Tech Tip

Moxi Systems vs Image-Based Counting

Overview

Cell counting is an essential task in every cell-based biological endeavor, whether research, biotech or advanced therapeutics. Most experiments that involve the use of cells require quantifying and measuring the number of cells that are alive or dead to determine viability. Ensuring these viability measurements are reliable is vital for confirmation that downstream steps in a biological workflow will be successful.

There are a variety of instruments that accomplish this task: hemocytometers, image-based cell analyzers, and Coulter principle based analyzers. Hemocytometers rely solely on user input and often have large amounts of variation depending on how the user manually counts cells, and thus is a very subjective method. Image based counters are an improvement over hemocytometers, but still have variable results because they rely on computer software to interpret static images of cells which are colorimetrically stained. Additionally, there is no flexibility in post-run data analysis which contributes to this variability. Because ORFLO's Moxi systems are based on the Coulter principle, each cell is directly measured by a physics-based method that objectively ensures every cell is properly analyzed and characterized to 0.1µm, significantly reducing variability. This, in combination with complete flexibility to manipulate the recorded data, ensures the highest accuracy and repeatability of any cell analyzer on the market.

Number of Cells Counted

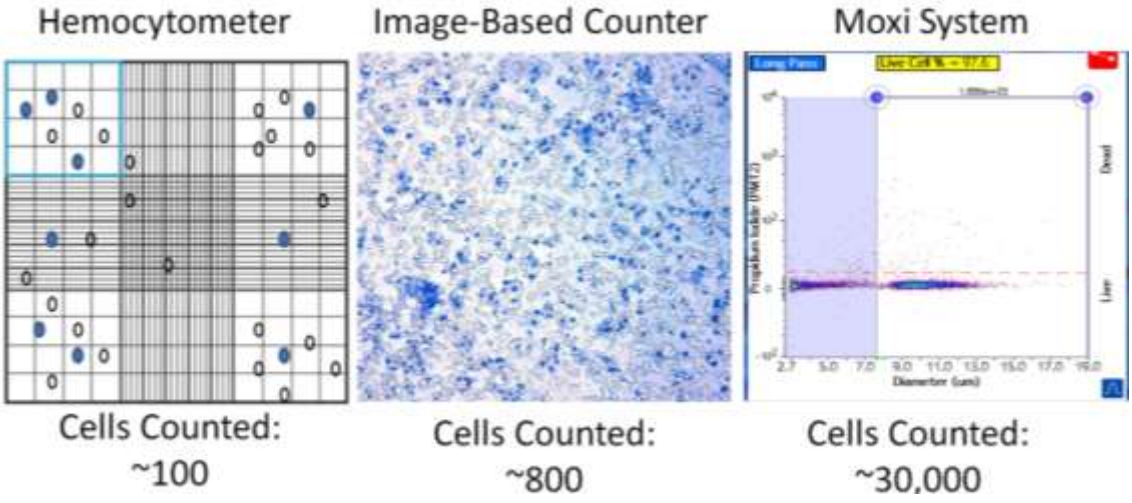


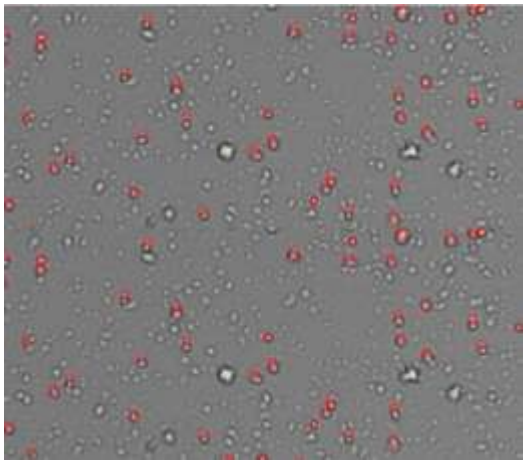
Fig. 1: Cell counting via different counting methods

Hemocytometers, the least accurate method and most time consuming, provide a manual estimate of the overall cell concentration using a small number of cells counted on a slide. Image based cell counters capture one or multiple images from a slide and computer software counts the cells in the images to give an extrapolated concentration estimate. This gives you a better estimation than a hemocytometer, but still only counts a few hundred cells per test. ORFLO's Moxi systems use of the Coulter principle provides direct measurement of the number and size of each cell as they pass through an electrical aperture. Due to the precise nature of this method and the ability to monitor minute electrical current changes in real-time, tens of thousands of cells can be passed through the aperture, recorded, and sized within seconds. These thousands of measurement instances are placed into a scatter plot or histogram that makes it easy to see the cells present in the sample and to manipulate the data on ORFLO's touch screen interface to obtain the most accurate results (Fig. 1).

Viability Staining

Various methods exist for determining cell viability. The two most commonly used substances for delineating which cells are alive or dead are trypan blue and propidium iodide (PI). Both dyes permeate only dead cells and either stain the cell blue (trypan blue) or emit orange light (PI) when excited with a laser, which then can be visualized in an image. Trypan blue is the method used for hemocytometers and some image based counters, while PI is the method used for other image based counters and ORFLO's Moxi V and Moxi GO II's direct fluorescence detection system.

Jurkat Cells	Moxi Calculated Concentration (20x Dilution)	Moxi Original Sample Concentration	Image Based Cell Counter Concentration
Live Cells	7.71E+04	1.54E+06	1.60E+06
Dead Cells	5.38E+04	1.08E+06	6.40E+05



This data shows the comparison between PI labeled viability measurements of the same sample, using both an image based counter and a Moxi GO II. The 20x dilution was part of this user's protocol (Fig.2), and the same volume was loaded into each instrument.

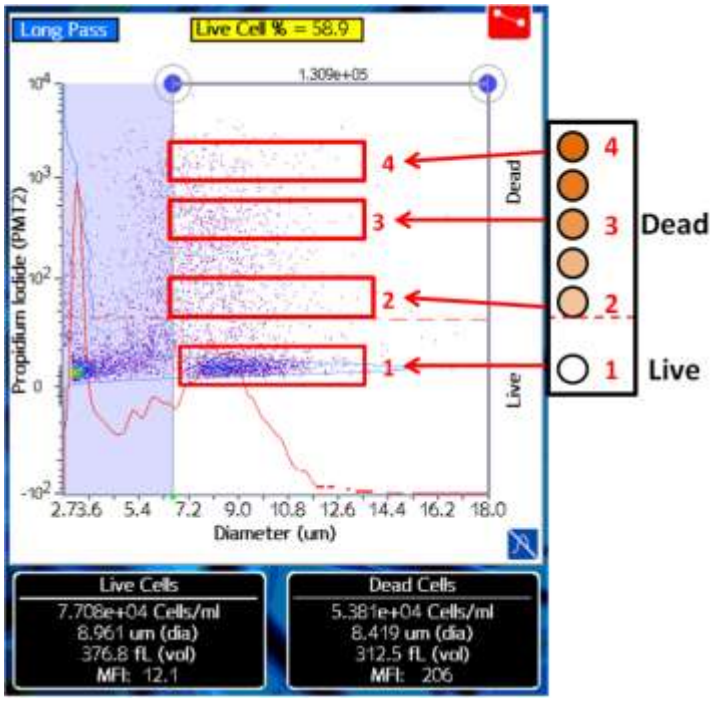


Fig. 2: Viability measurements taken with an imaging system vs. a Moxi system

The image based system and the Moxi system were relatively close in the concentration calculations for the live cell population; however, the image based system clearly underestimated the number of dead cells in the sample. This underestimation stems from the variation in staining color and/or fluorescence intensity that comes with any cell permeable stain like trypan blue or propidium iodide that is used for labeling dead cells. There will always be a distinctive population of cells that are dead but partially stained. The inability for users to adjust how an image based counter is determining the cutoff threshold for dead cells in these instruments means the dead cell count is often lower than it should be. Coupled with obtaining viability measurements from only a few hundred cells, and extrapolating that data to an original cell population of millions, means that image based viability results are often variable and inaccurate.

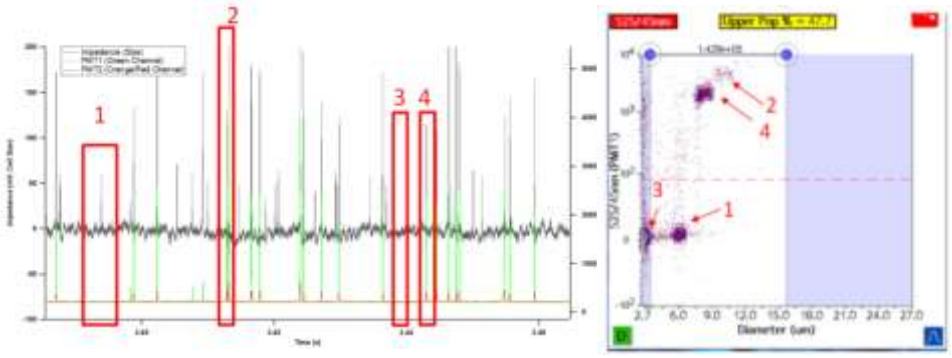


Fig. 3: Simultaneous recording of sizing and fluorescence data on a Moxi GO II

Unlike the image based analyzer, the Moxi V and Moxi GO II directly measure tens of thousands of cell events individually from the same sample amount you would add to an image based analyzer. The Moxi instruments simultaneously record the fluorescence signal and size information coming from the same cell event and match the sizing and fluorescence data together to get highly reliable data for each event (Fig. 3). The systems then display all the data points on a scatter plot similar to a flow cytometer output. The Moxi data analysis software enables users to manually adjust the cutoff threshold for fluorescently positive cells in a manner similar to gating cells via a flow cytometer. This gives the user the power to ensure that they are detecting even the partially stained cells as dead. Because Moxi systems analyze tens of thousands of cells, researchers are able to generate statistically accurate data and avoid estimation of cell counts and viability measurements.

Summary

A variety of options exist when counting cells and assessing the viability of a sample. Only ORFLO's Moxi systems provide the user the confidence that they are getting true cell counts and viability measurements, because only Moxi instruments have Coulter based counting principle, direct fluorescence detection, and fully modifiable post-data analysis interface.