Acridine Orange and Propidium Iodide (PI) Staining Protocol for Orflo's Moxi GO II – Next Generation Flow Cytometer

Overview:

Acridine Orange (AO) is a cell-permeant dye that stains nucleic acids (RNA and DNA), providing a means of generically labeling nucleated cells in samples. When bound to DNA, AO can be excited a 488nm laser and will fluorescently emit with a 525nm maximum, making it well-suited for detection with the PMT1 (525/45nm) channel of the Moxi GO II. The cells can be counterstained with Propidium Iodide (PI) to simultaneously determine the sample viability using the well-established membrane permeability metric.

Instrument/Cassettes:

- Orflo Moxi GO II Next Generation Flow Cytometer (Orflo Cat #MXG102)
- Type S+ Cassettes (Orflo Cat #MXC030/MXC032)

Reagents:

- Acridine Orange (AO, e.g. Sigma A6014-10g)
- Propidium Iodide (PI, e.g. Sigma P4170-10mg)
- Phosphate Buffered Saline (PBS, e.g. Thermo 10010023)
- Purified water (e.g. Sigma W4502)

Stock Solution Preparation

- Acridine Orange
  - Initial Dilution: 1mg/ml AO stock solution– Dissolve 100mg into 100ml of purified water.
  - 50µg/ml AO stock solution - Dilute 50µL of 1mg/ml AO stock solution into 950µL of water.
- Propidium Iodide (PI) Stock Solution (1mg/ml PI) – Dissolve 10mg into 10ml of purified water.

Staining Protocol

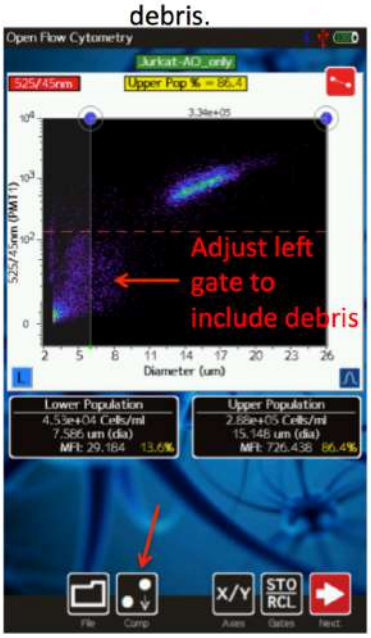
1. Suspend cells at density of 2-5 x 10^6 cells/ml in PBS.
2. Aliquot 494µL of cell suspension to a microcentifuge tube.
3. If using the 561nm/LP filter in the PMT2 slot, aliquot a second tube for use as a single stain positive control for compensation of the AO spillover into the PMT2 channel. (Note: This should not be necessary if you are using the 650nm/LP in the PMT2 slot)
4. Add 2 – 10 µL of 50µg/ml AO to each cell vial (final concentration 0.2 - 1µg/ml AO) – Note: It is recommended to perform a titration your sample to determine the optimal AO concentration. However, in the absence of that, it is recommended to try 0.1µg/ml AO as a starting point (add 1µL of 50µg/ml AO to 499µL cell suspension)
5. Add PI to the cells:
   a. If using the 561nm/LP filter, add 1µL of 1mg/ml PI to just one vial.
   b. If using the 650nm/LP filter, add 5µL of 1mg/ml PI to the cell vial

6. Gently Vortex (2-3 setting) and incubate in dark at room temperature for 5 minutes
7. Run on Moxi GO II using the GO Flow Assay with the fluorescent gain set to "Low":

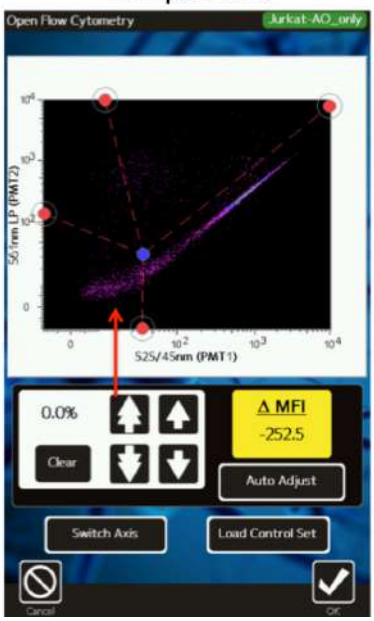
Compensation (needed for 561nm/LP filter only)

Prior to running your dual-stained (AO and PI) sample, run the single-stain AO control. Immediately following the test, compensate for the spillover as shown below:

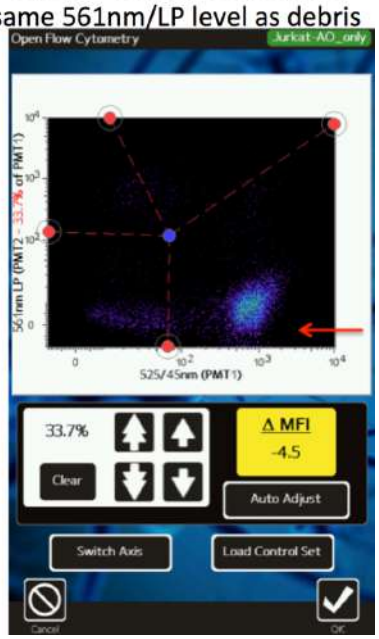
Debris can be used to compensate. Adjust the left gate to include some debris.



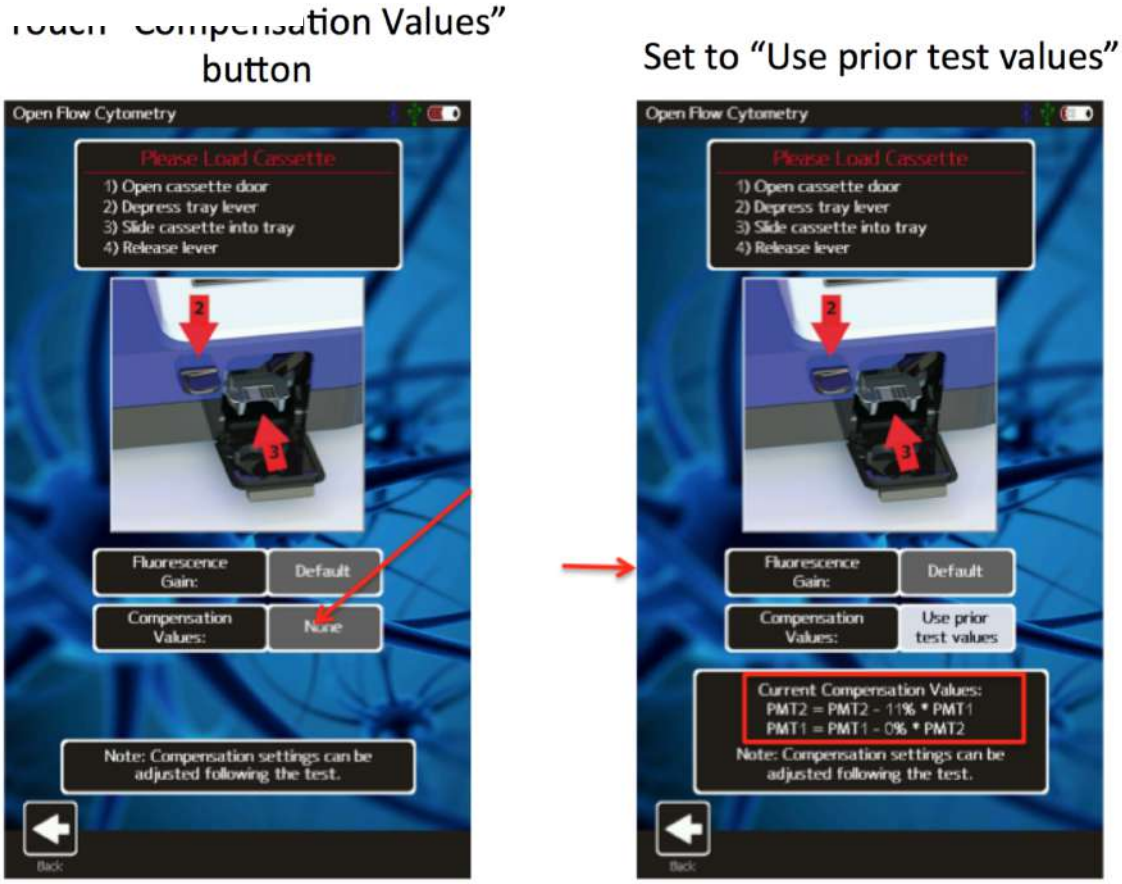
Touch "Comp" button, "Switch Axis" so that the 561nm/LP is the y-axis. Place debris in lower left quadrant



Place cell cluster in bottom right quadrant. Touch "Auto Adjust" - Cluster will lower to same 561nm/LP level as debris



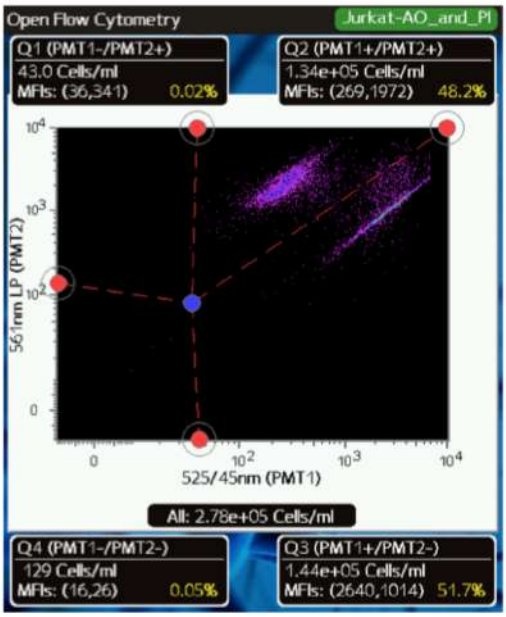
Then, when running your subsequent samples (AO and PI dual stain), make sure to select "Use Prior Compensation Values" (as shown below) to ensure that the compensation setting is applied to subsequent tests.



Example Output

The images below show the example output for a AO and PI labeled sample. In this case, a sample Jurkat cells were heat killed (60C, 15min) and mixed in a 50/50 ratio with healthy Jurkat cells to ensure that the output would include both live and dead cells for representative purposes. The cells were run using the GO Flow assay, with low fluorescent gain, and with the 561nm/LP filter installed in the PMT2 slot. The image on the left shows the sample without compensation applied and the image on the right shows it with applied compensation (33.7%). Note that, without compensation, you will see the characteristic narrow slanted cluster (rightmost cluster) that is a result of the direct correlation of the events due to the spillover. This artifact is eliminated post-compensation. However, also note that the magnitude of the AO signal decreases (shifts left in the image below/right) for the dead (PI+) cells. This is expected and due to PI quenching of the AO signal.

Uncompensated



Compensated