# Image Cytometry-Based High-Throughput Cell Concentration and Viability Detection Method using AO/PI Fluorescent Stains

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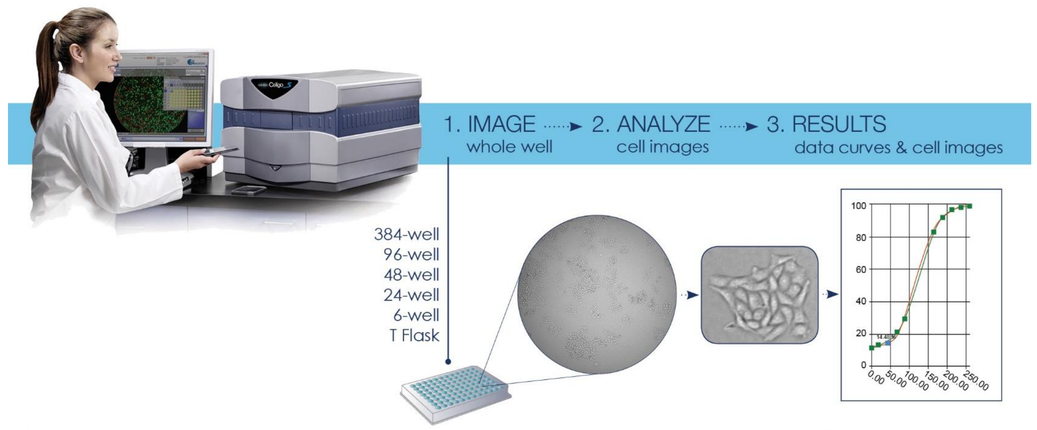
## 1. ABSTRACT

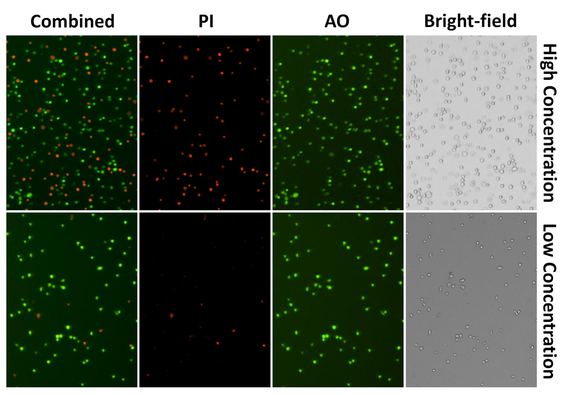
Cell concentration and viability measurement are two although simple but highly important parameters to ensure cell-based assays are performed properly. These two parameters have to be determined so that the final data can be normalized to generate meaningful and comparable results for downstream experiments. Cell-based assays performed in immuno-oncology, toxicology, or bioprocessing research fields often require measuring of multiple samples and conditions, thus the current automated cell counters that utilize disposable counting slides may not practical for a high-throughput screening setting. In the recent years, a plate-based image cytometry system has been developed for high-throughput biomolecular screening assays. In this work, we demonstrate a high-throughput AO/PI-based cell concentration and viability detection method using the Celigo image cytometer. First, the method was validated by comparing directly to the Cellometer automated cell counter. Next, cell concentration dynamic range, viability dynamic range, and consistency are determined. The high-throughput AO/PI method described here allows for 96-, 384-, and 1536-well microplates to be analyzed in less than 7 min, which can greatly reduce the time required comparing to the single sample-based automated cell counter. In addition, this method can improve the efficiency for high-throughput screening assays, where multiple cell counts and viability measurements are needed prior to performing assays such as flow cytometry, ELISA, or simply plating cells for cell culture.

## 2. CELIGO IMAGING CYTOMETRY FOR HT CELL COUNT AND VIABILITY ASSAY



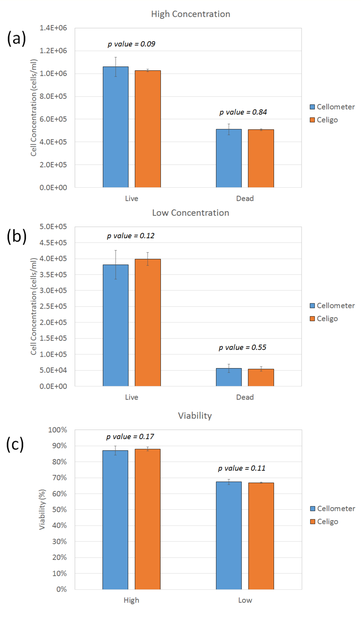

1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and captures bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. The measured parameters are used to generate cell proliferation kinetic data, viability, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, and ADCC cytotoxicity results

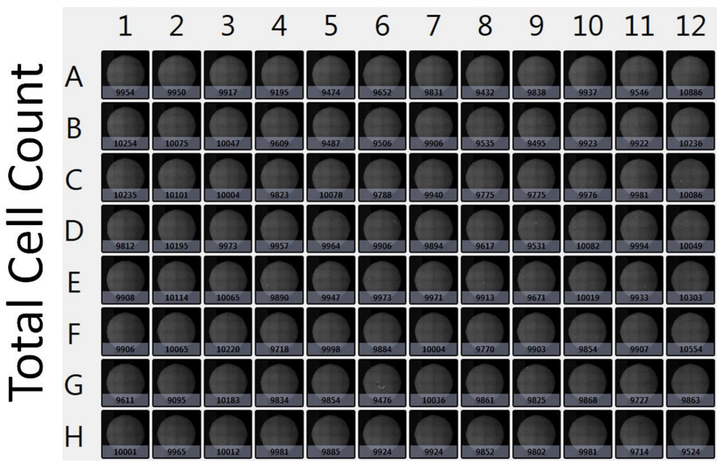
## 3. HIGH-THROUGHPUT CELL COUNT AND VIABILITY METHOD USING AOPI




- Using Greiner 655090 96-well microplates, 50 µL of 10X diluted AOPI staining solution from Nexcelom was pipetted into each well
- Next, 5 µL of each cell sample are added into each well
- Next, the microplate is centrifuged for 5 min at 1200 RPM to allow cells to settle to the bottom
- After centrifuge, the plate is inserted into Celigo
- The Celigo Image Cytometer then acquire images and count AO and PI stained cells to determine the concentration and viability

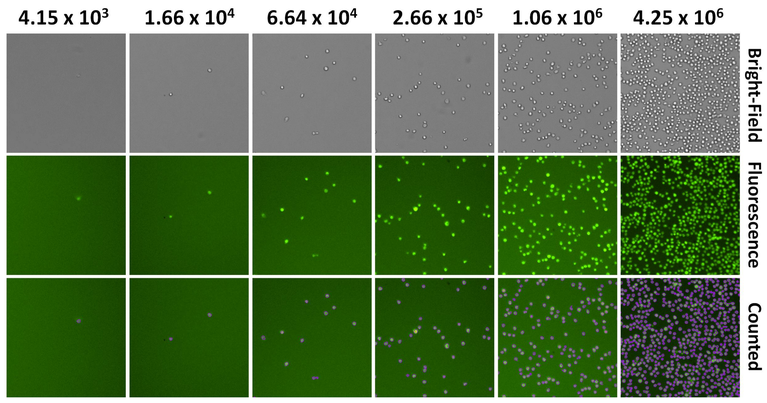
## 4. COMPARISON TO SINGLE COUNT AUTOMATED CELLOMETER CELL COUNTER

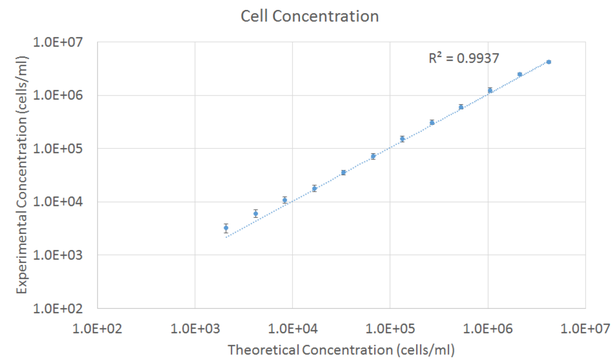



- Two different Jurkat cell samples were compared between Celigo and Cellometer
- The results showed that they were comparable for both cell concentrations as well as viabilities

## 5. CONCENTRATION LINEARITY EXPERIMENT AND RESULTS

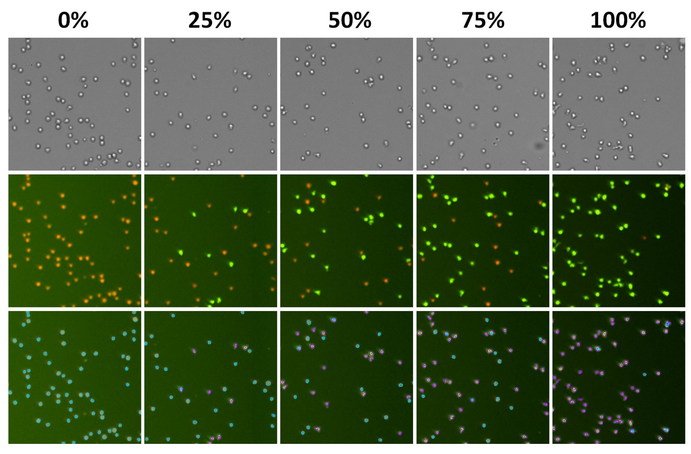


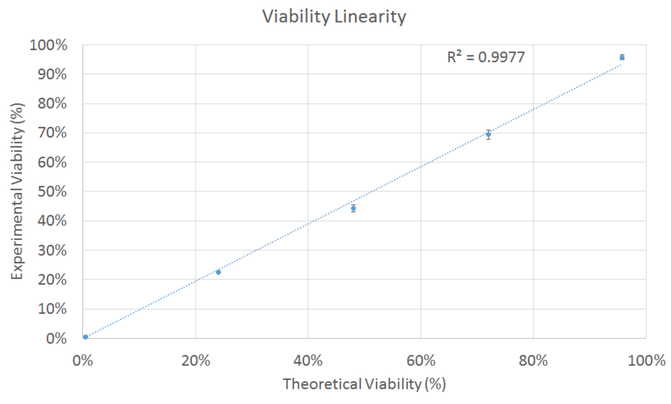




- A concentration series was performed to determine if Celigo Image Cytometry can accurately measure different cell concentrations in a dilution series
- 12 dilution points of Jurkat cells were prepared, the cells were stained with AOPI following the protocol described previously

- Bright Field, fluorescent and counted images captured on Celigo are shown above
- The cell density in the images showed increasing concentrations from left to right
- The AO positive cells are all counted accurately to generate the cell concentration for each well
- The concentration series results are plotted and shown in the graph above, showing linearity of $R^2$ value is calculated to be 0.9937

## 6. VIABILITY LINEARITY EXPERIMENT AND RESULTS




- A viability series was performed to determine if Celigo Image Cytometry can accurately measure different cell viabilities
- 5 viabilities of Jurkat cells were prepared by mixing fresh and heat-killed cells, the cells were stained with AOPI following the protocol described previously

- Bright Field, fluorescent and counted images captured on Celigo are shown above
- The cells showed increasing viability from left to right, where AO positive cells also showed increase as PI positive cells decreased
- The viability series results are plotted and shown in the graph above, showing linearity of $R^2$ value to be 0.9977

## 7. SUMMARY AND CONCLUSION

- We have demonstrated a plate-based high-throughput cell count and AO/PI viability detection method using the Celigo image cytometer
- The method was validated against the Cellometer automated cell counter
- The ability to analyze samples in a plate format reduced the assay time significantly from 60 to 3 min for 20 samples, which greatly increased throughput
- The proposed method can be used for assays involving cytotoxicity measurement for cancer research, simple cell counting for ELISA plating, for flow cytometry assays, or quality assurance for bioprocessing samples