Concentration and viability of PBMCs without lysing.

Importance of accurate PBMC counts

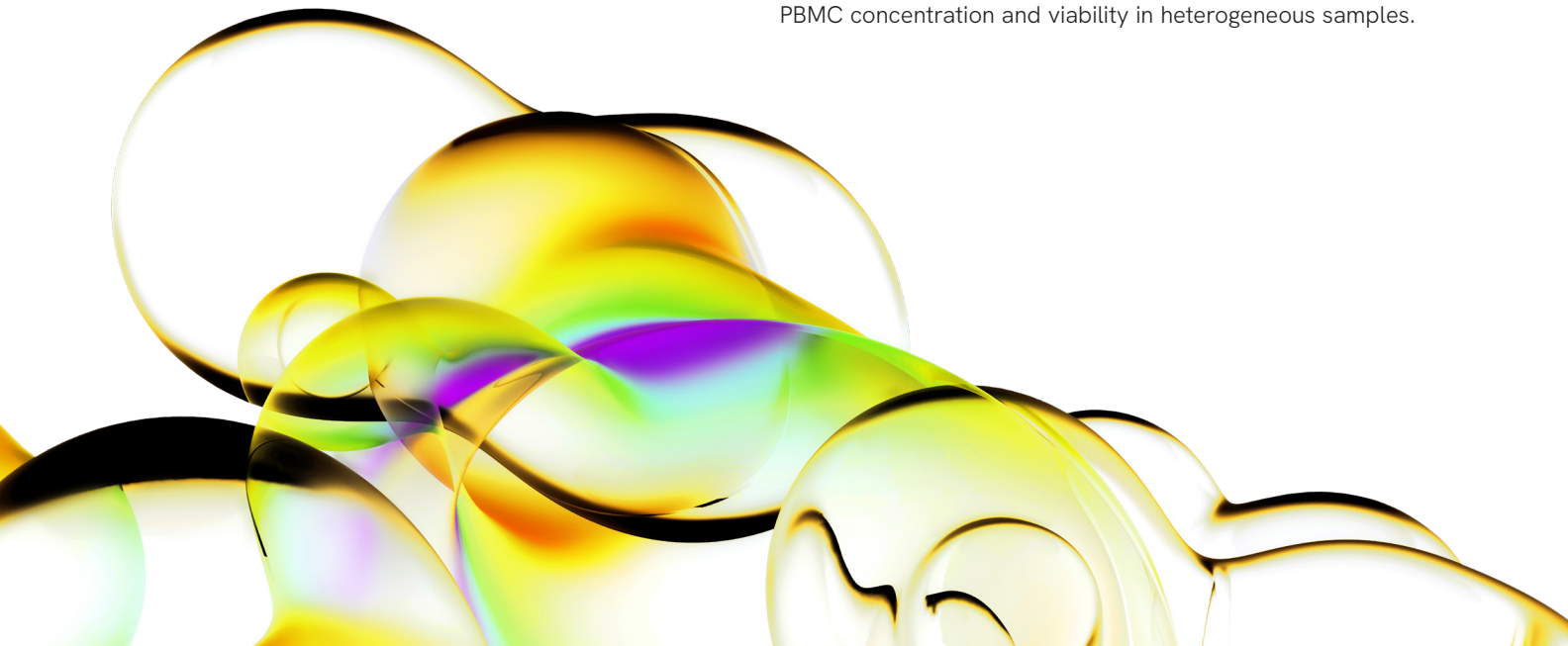
Peripheral blood mononuclear cells (PBMCs) are routinely used to measure immunological function through assays for cell proliferation, cytotoxicity, and cytokine expression. The use of cryopreserved PBMCs is necessary in clinical trials involving many patient samples collected over a period of time and even smaller research studies involving multiple time-points.

Ficoll separation is routinely used to isolate mononuclear cells from bone marrow, peripheral blood, and umbilical cord blood. Monocytes and lymphocytes form a buffy coat under a layer of plasma, where the cells are collected and washed. Typically, some residual platelets or red blood cells (RBCs) are mixed in with the mononuclear cells, which cause the separation quality to vary depending upon the patient sample and the operator.

Because RBC contamination in PBMC samples is common, the method for counting PBMCs must be designed to robustly exclude RBCs. Accurate PBMC cell concentration and viability data should be considered when evaluating results for functional assays. All samples should be evaluated and viability thresholds should be used in clinical trials in order to obtain reliable results.

Introduction: Cellometer image-based analysis of PBMCs

The Cellometer instruments combine image-based cell counting and dual-fluorescence detection to accurately determine PBMC concentration and viability in heterogeneous samples.



The Cellometer platforms including Cellometer K2 and Cellometer Auto 2000 offer pre-defined assay selections for simple and accurate analysis of PBMCs and nucleated cells. Cellometer Spectrum offers PBMC counting assays along with the added features of user-changeable fluorescence optics modules for additional staining / labeling options and enhanced cell based assays.

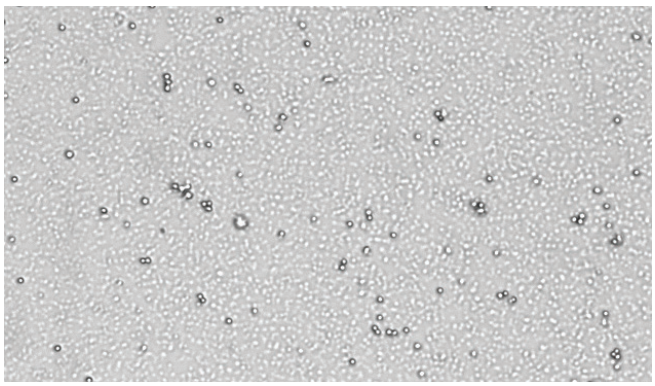
Experimental procedure

1. Combine 20µl of PBMC sample and 20µl of AO/PI dye mixture and mix well by pipetting up and down
2. Load 20µL of sample into the disposable counting chamber
3. Insert chamber into Cellometer instrument
4. Select assay from menu
5. Enter sample ID manually or scan in with bar code reader
6. Preview brightfield cell image and adjust focus (if necessary)
7. Click "Count" to begin analysis
8. Review images and counting results on-screen
9. Images, cell count, concentration, % viability, and cell diameter data are saved to a secure network location

Results

Brightfield imaging

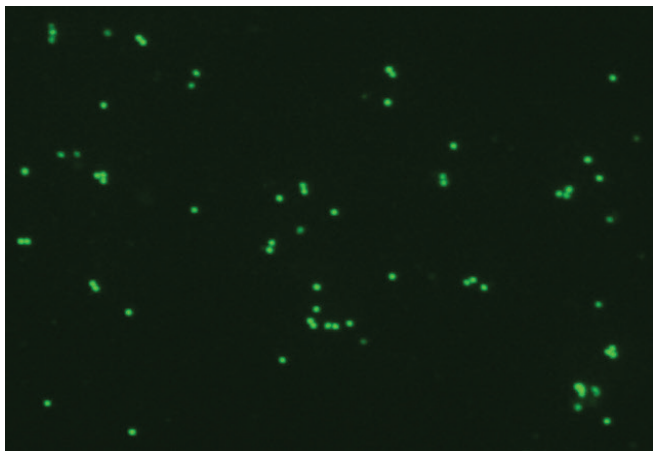
The Cellometer instrument acquires a brightfield image for each sample tested. The brightfield image allows researchers to verify cell morphology, evaluate the degree of homogeneity of the sample, and identify the presence of cellular debris.



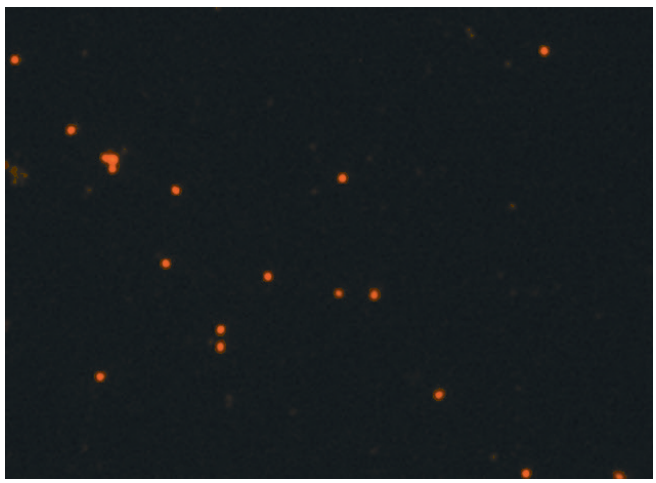
Brightfield image of PBMC sample following Ficoll separation

Fluorescent nucleated cell concentration and viability

Each PBMC sample is incubated with an acridine orange / propidium iodide (AO/PI) dye mixture. The AO dye is membrane-permeable and stains DNA in the cell nucleus of both live and dead cells. Propidium iodide DNA-binding dye is used to determine cell viability. Healthy cells are impermeable to the PI dye. Only dead (non-viable) cells with compromised membranes are stained. Additionally, the PI absorbs the green fluorescence from AO in the dead cells. Therefore, the green fluorescent signal identifies all the live nucleated cells and the red fluorescent signal identifies all the dead nucleated cells. Mature mammalian red blood cells don't contain nuclei, so only peripheral blood mononuclear cells are stained. The PBMC cell count is not affected by platelets, red blood cells, or cellular debris.



Fluorescent image showing viable (live) nucleated cells stained green with acridine orange.



Fluorescent image showing non-viable (dead) nucleated cells stained red with propidium iodide.

Assay: Immune cells, low RBC

Sample ID: new sample
Dilution Factor: 2.00

Count	Concentration	Mean Diameter
Total: 1428 cells	4.93x10^6 cells/mL	6.3 microns
Live: 996 cells	3.44x10^6 cells/mL	6.4 microns
Dead: 432 cells	1.49x10^6 cells/mL	5.9 microns

Viability: 69.8%

Cellometer Results Table

The Cellometer software automatically calculates total PBMC cell count, concentration, and % viability. The PBMC images and data table can be easily saved to a network for additional analysis or data archiving.

Overcome issues related to variability in sample preparation and manual cell counting with automated, dual-fluorescent analysis of PBMC concentration and viability. The Cellometers make it feasible to accurately screen a large number of PBMC samples over time for more accurate interpretation of immunological assay results.

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