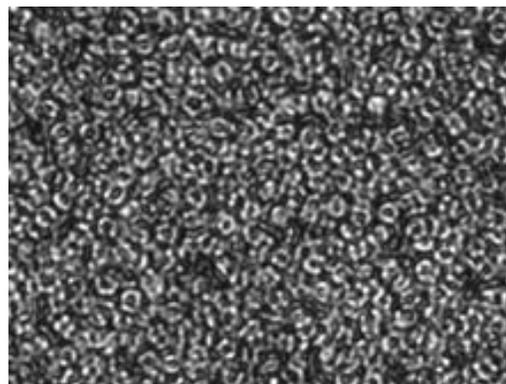


APPLICATION FOCUS: CELLOMETER VISION

Direct Count of White Blood Cells from Peripheral Blood Sample without Lysing Red Blood Cells



Brightfield image of whole blood. WBC's are not visible amongst RBC's



Fluorescent image showing AO stained WBC's. RBC's are not visible.

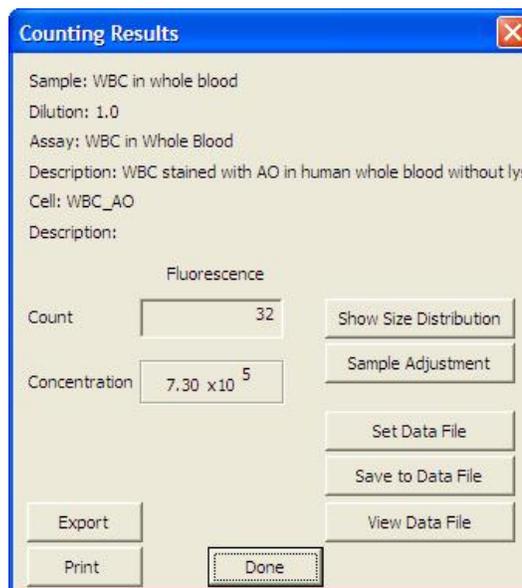
INTRODUCTION

Cellometer incorporates image based cell counting and fluorescence detection in a compact and easy-to-use instrument. With fluorescence detection capabilities, Cellometer Vision is an ideal solution for many complex cell population characterization assays such as rapidly counting white-blood-cells in whole blood.

As one of the most important cellular components in peripheral blood, the concentration of white blood cells is routinely used in researching various diseases, such as bacteria/virus infections, leukemia, or leucopenia. However, conventional methods to count white blood cells include a time consuming and tedious RBC lysis process.

Vision can be used to simplify traditional methods by mixing a cell membrane permeable DNA dye (acridine orange) with diluted whole blood sample that specifically stains white blood cells. To quantitatively analyze WBCs, we simply pipette 20µl of treated sample into a Cellometer Disposable Counting Chamber and place the chamber into the instrument. Since the counting chamber is disposable, no washing is required between samples, there is no risk of cross contamination of different samples, and the risk of exposure to biohazard materials is reduced.

Using proprietary algorithms, Cellometer Vision's robust operating software accurately analyzes cell images, and generates counting data in less than 60 seconds typically. Cell images and all analysis data, including cell size distribution histograms, can be saved for documentation. Data can also be easily exported to Microsoft Excel spreadsheets for further analysis.



Counting results box displays fluorescence cell count & concentration.

METHOD

Direct AO labeling of white blood cells:

1. Collect 1 drop of peripheral blood sample (20-50 μ l).
2. Take 20 μ l of blood sample and dilute the sample with 180 μ l of PBS.
3. Apply 5 μ l of Acridine Orange solution to 100 μ l diluted blood sample (final concentration 2-10 μ g/ml).

Running Assay:

1. Load 20 μ l of labeled sample into the Cellometer disposable counting chamber.
2. Insert chamber into Cellometer Vision.
3. Select assay from drop-down menu.
4. Enter Sample ID for this sample.
5. Preview cell images and click 'Count' to begin analyzing sample.
6. Review images and counting results.
7. Save or Export images and/or report data.

Step 3: Choose Assay from drop down menu

Step 4: Enter Sample ID

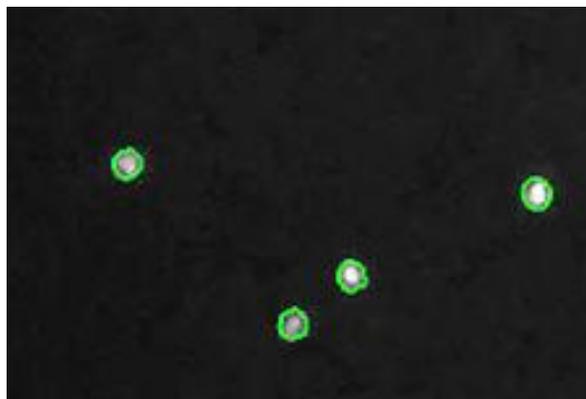


Figure 1. Fluorescent image showing counted AO stained WBC's. RBC's are not visible.

	A	B	C	D	E
1					
2	Sample Id	WBC AO 2.5 ug per ml			
3		11/28/2008 10:04			
4					
5	Assay Type	WBC AO			
6	Description	FL Only			
7	Cell Type	WBC AO			
8	Description	small size cells			
9					
10					
11	Fluorescent Field				
12					
13	Cell Count	203 cells			
14					
15	Concentration	3.64E+06 cells/ml			
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Figure 2. Example of data output to Excel.

Figure 3. All counting results can be instantly saved to a data table.

RESULTS

AO Stained WBC's are clearly visible in the acquired fluorescence image (Figure 1). The software indicates counted cells with a green circle (enlarged to show detail). Results display indicates fluorescence cells counted and automatically calculates concentration.

Cell and fluorescent size distribution histograms, scatter plots and data files can also be instantly generated. All experimental data can be instantly transferred to an Excel spreadsheet (Figure 2) or saved in a data table (Figure 3).

CELLOMETER Vision Trio SPECIFICATIONS:

Imaging Modes: Brightfield & 2 Fluorescence Channels

Filter Set 101: Excitation/Emission Peak: 475nm/535nm

Filter Set 202: Excitation/Emission Peak: 525nm /595nm

Dimensions: 6" x 8.5" x 14" (15cm x 22cm x 36cm)

Weight: 25lbs (11kg)

PC Specs: WinXP/1.8+GHz/1GB RAM/laptop included