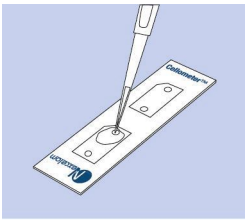
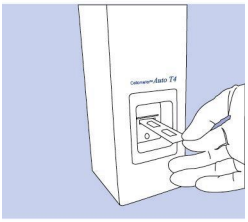
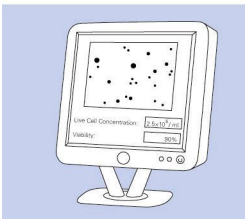
Application Notes No. 06001







Cellometer™ Auto T4: Technical Information

Summary

Cellometer™ Auto T4 is an imaging-based automatic cell counting system. This application note provides some details on methods of using the Cellometer™ system, and shows images and counting data to illustrate typical applications. Viability determination is based on trypan blue dye exclusion. Cell concentration is determined automatically based on total cell count and dilution factor. Comparison with traditional manual counting method using a hemacytometer shows same results for cell concentration and viability.

Introduction to Cellometer™ Auto T4

Cellometer™ Auto T4 is an imaging instrument that acquires cell data from multiple locations of Cellometer™ disposable counting chambers. It is connected to a computer via USB 2.0 cable. Auto T4 software automatically analyzes acquired cell images and measures cell concentration and viability.

Simplicity and compact size make this system an attractive product for automating cell counting operation. By using convenient cell type settings, the user can perform cell counting with high level of repeatability and accuracy. High quality disposable counting chambers, made of plastic materials, allow easy handling and disposal. Minimal sample amounts are used for counting to a total number much higher than hand-count, thus reducing counting error. Crisp and intuitive graphic user interface assists simple operation.

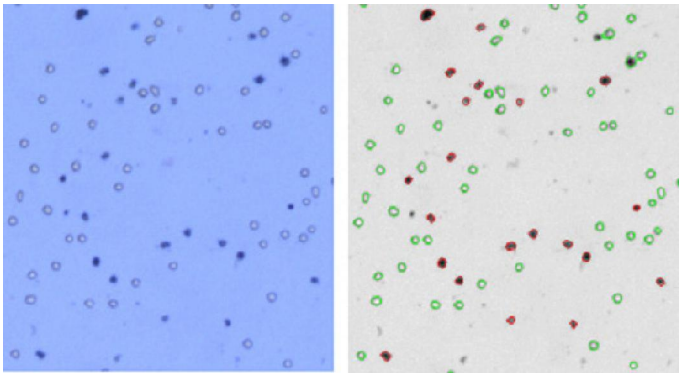


Figure 1. Images of hybridoma cells from Cellometer™ Auto T4. (a) Image of hybridoma cells stained with trypan blue in the Cellometer™ disposable counting chamber. This image shows the cell morphology for observation prior to the automatic counting. (b) Image of the same area as in (a) after counting. The red circles indicate dead cells and the green circles indicate live cells.

Cell Counting Procedure

There are three simple steps for cell counting with Cellometer™ Auto T4.

Step1: Pipette cells into Cellometer™ disposable cell counting chamber.

Step 2: Insert the disposable counting chamber into AutoT4 instrument.

Step3: Inspect cell images; determine concentration and viability automatically.

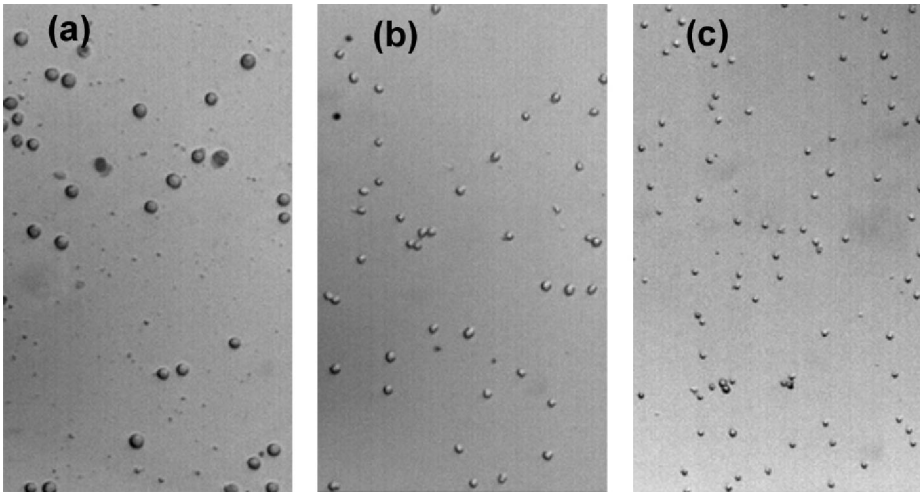


Figure 2. Typical images of large, medium, and small mammalian cells. Estimated diameters for these cell sample, as determined by Cellometer™ Auto T4, are approximately (a)16 µm, (b)10 µm and (c)6 µm, respectively.

For measuring total cell concentration, cells may be suspended in regular growth media or PBS. No special treatment or reagent is necessary. For detemining cell viability, cells need to be suspended in diluted trypan blue solution prior to be loaded into Cellometer™ disposable counting chamber.

Required sample volume is 15 to 20 microliters for each count.

By using disposable counting chambers, no washing step is involved after each sample. In addition, since sample is completely contained within the chamber, there is no cross-contamination.

Total Cell Count: Comparison to Hemacytometer

Figure 3 depicts a typical comparison study between manual hemacytometer and Cellometer™ Auto T4. The data indicates that same cell concentrations result from these two measurement techniques.

SKBr-3 cells were suspended in media. For hemacytometer measurements, cells in all four-corner squares were counted. The following formula was used to determine concentration.

Cell concentration (cells/ml)
= (total cell count / 4) x 10^4

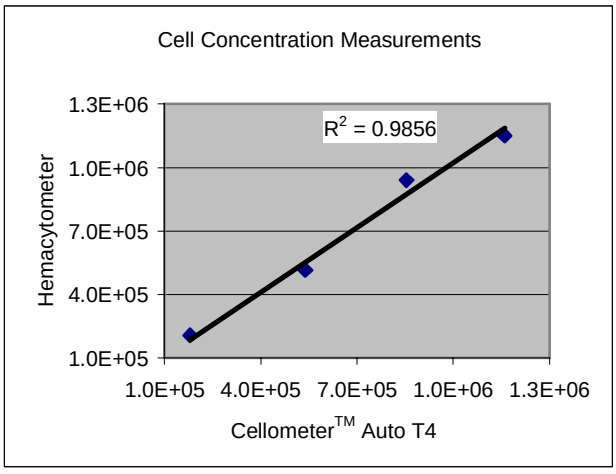


Figure 3. Comparison of SKBr-3 cell concentrations measured by hemacytometer and Cellometer™ Auto T4.

Viability: Comparison to Hemacytometer

Figure 4 shows results from a viability comparison study. Three samples of mouse B cells with difference viability were measured using hemacytometer and Cellometer™ Auto T4. The viability ranged from approximately 27% to 87%.

The results indicate that viability data measured by both methods are the same over a wide range of cell conditions.

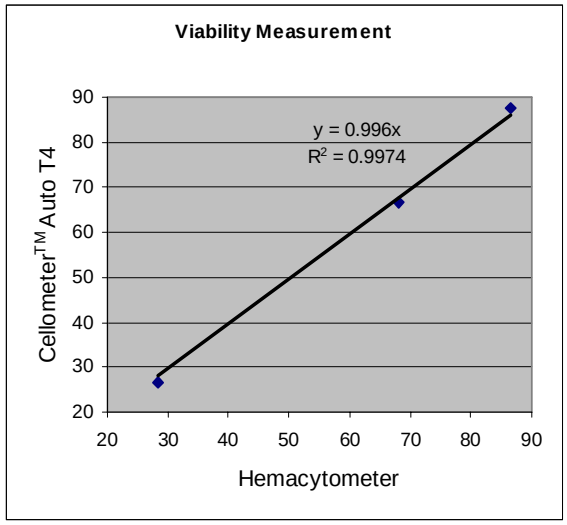


Figure 4. Comparison of primary mouse B-cell viability measured by hemacytometer and Cellometer™ Auto T4.

Heterogeneous Cell Analysis

Cellometer™ Auto T4 can also be used to perform heterogeneous cell analysis. The software algorithms allow specification of multiple parameters related to cell measurements. Based on size, shape, and contrast, cells of different types can be counted in a complex sample. Figure 5 shows an example of human PBMC cells with a background of a large number of platelets. In this case, PBMC cells are identified by size and counted, while platelets are excluded.

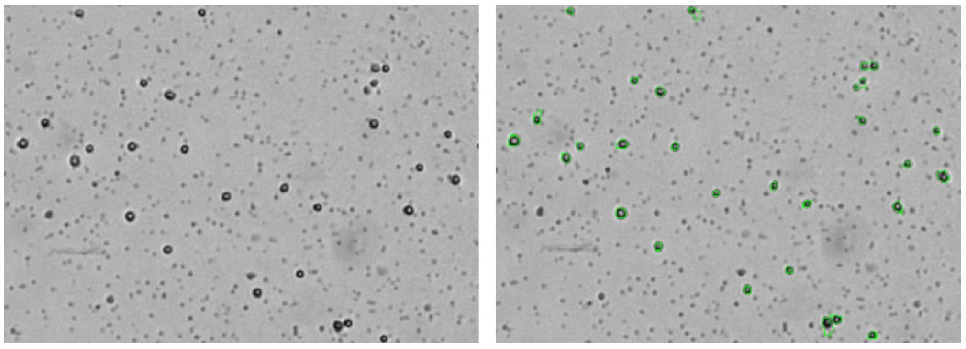


Figure 5. Human PBMC with large numbers of platelet background. Image (A) is the raw image, while image (B) shows the counting PBMC cells as indicated by green circles.

Instrument Specifications

Weight	9.0 lb
Size	3.5” x 4.0” x 12”
Wall adaptor	100-120 AC, 50-60 Hz, 0.5A
Input to instrument	12 V DC1.5 A Max

Computer Requirements

- Windows XP
- 2.4 GHz or higher speed processor
- 512 MB Memory
- USB 2.0 Port

Order Information

Model #	Description	Unit
Auto T4	Cellometer™ automatic cell counting system, with operating software, power supply and USB cable	each
CHT4-003	Cellometer™ all plastic, disposable cell counting chamber-slide for use with AutoT4 instrument only. (2 counting chambers/slide)	1000 counts (500 slides) / case