

Improving downstream results with the Countess[®] Automated Cell Counter

Introduction

Accurate cell enumeration is a critical parameter for a variety of cellular and biochemical applications. This technical note compares results using both the Countess[®] Automated Cell Counter (Figure 1) and a hemocytometer for three assays typical of high-throughput screening, high-content screening, and viral vector transduction experiments. Variability was determined in two application studies: one in which the Countess[®] cell counter and a hemocytometer were used to count cells prior to transduction with recombinant BacMam virus containing the Premo[™] Cameleon calcium sensor, and the other a high-throughput screening experiment using the FluxOR[™] potassium ion channel assay. In all cases, variation was minimized when the Countess[®] Automated Cell Counter was used to determine cell concentration.

In these applications, maintaining an accurate cell count results in more consistent and reproducible data. The error associated with traditional manual cell counting is introduced by both the device and the user. The error of a conventional hemocytometer counting procedure is as high as 70%, while automated counting with the Countess[®] counter reduces this high variability to less than 1%. A more accurate and consistent cell count improved downstream results in the following assays:

- In a homogeneous, cell-based assay for potassium ion channel function in human U2OS cells, automated cell counting resulted in higher Z' values and decreased assay-to-assay variability compared to manual counting.
- In an image-based microplate assay for phospho-H2AX in HeLa cells, automated cell counting yielded fewer discounted wells due to fewer samples being statistical outliers.
- In a viral vector transduction experiment evaluating infection efficiency of a baculovirus containing a GFP-signaling peptide fused gene into U2OS cells, the coefficient of variation (CV) was minimized.

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



Figure 1. The Countess[®] Automated Cell Counter. The cell counter was used as the standard automated cell counter for these experiments. The Countess[®] cell counter uses the trypan blue exclusion method to calculate cell concentration and provide viability information.

Results from Premo[™] Cameleon BacMam assays

During a 3-day experiment, two cell types (HeLa and U2OS) were separately transduced with a BacMam virus containing the Premo[™] Cameleon calcium sensor, then incubated overnight in a 96-well plate at 37°C and 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 at 538 nm (medium gain, 6 reads/well). The average fluorescence of 56 wells of each cell type was determined for each date, and then the average and percent coefficient of variation (% CV) of the three experiments were determined. The fluorescence signal was then normalized to the Countess[®] counter data (Figure 2).

These data show that the plates quantified using the automated cell counter prior to plating have a smaller % CV than the plates that were estimated visually. This indicates that the Countess[®] Automated Cell Counter lowers variation from experiment to experiment by allowing the researcher to plate a consistent number of cells from plate to plate, and day to day.

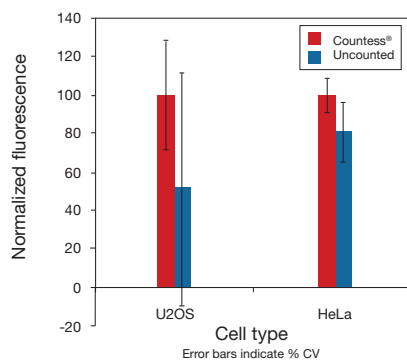


Figure 2. The Countess® Automated Cell Counter delivers more consistent results in Premo™ Cameleon BacMam assays. Red columns indicate plates that were transduced after counting cells with the Countess® Automated Cell Counter. These cells were plated at 10,000 cells/well and 200 viruses/cell. Blue columns indicate cells plated after being estimated by eye and transduced with 1% virus.

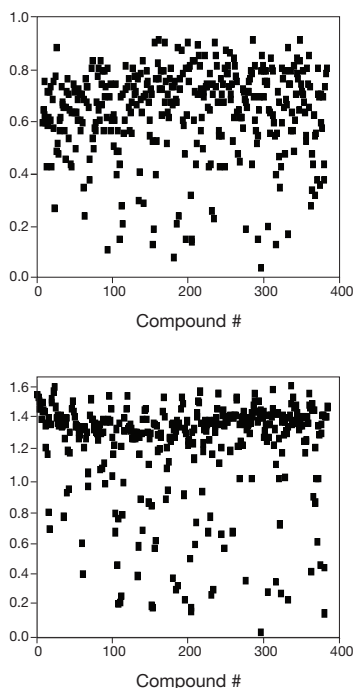


Figure 3. The Countess® Automated Cell Counter improves Z' values in an HTS experiment. (A) Human U2OS cells expressing BacMam hERG were plated by hand with estimated cell densities of 5,000 cells/well prior to running the FluxOR™ assay. A Z' value of .55 was determined for the plate shown. (B) Cells were prepared identically to those in (A), but quantified with the Countess® Automated Cell Counter at 5,000 cells/well. A Z' value of 0.75 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 Tocris® miniscreen compound collection.

Results from high-throughput screening (HTS) experiment

The results (Figure 3) show that automated cell counting improves data quality and signal fidelity in the FluxOR™ potassium ion channel assay—more consistent data results in higher Z' values by reducing the statistical outliers associated with manual counting.

Optimizing cell count using the Countess® Automated Cell Counter

In this experiment (Figure 4), human U2OS cells expressing BacMam Kir2.1 (KCNJ2) were counted with the 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 2 hours before the FluxOR™ assay.

Conclusion

These experiments show that the Countess® Automated Cell Counter improves results by providing consistent and accurate data important for reproducibility and statistical confidence.

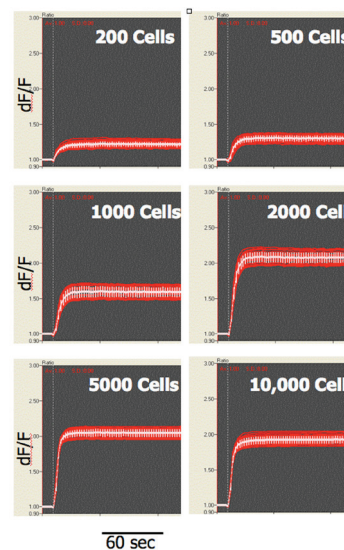


Figure 4. Optimizing cell density in the FluxOR™ potassium ion channel assay with the Countess® Automated Cell Counter. White lines indicate average and standard deviation values.