

# Comparison of cell counting instrumentation: Flow cytometry vs. the Countess™ Automated Cell Counter

Many cell-based research studies require the counting of cells prior to beginning an experiment. This allows standardization of cell concentration between samples to minimize error and variation in downstream results. Several different cell counting instruments are available:

- Glass and disposable hemocytometers
- Flow cytometers
- Benchtop counters

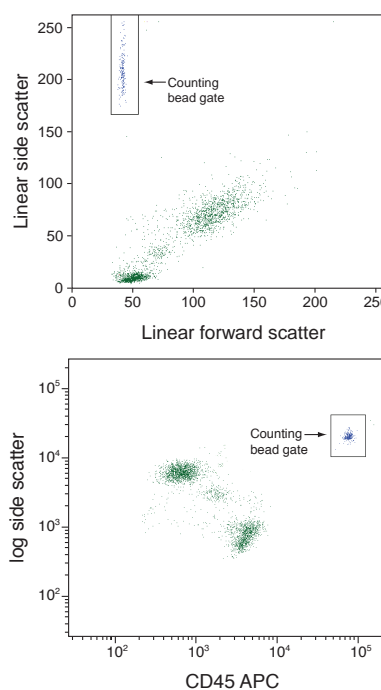
This analysis compares the flow cytometry cell counting method to the benchtop Countess™ Automated Cell Counter, examining techniques, accuracy, and precision. We demonstrate that both methods provide reliable and reproducible results, but the Countess™ instrument is less expensive and does not require additional maintenance or data processing.

## Flow cytometry method of cell counting

Flow cytometry allows rapid enumeration of objects. Flow cytometry instruments incorporate three main components into sample movement and analysis: fluidics to create movement, optics to excite and detect fluorochromes, and electronics to amplify and display fluorescence signals. The fluidics system positions objects using air or fluid into a single-file line from the sample container to the machine and into the path of the laser. The laser, tuned to a specific wavelength, excites the fluorochrome, which releases photons that pass through dichroic and emission filters to a photomultiplier tube (PMT). There, the signal is amplified and output signals are displayed.

Object counting on the flow cytometer is conducted by using a standardized internal bead control and calculating the ratio of beads to objects in the sample, or by analyzing an exact volume of sample and calculating the number of objects per volume. The objects may be stained with fluorescent dyes or labeled with fluorochrome-tagged antibodies to provide additional differentiation. Objects also may be differentiated by size and

internal granularity. These distinguishing features allow the operator to identify different populations of objects (e.g., beads vs. cells, or CD45<sup>+</sup> vs. CD45<sup>-</sup>) and to select those specific regions for analysis. Calculated output may be expressed as total objects/mL or a specific subset of objects/mL, depending upon the differential staining or labeling (Figure 1).



**Figure 1. Counting bead gating with no-wash/lysed whole blood and CD45 gating.** Peripheral blood lysed with Cal-Lyse™ Lysing Solution was stained in a no-wash assay format with anti-CD45 allophycocyanin before adding CountBright™ Absolute Counting Beads. A plot of CD45-positive cells vs. logarithmic side scatter shows the counting bead gate.

Flow cytometry counting is advantageous in terms of the speed of analyzing a statistically relevant sample size (>10,000 objects), the number of replicates that may be analyzed, and the accurate measurements obtained by an experienced user. Disadvantages include the up-front cost of an instrument, maintenance and experimental setup, object identification and gating, software interface and data analysis features, and overall time to results.

### 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 concentration, 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 2).

Sample preparation for the Countess™ Automated Cell Counter is similar to that of a hemocytometer. The cells are collected and suspended to a concentration of approximately  $1 \times 10^6$  cells/mL, an aliquot is mixed with an equal aliquot of 0.4% trypan blue, and 10  $\mu$ L of the mixture is transferred to a non-gridded disposable Countess™ Chamber Slide. The slide is inserted into the Countess™ instrument, and image adjustments are made using the zoom function and fine focusing knob to bring the cells into proper focus. Then, a single touch of a button on the touch screen starts the automatic process of acquiring and analyzing the image, resulting in a readout of total cells/mL, live cells/mL, dead cells/mL, and percent viability. This entire procedure—from mix to result—is performed in under a minute.

The advantages of the Countess™ Automated Cell Counter over a flow cytometry system are the imaging-based object identification mode and rapid time-to-results without needing an expensive, maintenance-intensive instrument (the Countess™ instrument costs less than \$5,000). Instead of dots on a computer screen generated by a flow cytometer, the Countess™ instrument displays an image of the cells. The Countess™ instrument needs no maintenance or daily calibration/validation, and immediately provides answers without requiring the user to identify specific populations, perform gating, download statistics, or perform calculations.

### Instrument comparison

To compare performance, cell counting, and viability measurements between the flow cytometer and the Countess™ Automated Cell Counter, experiments were set up using a Becton Dickinson BD FACSCalibur instrument with CountBright™ Absolute Counting Beads, and the Countess™ instrument. Untreated and heat-treated K562 cells were mixed at different ratios to obtain theoretical 100%, 75%, 50%, 25%, and 0% percent viability.

Measurements were obtained on the Countess™ Automated Cell Counter according to the manufacturer's instructions. For flow cytometry, 500  $\mu$ L of each sample was mixed with 50  $\mu$ L of counting beads and 1  $\mu$ L of 1 mg/mL propidium iodide (PI) and analyzed on the BD FACSCalibur instrument. A tube containing only beads identified the bead population. Regions were drawn around PI-positive and PI-negative cell-objects and another region around the bead population. Final concentrations and percent viability were calculated using the bead concentration value of  $5.18 \times 10^5$ /50  $\mu$ L and the equation shown in Figure 3.



**Figure 2. 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 eyestrain, and minimizes subjective judgments that can lead to error. It takes 3 simple steps: **(A)** Mix 10  $\mu$ L of sample with 10  $\mu$ L of trypan blue, and pipet into a Countess™ Chamber Slide. **(B)** Insert slide into the instrument. **(C)** Press the "Count cells" button, and results are displayed in 30 seconds.

Figure 4 compares the cell counts obtained by the flow cytometer and Countess™ instrument. Cell counts were within one standard deviation, and there was no significant difference between the counting measurements (two-tailed p-value of 0.723). Figure 5 shows a correlation of viability data for five data points along a viability curve. The data demonstrate a very strong correlation in viability readings between the two instruments (R<sup>2</sup> value of 0.9984).

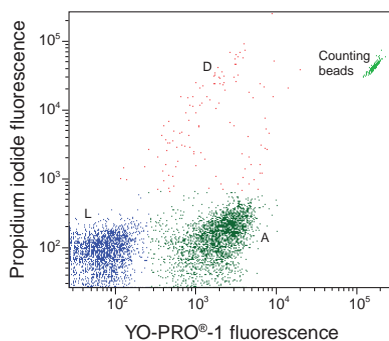
### Summary

The flow cytometer and Countess™ Automated Cell Counter both provide reliable and reproducible counting and viability measurements. The differences between the two methods lie primarily in instrument cost and the additional maintenance and data processing required for the flow cytometer. Flow cytometers are expensive (>\$30,000), require sample controls for data interpretation, data processing and calculation steps, and more consumables including counting beads, sheath fluid, and disposable sample tubes. The Countess™ Automated Cell Counter, on

**Calculation (example):** A 1,000 µL volume of cells was stained, and 50 µL of CountBright™ Absolute Counting Beads was added.

$$\frac{1,700 \text{ cells}}{1,030 \text{ beads}} \times \frac{49,500 \text{ beads}}{1,000 \mu\text{L}} = 81.7 \text{ cells}/\mu\text{L}$$

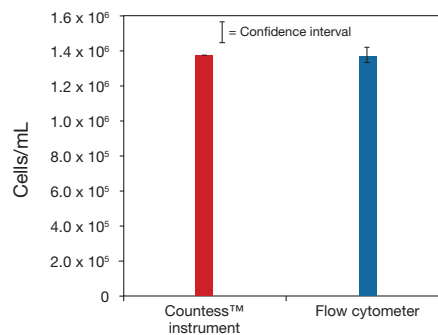
**Note:** The calculation should be corrected if the sample is diluted or if a different volume of CountBright™ beads is used.



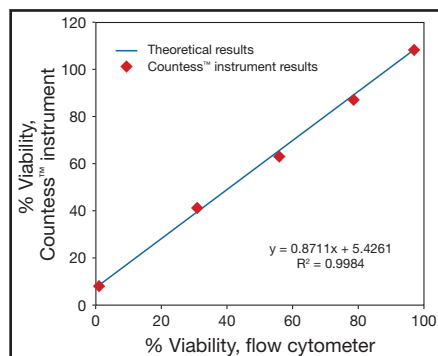
**Figure 3. Flow cytometry cell counting.** Samples were mixed with counting beads and 1 µL of 1 mg/mL propidium iodide (PI) and analyzed on the BD FACSCalibur instrument. A tube containing only beads identified the bead population. Regions were drawn around PI-positive and PI-negative cell-objects and another region around the bead population. Final concentrations and percent viability were calculated using the equation shown.

the other hand, is less expensive than flow cytometers (<\$5,000), and does not require daily maintenance or additional data processing. Sample preparation is less cumbersome, and fewer consumables (only slides and trypan blue) are needed. Skilled operators are also not necessary. The two instruments may actually complement each other; the Countess™ instrument may be used for rapid cell counting and sizing prior to setting up a flow cytometry experiment. The Countess™ instrument may replace the hemocytometer or Coulter counter and become the instrument of choice to count and standardize cell concentrations to the typical 100,000 cells/tube for a flow cytometry experiment.

Learn more about the Countess™ Automated Cell Counter at [www.invitrogen.com/countess](http://www.invitrogen.com/countess).



**Figure 4. Cell count comparison between the Countess™ Automated Cell Counter and flow cytometer.** Cell counts were within one standard deviation, and no significant difference is observed between the two methods (two-tailed p-value of 0.723).



**Figure 5. Correlation of viability data between the Countess™ Automated Cell Counter and flow cytometer.** The data demonstrate a very strong correlation in viability readings between the two instruments (R<sup>2</sup> value of 0.9984).

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