Cell counting

Comparison of cell counting using Countess 3 and Countess 3 FL Automated Cell Counters vs. hemocytometers

Introduction

Many research studies require the counting of cells prior to beginning an experiment. For example, counting may be necessary to standardize the cell concentration between samples to minimize error and variation in downstream results. The most common methods for counting cells make use of microscopes and hemocytometers. Advances in imaging technology have enabled the automation of cell counting, providing improved accuracy and reliability with much less time and effort. Unfortunately, many laboratories have been unable to implement automated cell counting. Invitrogen™ Countess™ 3 and Countess™ 3 FL Automated Cell Counters were developed to remove some of the barriers to switching to an automated cell counter. A comparison of these cell counting methods is presented here.

Accuracy

Variability due to subjectivity

Reliance on operator judgment, regardless of the cell counting method, contributes to error in results. Light intensity and focus on microscopes and some automated cell counters are set subjectively, which can lead to variability. The operator must also determine which objects to include and exclude in the cell count. If measuring viability, the operator must decide which cells will be counted as dead or live. The number of subjective variables in manual counting can thus lead to wide variation in results between operators.

Countess 3 and Countess 3 FL cell counters can remove many of these subjective variables and save time. Countess 3 and Countess 3 FL instruments use a sophisticated algorithm to determine optimal focus and light intensity. The ability to gate cells based on cell size, brightness, and circularity using quantitative measurements rather than operator judgment also helps reduce error due to subjectivity and allows improved repeatability between samples and users (Figure 1).

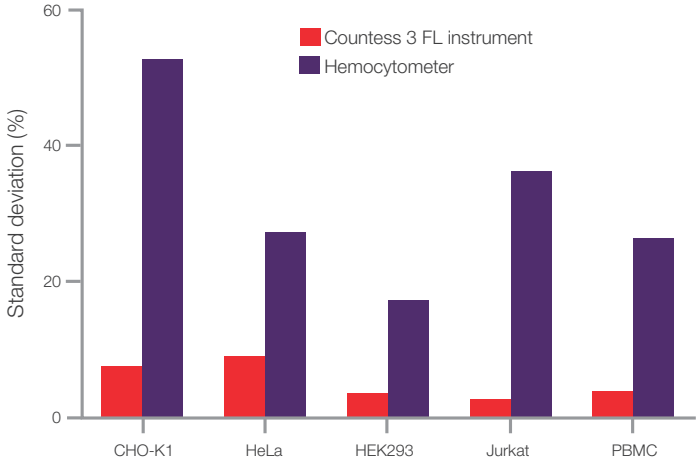

Figure 1. Comparison of user variability in cell counting with a hemocytometer and the Countess 3 FL instrument. A single sample each of PBMCs and CHO-K1, HeLa, HEK293, and Jurkat cells was counted by three different operators using the Countess 3 FL cell counter. The cells were then counted manually using a hemocytometer and microscope. User-to-user variability was much higher with the hemocytometer than with the Countess 3 FL instrument.

Number of cells counted

In practice, many researchers do not count enough cells, leading to unreliable cell counts with high standard deviations. To save time during manual counts, many users count only one or two of the squares on a traditional hemocytometer grid. Counting more squares (i.e., a greater area) will typically provide more consistent results, but at the cost of additional time. Countess 3 and Countess 3 FL Automated Cell Counters interrogate the equivalent of nearly four traditional hemocytometer squares during the 10-second counting time, thereby increasing count-to-count consistency by reducing the effect of cell distribution variation (Figure 2).

Replicates

Regardless of the cell counting method, variance can be reduced by performing multiple cell counts on a sample. Several replicates can be analyzed on Countess 3 and Countess 3 FL instruments in the time it takes to perform one count with a hemocytometer (up to 5 minutes). This helps increase accuracy and confidence in results, as replicates are often omitted when using tedious manual counting methods, due to time constraints, especially when multiple cell samples must be counted.

Clumpy cells

Obtaining accurate results with samples containing clumpy cells can be difficult with manual cell counting as well as with some automated cell counters. A common challenge with clumpy cells is the ability to discern borders between the cells to determine the number of cells contained in a clump. Some automated cell counters are limited to accurately counting only clumps of five or fewer cells. The advanced counting algorithms of Countess 3 and Countess 3 FL instruments can clearly identify cell boundaries within complex clumps of cells, resulting in accurate cell counts (Figure 3).

In addition to identifying singlets in cell clumps, Countess 3 and Countess 3 FL Automated Cell Counters can detect cell clusters and measure the total percentage of aggregation within a sample. The on-screen display uniquely distinguishes aggregates from singlet cells with different colors to reflect their identification, and brightfield mode provides visual confirmation of cells within each aggregate.

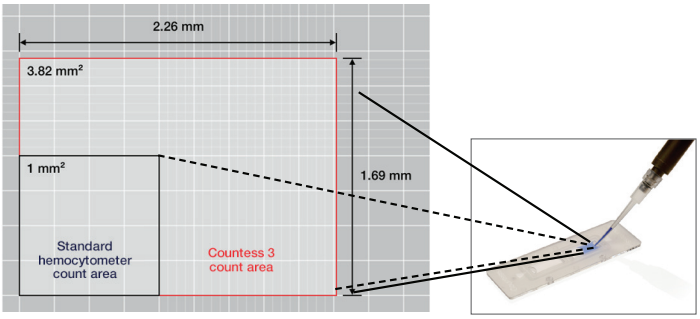

Figure 2. Counting area. The counting area of the Countess 3 instrument compared to that of a standard hemocytometer.

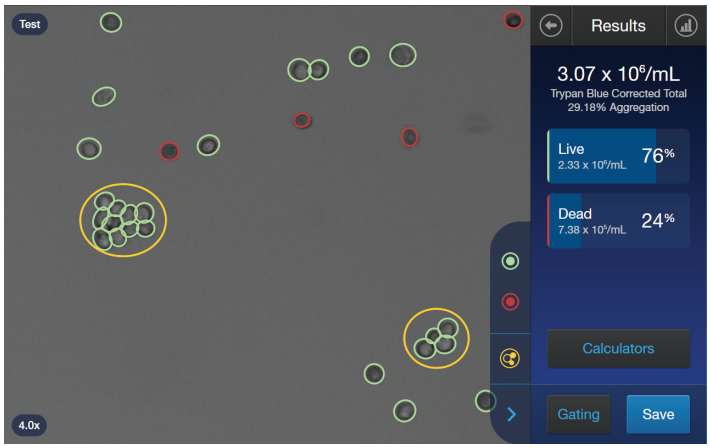

Figure 3. Accurate cell counts, even with clumpy samples. The segmentation and counting algorithms of the Countess 3 cell counter were able to resolve clumps in this U2OS human osteosarcoma cell sample and show the percent aggregation within the sample.

Fluorescent cell counting

Fluorescence-based cell counting offers several advantages over non–fluorescence-based methods. It provides higher contrast against the background, making it easier to identify and count cells, and it allows the use of different fluorescent markers to differentiate and count various cell types. Fluorescence-based counting also reduces the likelihood of misidentifying debris as cells, which helps ensure more accurate results.

The Countess 3 FL Automated Cell Counter has two optional, interchangeable fluorescence channels. The colors it collects are determined by inserting individual light cubes, and more than 20 light cubes are available. The Countess 3 FL Automated Cell Counter will automatically determine the best lighting, and it can count fluorescent cells in two modes: brightfield and fluorescence. Brightfield-based counts use the brightfield image to identify cells, and report the percentage of fluorescence-positive cells quickly and easily. Fluorescence-based counts use one or both fluorescence channels to count all fluorescent objects observed and report the total count as well as which cells are fluorescent in each channel or both channels (Figure 4).

Cost savings

A key factor contributing to reluctance to adopt automated cell counting is the ongoing cost of proprietary, one-time-use disposable slides that are required with automated systems. A hemocytometer used for manual cell counting can be washed and reused indefinitely. Countess 3 and Countess 3 FL Automated Cell Counters are designed to work with reusable glass slides (Figure 5) to help reduce costs, but they also work with convenient disposable slides. This innovation now makes it affordable for labs to incorporate this excellent cell counting method without the ongoing cost of consumables associated with other automated cell counters.

Time savings

The additional time it takes to manually count cells (up to 5 minutes) compared to counting with Countess 3 and Countess 3 FL Automated Cell Counters (10 seconds) is often overlooked as an added cost. An individual counting five slides per day (two samples per slide) can save ~10 hours per month by switching to an automated counter with a reusable slide. The time saved increases to ~15 hours per month when using disposable slides (Figure 6). This additional time can be applied to other activities in the lab, resulting in a significant advantage when switching from manual to automated cell counting.

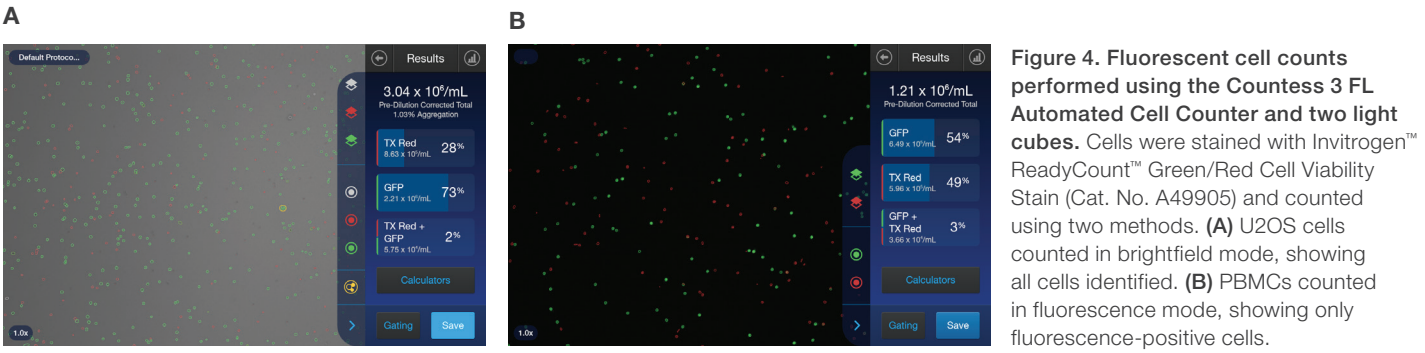

Figure 4. Fluorescent cell counts performed using the Countess 3 FL Automated Cell Counter and two light cubes. Cells were stained with Invitrogen™ ReadyCount™ Green/Red Cell Viability Stain (Cat. No. A49905) and counted using two methods. **(A)** U2OS cells counted in brightfield mode, showing all cells identified. **(B)** PBMCs counted in fluorescence mode, showing only fluorescence-positive cells.

Figure 5. The reusable slide for the Countess 3 FL Automated Cell Counter helps reduce the cost of consumables.

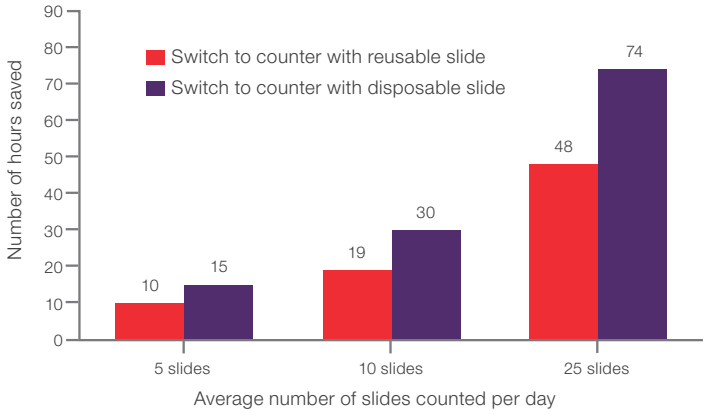

Figure 6. Estimated hours saved per month by switching from manual cell counting to using an automated cell counter.

Information obtained

Information that can be obtained with a hemocytometer is limited to the total number of cells, number of live cells, and number of dead cells present in the sample. Additional data can be obtained with an automated cell counter, including the average cell size and a histogram showing the distribution of cells based on fluorescence intensity or live and dead cell size (Figure 7). With Countess 3 and Countess 3 FL instruments, the information collected can be saved as a CSV file to a USB drive for further analysis. The raw image as well as the results screen can also be saved to a USB drive and transfered to a computer.

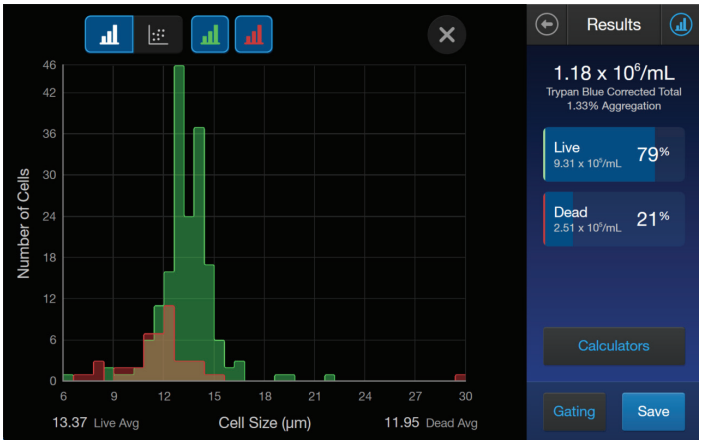

Figure 7. Results showing the average cell size and cell size distribution help target a specific population of cells.

Environmental impact

Use of an automated cell counter with disposable slides is convenient and saves time, but it also increases the amount of waste that goes into landfills. Using a reusable glass slide with the Countess 3 or Countess 3 FL cell counter could potentially offset the environmental impact of disposing of up to 1,200 disposable slides per year for each instrument that replaces an automated counter that uses disposable slides.

Summary

The benefits of automated cell counting compared to manual cell counting are well documented and include increased accuracy, simplified workflows, and significant time savings. Despite these benefits, the cost of disposable slides has been a barrier to adoption of automated cell counting. The ability to use reusable slides with Countess 3 and Countess 3 FL Automated Cell Counters has reduced the cost associated with automated cell counting, making it available to labs that may have been unable to afford this advanced technology that enables faster counting and more accurate results.

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