

Comparison of image-based cell counting methods:

Countess™ Automated Cell Counter vs. the hemocytometer

Introduction

Many cell-based research studies require the counting of cells prior to beginning an experiment. This allows standardization of cell concentration between samples, minimizing error and variation in downstream results. The invention of the microscope and hemocytometer made cell counting possible, and is the most commonly used method to date. Fortunately, advancements in imaging technology have enabled the automation of cell counting, providing the best promise for improving experimental accuracy, reliability, and time-to-results, with much less time and effort.

This technical note compares the Countess™ Automated Cell Counter to the hemocytometer method of cell counting, examining methodology, accuracy, precision, and dynamic range. The Countess™ method provides comparable accuracy and precision to the most accurate hemocytometer method, while offering the following additional benefits:

- Simple, automated procedure
- Faster time-to-results
- Broader concentration range measured

Countess™ Automated Cell Counter

The Countess™ Automated Cell Counter is a benchtop instrument that uses the standard trypan blue technique for cell viability determination; digital image capture and a sophisticated image analysis program determine the cell count, cell size, and percent viability of a cell population. The instrument contains a small microscope, digital camera, and computer that together enable the user to view the sample, adjust the image for optimal alignment, acquire an image, and rapidly obtain cell count and viability results—without having to manually count the cells or perform tedious calculations (Figure 1).

The Countess™ instrument generates an image of the actual cells, increasing the researcher's confidence in the results. The image and associated data can be saved on a USB drive, and can be further analyzed using Countess™ PC Software, if desired. The software can then generate a printed report for archiving in a lab notebook. The image is also saved as a .jpeg file and the numerical data in CSV format, which allows results from many samples to be analyzed using common spreadsheet and image processing programs.



Figure 1—Countess™ Automated Cell Counter. The Countess™ Automated Cell Counter eliminates the tedium and subjectivity of manual cell counting. Automated counting frees up your time, reduces eye strain, and minimizes subjective judgments that can lead to error. It takes 3 simple steps: **(A)** Mix 10 μ L of sample with 10 μ L of trypan blue, and pipet into Countess™ chamber slide. **(B)** Insert slide into the instrument. **(C)** Press the “Count cells” button, and results are displayed in 30 seconds.

Hemocytometer method of cell counting

The hemocytometer cell counting method uses a specially crafted gridded-glass slide consisting of two chambers, each divided into nine 1 x 1 mm squares. A glass cover is supported by the grid, and the cell suspension is loaded via capillary action into the chamber (Figure 2). The cells lie over the grid occupying a volume of 0.1 μ L for each 1 x 1 mm square, and are counted manually. The number of cells counted in a specific number of squares and the dilution factor are used to calculate the original concentration in cells/mL.

Proper sample preparation technique is critical when working with the traditional hemocytometer. The glass hemocytometer, with its free cover slip, is subject to over-filling, resulting in cell numbers that are often higher than those produced by disposable, fixed cover slip hemocytometers. Careful, consistent sample loading must be performed to avoid over-filling. The cell suspension must also be properly diluted so that a reasonable number of cells (25 to 250) are placed in each square. In practice, most researchers do not count enough cells, leading to unreliable cell counts with high standard deviations.

Another factor contributing to error is the reliance on operator judgement. The operator must determine which objects are included in the count, and which objects to exclude as debris. If measuring viability, the operator must also decide which cells will be counted as “dead” and which will be counted as “live”. This often leads to inter-operator variability and inaccurate results.

The advantages of using a hemocytometer include the ability to make immediate judgments and decisions about the sample analysis. This is useful when the sample is full of debris and contains multiple object shapes and sizes. However, the subjective nature of human manual counting can lead to wide

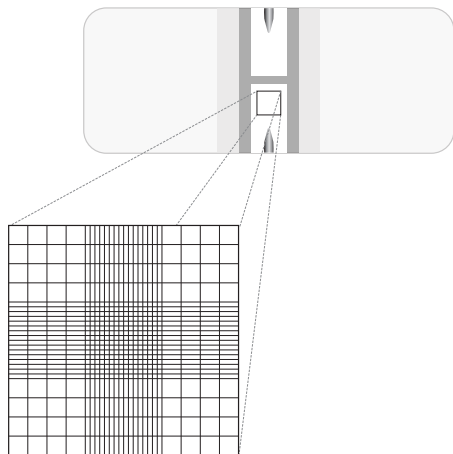


Figure 2—The hemocytometer.

variation in results. In addition, the procedure is tedious and requires careful cleaning and handling of the hemocytometer parts. In practice, the tedium of manual counting means that the counting step is often avoided, sometimes at the cost of precision and reliability of downstream experiments.

The Countess™ Automated Cell Counter compared with the hemocytometer method

Sample preparation

Sample preparation for the Countess™ Automated Cell Counter is similar to that of the hemocytometer, but the procedure is simpler and takes far less time to master. Like the hemocytometer, the instrument provides best results with fresh, homogeneous cell samples. The cells are collected and suspended to a concentration of approximately 1×10^6 cells/mL. An aliquot of cells is mixed with an equal volume of 0.4% trypan blue, and 10 μ L of the mixture is transferred to a non-gridded, disposable Countess™ chamber slide. The slide is inserted into the instrument, and the image that appears on the screen represents the same area as four 1 x 1 mm squares on a hemocytometer. Using the zoom function and fine focusing knob, image adjustments are made to bring the objects into proper alignment. Then, a single touch of a button on the touchscreen starts the automatic process of acquiring and analyzing the image, resulting in readout of total cells/mL, live cells/mL, dead cells/mL, and percent viability. This entire procedure—from sample preparation to results—is performed in less than 1 minute.

Time-to-results

Several replicates can be analyzed on the Countess™ instrument in the time it takes to perform one hemocytometer count (up to 2 minutes). This increases accuracy and confidence in results, because in practice, replicates are often avoided when using tedious manual counting methods.

Accuracy and precision

Accuracy and precision were evaluated for the Countess™ Automated Cell Counter and compared to both glass and disposable versions of the hemocytometer. For this experiment, a standard bead solution of known concentration (9.57×10^5 beads/mL) was used as the starting material for concentration measurements. The beads were mixed 1:1 with 0.4% trypan blue, and triplicate samples were measured using a G-chip disposable hemocytometer (Beckman Coulter) and a Bright-Line hemocytometer (Hausser Scientific).

Statistical significance between the methods was determined using two different tests. An F-test indicated whether differences in variance were significant across all three methods tested, and a 2-sample t-test indicated whether the difference between the Countess™ method and the most accurate hemocytometer method was statistically significant.

The results show that the Countess™ Automated Cell Counter was comparable in accuracy and precision to the disposable hemocytometer. However, the glass hemocytometer method was significantly less accurate, giving higher than expected bead counts (Figure 3). Similar results were obtained with all cell lines tested (data not shown).

Effective concentration range

Additional experiments were performed to test the range of cell concentrations that can be effectively measured using each method. Using 20 different cell lines (Table 1), cells were concentrated, serially diluted to a very low concentration (<1 x 10⁴ cells/mL), and concentrations were measured using the

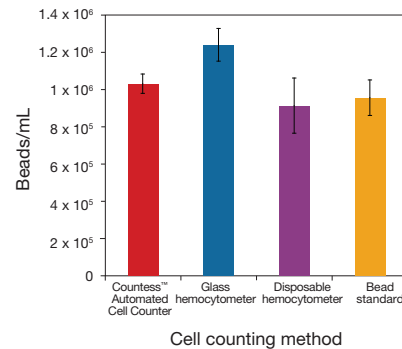


Figure 3—Accuracy and precision of the Countess™ Automated Cell Counter compared to glass and disposable hemocytometer methods. A standard bead solution of known concentration (9.57 x 10⁵ beads/mL, Beckman Coulter) was used as the starting material for counting measurements using the Countess™ Automated Cell Counter, Bright-Line glass hemocytometer (Hausser Scientific), and C-Chip disposable hemocytometer (Digital Bio). The beads were mixed 1:1 with 0.4% trypan blue, and triplicate samples were measured according to each manufacturer's instructions. Accuracy was evaluated by comparing the mean cells/mL measured by each method to the expected concentration of the standardized bead solution (last bar: 9.57 x 10⁵ ± 10% beads/mL). Precision is indicated by the standard deviations; error bars represent one standard deviation. Accuracy and precision are comparable between the Countess™ Automated Cell Counter and the disposable hemocytometer. The glass hemocytometer is significantly less accurate.

Table 1—Cell lines validated on the Countess™ Automated Cell Counter. For more information on our findings, visit www.invitrogen.com/countess.

| Cell type | Vendor number | Animal | Organ |
|--|----------------------|-----------------|----------------------------|
| HEK-293 | ATCC CRL-1573 | Human | Kidney |
| A431 | ATCC CRL-2592 | Human | Skin |
| CHO-M1WT2 | ATCC CRL-1984 | Chinese hamster | Ovary |
| CHSE | ATCC CRL-1681 | Chinook salmon | Embryo |
| COLO-205 | ATCC CCL-243 | Human | Colon |
| COS-7 | ATCC CRK-1651 | African monkey | Kidney |
| HeLa | ATCC CCL-2 | Human | Cervix |
| HepG2 | ATCC CRL-10741 | Human | Liver |
| HL-60 | ATCC CCL-240 | Human | Blood |
| J774A.1 | ATCC TIB-67 | Mouse | Blood |
| Jurkat | ATCC TIB-152 | Human | Blood |
| MCF7 | ATCC HTB-22 | Human | Breast |
| MRC-5 | ATCC CCL-171 | Human | Lung |
| NIH/3T3 | ATCC CRL-1658 | Mouse | Embryo |
| PC-12 | ATCC CRL-1721 | Rat | Adrenal gland |
| SF-21 | Invitrogen 12682-019 | Insect | Ovary |
| U266 | ATCC TIB-196 | Human | Blood |
| U-2 OS | ATCC HTB-96 | Human | Bone |
| K562 | ATCC CCL-243 | Human | Bone marrow |
| Adipocytes | Invitrogen R7788-110 | Human | Adipose-derived stem cells |
| Human aortic smooth muscle cells | Invitrogen C-007-5C | Human | Smooth muscle |
| Human pulmonary artery endothelial cells | Invitrogen C-008-5C | Human | Blood vessel |
| Human pulmonary artery smooth muscle cells | Invitrogen C-009-5C | Human | Smooth muscle |
| Human umbilical vein endothelial cells | Invitrogen C-015-5C | Human | Blood vessel |
| Whole lysed blood | Donor | Human | Blood |

Countess™ Automated Cell Counter and the disposable hemocytometer (Figure 4).

The measurement range of the hemocytometer is effectively one order of magnitude (~2.5 x 10⁵ cells/mL to ~2.5 x 10⁶ cells/mL). This concentration range for manual cell counting is limited by the number of cells that can be reliably counted by the human eye, and by the volume requirements for filling the chamber. By comparison, the effective concentration range for the Countess™ instrument extends to twice the range of the hemocytometer, because this automated method is not subject to the same counting criteria as the hemocytometer, and is not subject to the limitations of the human eye. These results were consistent over the 20 different cell types tested.

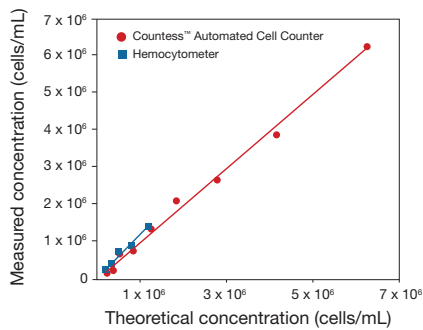


Figure 4—Countess™ data extend further to higher cell concentrations than hemocytometer readings. Cells were concentrated, serially diluted, and measured using the Countess™ Automated Cell Counter and the C-Chip disposable hemocytometer (Digital Bio). Representative data are shown for human adipose-derived stem cells. Data points represent 3 measurements on the Countess™ instrument, and 1 measurement on the hemocytometer. As shown, the Countess™ Automated Cell Counter has a significantly higher effective concentration range than the hemocytometer. This difference is due to the use of digital image capture and computer analysis for counting, as opposed to a reliance on the human eye.

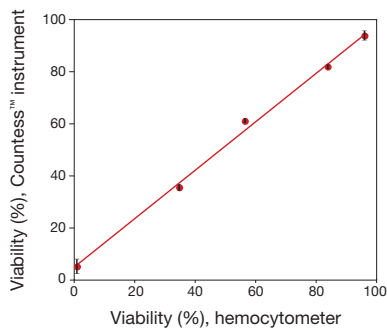


Figure 5—Viability range comparison between the Countess™ Automated Cell Counter and C-chip disposable hemocytometer. Linear correlation plot of percent viability as measured on the Countess™ Automated Cell Counter vs. the hemocytometer. Data points are the mean of three samples using human adipose-derived stem cells. Error bars represent one standard deviation.

Viability range

To test the range of percent viability that can be effectively measured with each instrument, two 5 mL samples of 1 x 10⁶ cells/mL were prepared in buffer, and one sample was heat-treated (60°C) for 1 hour. Five mixtures were then prepared to yield different ratios of untreated:treated cells (100:0, 75:25, 50:50, 25:75, 0:100), and mixed with trypan blue. Cell counts and percent viability were measured on the Countess™ Automated Cell Counter and the hemocytometer.

The viability range that was effectively measured by the Countess™ instrument was highly correlated with that of the hemocytometer; the Countess™ Automated Cell Counter can determine viability from 100% down to 0%, unlike other automated cell counters (Figure 5). For viability measurements using cultured cells at ~1 x 10⁶ cells/mL, the Countess™ instrument reproducibly measured cell viability within a difference of 10% of the C-Chip disposable hemocytometer (Figure 6).

Automated cell counting with accuracy you can rely on

These comparisons demonstrate that the Countess™ Automated Cell Counter is a reliable alternative to the laborious manual hemocytometer counting method. Total cell count and viability results from the Countess™ instrument correlate well with the most accurate disposable hemocytometer method. The Automated Cell Counter provides the additional benefits of faster time-to-results and an easier procedure, and enables the measurement of higher cell concentrations compared to the manual method.

The Countess™ Automated Cell Counter represents an important technological advance in automated cell counting, providing the researcher with high-quality cell count data for significantly less time and effort.

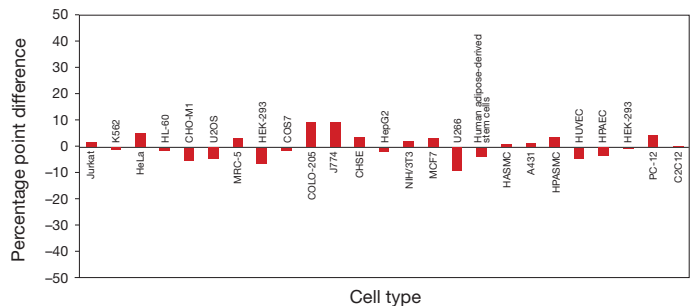


Figure 6—Viability measurement differences across various cell types. The Countess™ Automated Cell Counter consistently measured cell viability within 10 percentage points of the C-Chip disposable hemocytometer for each of the cell types shown.

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