# Moxi Cyte Two Color Viability Reagent (MXA069) User's Guide

**(Rev – 20250206)**

**FOR RESEARCH USE ONLY**
**For Moxi GO II Systems**

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# Moxi Cyte Two Color Viability Reagent (MXA069)

## Overview

The Moxi Cyte Two Color Viability Reagent (MXA069) is an Acridine Orange (AO) and Propidium Iodide (PI) – based staining reagent specially formulated for use with ORFLO's Moxi GO II systems to provide rapid assessment of the viability of a cell sample. The assay is based on the combination of a permeable stain mixed with the general membrane-integrity/dye-exclusion method of assessing cell viability. AO is a cell permeable dye that fluoresces green when bound to DNA. PI is a cell membrane-impermeant, DNA-intercalating dye that increases fluorescence over 50x upon binding to DNA. For all cells, AO will penetrate into the cells and the cell will fluoresce green. For healthy cells, PI cannot penetrate the cell membrane and therefore cannot bind to its DNA and fluoresce. As a result, no orange/red fluorescence signal is recorded as this cell is counted. However, as cells lose membrane integrity through necrosis or apoptosis, the PI in this reagent permeates the cell, and as such both green and orange/red fluorescence signal is generated for dead cells.

## Components

- 111 test (15ml) vial of ORFLO Moxi Cyte Two Color Viability Reagent (MXA069)

## Storage and Handling

- Store the kit at 2-8°C
- Use product aseptically
- Minimize light exposure.

## Moxi Cyte Two Color Viability Reagent (MXA069)

**Protocol**

1. Prepare cells into a single-cell suspension free of large (>30µm) particulate.
    a. Notes:
        i. Primary harvests (e.g. blood or tissue extractions):
            1. If large extracellular debris is present, pass sample through a 40µm cell strainer to remove it.
            2. If large amounts of RBCs are present in the sample, an appropriate RBC lysis method must be applied to get proper counts (RBCs will mask the signal of the cell population of interest due to their extremely high concentration levels).
        ii. Cell aggregation/Clustering – Clusters and aggregates can be dissociated through pipette trituration (repeatedly, e.g. 10x, pull a substantial fraction of the cell sample in and out of a pipette tip). If a dissociation treatment was used, perform this step while cells are still suspended in the dissociation reagent.

2. Mix cell suspension and Moxi Cyte Two Color Viability Reagent (invert several times to mix) according to the following table:

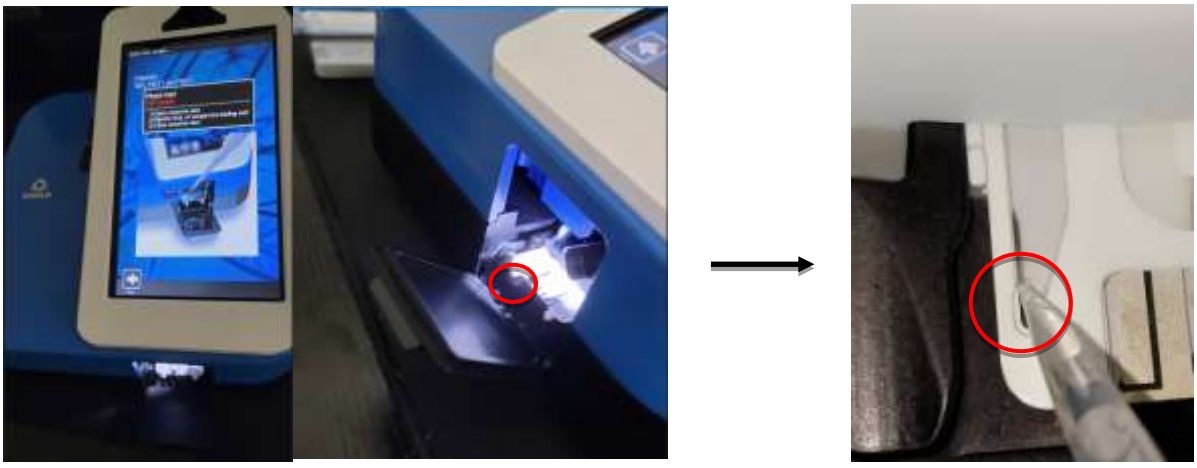
| Concentration Of Original Cell Suspension | Moxi Cyte Viability Reagent Volume | Cell Suspension Volume | Dilution Factor |
|---|---|---|---|
| 1 x $10^5$ to 3 x $10^6$ cells/ml | 135 µL | 15 µL | 10x |
| 3 x $10^6$ to 6 x $10^6$ cells/ml | 190 µL | 10 µL | 20x |
| 6 x $10^6$ to 1 x $10^7$ cells/ml | 290 µL | 10 µL | 30x |
| >1 x $10^7$ cells/ml | 490 µL | 10 µL | 50x |

3. Incubate for 5 minutes in the dark at room temperature (25°C).
4. Analyze with the Moxi GO II using the "*GOFlow*" app **with the fluorescent gain set to "Low"** within 20 minutes of initial mixing/staining.

**Running a Test** ***(Note: For detailed Moxi GO II operation instructions and images, please see the latest User Guides at https://www.orflo.com/user-manuals/ as well as virtual demos for the Moxi Go II)***

1. Prepare stained cell sample as described above.
2. Check to make sure the proper filters are installed on the side of the system.
    a. **Note: Using the 525/45nm filter (PMT1) and 646nm/LP filter (PMT2) are recommended. Use of the 561nm/LP filter in place of the 646nm/LP filter is okay but will require compensation (see below section)**
3. Turn unit on and select the "*GOFlow*" app.
4. Open the door fully, insert an unused cassette, close the door, and wait for the laser cassette alignment to complete.

5. When the alignment completes (and prompted to "Enter the Sample" at the top left black bar), open the door to the first stop (45° angle) and pipette 60µL of the sample into the loading well in one fluid motion.

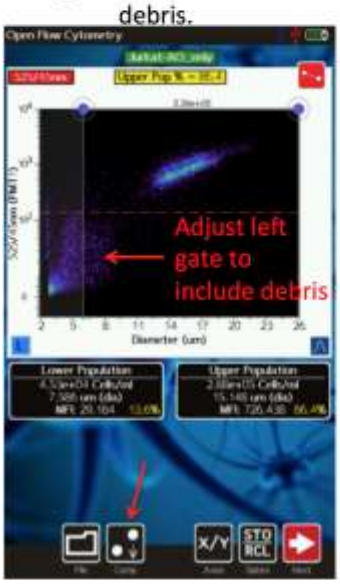


6. Immediately close the door (try not to delay longer than a second or two or the sample will begin to settle). Once the door is closed, the test will automatically run.

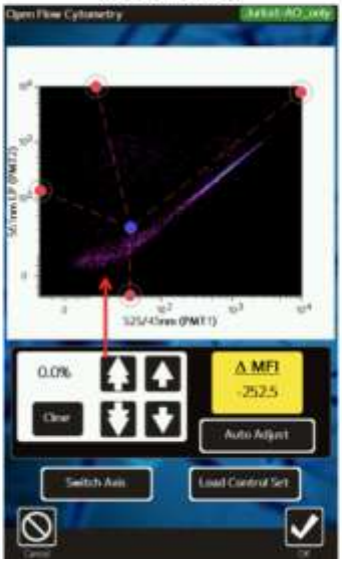
**Compensation (needed for 561nm/LP filter only)**

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:

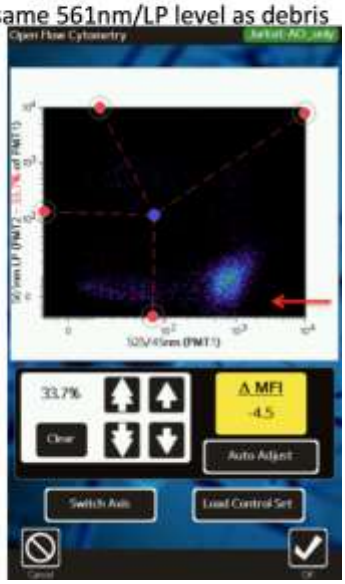
Debris can be used to compensate. Adjust the left gate to include some debris.




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

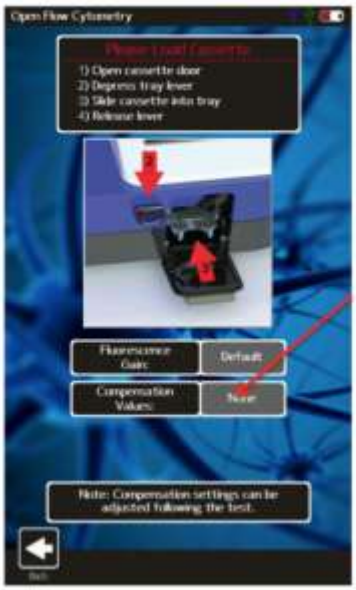



Place cell cluster in bottom right quadrant. Touch "Auto Adjust" - Cluster will lower to same 561nm/LP level as debris

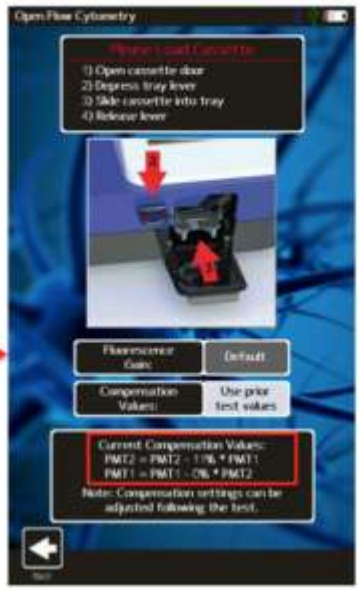



Then, when running your subsequent samples (AO and PI dual stain), make sure to select "Use Prior Compensation Values" (as shown below) to ensure that the compensation setting is applied to subsequent tests.

Touch "Compensation Values" button

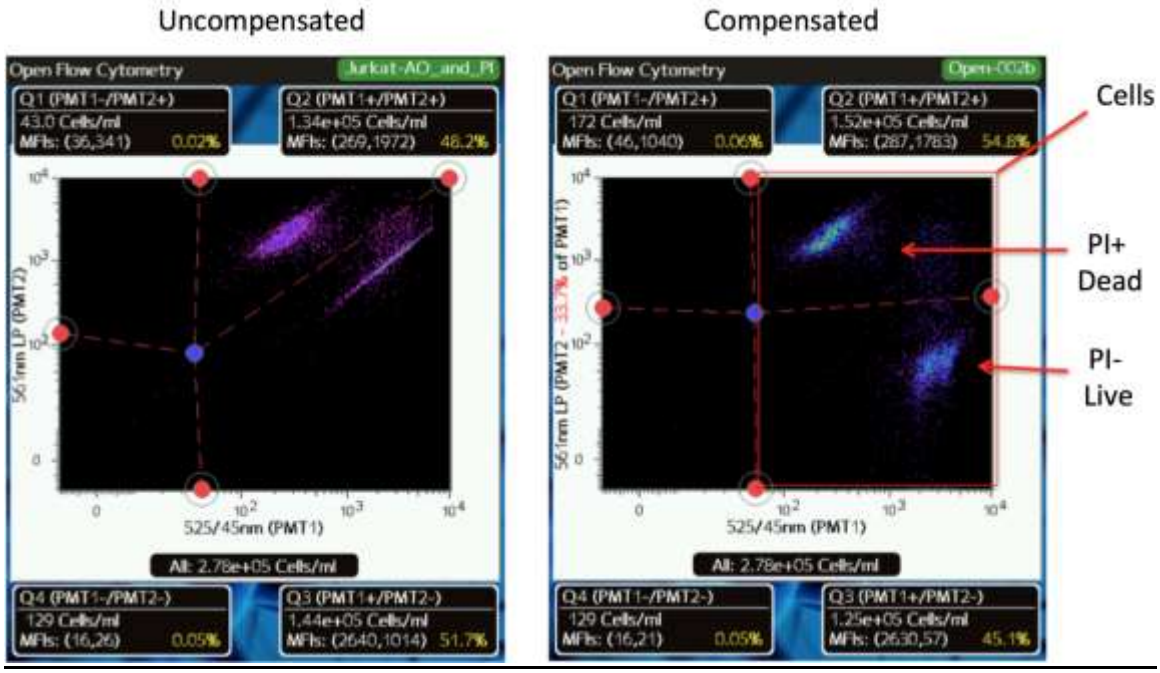



Set to "Use prior test values"



## Example Output

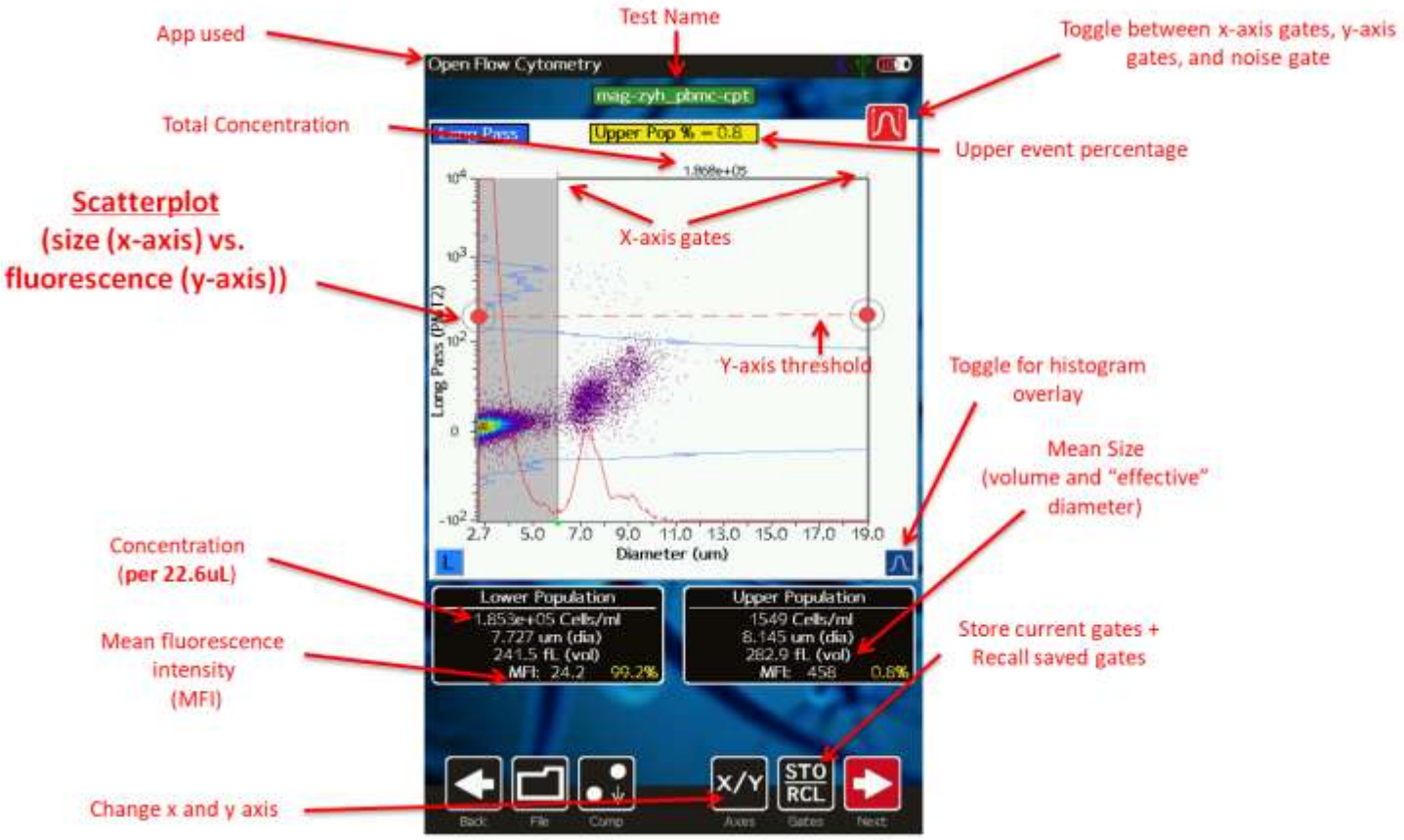
The images below show the example output for an AO and PI labeled sample. In this case, a sample of Jurkat cells were heat killed (60˚C, 15min) and mixed in a 50/50 ratio with healthy Jurkat cells to ensure that the output would include both live and dead cells for representative purposes. The cells 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.



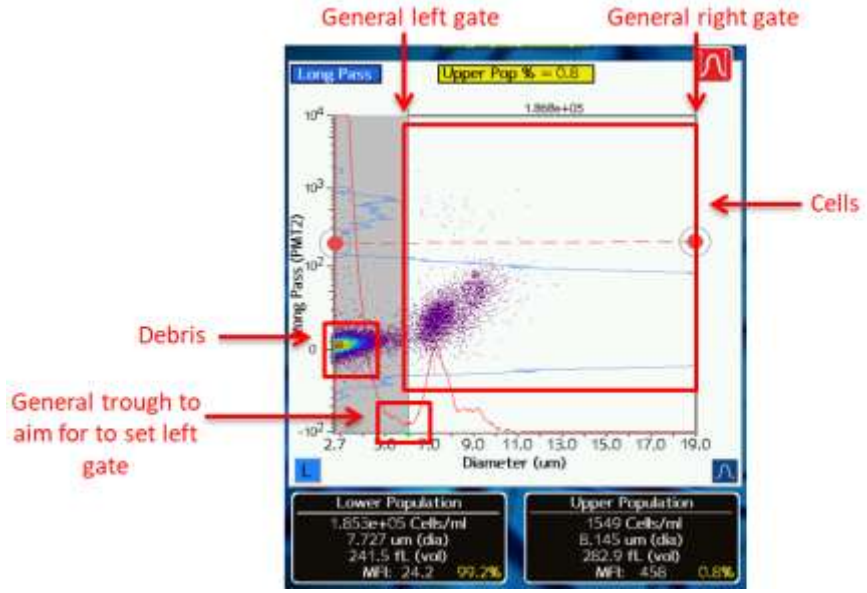

## Analyzing Results *(Note: For detailed Moxi GO II operation instructions and images, please see the latest User Guides at https://www.orflo.com/user-manuals/ as well as virtual demos for the Moxi Go II)*

1. Results are initially displayed as a Scatter/Dot Plot, Log[PMT 1] vs. Diameter (µm), with cell population gates set from the prior test run.
2. Refer to the image below for adjustments on the post-run data analysis screen
3. If it is difficult to view the cell population, touch the rescale icon to "zoom in" until you can clearly see the cells. This is only necessary for smaller particles, e.g. isolated nuclei.
4. Make sure to press the histogram overlay toggle (blue button at the bottom right of the scatterplot), as this will allow for significantly easier and more intuitive sample gating.

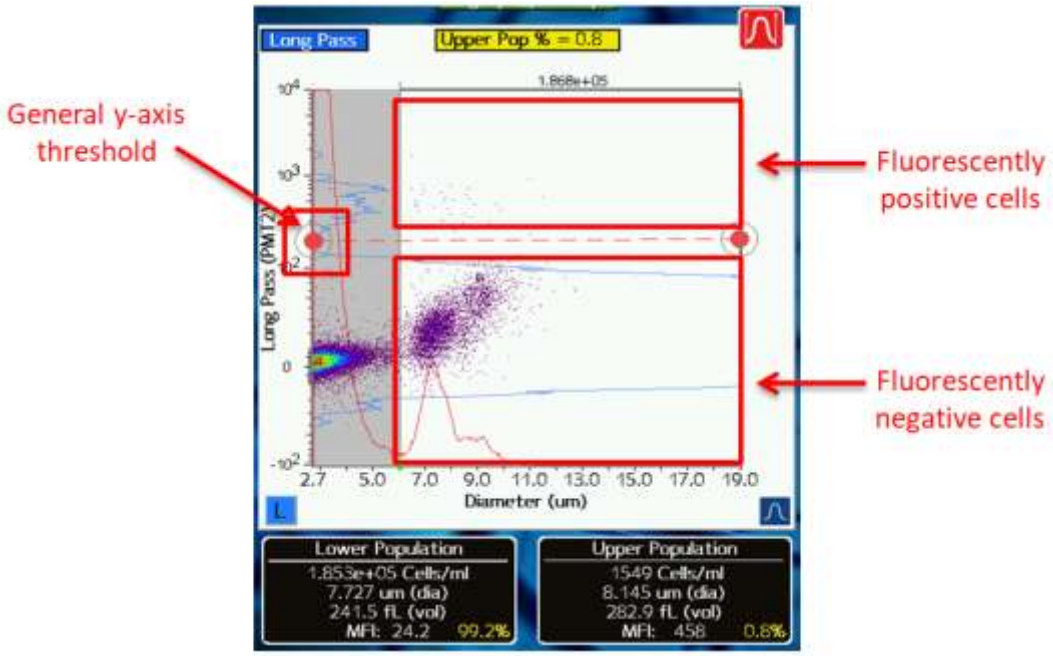
## Moxi Cyte Two Color Viability Reagent (MXA069)




5. Adjust the size gates to include the cell region by dragging the blue markers to define the appropriate size region (image below). For general gating, the left x-axis gate should be set at the trough between the debris peak (~3µm) and the cells. The right x-axis gate should be set all the way to the far right to include all cells. If auto-gating is enabled, the system will do this gating automatically. Note: The left marker can be angled if needed by toggling the left gate pivot (touch the red or green dot at the bottom of the gate to toggle).



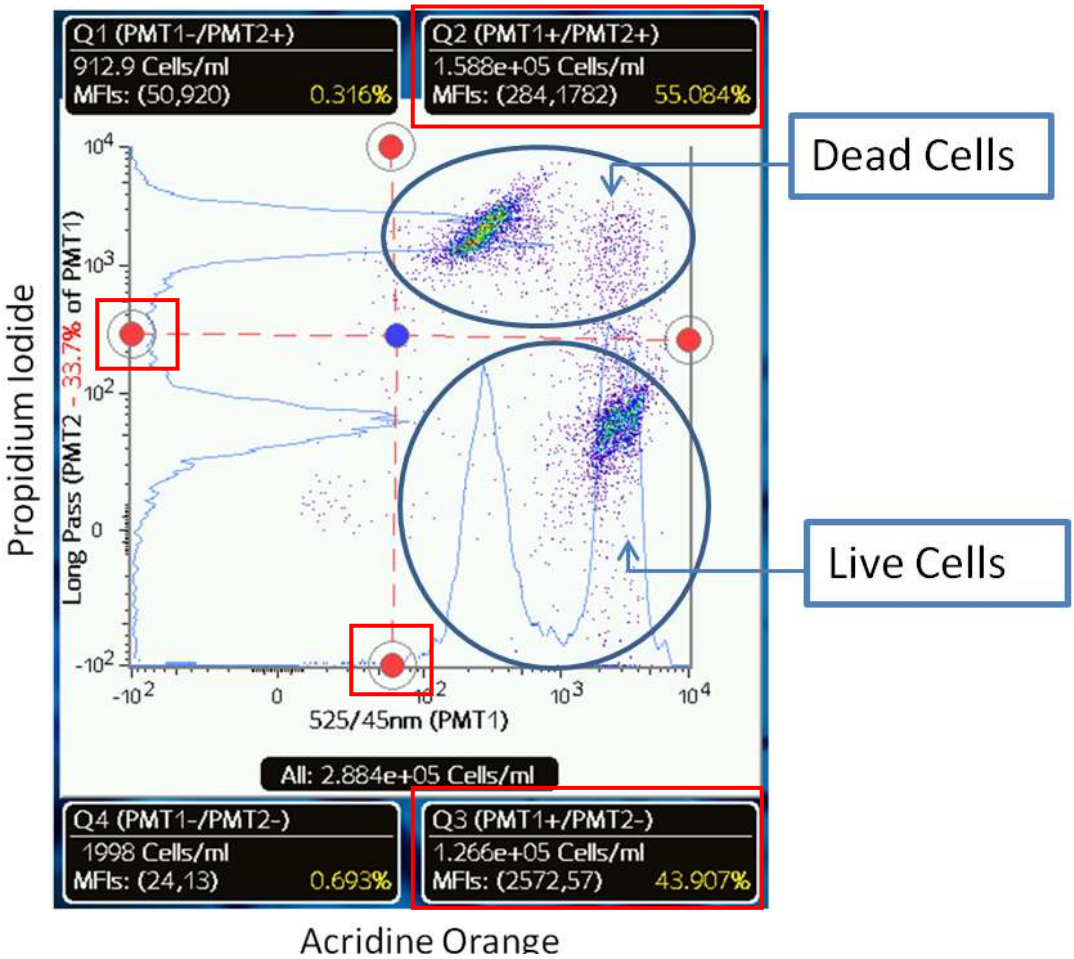
6. Touch the red gate icon (top right of scatter plot, indicated in the left image below) to enable fluorescent gating (y-axis). For general gating for viability, set the gate at the beginning of the base of the non-fluorescent population (meaning it's including only clearly living cells) by using the histogram overlay.




7. Drag the red markers to position the dashed red live/dead (fluorescence) gating marker between the live and dead clusters. If auto-gating is enabled, the system will do this gating automatically.

8. ***Sample/Test information*** (refer to image at top of page 6):
    a. *Total Cell Concentration*: listed above the scatter/dot plot.
    b. *Upper Population Percentage*: listed in the yellow box above the scatter/dot plot.
    c. *Lower Cell Population Concentration*: Listed in the black box at the bottom-left of the scatter/dot plot.
    d. *Lower Cell Population Mean Diameter*: Listed in the black box at the bottom-left of the scatter/dot plot.
    e. *Upper Cell Population Concentration*: Listed in the black box at the bottom-right of the scatter/dot plot.
    f. *Upper Cell Population Mean Diameter*: Listed in the black box at the bottom-right of the scatter/dot plot.
    g. *Mean Fluorescence Intensity (MFI)*: Listed in the black box at the bottom for each cluster (Live/Dead).

9. Change the X and Y axes such that PMT2 is on the Y axis and PMT1 is on the X axis. This will allow for two-dimensional analysis of cell using both AO (green; PMT1) and PI (orange/red; PMT2).




10. Re-adjust the quadrants if necessary by moving the central blue dot to the desired place. It is also possible to move the individual red dots on the edges as well. Use the histogram overlays to ensure proper quadrant placement. In general, the X-axis (AO; PMT1) dotted red line should be placed just before the first histogram peak (see above). Because there are fluorescently negative and fluorescently positive cell populations on the Y-axis (PI; PMT2), the dotted red line should be placed in the trough in between the two peaks.

11. Data should be recorded for **live cells** from **only Q3** (AO+PI-; green positive, orange negative) and data should be recorded for **dead cells** from **only Q2** (AO+PI+; green positive, orange positive)

## Troubleshooting

- No/poor fluorescent separation:
    a. Check total cell concentration – Increase or decrease? dilution factor (see table on Page 2) to achieve optimal concentrations.
    b. Reagent may have expired or degraded – Optimal kit storage conditions are **2-8˚C in the dark**. Always use a clean pipette tip to avoid contamination of the reagent. If you suspect the reagent integrity may have been compromised, re-order new reagent.
- Incomplete test or poor fluid flow:
    a. Over-concentration of samples can result in clogging. Increase dilution factor (see table on Page 2) to achieve optimal concentrations.
    b. Remove large particle/particulate by straining sample with a cell strainer (e.g. 40 µm).
    c. Break apart cell clusters/aggregates with pipette trituration.
- Cell population not visible/discernible:
    a. Re-scale the x-axis after the test to better view smaller-diameter cell populations.
    b. Ensure that the minimum concentration (>1 x $10^5$ cells/ml) of cells are present in the sample.
    c. Reduce potentially interfering cell size populations (e.g. peripheral cells, RBCs) via cell lysis, gradient centrifugation, or pre-plating techniques.