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

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 V 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 V 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 V System (Orflo Prod#MXV102)
- Type S+ Cassettes (Orflo Prod #MXC030)

Quick Reference Protocol Table (more detail below)

Step	Bone Marrow - ASPIRATE	Check
1 (PBS)	Add 930µl PBS to microcentrifuge tube	
2 (Zap)	Add 15µL Zap-oglobin II to tube	
3 (PI)	Add 5uL PI (1mg/ml) to tube	
4 (Cells)	Add 50µL of Cell Sample to tube	
5 (Mix)	Inversion Mix – 10x	
6 (Incubate)	5 min (RT, Dark)	
7 (MGII Test)	Run Cell Counts (Size +Viability) Assay	
DILUTION	**20x**	

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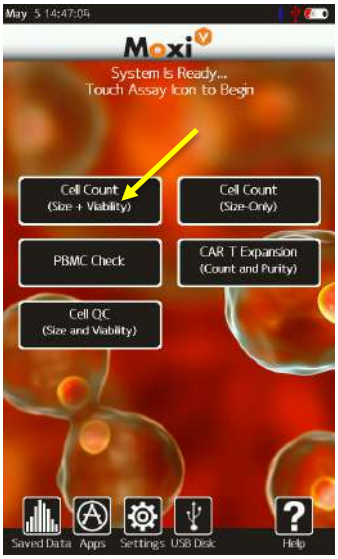
Detailed Protocol

1. Add 921µL 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 24µL (~1 drop) 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. *For the highest accuracy, use a pipette to measure Zap-oglobin quantity. Remove Zapoglobin II bottle nipple so that a pipette can be used for an exact sampling of the Zapoglobin II*
3. *Optional:* 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.

Size + Fluorescence (if PI used) – Test and Results



1. Turn on the Moxi V system
2. After the above 5 minute incubation steps complete, select the "Cell Count (Size + Viability)" assay (see yellow arrow in the image to the right)
3. Load a Type S+ cassette into the system
4. The System will automatically align to the cassette (and for the first test of the session with find the laser "home" position)
5. 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
6. Following the test, gate the sample:
    a. Optional: For optimal accuracy, rescale the output to the 2.7-11µm scale by touching the rescale button (yellow arrow) as shown below.

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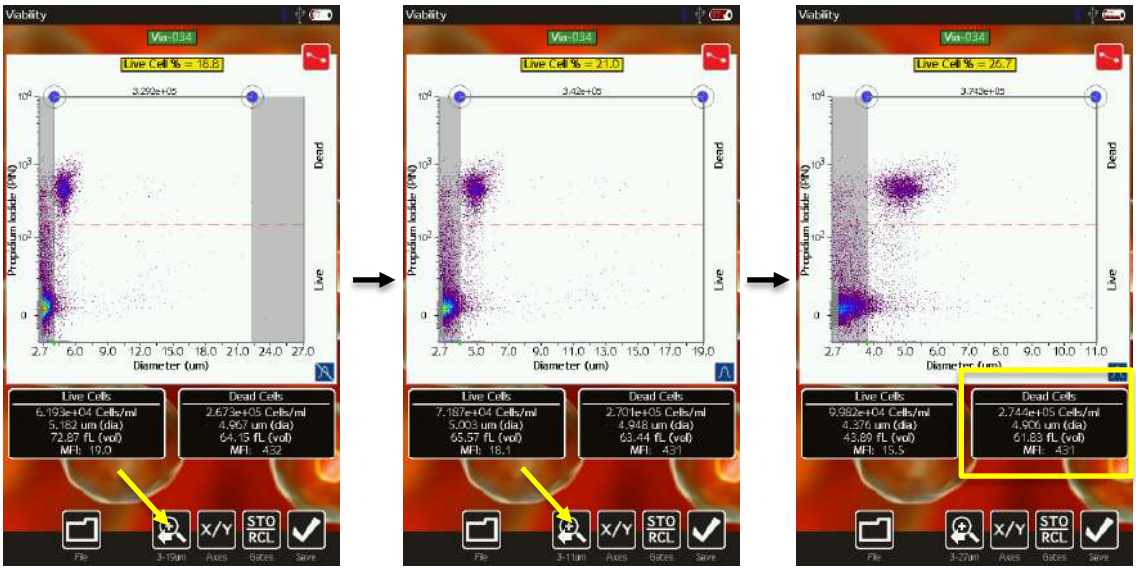
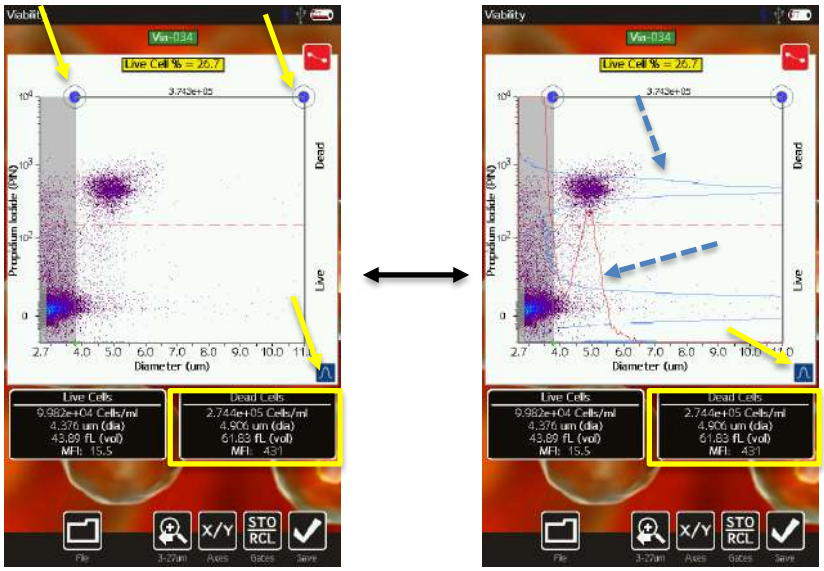
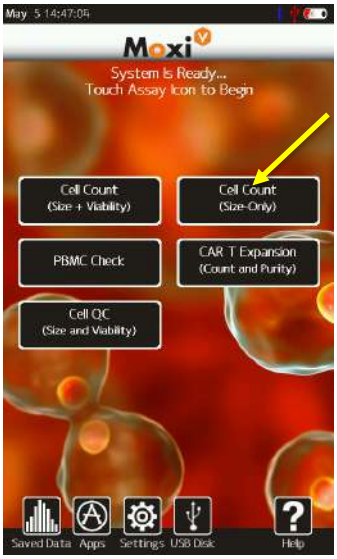


b. Touch-and-drag the right blue/size gate all the way to the right of the scatter plot (as shown below)
c. Position left blue/size gate to the left of the bright/upper population dragging the blue dot (see images below)
d.
e. Touch the red gate toggle icon to switch to the red/fluorescence gating
f. 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)*



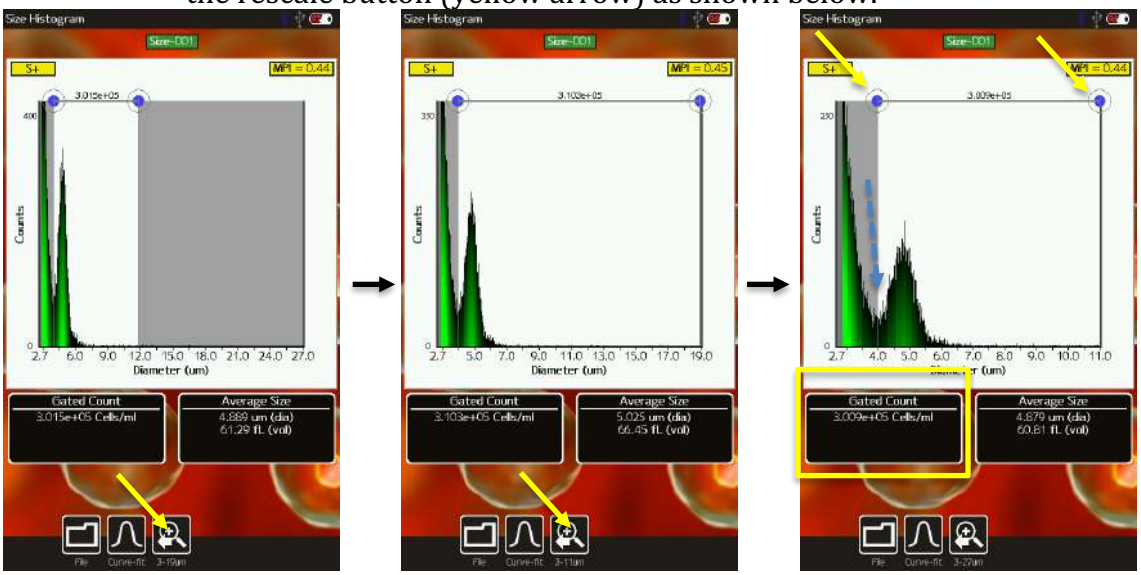
g. Following gating, record the total nucleated cell concentration. The measured total nuclei / nucleated cell concentration is listed in the "Dead Cells" black box (highlighted with yellow boxes in the above figures, e.g. 2.744e5 nuclei/ml in the above example) to the bottom/right of the scatter plot.

h. To get the dilution adjusted nuclei /nucleated cell concentration, simply multiply this number by 20, e.g. 2.744e5 cells/ml * 20 = 5.488e6 cells/ml



Size Only (no PI)– Test and Results

1. Turn on the Moxi V system
2. After the above 5 minute incubation steps complete, select the "Cell Count (Size only)" assay (see yellow arrow in the image to the right)
3. Load a Type S+ cassette into the system
4. ***Dispense 60µL of the cell prep*** (Zapoglobin II + PI tube) into the cassette loading well and close the door of the system
5. Following the test, gate the sample:
   a. Optional: For optimal accuracy, rescale the output to the 2.7-11µm scale by touching the rescale button (yellow arrow) as shown below.



   b. Touch-and-drag the right blue/size gate all the way to the right of the scatter plot (as shown above)
   c. Position left blue/size gate in the valley between the noise and the nuclei peak (see dashed blue arrow in image above)
   d. Following gating, record the total nucleated cell concentration. The measured total nuclei / nucleated cell concentration is listed in the "Gated count" black box (highlighted with yellow box in the above

figures, e.g. 3.009e5 nuclei/ml in the above example) to the bottom/left of the histogram.

e. ***To get the dilution adjusted nuclei /nucleated cell concentration, simply multiply this number by 20, e.g. 2.744e5 cells/ml * 20 = 6.018e6 cells/ml***