Tech Tip

Nuclei Counting

Overview

For some sample types such as blood that include cells like red blood cells (RBCs) that do not contain nuclei, it's sometimes useful to isolate the nuclei for proper analysis of the cells of interest. This method removes all of the unwanted cells like RBCs since only immune cells in a blood sample will have nuclei. This gives a more accurate picture of the total white blood cell (WBC) count.

Many single cell genomics samples will isolate nuclei during the library preparation stage. Reliable analysis of the number of isolated nuclei is a critical step to ensure that the downstream single cell run is successful.

Analysis of nuclei is easy and straightforward with any Moxi system from ORFLO Technologies. The Coulter principle-based microfluidics technology at the core of ORFLO's proprietary cassettes in combination with the intuitive instrument software allows for simple isolation of nuclei from debris by size alone. Staining with propidium iodide (PI) provides the ability of a second level of analysis for nuclei counting and is possible with the Moxi V or Moxi GO II.

Nuclei Counting via Sizing

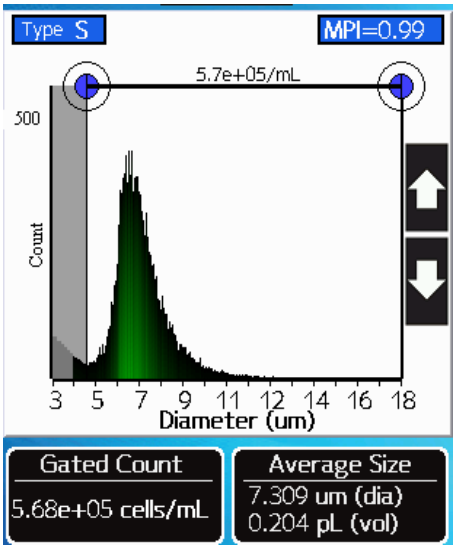

Fig. 1: Nuclei counting by size on a Moxi Z

Nuclei are a specific diameter (5-9um), and as such will show up on a Moxi system within that window on the x-axis (Fig. 1). The instrument software on all Moxi systems is able to adjust the gating on the x-axis to include only the recorded events of a desired size range. This allows for easy debris removal (events <3um) and guarantees that only nuclei are being counted.

Nuclei Counting via Sizing + Fluorescence

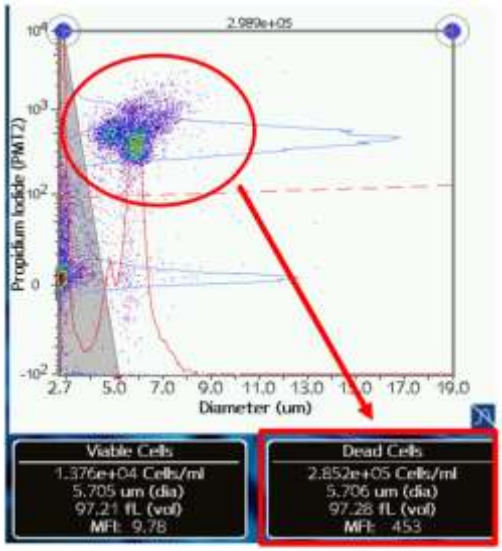

Fig. 2: Nuclei counting by size + fluorescence on a Moxi GO II

Fluorescent dyes such as propidium iodide (PI) bind to DNA and emit light that can be recorded with an orange/red filter. While live cells do not allow for PI to enter, isolated nuclei readily uptake PI and will fluoresce orange/red. This allows for a second level of nuclei isolation and provides the best method for reliable and accurate counting. The Moxi V and Moxi GO II systems record both sizing and fluorescence information for each of the tens of thousands of events that pass through the cassette during a sample run and produce an intuitive scatter plot similar to a flow cytometry output (Fig. 2). The x-axis can be adjusted to gate out unwanted debris and/or residual live cells and the y-axis can be adjusted to delineate events that are fluorescently positive. By gating the x-axis for events >5um and gating the y-axis on fluorescently positive events (typically $>10^2$ for PI staining), the user will receive reliable and repeatable nuclei counting results every time.

Nuclei Counting for PBMC Samples

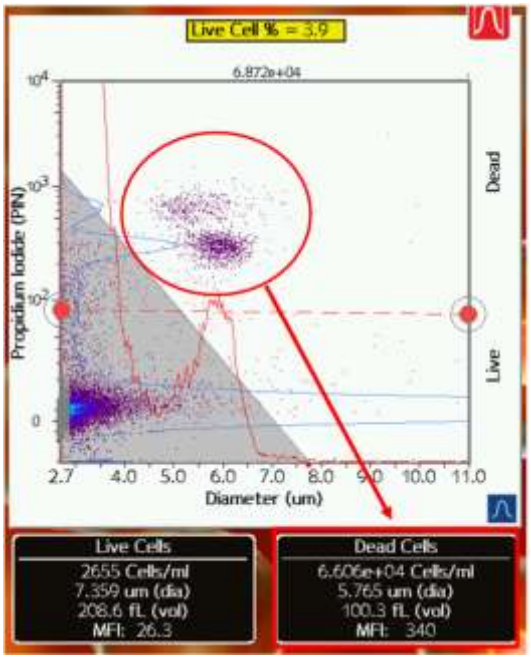

Fig. 3: Nuclei counting of a lysed PBMC sample using a Moxi V

This data was generated by a Moxi V user isolating nuclei from a PBMC sample for counting of the white blood cell (WBC) population (Fig. 3). The two-dimensional analysis provided by sizing and fluorescence highlights the Moxi systems' ability to properly isolate nuclei from debris and residual un-lysed RBCs or WBCs. Visual inspection of the recorded events helps show the effectiveness of the lysis of the sample as residual RBCs and WBCs will show up as events that are >3um but are fluorescently negative. Adjusting the gates to isolate these unlysed cells allows for additional quantification possibilities to determine the exact percentage of lysed cells if desired.

Nuclei Counting for Single Cell Genomics

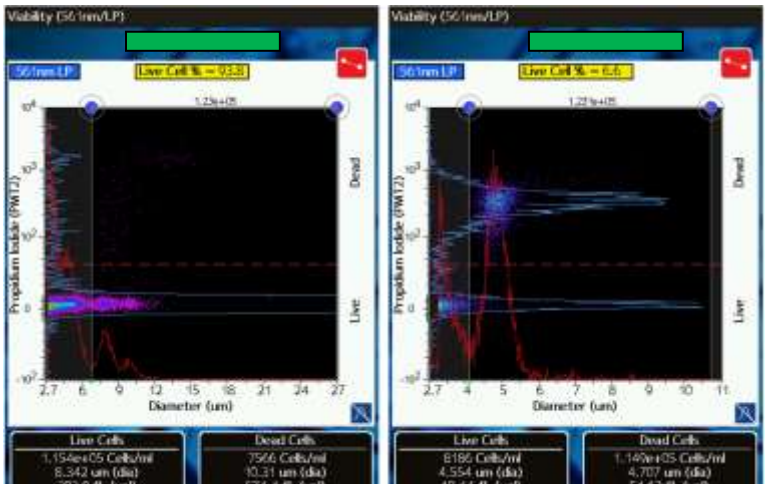

Fig. 4: Nuclei isolated for single cell library prep on a Moxi GO II.

This data was taken from a Moxi GO II user isolating nuclei from a sample during the library preparation stage prior to single cell sequencing. Accurate concentration calculations of nuclei when compared to the unlysed sample (Fig 3, right), in addition to easy identification of nuclei via PI staining, show the effectiveness of Moxi instruments in giving a fast, easy, reliable check of nuclei before sending it off for sequencing.

Summary

For any sample that requires isolation of nuclei, Moxi systems provide a reliable method of nuclei counting with unbeatable reproducibility of <5% CV. The post-run analysis software enables the user to either manually adjust gating or store pre-set gating parameters to ensure that nuclei are being properly isolated and quantified every time. This gives the flexibility to adapt to different samples as well as enable easy reproducibility between runs. Two-dimensional sample analysis with the Moxi V and Moxi GO II provides unbeatable reliability and gives the most accurate nuclei analysis of any cell counter on the market.