Moxi Cyte Single Color Viability Reagent (MXA055) User's Guide

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FOR RESEARCH USE ONLY
For the Moxi V or Moxi GO II Instruments

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Moxi Cyte Single Color Viability Reagent (MXA055)

Overview

The MoxiCyte Viability Reagent (MXA055) is a Propidium Iodide (PI) – based staining reagent has been specially formulated for use with Orflo's Moxi V or Moxi GO II instruments to provide rapid assessment of the viability of a cell sample. The assay is based on the general membrane-integrity/dye-exclusion method of assessing cell viability. PI is a cell membrane-impermeant, DNA-intercalcating dye that increases fluorescence over 50x upon binding to DNA. For healthy cells, the dye cannot penetrate the cell membrane and therefore cannot bind to its DNA and fluoresce. As a result, no fluorescence signal is recorded as this cell is counted. However, as cells lose membrane integrity through necrosis or apoptosis, this PI-based reagent permeates the cell and a strong fluorescence signal is generated.

Components

- 111 test (15ml) vial of Orflo MoxiCyte Viability Reagent (MXA055)

Storage and Handling

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

Protocol

1. Prepare cells into a single-cell suspension free of large (>30um) particulate.
    a. Notes:
        i. Primary harvests (e.g. blood or tissue extractions):
            1. If large extracellular debris is present, pass sample through a 40um cell strainer prior 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 MoxiCyte Viability Reagent (invert several times to mix) according to the following table:

Concentration Of Original Cell Suspension	MoxiCyte 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

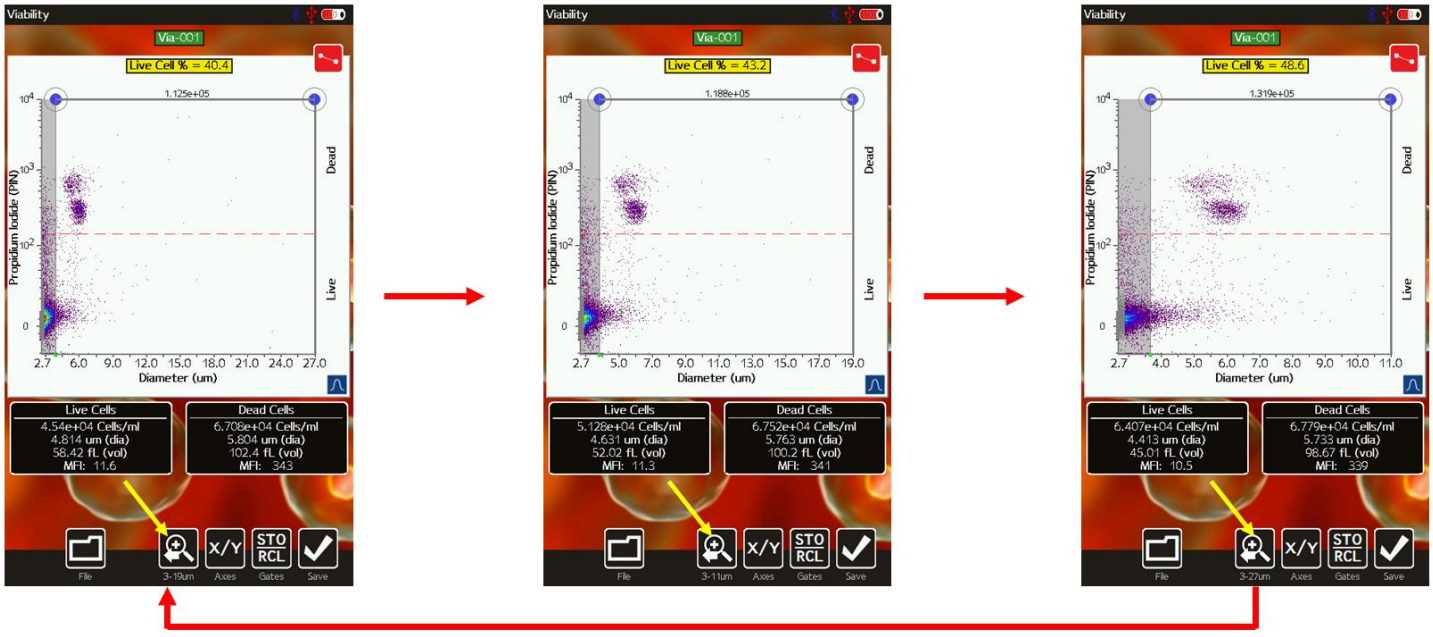
3. Incubate for 5 minutes in the dark at room temperature (25°C).
4. Analyze with the Moxi V or Moxi GO II using the "*Cell QC*" or "*Cell Count (Size + Viability)*" app within 20 minutes of initial mixing/staining.

Running a Test ***(Note: For detailed Moxi V and 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 V and Moxi Go II)***

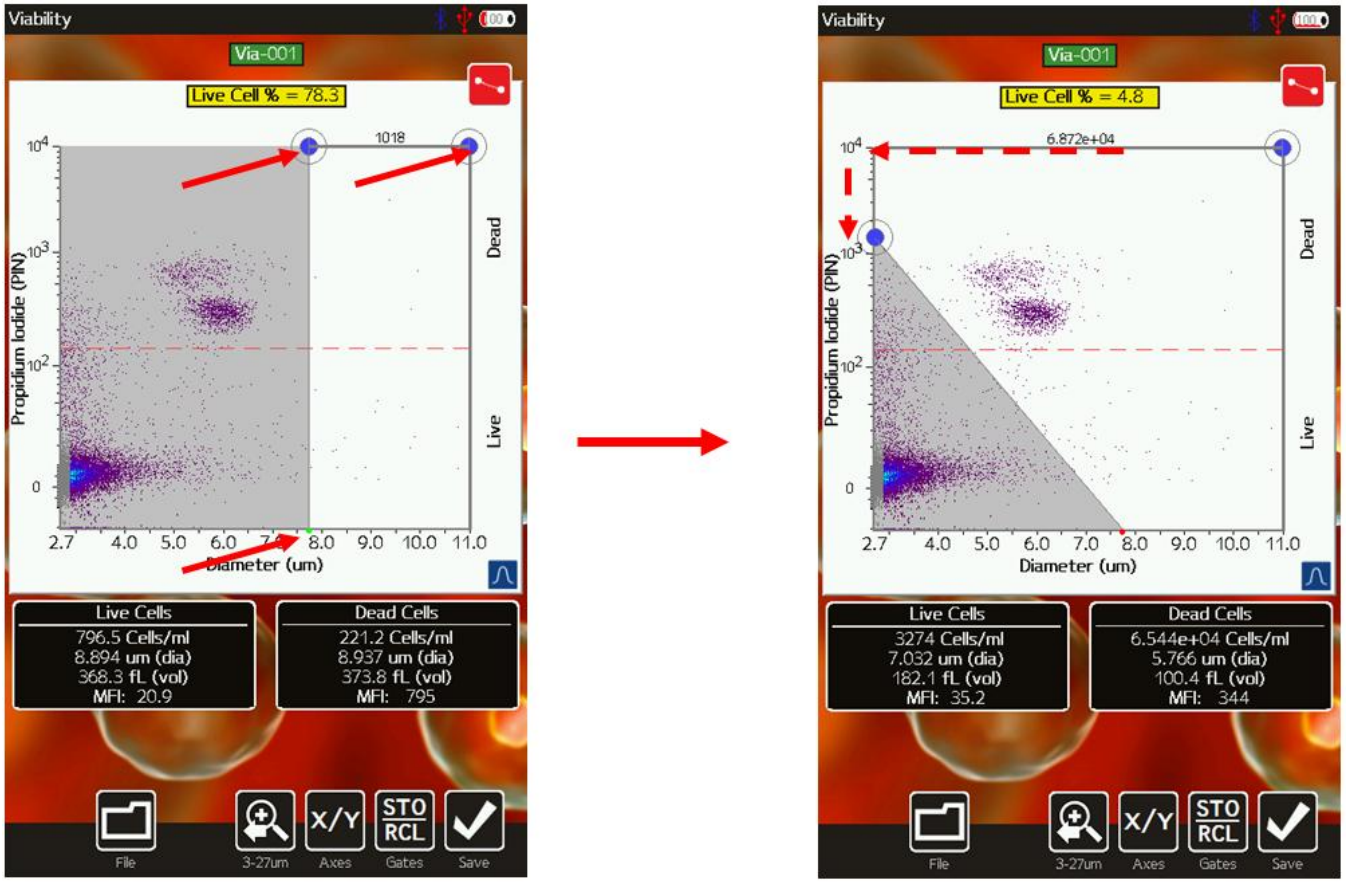
1. Prepare stained cell sample as described above.
2. Turn unit on and select the "*Cell QC*" or "*Cell Count (Size + Viability)*" app.
3. Open the door of the Moxi V/GO II unit all the way to depress the tray and insert an unused cassette.
4. Close the door and wait for the laser cassette alignment to complete.
5. Open the door only partially until you feel slight resistance (~45° angle). Mix sample well after incubation to ensure single-cell suspension and pipette 60 µL of sample into the cassette entry well.
    a. Notes:
        i. Avoid allowing pockets of air interspersed with fluid (pipette fast enough to prevent this).
        ii. It is OK to have a bead of fluid form on the entry well.
6. Close the door and test will automatically run.

Analyzing Results ***(Note: For detailed Moxi V and 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 V and Moxi Go II)***

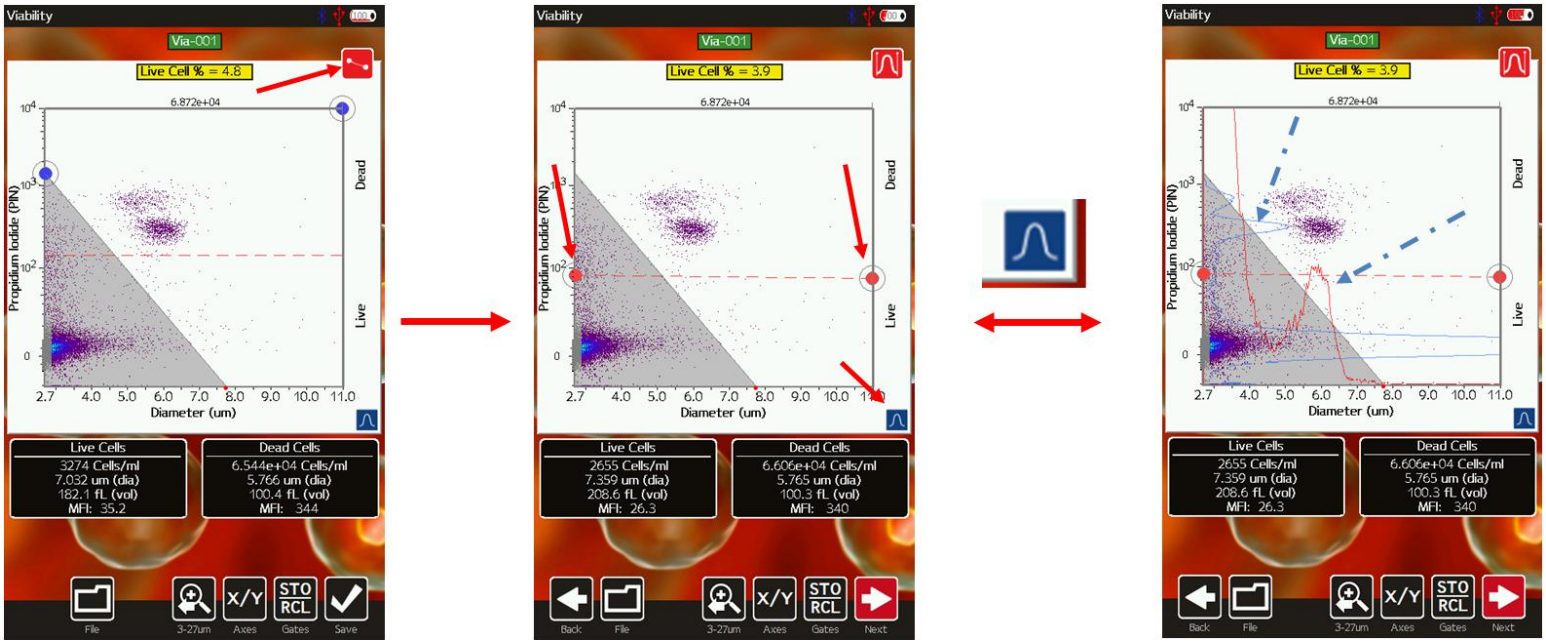
1. Results are initially displayed as a Scatter/Dot Plot, Log[PMT V] vs. Diameter (µm), with cell population gates set from the prior test run.
2. If it is difficult to view the cell population, touch the rescale icon to "zoom in" until you can clearly see the cells (image below). This is only if needed for smaller particles, e.g. the nuclei shown



3. Adjust the size gates to include the cell region by dragging the blue markers to define the appropriate size region (image below). 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).



4. Touch the red gate icon (top right of scatter plot, indicated in the left image below) to enable fluorescent gating (y-axis).



5. Drag the red markers to position the dashed red live/dead (fluorescence) gating marker (above right) between the live and dead clusters. Histogram overlays can be used to help with more complicated samples by pressing the blue curve button (bottom right of scatter plot, indicated in the middle image above). If auto-gating is enabled, the system will do this gating automatically.
6. ***Sample/Test information*** (refer to image on the above/right):
    a. *Total Cell Concentration*: listed above the scatter/dot plot.
    b. *Viability Percentage*: listed in the yellow box above the scatter/dot plot.
    c. *Live Cell Concentration*: Listed in the black box at the bottom-left of the scatter/dot plot.
    d. *Live Cell Mean Diameter*: Listed in the black box at the bottom-left of the scatter/dot plot.
    e. *Dead Cell Concentration*: Listed in the black box at the bottom-right of the scatter/dot plot.
    f. *Dead Cell 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).

Troubleshooting

- No/poor fluorescent separation:
    a. Check total cell concentration – Increase dilution factor (see table above) 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 samples can result in clogging. Increase dilution factor (see table above) 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.