Comparison of cell counting using Countess II Automated Cell Counters vs. hemocytometers

Introduction

Many research studies require the counting of cells prior to beginning an experiment. This may be needed, for example, in order to standardize cell concentration between samples to minimize error and variation in downstream results. The most common method for counting cells uses a microscope and hemocytometer. Advancements in imaging technology have enabled the automation of cell counting, providing improved accuracy and reliability, with much less time and effort. Unfortunately, many labs have been unable to implement an automated cell counter. The Invitrogen™ Countess™ II 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 settings on a microscope and some automated cell counters are subjective, and therefore 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.

The Countess II instruments can remove many of these subjective variables, while also saving time. The Countess II instruments use a sophisticated algorithm to determine the 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 errors due to subjectivity and allows increased repeatability between samples and users (Figure 1).

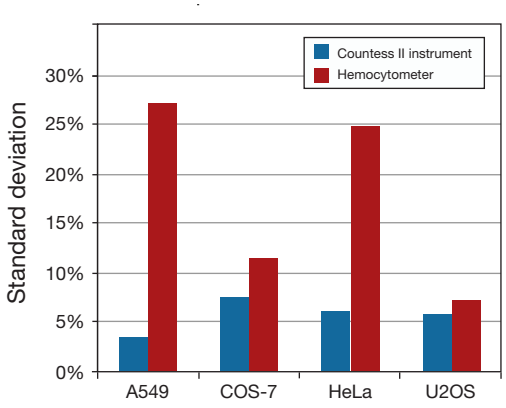

Figure 1. User variability counting with a hemocytometer compared to using a Countess II instrument. Identical samples of A549, COS-7, HeLa, and U2OS cells were counted by three different operators using a Countess II Cell Counter and then manually with a hemocytomer and microscope. The user-to-user variability for the hemocytometer is much higher than for the Countess II 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 II 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).

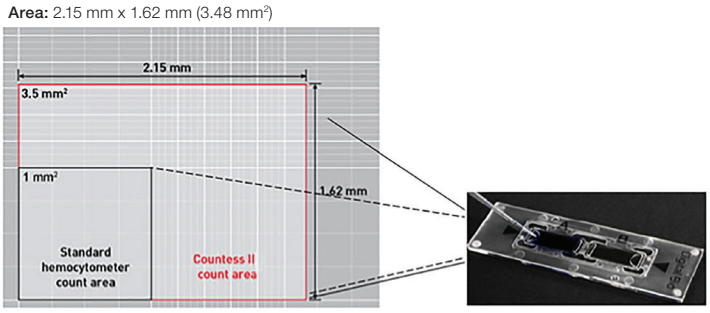

Figure 2. Counting area. The counting area of the Countess II instruments compared to a standard hemocytometer.

Replicates

Regardless of cell counting method, a reduction in variance can be obtained by performing multiple cell counts for a sample. Several replicates can be analyzed on the Countess II 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 avoided when using tedious manual counting methods due to time constraints, especially when multiple cell samples must be counted.

Clumpy cells

Obtaining accurate results from 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 the Countess II instruments can clearly identify cell boundaries within even complex clumps of cells, resulting in accurate cell counts (Figure 3).

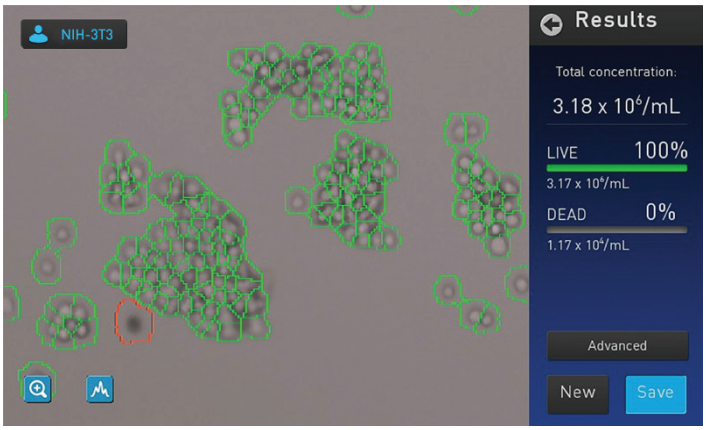

Figure 3. Accurate cell counts, even with clumpy samples. The segmentation and counting algorithms of the Countess II cell counters are able to resolve clumps of cells in this NIH/3T3 cell sample.

Fluorescent cell counting

Flow cytometry and manual cell counting with a hemocytometer and microscope are the gold standards for fluorescent protein expression analysis, but both require advanced training for users and relatively expensive instrumentation. The Countess II FL Automated Cell Counter has two optional, user-interchangeable fluorescent channels. The colors collected are determined by the insertion of individual light cubes; more than 20 light cubes are available.

The Countess II FL Automated Cell Counter is able to count fluorescent cells with comparable accuracy, but with faster turnaround and greater cost-effectiveness while reducing user-to-user variability (Figure 4).

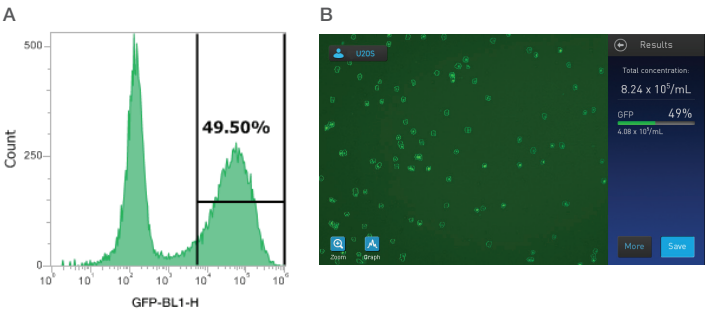

Figure 4. Fluorescent cell counts similar to those obtained by flow cytometry can be easily acquired with the Countess II FL Automated Cell Counter, with a single light cube. U2OS cells were transduced using Invitrogen™ CellLight™ Nucleus-GFP, BacMam 2.0 (Cat. No. C10602), and allowed to incubate for 36 hours. The cells were evaluated for GFP by **(A)** flow cytometry and **(B)** the Countess II FL Automated Cell Counter. The proportions of transduced cells determined by the two methods are nearly identical.

Cost

A key factor leading 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. The Countess II FL Automated Cell Counter was designed to work with a reusable glass slide (Figure 5) to help significantly reduce costs, but also works with convenient disposable slides. This innovation now makes it affordable for labs to incorporate this superior cell counting method without the ongoing cost of consumables associated with other automated cell counters.

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

Time savings

The additional time it takes to manually count cells (up to 5 minutes) compared to counting with the Countess II 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 savings 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.

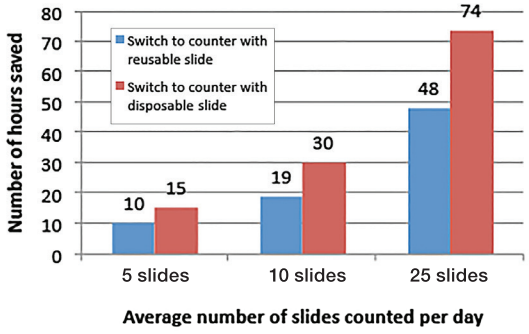

Figure 6. Estimated hours saved per month when switching from manual cell counting to using an automated cell counter.

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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 the Countess II 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.

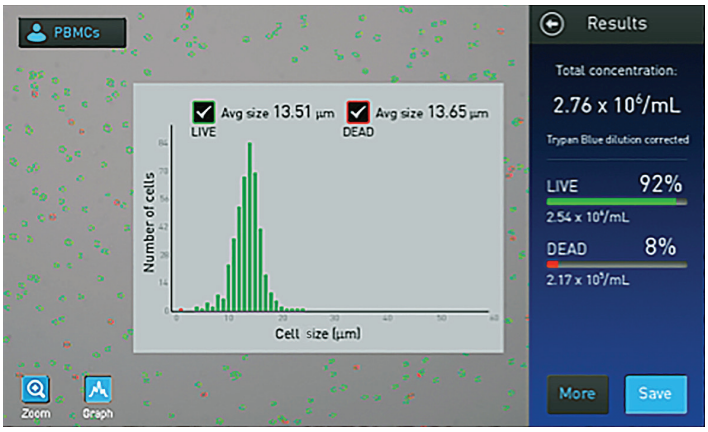

Figure 7. Results showing the average cell size and cell size distribution helps target a specific population of cells.

Environmental impact

Use of an automated cell counter with a disposable slide is convenient and saves time, but also increases the amount of waste that goes to landfills. Using a reusable glass slide with the Countess II FL counter could potentially displace 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. The cost of disposable slides has been a barrier to adoption of automated cell counting despite these benefits. The ability to use a reusable slide with the Countess II FL Automated Cell Counter 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.

Find out more at **thermofisher.com/countess**

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