

Comparison of automated cell counting methods: Countess™ Automated Cell Counter vs. the Coulter counter

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. There are several different counting methods and instruments available to accomplish this task, including glass and disposable hemocytometers, flow cytometers, and benchtop counters.

This technical note compares the Countess™ Automated Cell Counter to the Beckman Coulter Z1™ Series Coulter Counter® Cell and Particle Counter, examining methodology and performance. The Countess™ method provides comparable performance to the Coulter counter method, while offering the following additional benefits:

- Easier methodology, setup, and maintenance
- Provides cell viability and size data in addition to cell counts
- Actual results are visualized, increasing confidence
- More affordable

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 1).

The Coulter Principle of cell counting

The Coulter counter instrument was originally developed as a means to count blood cells rapidly. The instrument uses the Coulter Principle for particle counting, also known as the Electrical Sensing Zone method. This sensing method works by using two electrodes, one in a tube with a specific aperture size, and another outside the tube and immersed in a beaker containing the particles in a low concentration electrolyte solution. An electrical current path is created by the electrolyte solution when an electrical field is applied, and the impedance between the

two electrodes is measured; this creates the “sensing zone”. As the particles pass through the aperture, they cause a short-term change in the impedance; this change is measured as a voltage pulse and the pulse height is proportional to the volume of the electrolyte solution displaced by the particle. The particles are counted, and if a controlled volume of liquid passes through the aperture, particle concentration can be calculated.

In comparison to manual cell counting, the use of the Coulter Principle is advantageous in terms of its speed, accuracy, precision, and size gating. However, upfront cost of the instrument, time-consuming instrument maintenance and setup, and lack of a viability measurement are disadvantages of Coulter Principle using instruments such as the Beckman Coulter Z1™ Series Coulter Counter® Cell and Particle Counter.

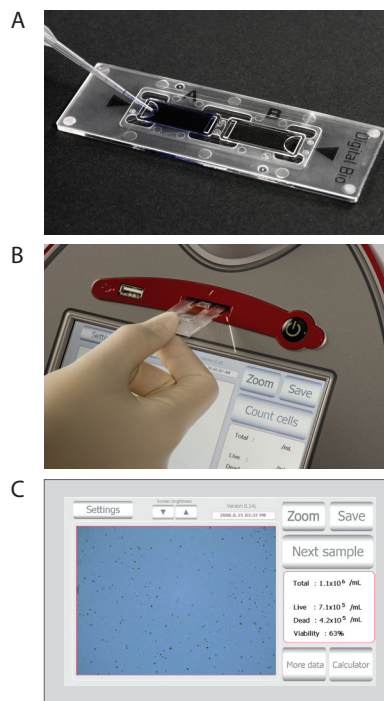


Figure 1—Countess™ Automated Cell Counter. The Countess™ Automated Cell Counter eliminates the tedium and subjectivity of manual cell counting. Automated counting frees up your time, reduces eye strain, and minimizes subjective judgments that can lead to error. It takes 3 simple steps: (A) Mix 10 μ L 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.

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 1×10^6 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.

The Countess™ Automated Cell Counter compared with the Coulter method

Obtain viability data

Due to the nature of the electrical sensing process used by the Coulter counter instrument, the method is unable to produce a viability measurement. By comparison, the Countess™ Automated Cell Counter uses an image analysis system that offers the added benefit of distinguishing live cells from dead cells. The instrument identifies objects in the field as cells based on their similarity to the average size of all the objects in the image, and their degree of circularity. Live cells are then identified as objects with bright centers and dark edges, whereas dead cells are objects that are dark throughout. This image analysis system is similar in principle to manual counting methods, but with the added advantages of minimizing labor and eliminating the potential bias of human interpretation.

Visualize results and increase confidence

Because the Coulter counter does not use image-based technology to count cells, results cannot be visualized as an image. The Countess™ instrument generates an image of the actual cells, increasing the researcher's confidence in 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.

The Coulter counter also requires an assumption about the size of particles in a sample to determine the appropriate size-gating parameters for counting. Because it only counts particles, clumps of cells can only be estimated. In contrast, the Countess™ instrument does not require size-gating and has a much more sophisticated method of counting cells in clumps.

See Table 1 for a full comparison between the Countess™ and Coulter counter methods of cell counting.

Faster, easier cell counting without sacrificing performance

To compare performance between the Coulter counter instrument and the Countess™ instrument, K562 cells were counted using a Beckman Coulter Z1™ Series Coulter Counter® Cell and Particle Counter and the Countess™ Automated Cell Counter.

Measurements were obtained from replicate samples with the Countess™ Automated Cell Counter according to the manufacturer's instructions. Replicate samples were prepared for analysis on the Coulter counter instrument by diluting 100 μ L of K562 cells into 10 mL of Isoton® II Diluent, mixing gently, and placing the vials into the instrument. The appropriate dilution factor and threshold were set. Readouts for both instruments were expressed in total cells/mL.

Table 1—Comparison between the Countess™ Automated Cell Counter and Coulter counter methods of cell counting.

Feature	Countess™ Automated Cell Counter	Coulter counter
Viability data	Provided	None provided
Cell size data	Provided	None provided
Visualize results	Provided	None provided
Setup	Easy	Difficult
Calibration/maintenance	None required	Required daily/monthly

In terms of performance, the two instruments gave similar results (Figure 2). Cell counts were within one standard deviation, with no significant difference between them (two-tailed p-value of 0.297). Both methods are highly accurate and reproducible, but the Countess™ Automated Cell Counter has the added benefits shown in Table 1.

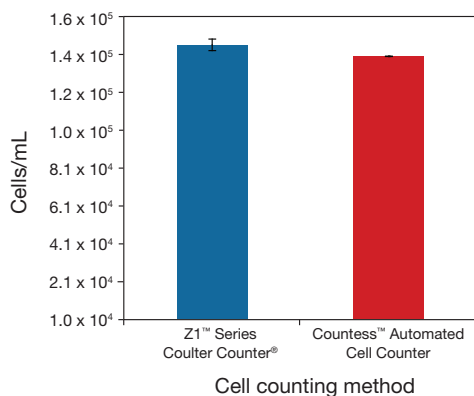


Figure 2—Equivalent results between Countess™ Automated Cell Counter and Coulter counter methods of cell counting. K562 cells were counted using the Beckman Coulter Z1™ Series Coulter Counter® Cell and Particle Counter, and the Countess™ Automated Cell Counter. Cell counts from replicate samples were obtained on the Countess™ Automated Cell Counter according to the manufacturer’s instructions. Replicate samples were prepared for analysis on the Coulter counter instrument by diluting 100 µL of K562 cells into 10 mL of Isoton® II Diluent.

Automated cell counting with accuracy you can rely on

While the Coulter counter and the Countess™ Automated Cell Counter both give reliable and reproducible cell counts, the imaging-based object identification method used by the Countess™ instrument enables viability determination and rapid time-to-results without requiring an expensive, maintenance-intensive instrument.

Since the Countess™ Automated Cell Counter is an imaging-based instrument, the user can rely on familiar counting methods and can see the cells counted, providing a higher level of confidence in the results. In addition, the Coulter counter can be quite labor-intensive, requiring daily QC as well as monthly cleaning to ensure all parts are in working order and measurements are reliable. The Countess™ Automated Cell Counter requires only occasional updating, including maintenance of the most current version of firmware, and the setting of parameters for the specific lot or dye used for viability.

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 cost and effort.

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