Rapid Image Cytometry Method for Measuring Concentration and Viability of Primary Cells used in Cellular Therapy

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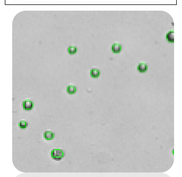
1. ABSTRACT

Cellular therapy has become a major clinical research field that creates tailor-made medical treatments for many human diseases. Primary cells obtained from patients and mouse models often contain nonspecific particles such as red blood cells (RBC), platelets, and cellular debris, which can make the cell sample analysis difficult. To remove nonspecific particles that can interfere with analysis, a ficoll gradient separation or RBC lysis is routinely performed. Measurements of concentration and viability of the cell sample are necessary for clinical researchers to qualify the collected patient samples for research and downstream processing. In this work, we validated a fluorescence-based image cytometry method using acridine orange (AO) and propidium iodide (PI) to rapidly measure concentration and viability without the need for tedious purification protocols. Using the Cellometer Vision instrument we performed the viability and concentration analysis and validated our results using the traditional hemacytometer method for samples including but not limited to peripheral blood mononuclear cells, mononuclear cells, Leuko Pac, bone marrow, cord blood, whole blood, bronchoaveolar lavage, and primary murine samples. This image-based cytometer method can increase clinical research efficiency by eliminating the need for purification steps and for manual counting. Furthermore, it can eliminate the user-to-user variation, thus improving the accuracy of the cell analysis.

2. CELLOMETER IMAGE CYTOMETRY FOR AUTOMATED PRIMARY CELL COUNTING AND VIABILITY

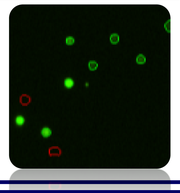
Pipette 20 µL of sample into disposable counting chamber

Bright-field (BR)



Insert chamber in Cellometer

Fluorescent (FL)



Results Table

Assay: A_Immune Cells_Low RBC

Cell Type F1: A_Immune Cells_Low RBC (AO)
Cell Type F2: A_Immune Cells_Low RBC (PI)

Sample ID: PBMC AOPI-2
Dilution: 2.00

Results:

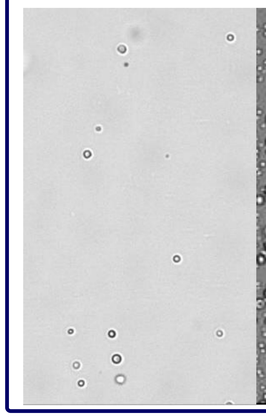
Count	Concentration
Total: 1035	1.16x10^7 cells/mL
Live: 954	1.07x10^7 cells/mL
Dead: 81	9.06x10^5 cells/mL

Viability: 92.2%

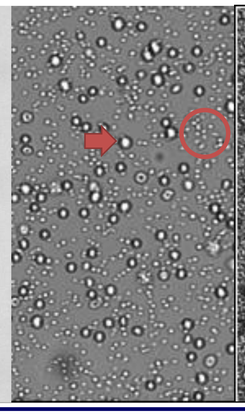
3. WHAT IS A HETEROGENEOUS CELL POPULATION?

Homogeneous

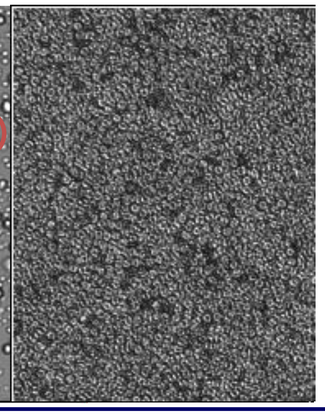
Purified CD34+ cells



Ficolled PBMCs



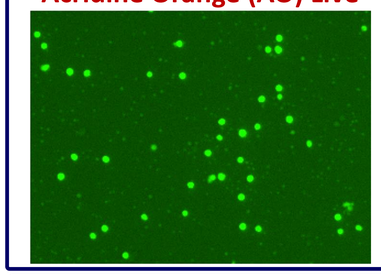
Whole blood



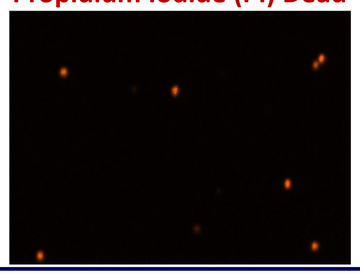
- A heterogeneous cell population contains target cells and other particles such as RBCs, Platelets, and tissue debris
- A homogeneous cell population (**LEFT**) is a purified CD34+ cell sample, where only target cells remained by separation
- Ficolled PBMCs is a heterogeneous cell population (**MIDDLE**) that contains residue RBCs, Platelets, and some debris
- Whole blood is a heterogeneous cell population (**RIGHT**) with mostly RBCs and Platelets. The WBCs are in much lower percentages in comparison to RBCs

4. DUAL-FLUORESCENCE ACRIDINE ORANGE/PROPIDIUM IODIDE CELL VIABILITY METHOD

Acridine Orange (AO) Live



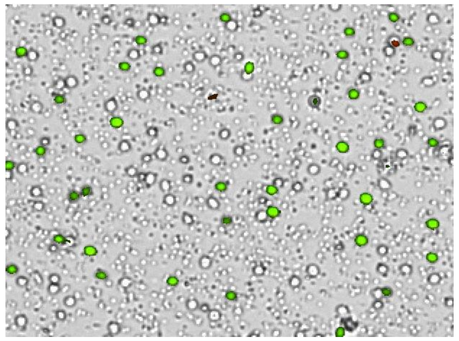
Propidium Iodide (PI) Dead



- Initial development was based on AO/EB dual-staining method from Professor Lenoard Herzenberg's Lab at Stanford
- AO is permeable to both live and dead cells
  - AO binds to DNA and fluoresces bright green
- PI can only enter dead cells
  - Binds to DNA of dead cells and absorbs AO fluorescence
- No signal is generated from non-nucleated cells

5. VARIATION IN LEUKAPHERESIS CELL SAMPLES USING MANUAL TRYPAN BLUE METHOD

Bright-Field + Fluorescence Overlay

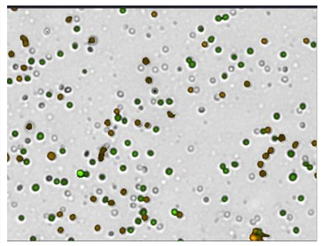


- A Leukapheresis cell sample when measured using manual trypan blue showed
  - 119 total cells, while only 39 are fluorescent when stained with AO/PI
  - Therefore, there is an over-counting of 3X by manual counting of nucleated cells
- 6 Leukapheresis cell samples were compared between trypan blue manual counting and using AO/PI with Cellometer Vision
  - The results showed that manual counting could have as high as 12X difference
- It means that using manual counting could generate inaccurate nucleated cell counting that can affect assays and experiments downstream

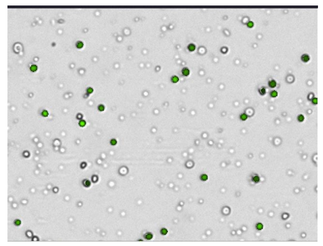
Leukapheresis Sample	A	B	C	D	E	F
Ratio (Manual counting # to Cellometer AO/PI #)	7X	5X	12X	3X	7X	5X

6. EXAMPLES OF COMMONLY USED PRIMARY CELLS STAINED WITH AO/PI DUAL-STAIN

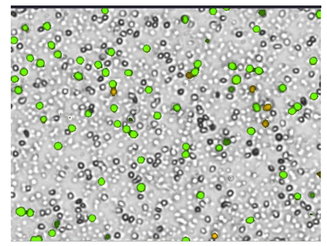
Mouse Splenocytes



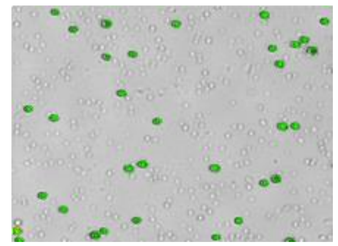
Human MNCs



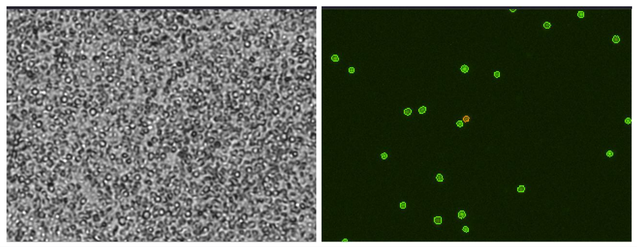
Human LeukoPacs



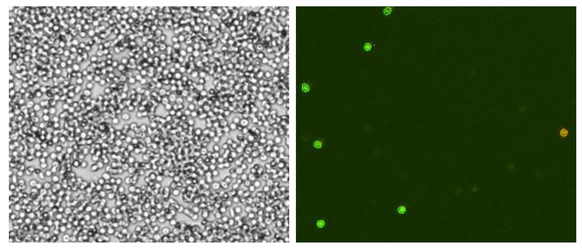
Human PBMCs



Human Bone Marrow



Human Cord Blood



7. COMPARISON OF MANUAL TRYPAN BLUE TO AUTOMATED AO/PI BY ALLCELLS

1. Whole bone marrow, unprocessed cord blood, and apheresis peripheral blood were measured using manual counting and automated AO/PI counting
2. For manual trypan blue method, to prevent counting RBCs, Platelets, or other debris, cells are lysed with a lysing buffer containing crystal violet to stain the free nuclei, and the nuclei are manually counted
3. Each sample is then stained with AO/PI to automatically count all the nucleated cells without the lysing step

Sample Name	Concentration manual	Concentration automated	Differences
Whole bone marrow	33.5 x 10^6 cells/ml	34.2 x 10^6 cells/ml	2.0%
Unprocessed cord blood	6.8 x 10^6 cells/ml	6.9 x 10^6 cells/ml	1.4%
Apheresis peripheral blood	61.0 x 10^6 cells/ml	63.2 x 10^6 cells/ml	3.5%