ORFLO

Tech Tip

PBMC Counting and Viability

Overview

Blood extraction and analysis is routinely performed for medical testing and disease monitoring. It's a simple, relatively non-invasive procedure that provides sufficient sample volume for a variety of tests. Well-established protocols exist to isolate peripheral blood mononuclear cells (PBMCs) and to remove red blood cells (RBCs) to obtain optimally purified immune cells for downstream purposes. But one critical step is to know how many cells have been isolated. Cell counting, therefore, is essential for determining how to move forward with either patient treatment or research experiments.

The only way to overcome the challenges in counting complex cell suspensions like blood samples is to find a way to first, count more cells and then find a way to avoid the errors inherent in imaging-based instruments to distinguish real cells from debris. ORFLO's Moxi line of instruments solves these challenges. All ORFLO Moxi Coulter counting systems measure the actual cell population using electrical impedance rather than using images and algorithms to arrive at an average cell count (> 10,000 cells). This means that according to the law of large numbers, you will be much closer to the true mean value even with a single sample run. While triplicate runs can be done to ensure accuracy, the variance will be a fraction of that obtained using a non-Coulter counting system.

Easy Debris Removal

Debris is one of the largest inhibitors of accurate cell counts in blood samples. It can obscure the fluorescence signal of certain cells in an image and make it significantly more difficult for an image-based counter's algorithm to properly segment out and identify cells. Moxi instruments completely circumvent this problem because the counting technology obtains size information for every event. Debris particulates are significantly smaller than real cells and will therefore be separated automatically when the events are counted on Moxi instruments. This makes determining what events are debris and what events are cells a simple process.

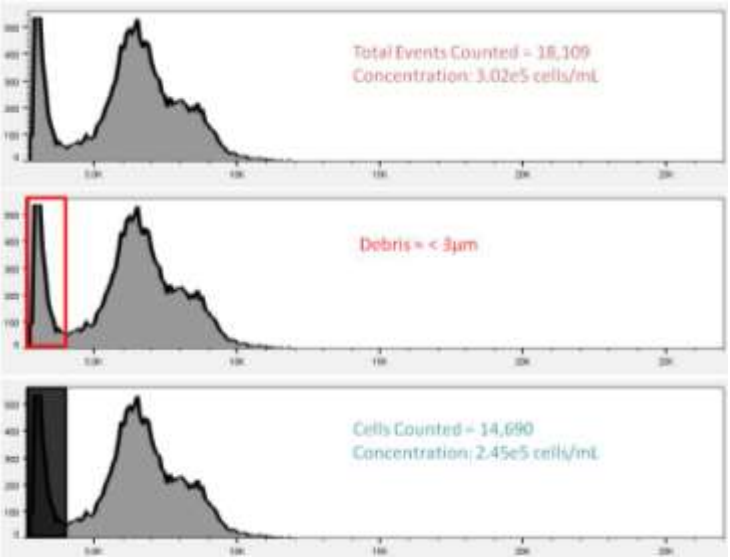

Fig. 1: Calculation of real PBMC cell concentration via debris removal on a Moxi Z

In a PBMC sample run on a Moxi machine, over 18,000 events (cells + debris) were recorded (Fig. 1). However, the associated concentration that the instrument calculates includes debris particles. On the Moxi system output histogram of size vs count, the peak that appears at < 3 µm (red square to the left) is cell debris. Using the gating feature on a Moxi system, the user can optimize the accuracy by removing the debris events from the total counts and ensure that only real cells are included in the concentration calculation.

Counting Specific Sub-Populations of Cells

The general consensus from published literature shows a wide size range for RBCs and lymphocytes that almost entirely overlap. The upper boundary of the lymphocyte size range is also essentially the same size as the monocytes. But with a Moxi instrument, these ranges become significantly smaller and it becomes possible to distinguish these two cell types.

PBMC Cell Sizes		
Cell Type	Average Size (literature)	Average Size (Moxi)
Red Blood Cell	6-8µm	5.5µm
Lymphocyte	6-9µm	7.5µm
Monocyte	9.5µm	9.5µm

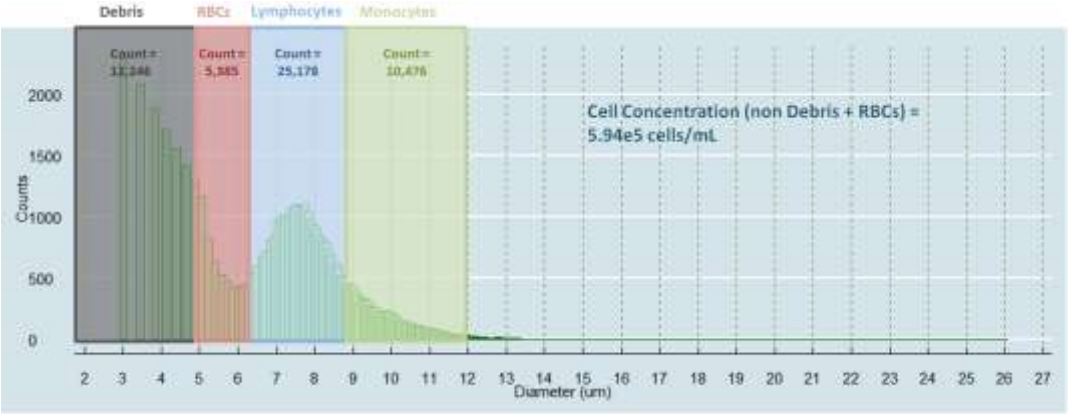

Fig. 2: Accurate counting of specific PBMC sub-populations using a Moxi Z

Because the Moxi instruments can be as precise as 0.1µm, RBCs can be clearly distinguished at 5.5µm, Lymphocytes can be distinguished at 7.5µm, and Monocytes can be distinguished at 9.5µm in the same sample (Fig. 2).

Viability Measurements

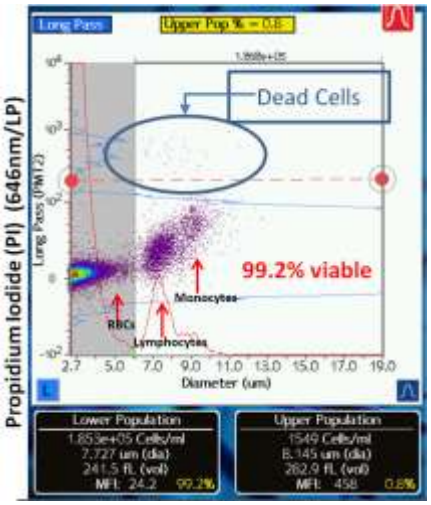

Fig. 3: Viability measurements of PBMCs using PI on a Moxi GO II

Moxi V and Moxi Go II systems identify both the cell sizes and counts of specific cell populations in PBMC samples, and can also determine viability percentages of live cells in the same run. Moxi V and Moxi GO II provide the ability to resolve cells in two dimensions, by size on the x-axis and by fluorescence intensity on the y-axis. This is made easy by the software's histogram overlay feature. Viability analytics can be obtained at the same time as total PBMC cell counts by using fluorescent dyes such as propidium iodide (PI) or ORFLO's premade PI-based viability reagent (MXA055)(Fig. 3).

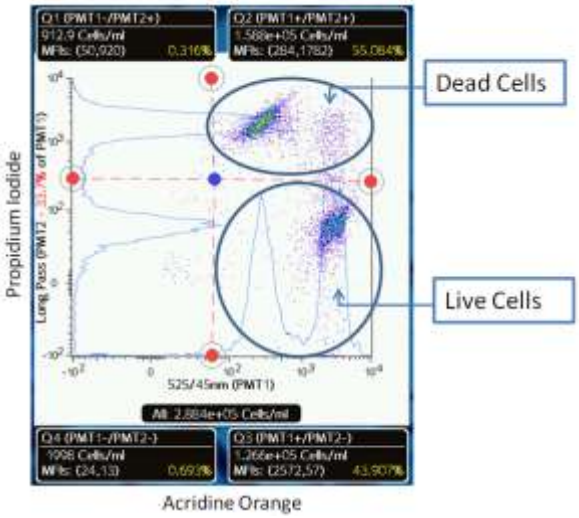

Fig. 4: Viability measurements of PBMCs using AO and PI on a Moxi GO II

Two-color viability measurements on the Moxi GO II provide an extra level of accuracy with conclusive viability results. By coupling acridine orange (AO) and propidium iodide (PI) measurements together, all truly dead cells will be easily identified (Fig. 4). The user first uses gating to remove the debris and RBCs based on size, and then uses an intuitive two-dimensional output similar to a flow cytometer to truly isolate and quantify the live PBMC cells. Both of these steps are made easy by using the software's histogram overlay feature. Viability measurements have never been more reliable when coupling ORFLO's sizing technology with a two-color fluorescent dye combination using ORFLO's premade AO + PI-based two-color viability reagent (MXA069).

Summary

Inaccuracy when analyzing PBMC samples comes from the inadequate counting of the cells of interest, usually because of debris or lack of robust counting. By circumventing imaging and employing the gold-standard and physics-based Coulter principles, Moxi instruments give unparalleled abilities to generate the truest cell count. With the added opportunities for fluorescence-based detection with a Moxi V and Moxi GO II, PBMC viability checks have never been more accurate. Avoid wasting precious samples and ensure accurate cell counts the first time by relying on a Moxi instrument.