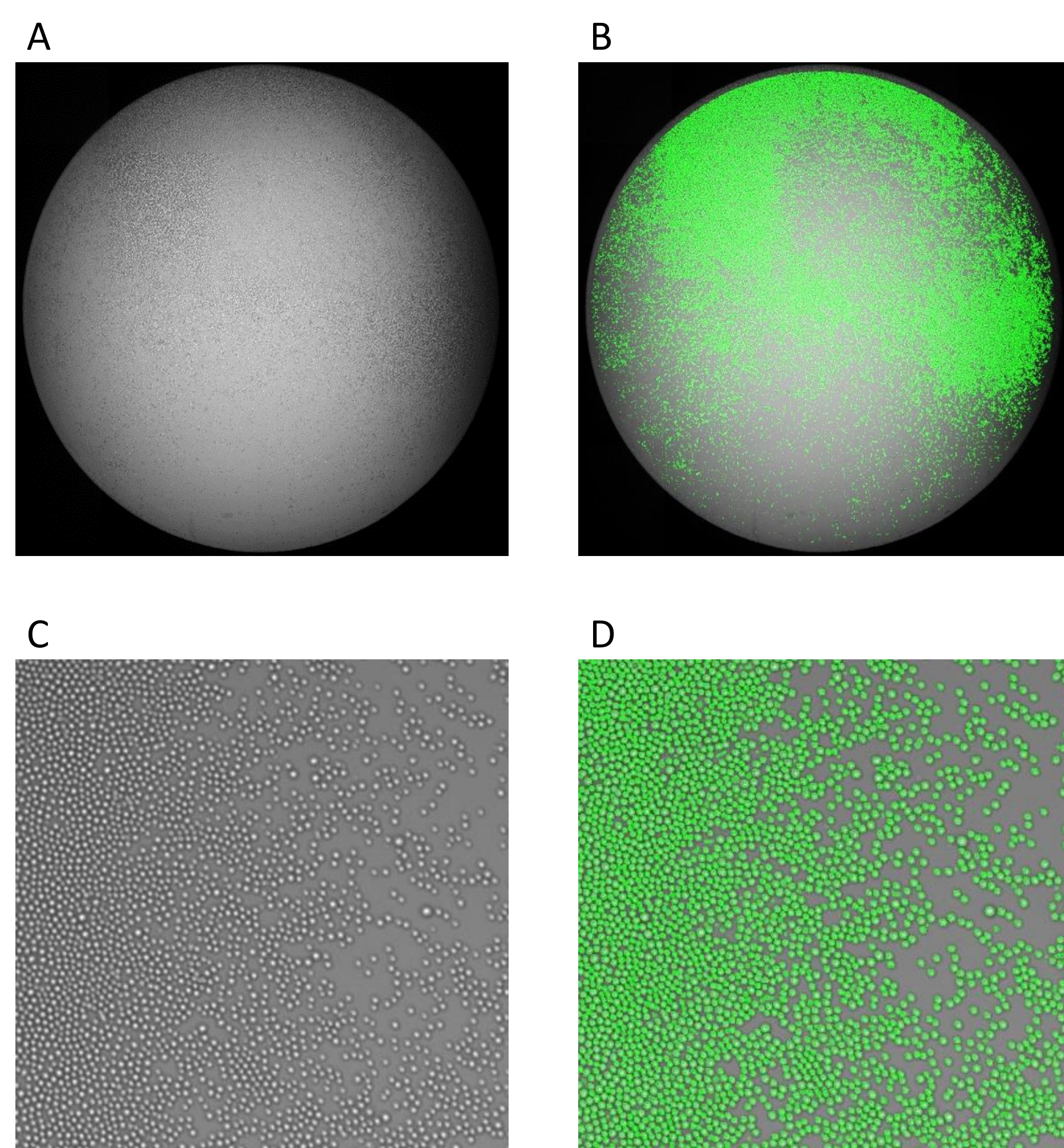
## 1. ABSTRACT

Traditionally, cell proliferation has been assessed using detection methods such as CellTiter-Glo® or MTT assay. However, these methods require multiple steps, expensive reagents, and are fixed end-point assays, prohibiting the same cells from being repeatedly assessed over a time course. There has been a need for plate-based label-free cell proliferation and cytotoxicity detection method to directly determine the number of cells per well in order to eliminate the issues with the traditional methods. In this work, we demonstrated the label-free cell proliferation and cytotoxicity detection method using the Celigo® Image Cytometry by measuring the effect of 4 proprietary compounds on the proliferation of 4 proprietary suspension cell lines from Ignyta. The same experiment was performed simultaneously using the CellTiter-Glo® Assay, and the proliferation results were compared directly to Celigo. 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) on Day 0. On Day 3, the Celigo was used to image and analyze cell proliferation using the bright-field application. The CellTiter-Glo method was used to determine cell proliferation on the same plate imaged and analyzed by Celigo, which means that the same wells were analyzed by both methods for a true comparison. Dose-response curves and IC<sub>50</sub> values were calculated and compared between the two proliferation detection methods. Cell proliferation results between the two methods of Celigo and CellTiter-Glo were highly comparable with a correlation factor of r<sup>2</sup>=0.998. The label-free image cytometry method is a simple, rapid, and reagent-free approach to determine cell proliferation in suspension cells. In addition, captured cell images can be used to verify proliferation results obtained to eliminate uncertainties in the traditional methods. Most importantly, the proposed method can image and analyze cells in a time course study, so the same cells can be assessed repeatedly over multiple time points throughout an experiment rather than single, fixed end points, thereby reducing time, supplies, and cost.

## 2. 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 CellTiter-Glo protocol, meaning the same wells were analyzed by both methods for a true comparison.
- Dose-response curves and IC<sub>50</sub> values were calculated and compared between the two proliferation assessments.

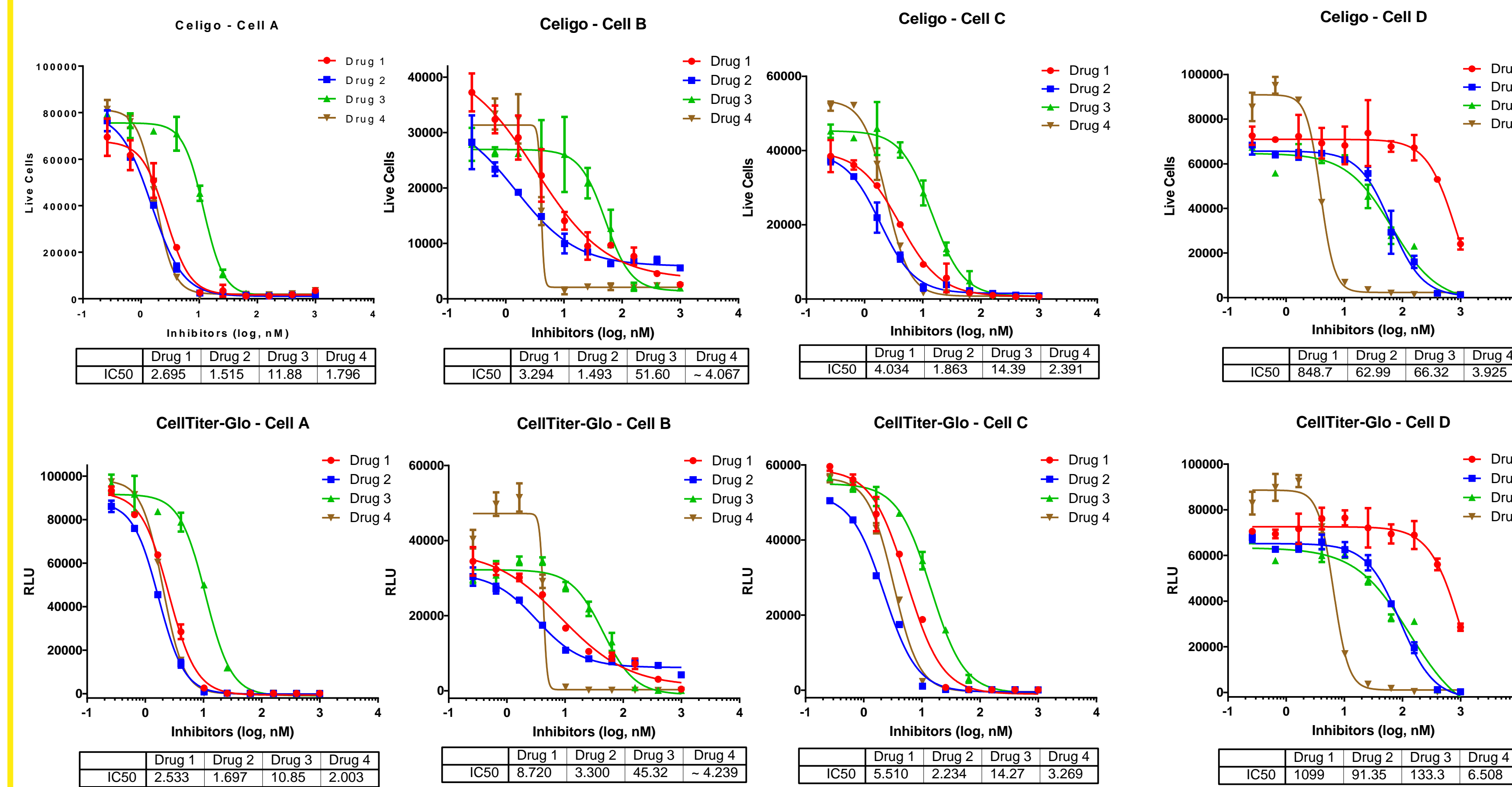
## 3. CELIGO BRIGHT-FIELD IMAGING



**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.

- The four cells lines used in this study were suspension cells and looked identical.
- The Celigo was able to image and quantify all the cells in the wells.
- The data was subsequently used to compare the cell counts with the CellTiter-Glo data.

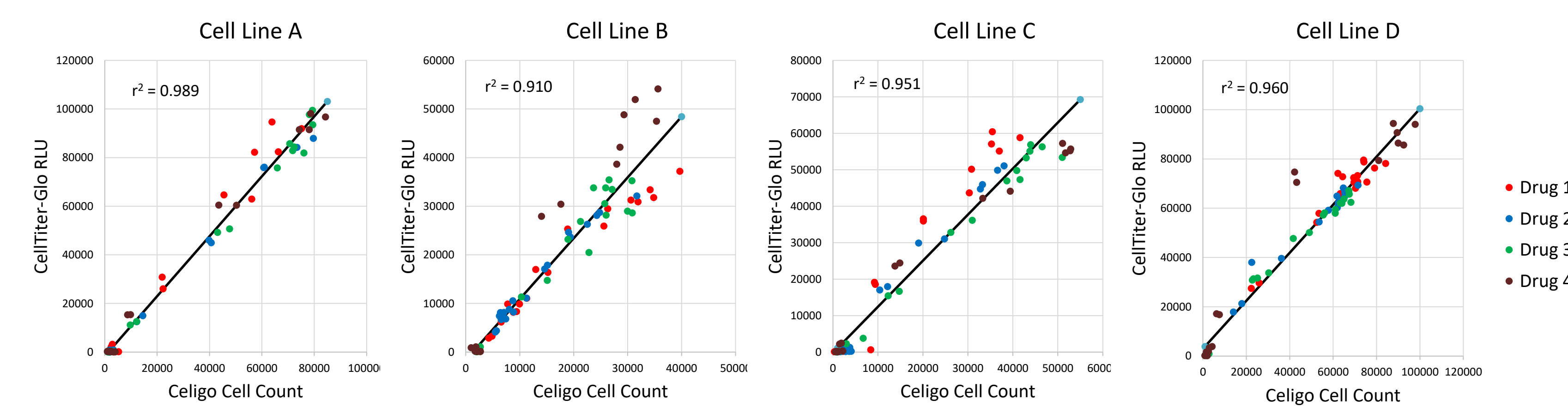
## 4. COMPARISON OF IC<sub>50</sub> CURVES FOR CELIGO CELL COUNTS AND CELLTITER-GLO



**Fig. 2:** Side-by-side comparison of IC<sub>50</sub> curves for cell types A to D treated with Drugs 1-4 between the Celigo (top row) and CellTiter-Glo (bottom row). Data was processed using PRISM GraphPad software and a four-parameter fitting algorithm was applied to calculate IC<sub>50</sub> values. All concentrations in nM.

- Celigo cell counting data generated IC<sub>50</sub> curves very similar to the CellTiter-Glo data.
- The potency of the drugs used in this study was equivalent in the Celigo and CellTiter-Glo data.

## 5. CELIGO CELL COUNTS AND CELLTITER-GLO CORRELATION



**Fig. 3:** For each cell line, the well CellTiter-Glo data point was plotted against the identical well Celigo cell counts. In each graph, all four drugs data points were colorized separately. Finally, the correlation for all data points for each cell line (including all four drugs) was reported.

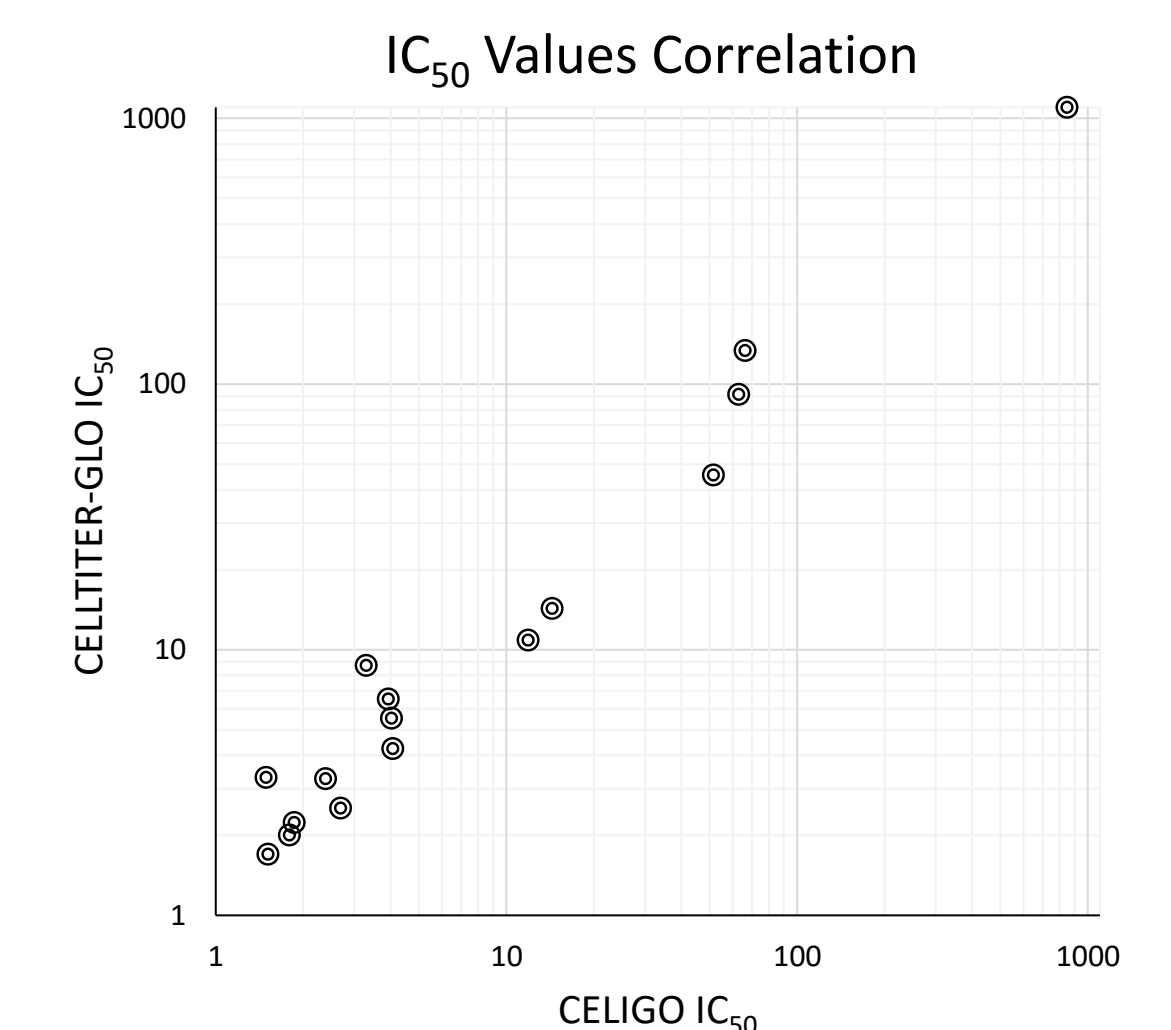
- Celigo cell counts and CellTiter-Glo data correlated very well regardless of the cell line or the drug that was used.
- The Celigo provides an alternative to the expensive CellTiter-Glo reagent to monitor the effect of drug on cell proliferation.

## 6. COMPARISON OF IC<sub>50</sub> FOR 4 DRUGS

	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

**Table 1:** Side-by-side comparison of IC<sub>50</sub> values (nM) for cell types A-D with drugs 1-4 between the Celigo and CellTiter-Glo. CTG= CellTiter-Glo.

- For each cell type and drug combination, the IC<sub>50</sub> values for the Celigo cell count and CellTiter-Glo were very similar.
- IC<sub>50</sub> values correlated very well between the two assays.



**Fig. 4:** Correlation of IC<sub>50</sub> values generated from Celigo cell counts and CellTiter-Glo.

## 7. SUMMARY AND CONCLUSION

- Cell proliferation results between the two methods of Celigo and CellTiter-Glo were comparable and the generated data values were highly correlated (r<sup>2</sup>=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.

## 8. REFERENCES

- 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
- Crouch, SP et al. (1993) The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *J. Immunol. Methods* 160, 81-8.

