

Improving Downstream Results with Automated Cell Counting

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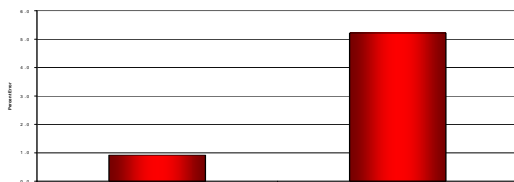
Abstract

Accurate cell enumeration is a critical parameter in a variety of cellular and biochemical applications. Here, we compare results obtained using both automated and manual cell counting methods for three assays typical of high throughput screening, high content screening and viral vector transduction experiments. In these applications, maintaining an accurate cell count has proven to result in more consistent and reproducible data. The error associated with traditional methods of manual cell counting is introduced by both the instrument and the user. The error of a conventional hemocytometer counting procedure is as high as 70% while automated cell counting greatly reduces the error by as much as 50% by simply eliminating the variability introduced by the user. A more accurate and consistent cell count improved the downstream results in the following assays. In a homogenous, cell-based assay for potassium ion channel function in human U-2 OS cells, we found that automated cell counting resulted in higher Z' values than numbers obtained by manual counting and decreased assay-to-assay variability. In an image-based microplate assay for phosphor-H2AX in HeLa cells, we found that automated cell counting yielded fewer discounted wells due to fewer samples being statistical outliers. In a viral vector transduction experiment for evaluation of infection efficiency of a baculovirus containing a GFP signaling peptide fused gene into U-2 OS cells, we found the coefficient of variation was minimized. As is evident from these experiments, automated cell counting improves results by providing consistently accurate data that is important for reproducibility and statistical confidence.



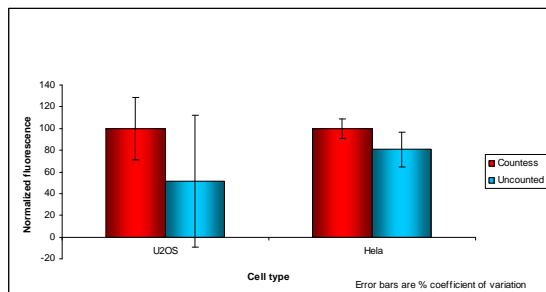
The Countess™ Automated Cell Counter was used as the standard automated cell counter for these experiments. The Countess™ automated cell counter uses the trypan blue exclusion method to calculate cell concentration and provide viability information.

Figure 1- Automated cell counting decreases inter-user error to less than 1%



In the above experiment, we compared the Countess™ automated cell counter with a disposable hemocytometer. Three separate chambers were loaded by three different, trained users. Each chamber was counted by each of the users. The same work flow was followed for the hemocytometer counts. As is depicted in the chart above, the variation between users is as much as 5% when using the hemocytometer, and less than 1% when using the Countess™ automated cell counter.

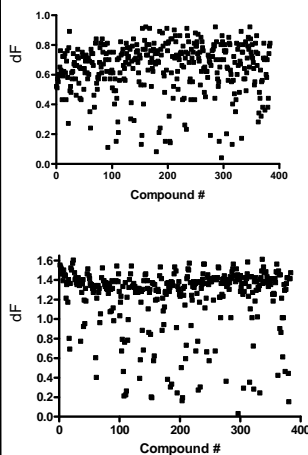
Figure 2-Automated cell counting results in more consistent results in Premo™ Cameleon BacMam assays



Over three days, two cell types (HeLa and U-2 OS) were separately transduced with a BacMam containing the Premo™ Cameleon calcium sensor then incubated overnight in a 96-well plate at 37°C, 5% CO₂. On the following day, the resulting unstimulated cellular fluorescence was measured on a Flexstation® fluorescent plate reader (MDS Analytical Technologies) with excitation at 485 nm and emission 538 nm (medium gain, 6 reads/well). The average fluorescence of 56 wells of each cell type was determined for each date, then the average and percent coefficient of variation (% CV) of the three experiments were determined. The fluorescence signal was then normalized to the Countess™ data. The red columns indicate plates that were transduced after counting cells with the Countess™ automated cell counter. These cells were plated at 10,000 cells/well and 200 viruses/cell. The blue columns indicate cells that were plated after being estimated by eye and transduced with 1% virus.

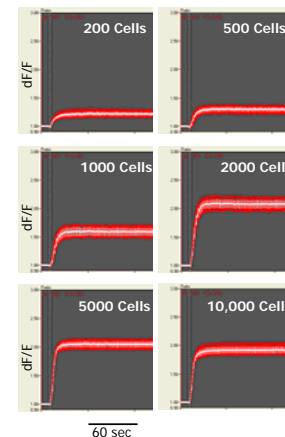
These data show the plates that were quantified using the automated cell counter prior to plating have a smaller %CV than the plates that were estimated visually. This indicates that using the Countess™ automated cell counter can result in more consistent day-to-day results.

Figure 3 – Automated cell counting improves Z' values in HTS experiment



Automated cell counting improves data quality and signal fidelity in the FluxOR™ potassium ion channel assay. Top panel: Human U-2 OS cells expressing BacMam hERG were plated by hand with estimated cell densities of 5,000 cells per well prior to running the FluxOR™ assay. A Z' value of .55 was determined for the plate shown. Bottom panel: Cells were prepared identically to top panel, but quantified with the Countess™ Automated Cell Counter at 5,000 cells per well. A Z' value of .75 was determined for the plate shown. Built-in DMSO and cisapride controls were used to determine assay window and Z' values for a series of 384 compounds pulled from the Trocris® miniscreen compound collection.

Figure 4 – Optimizing cell count using Countess™ automated cell counter



Automated cell counting used to optimize cell density in the FluxOR™ potassium ion channel assay. Human U-2 OS cells expressing BacMam Kir2.1 (KCNJ2) were counted with an Invitrogen Countess™ automated cell counter prior to plating at the indicated cell number per well in a Greiner Poly-D-Lysine coated 384 well microplate. Cells were allowed to adhere for two hours before FluxOR™ assay. White lines indicate average and standard deviation values.

Results

Due to the critical nature of accurate cell counts in biochemical and cellular assays, it is important that the cell counts hold steadfast from day to day and user to user. We have shown that inter-user variation can differ dramatically when using a hemocytometer. We have also shown that when using the Countess™ automated cell counter, inter-user variation decreases to less than 1%. Here, the Countess™ automated cell counter streamlines the enumeration of cells to be used in the stated assays. In the transduction of two cell types by Premo™ Chameleon BacMam, we have shown that the Countess automated cell counter lowers the variation from experiment to experiment by allowing the researcher to plate a consistent number of cells from plate to plate, and day to day. In the FluxOR™ potassium ion channel assay, we have shown that more consistent data results in higher Z' values by reducing the number of statistical outliers associated with manual counting.

Conclusions

As is evident in these experiments, automated cell counting improves results by providing consistent and accurate data that is important for reproducibility and statistical confidence.



The Countess™ automated cell counter has a user friendly interface that has been developed to represent the familiar hemocytometer that researchers typically use to count cells.