Nuclei Counting with Coulter-Principle Sizing and Propidium Iodide (PI) on the Moxi GO II

Overview

This protocol describes an approach to achieve rapid, highly-accurate nuclei / nucleated-cell counts by: 1.) First, lysing the cells to isolate the nuclei and 2.) Second, labeling the nuclei with propidium iodide (optional, for added accuracy), and 3.) Third, counting the nuclei using the Moxi GO II system. This approach can be applied to a variety of samples (whole blood, stromal vascular fraction, bone marrow extracts, etc.) in which enumeration of nucleated cell may be difficult due to high levels of red blood cells (RBC's) or extracellular debris. While it includes a specific lysis protocol using Zap-oglobin II, it can be applied to any nuclei preparation. With the efficiency of the Zap-oglobin II lysis, and the ability of the Moxi GO II to size-resolve (Coulter Principle) particles down to 3µm in diameter, the system can resolve the nuclei without the PI label. The PI label allows for a second dimension of distinguishing the nuclei counts, improving the accuracy.

Reagents

- Zap-oglobin II Lytic Reagent (Beckman Coulter, Cat # 7546138)
- Ca^{2+}-free and Mg^{2+}-free PBS or the equivalent (e.g. Thermo, Cat #10010023)
- Cell or Nuclei Sample (Note: for samples that are prone to clotting, they should be collected into an anti-coagulant, e.g. Heparin preferred, coated collection tube)
- Propidium Iodide (PI), 1mg/ml stock solution (e.g. Thermo, Cat #P3566)

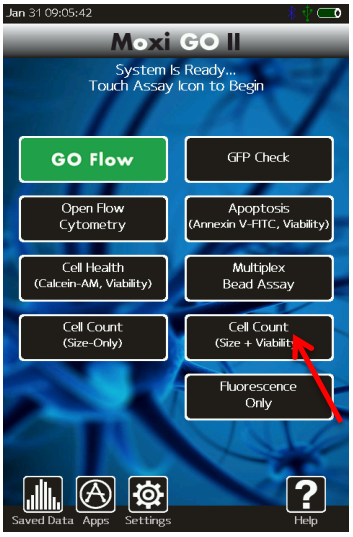
Instruments and Cassettes

- Moxi GO II System (Orflo Prod#MXG102)
- Type S+ Cassettes (Orflo Prod #MXC030)

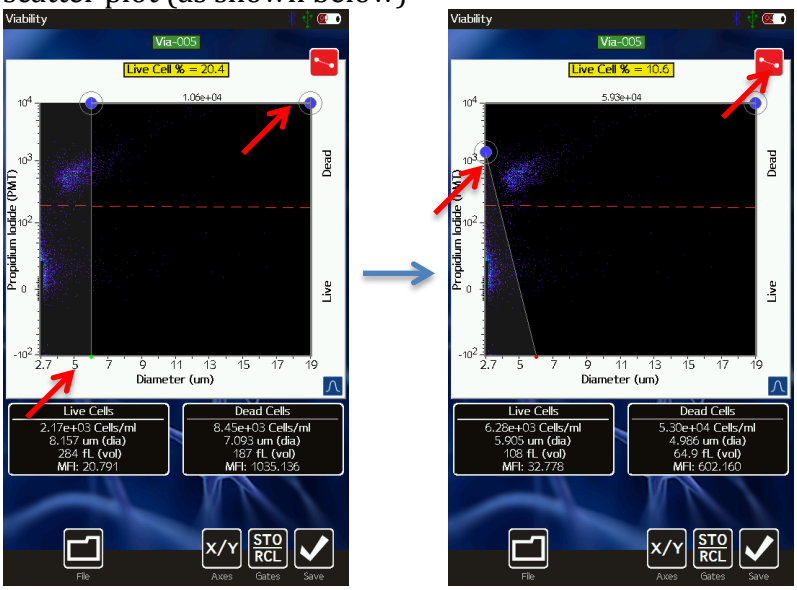
Protocol

1. Add 930µL of PBS (or 945µL if the nuclei were already isolated, i.e. no lysis step is needed) to a new micro-centrifuge tube or other small-volume tube
2. ***If lysis is required to isolate the nuclei***: Add 15µL of Zapoglobin II to PBS.
   Notes:
   a. *Mix Zapoglobin II vial well*
   b. *Make sure there are not visible precipitates in the Zapoglobin II vial (if there are, a new vial may need to be ordered)*
   c. *Remove Zapoglobin II bottle nipple so that a pipette can be used for an exact sampling of the Zapoglobin II*
3. Add 5µL of PI (1mg/ml) to the Zapoglobin II / PBS vial
4. Add exactly 50µL of the cell/nuclei sample to the above vial. Notes:
   a. Mix the sample well before sampling by inversion mixing (turn vial upside down/right-side up 20x) to ensure proper dispersion of cells./nuclei.
   b. After dispensing, pipette up-and-down in the media to ensure residual cells on the pipette tip are dispensed as well.

5. Mix the sample several times (10x) through inversion-mixing and allow the sample to sit in the dark at room temperature for 5 minutes.
6. Turn on the Moxi GO II system
7. After the above 5 minute incubation steps complete, select the "Cell Count (Size + Viability)" assay (see red arrow in the image to the right)

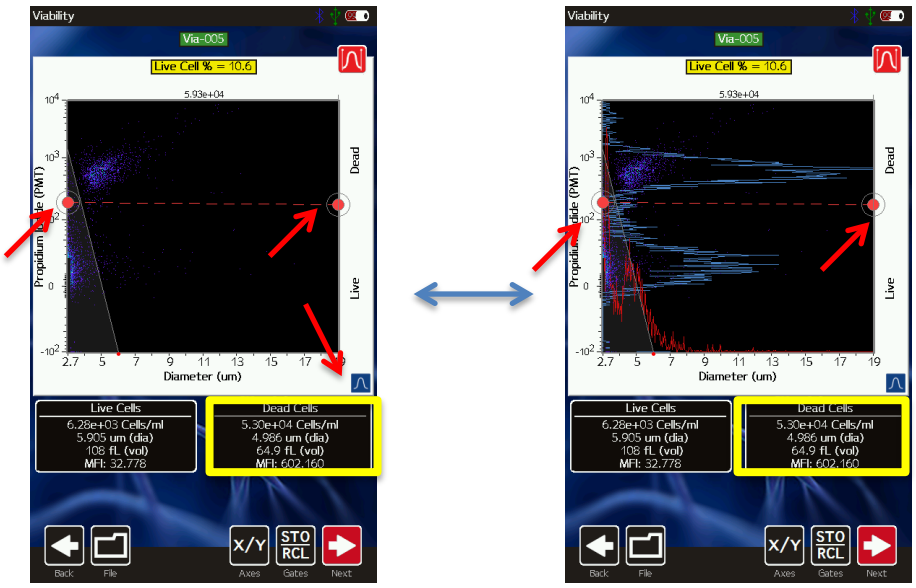


8. Load a Type S+ cassette into the system (door can be left open)
9. The System will automatically align to the cassette (and for the first test of the session with find the laser "home" position)
10. When that step completes (black bar at top will indicate "Enter the sample…" and "Close door when ready.", ***dispense 60µL of the cell prep*** (Zapoglobin II + PI tube) into the cassette loading well and close the door of the system
11. Following the test, gate the sample:
    a. Touch-and-drag the right blue/size gate all the way to the right of the scatter plot (as shown below)



    b. Position left blue/size gate at the tick between 5µm and 7µm by touching and dragging the blue dot (see image to above/left)
    c. Touch the green dot at the bottom of the gate to lock the bottom position (above/left image)
    d. Touch-and-drag the blue dot to the left to include the upper cell cluster (note the blue/dot gate can wrap around the side axis, as shown, if necessary, image above/right)
    e. Touch the red gate toggle icon to switch to the red/fluorescence gating

f. Touch-and-drag the red fluorescence gates to place the red line just under the fluorescent cluster.



Note: Turning on the histogram overlays can help in the gate placement.

1. *Touch the blue icon at the bottom right of the scatter plot*
2. *Swipe left/right if necessary to rescale the blue fluorescent histogram*
3. *Place the red markers just underneath the main fluorescent peak (as shown below)*

g. Following gating, record the total nucleated cell concentration.

i. The measured total nuclei / nucleated cell concentration is listed in the "Dead Cells" black box (highlighted with yellow boxes in the above figures, e.g. 5.3e4 cells/ml in the above example) to the bottom/right of the scatter plot.

h. To get the dilution adjusted nuclei /nucleated cell concentration, simply multiply this number by 20, e.g. 5.3e4 cells/ml * 20 = 1.06e5 cells/ml