

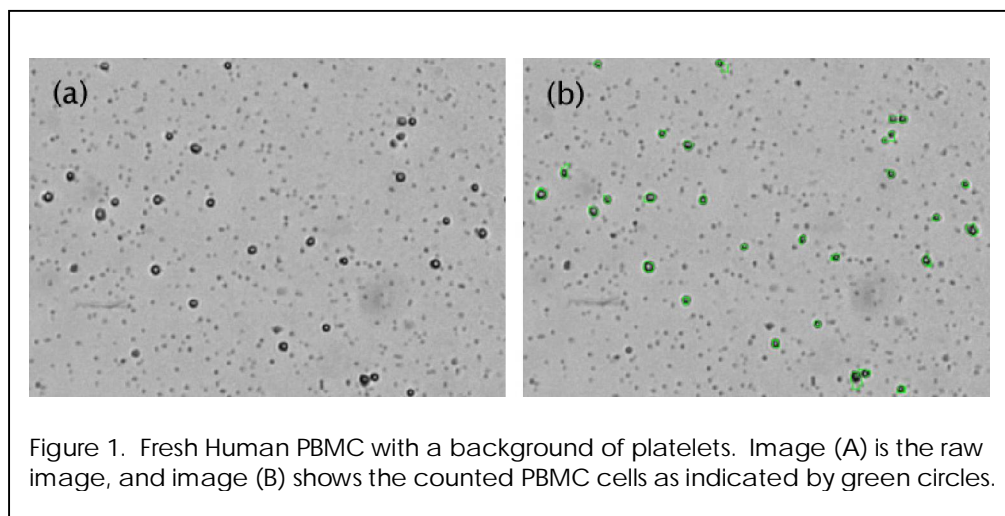
## Counting Cells for Immunology Using Cellometer™ Auto T4 Cell Counter

### Summary

Cellometer™ Auto T4 is routinely used for counting cells used in immunology research. This application note provides some details on methods of using the Cellometer™ system, and shows images and counting data to illustrate a typical application. Viability determination is based on trypan blue dye exclusion. Cell concentration is determined automatically based on total cell count and dilution factor. Cell size exclusion is used to count mature DC cells cultured from PBMC.

### 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.



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.

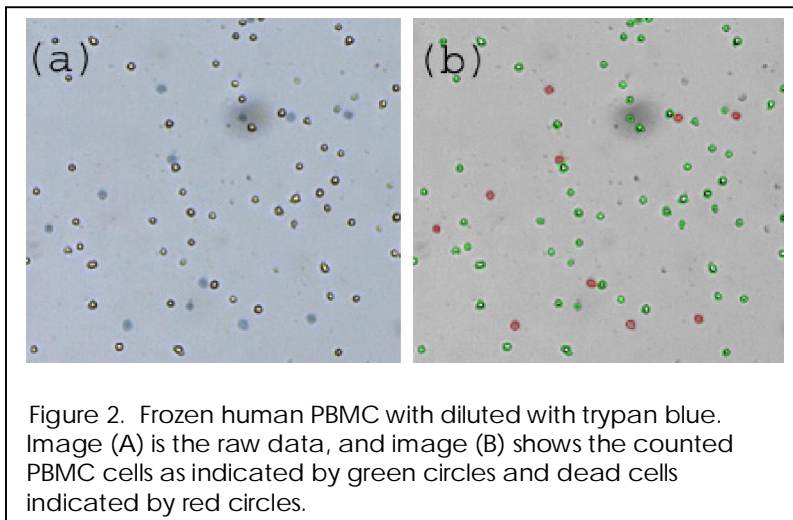


Figure 2. Frozen human PBMC with diluted with trypan blue. Image (A) is the raw data, and image (B) shows the counted PBMC cells as indicated by green circles and dead cells indicated by red circles.

## Measure 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.

## Image of Cells Used in Immunology Research

Figure 3 shows images and counting data for activated T cell, macrophage, and mouse lymphocyte. Activated T cells exhibit irregular shapes. Mouse macrophage cells cluster together. For both special situations, the Auto T4 cell counting software is able to identify individual cells successfully.

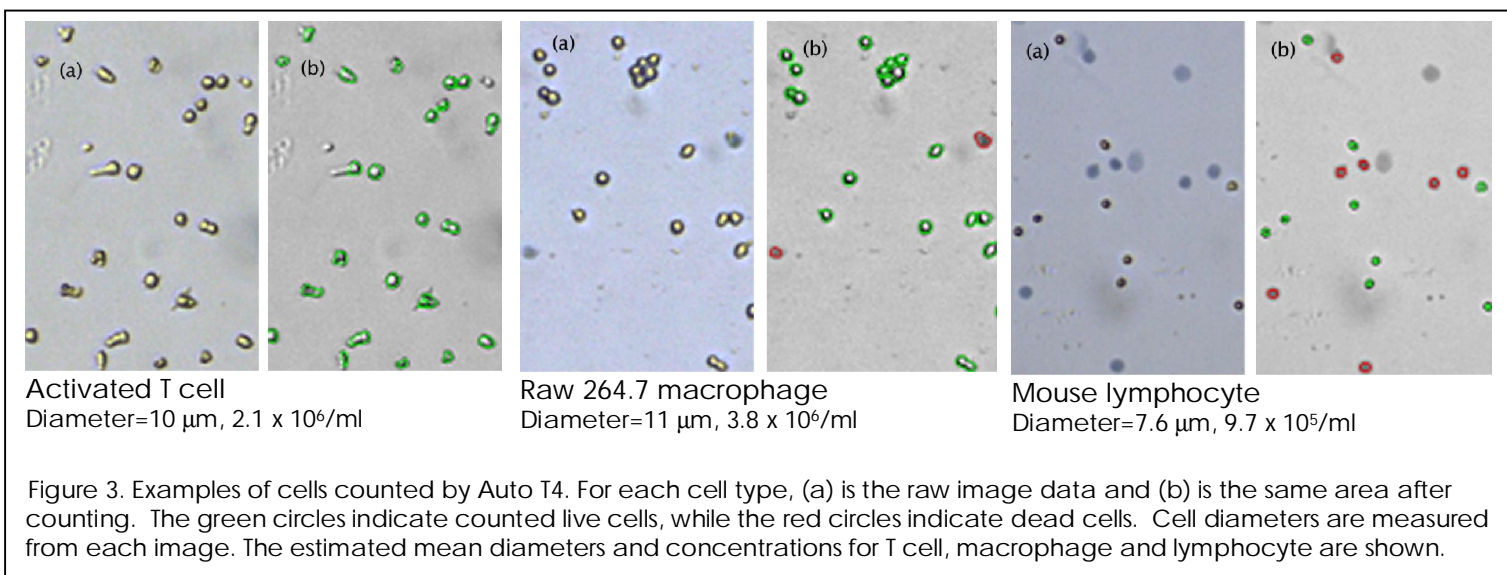
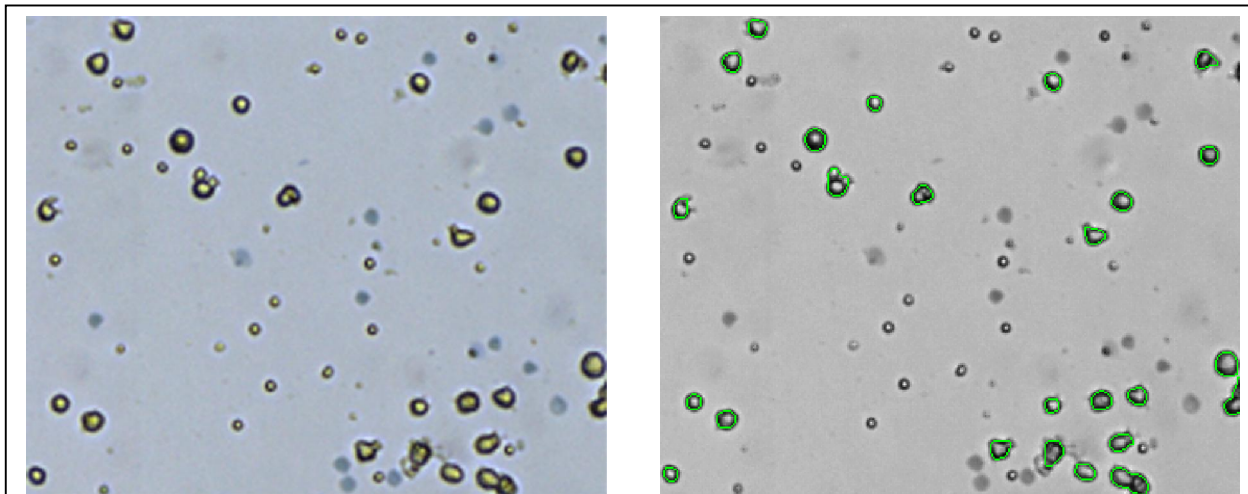


Table 1. List of cells counted for immunology research using Cellometer™ Auto T4.

T cell_CD4	T cell_CD8
B cell	Activated T cell
Fresh human PBMC	Fresh cyno PBMC
Mouse lymphocyte	Mouse thymocyte
Mouse marrow	Fish kidney marrow
Macrophage	Monocyte
NK cells	Mast cell
DC	Mature DC cultured from PBMC

## Count Cells in a Heterogeneous Sample



(a) Raw image of mature DC cells mixed with other cell types, smaller in size.

(b) Counted mature DC cells indicated by green circles.

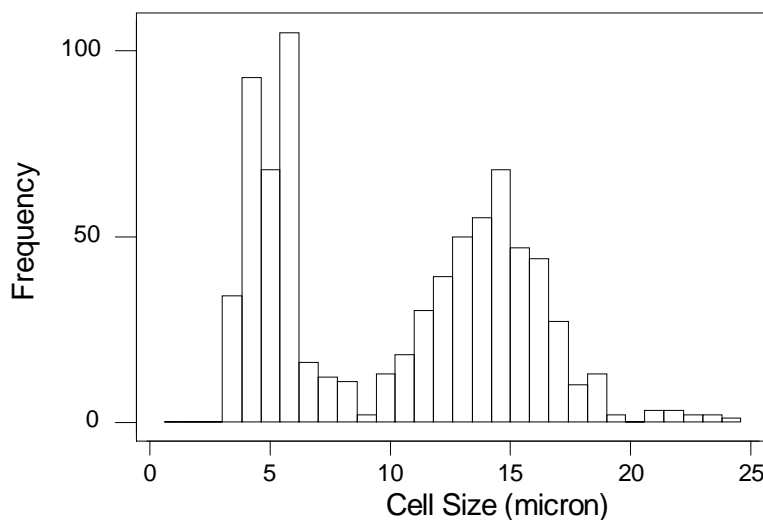


Figure 4. Images and cell size distribution measured by Auto T4.