ORFLO | Personal Cell Analysis

PBMC Counting + Sizing with Moxi Instruments

Introduction

Blood extraction and analysis is a routine practice for a lot of medical tests and disease monitoring. It's a simple, relatively non-invasive procedure that gives a sufficient volume of sample to work with for whatever tests need to be run for the patient. More recent advances in genomics and particularly immune-based genomic therapies have provided an entirely new avenue for using collected blood samples. The subset of cells called the peripheral blood mononuclear cells (PBMCs) contains all of the non-red blood cells with a round nucleus (i.e. lymphocytes, monocytes, natural killer cells (NK cells) or dendritic cells). These are the cells of interest for these types of therapies as the immune cells will be re-programmed to both study and treat disease.

There are well-established protocols to isolate PBMCs and to remove red blood cells (RBCs) to obtain optimally purified immune cells for downstream purposes. But one critical step is knowing how many cells have been isolated. This is where cell counting comes into play, as this check will determine how to move forward with either patient treatment or research experiments.

For prepping downstream experiments, confidence in the concentration of cells received when counting is vital. Because the patient sample is often a limited resource and multiple counting runs whittle away at the precious sample volume, getting a concentration value that is reliably close to the true mean with the fewest number of replicates possible is ideal.

Moxi Cell Analyzers

The only way to overcome the challenges that still persist in counting complex cell suspensions like blood samples is to find a way to count more cells and find a way to remove the reliance on an image to distinguish what is a real cell. This is where ORFLO's Moxi line of products comes in. All three of the instruments (Moxi Z, Moxi V, and Moxi GO II) count the number of cells you want (> 10,000 cells). This means that, according to the law of large numbers, you will be much closer to the true mean even with just one run. While triplicate runs can be done to ensure accuracy, the variance will be a fraction of that obtained using another method.

Normally, accuracy is negatively impacted by acquisition time. However, by leveraging the current gold standard Coulter Principle, we bring you both with no compromises to either. All of this in a self-contained, easy-to-use, benchtop-sized machine.

Microfluidics Meets Cell Counting

Don't like wasting half of your sample on figuring out whether your experiment worked correctly? Don't like spending time and money on a large-scale flow cytometry run for a simple transfection efficiency or cell viability check? Our innovative microfluidics cassettes have created a simple, effective, and self-contained way to get everything you need, all for the low, low cost of 60µL! Just insert the cassette, pipette in a small amount of your precious sample, and let the microfluidics and our proprietary cell counting technology do the rest.

Gold Standard Count Accuracy

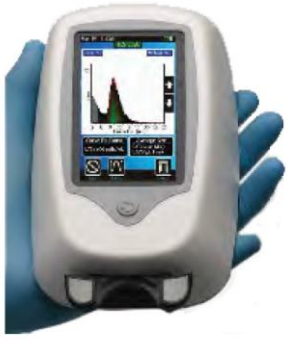
All Moxi instruments were compared to the gold standard Beckman-Coulter Z2 system, an image-based cell counter, and a hemocytometer, with respect to counts of serial dilutions of a range of particle types (beads, yeast, mammalian cells) and sizes (~4µm to ~16µm in mean diameter). The results showed that the count-to-count variability, as measured by the coefficient of variation (CV), was on the order of 20-30% for the imaging system, compared to 4% for the Moxi system.

Moxi GO II - Next Generation Dual-Channel PMT Fluorescence Cell Analyzer

With our Moxi Go II system, we have coupled our cell counting technology with the ability to detect two channels of fluorescence to revolutionize the way that cell counting is done. Our 488nm laser and Photo Multiplier Tubes (PMTs) gives the accuracy and detection capabilities of a normal flow cytometer for both a 525/45nm (Green) channel and 561nm/LP (Orange/Red) channel. This combination gives you unparalleled benchtop power when working with any cell types marked with any of your favorite fluorescent cell markers!

Moxi V – Mini Automated Single-Channel Fluorescence Cell Counter

Don't need two channels, but want some fluorescent capabilities? We have you covered with our single 561nm/LP (Orange/Red) cell viability instrument in the Moxi V. Moxi V provides true cell viability counts, 50-100x more sensitive than vision counters. Get precise cell volumes with CVs less than 3%, without the need for triplicates and a self-contained, easy to use, and affordable machine.



Moxi Z – Ultra-small Cell Counter

Love our cell counting technology but don't need any fluorescence? Then our Moxi Z is the right machine for you. All the power of our microfluidic cell counting literally in the palm of your hand, and at a low, cost-effective rate. It provides the same robust cell counting technology of the Moxi Go II and Moxi V, with the added ability to assess cell culture health without the use of dyes.

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. This is particularly true without a fluorescent stain, as the bright field image of a fresh unfiltered PBMC sample is going to be impossible to count properly.

Moxi instruments completely circumvent this problem because the counting technology by default obtains size information for every event. Because they do not rely on an image and because debris is significantly smaller than real cells, determining what is debris and what is cells is simple.

In a real-world example of a PBMC run on a Moxi machine, over 18,000 events (cells + debris) was recorded (**Fig. 1**).

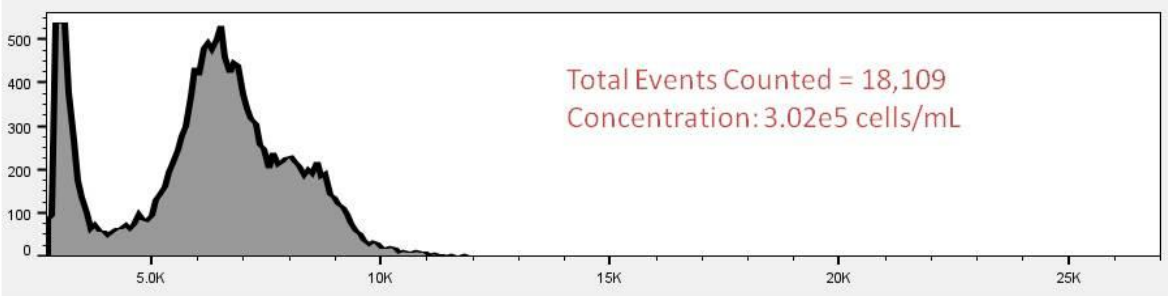


Fig. 1: Experimental data from a PBMC sample run on a Moxi Z. X-Axis delineates particle/cell size and Y-Axis delineates number of events recorded.

However, this concentration that the instrument gives is inaccurate because it includes debris particles in its calculation. On the output histogram of size vs count (which can be obtained on any Moxi instrument), the peak that appears at < 3 µm (red square below) is all of the smaller particles, which in this case is debris (**Fig. 2**).

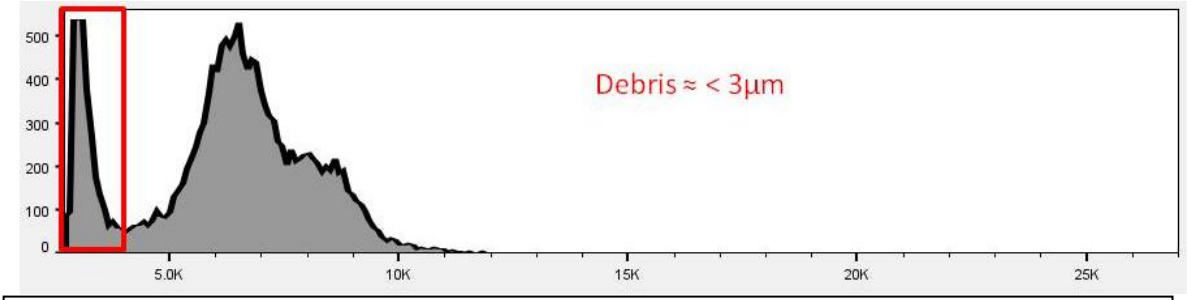


Fig. 2: Experimental data from a PBMC sample run on a Moxi Z. X-Axis delineates particle/cell size and Y-Axis delineates number of events recorded. Debris events that were recorded are highlighted in red.

Using the gating feature on a Moxi system enables the removal of those debris events from the total counts and ensures that only real cells are included in the concentration calculation (**Fig. 3**).

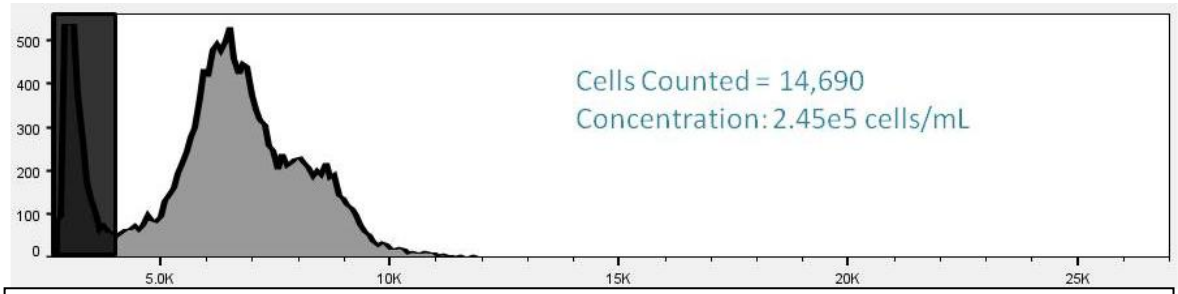


Fig. 3: Experimental data from a PBMC sample run on a Moxi Z. X-Axis delineates particle/cell size and Y-Axis delineates number of events recorded. Debris events that are gated out are in black.

As an additional benefit, by taking the percentage of counts that remain post-debris in comparison to the total counts including debris, an estimation of debris percentage of your sample can be obtained. This can be particularly useful for monitoring debris removal during a cleanup step before downstream experiments or analyses.

Counting Specific Sub-Populations of Cells

Peripheral blood mononuclear cells (PBMCs) are the subset of blood cells with a round nucleus (i.e. lymphocytes, monocytes, natural killer cells (NK cells) or dendritic cells). The underlying principle of the Moxi instruments by default provides information on particle size and volume, as the same electrical displacement that allows for an event to be recorded can be used to determine how large the particle was as well. The charge displacement is linearly related to the size of the cell that passed through the aperture. This allows for incredibly accurate sizing calculations down to 0.1µm in fractions of a second (**Fig. 4**).

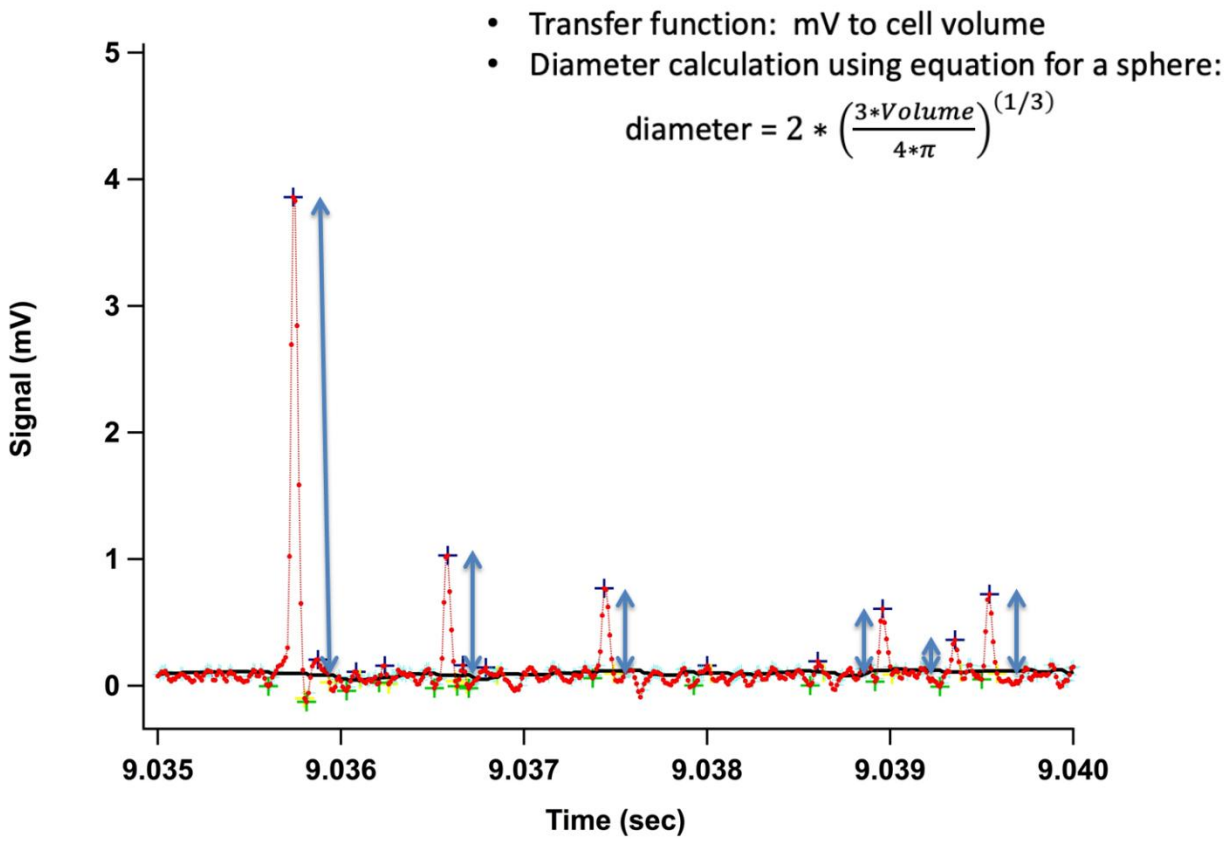


Fig. 4: Subset of event data recorded from a sample run on a Moxi Z. X-Axis delineates time and y-axis delineates the displacement voltage as the particle passes through the instrument's detection aperture. Particle event size is linearly correlated with the height of the peak and calculated using the equation (top right).

These diameter measurements all recorded as the counting is going on, and an output graph of "Diameter" vs "Counts" is generated on all three instruments. An example output of a Moxi Z shows how both robust counting and cell sizing is performed simultaneously and output on an intuitive graph and user interface (**Fig. 5**).

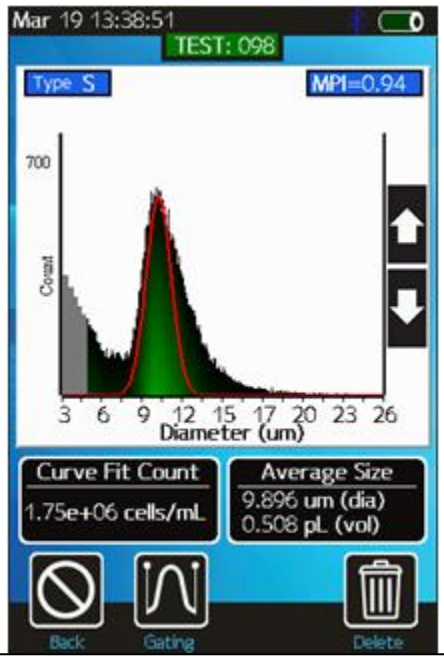


Fig. 5: Example output of a PBMC sample run on a Moxi Z. X-axis delineates particle size and Y-axis delineates the number of events recorded. The total concentration calculated from the Moxi Z's "curve-fit" mode can be seen on the bottom left. The average particle size and volume can be seen on the bottom right.

The general consensus from the 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 monocytes. But with a Moxi instrument, these ranges become significantly smaller and it becomes possible to distinguish these cell types (**Table 1**).

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

Table 1: Average cell size for PBMC cell types. The literature ranges were taken from reported papers and online references. The Moxi ranges were calculated from a repository of PBMC data recorded on all of our Moxi instruments

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. 6**).

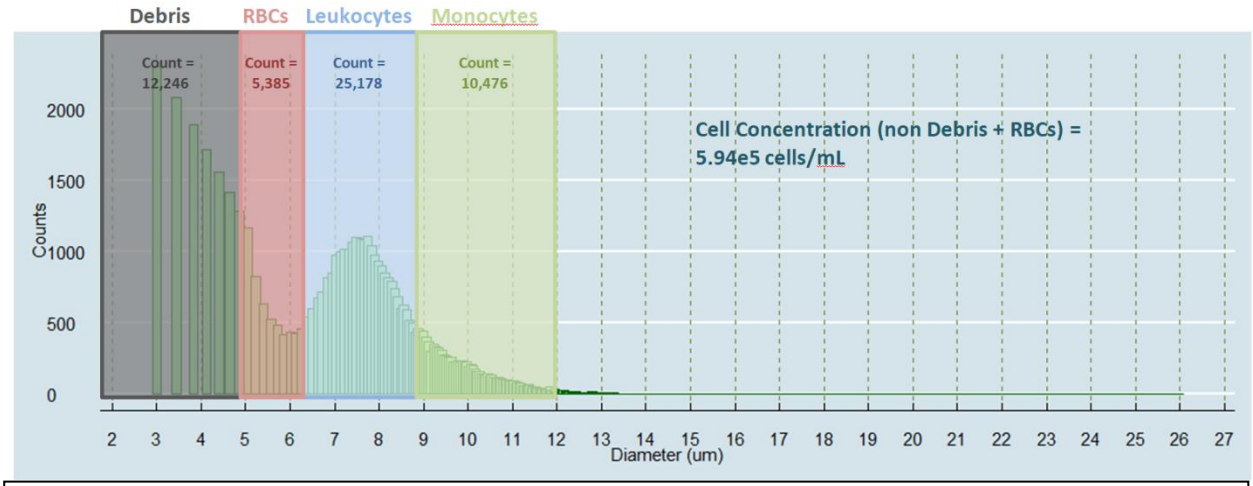


Fig. 6: Real PBMC data obtained from a PBMC sample run on a Moxi Z.

This real-world PBMC data was generated from a Moxi Z in a research laboratory. By reporting events by size, it is a simple matter to gate around specific subpopulations to either remove them from the overall counts (as in the case of debris or RBCs) or count them specifically (Leukocytes + Monocytes). This allows for easy removal of undesirable cells/debris from the calculations and gives an accurate concentration for just real cells right on the instrument's touch-screen interface. Because the sizing can be so specific, it is possible to distinguish between cell types much more accurately and give cell counts and concentrations for each of those as well. In this sample, an elevated peak at ~5µm can be observed, indicating potential RBC contamination in the sample.

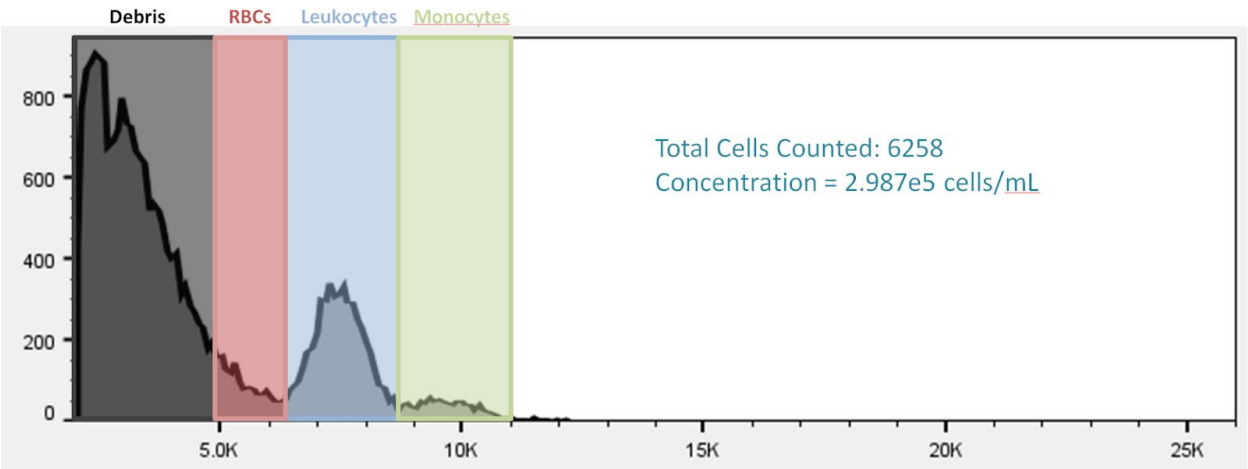


Fig. 7: Real PBMC data obtained from a PBMC sample post-RBC lysis treatment run on a Moxi GO II.

This second real-world PBMC data generated from a Moxi Go II by a cell therapy company looking to isolate their immune cells for downstream therapeutic purposes shows how you can easily monitor RBC treatment effectiveness and determine if they have been properly removed from the sample (**Fig. 7**). After treating with their RBC lysis solution, the peak ~5µm is gone indicating that the sample is properly purified. As seen before, it is also possible to distinguish between Leukocytes and Monocytes, proving that the level of sizing sensitivity persists across Moxi instruments.

Additional Measurements (Moxi V + Moxi GO II)

More Accurate Cell Count Measurements Using AO:

With the Moxi Go II, you can not only distinguish PBMC cells based on size, but easy Acridine Orange (AO) staining seamlessly separates real cells from debris and RBCs, guaranteeing the highest quality cell counts for each cell type (**Fig. 8**).

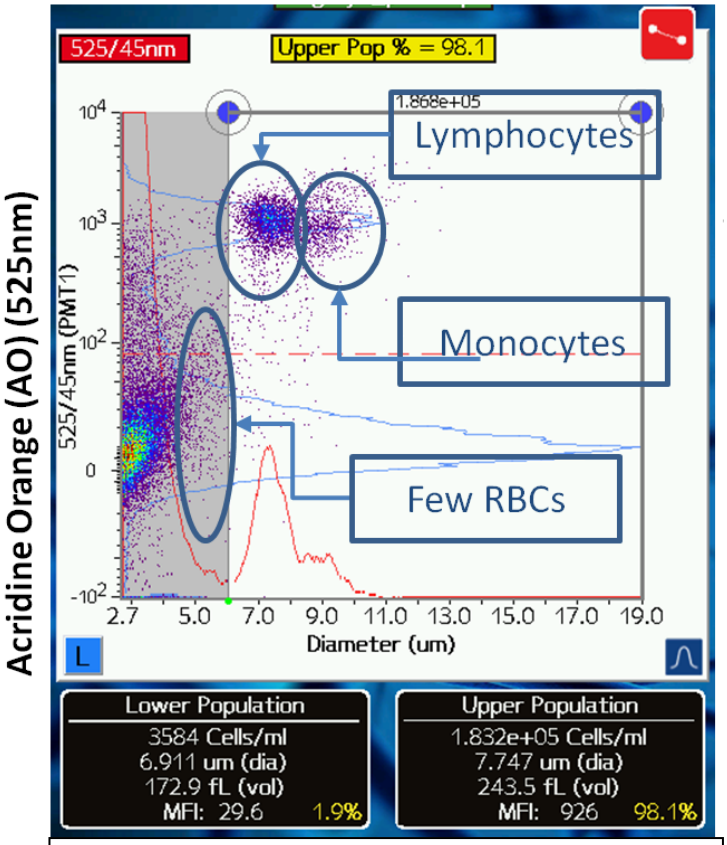


Fig. 8: Reliable delineation of various PBMC cell populations can be performed via the Moxi Go II. Data is the output taken from a Moxi Go II using the "Open Flow Cytometry" application. Acridine Orange staining was performed using our AO/PI Kit (MXA069) to distinguish cells from debris.

Viability Measurements Using PI:

The Moxi V and Moxi Go II are uniquely primed to address both the size-based identification and quantification of specific cell populations in your PBMC and determine percentages of live cells all in one run. With the ability to resolve cells in two dimensions by size on the x-axis and by fluorescence intensity on the y-axis, you can get viability analytics via fluorescent dyes such as propidium iodide (PI) or our premade PI-based viability reagent (MXA055) and total PBMC cell counts at the same time (**Fig. 9**).

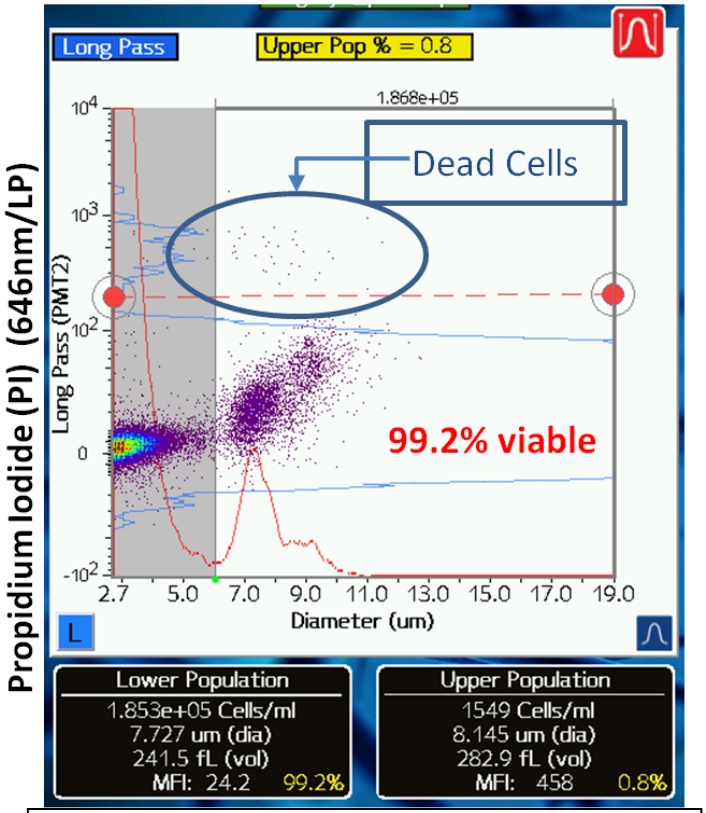


Fig. 9: Two-dimensional analysis of viability and cell size on a single sample run. Output image was generated on a Moxi Go II using the "Cell QC" application. PBMC cells were stained with Propidium Iodide (PI) to determine live vs dead cells.

Viability Measurements Using AO + PI:

Two-color viability measurements on the Moxi GO II provide an extra level of accuracy that gives the overall best viability results. By coupling acridine orange (AO) and PI, all truly dead cells will be easily identified (**Fig. 10**). Gate out the debris and RBCs based on size, and then use an intuitive two-dimensional output similar to a flow cytometer to truly isolate and quantify the live PBMC cells. By coupling our sizing technology with this two-color combination, either using your favorite AO and PI from your favorite vendor or our premade AO + PI-based two-color viability reagent (MXA069), viability measurement on your bench top have never been more reliable.

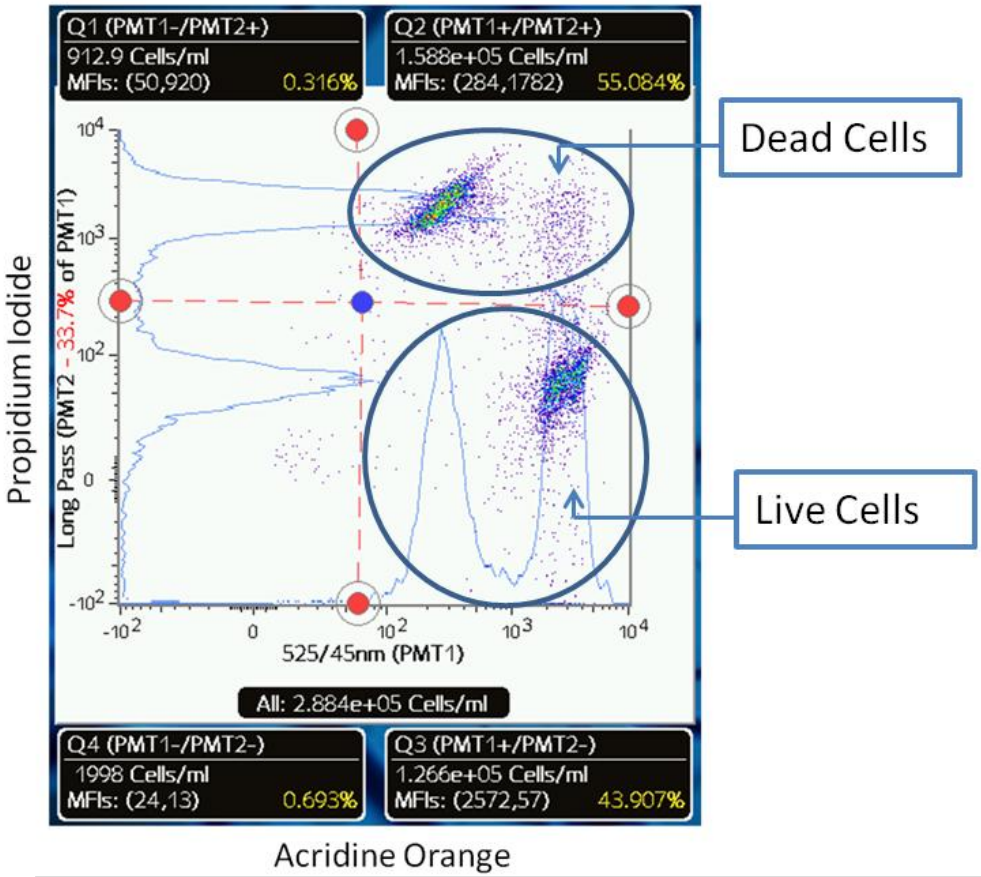


Fig. 10: Two-dimensional analysis of viability using AO (x-axis) and PI (y-axis). Output image was generated on a Moxi Go II using the "Open Flow Cytometry" application. PBMC cells were stained with AO and PI to determine live vs dead cells. Debris and RBCs were gated out before via size before looking at fluorescence.

Conclusion

For any type of work involving cells, Moxi cell analyzers are being used. Much of the hassle of 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 going back to gold-standard physics-based principles, every Moxi instrument gives unparalleled abilities in getting the best 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. Ensure your precious samples aren't wasted by making sure you get the quality checks right the first time by relying on a Moxi instrument.

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