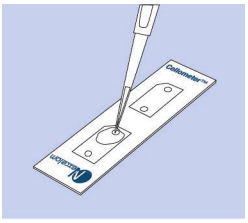
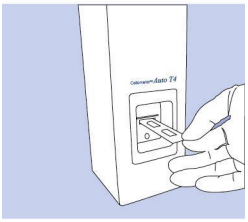
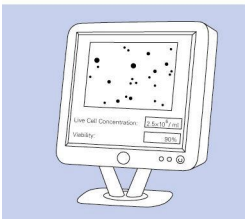
Application Notes No. 06003







PBMC Counting by Cellometer™ Auto T4

Summary

Cellometer™ Auto T4 is routinely used for counting peripheral blood mononuclear cells (PBMC). This application note provides some details on methods of using the Cellometer™ system, and shows images and counting data to illustrate a typical application. Both fresh and frozen PBMC samples can be counted. Interference due to platelet background in fresh PBMC is eliminated with Auto T4 cell counting software. Viability determination is based on trypan blue dye exclusion. Cell concentration is determined automatically based on total cell count and dilution factor.

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 a USB cable. Auto T4 software automatically analyzes acquired cell images and measures cell concentration and viability.

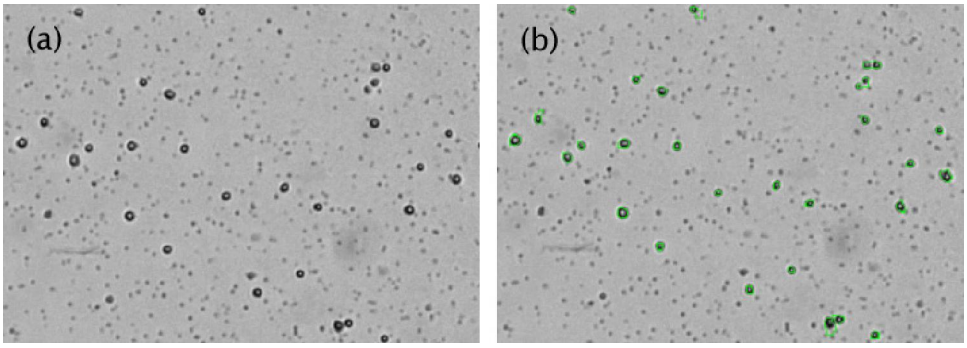


Figure 1. Fresh Human PBMC with a background of platelets. Image (A) is the raw image, and image (B) shows the counted PBMC cells as indicated by green circles.

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

Step1: Pipette 20 µl 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.

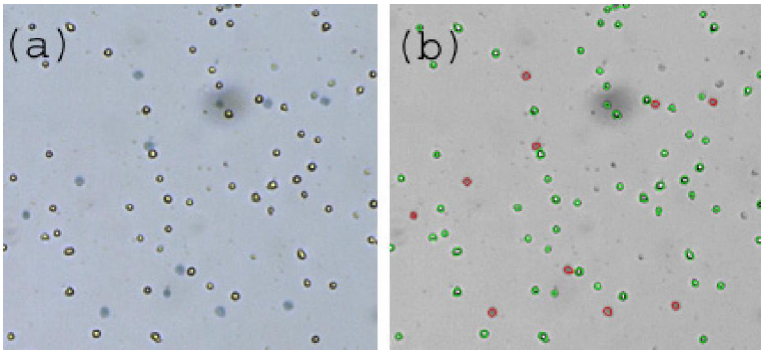


Figure 2. Frozen human PBMC with diluted with trypan blue. Image (A) is the raw data, and image (B) shows the counted PBMC cells as indicated by green circles and dead cells indicated by red circles.

Images of Fresh and Frozen PBMC

Fresh and previously frozen human PBMC samples are different in terms of their background. Fresh samples typically contain platelets, while the previously frozen samples typically have more dead cells. Figure 1 shows an example of fresh human PBMC cells with a background of platelets. In this case, PBMC cells are identified by size and counted, while platelets are excluded. Figure 2 shows images from PBMC cells that have been frozen. With trypan blue, live cells and dead cells are clearly differentiated. Dead cells are identified and indicated by red circles.

Red Blood Cell Exclusion

In some PBMC samples, there are some red blood cells left after sample preparation. Cellometer™ Auto T4 has been used to exclude red blood cells during cell counting. In Fig. 3, imaging analysis parameters for PBMC cell type are set up to exclude the red blood cells, which are indicated by white arrows.

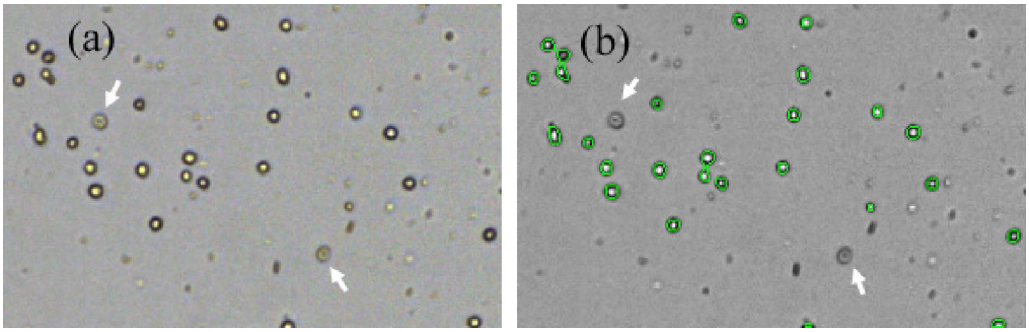


Figure 3. Red blood cell exclusion. (a) Raw data image. (b) The same image with green circle indicating counted PBMC. The white arrows point to the excluded red blood cells.

Plot Cell Concentration versus Sample Dilution

Figure 4 shows data of a set of PBMC dilution measurements. A total of 5 two-fold dilutions are used to generate the plot. The result shows excellent linearity for concentration versus sample dilution.

Procedure:

1. Prepare cell sample: fresh PBMC.
2. Dilute samples with PBS at different dilution factors.
3. Pipette 20 µl of solution into sample introduction port of a disposable counting chamber.
4. Insert loaded counting chamber into Auto T4.
5. Run Auto T4 software to obtain cell concentration results.

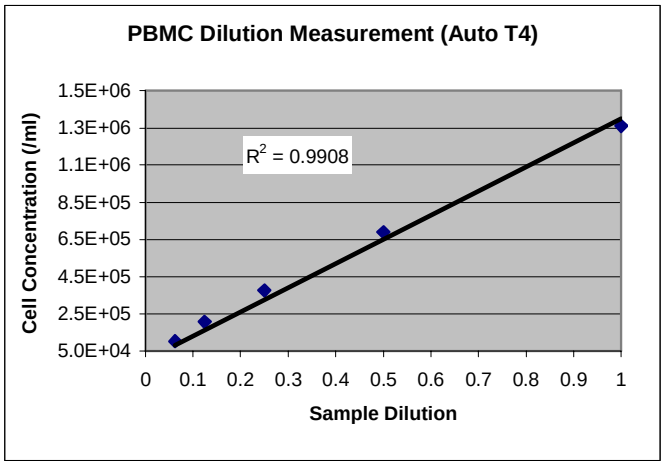


Figure 4. Cell concentration measurements for a PBMC sample dilution series.