MATERIALS REQUIRED

- Moxi GO II (Orflo Cat #MXG102)
- System Check Beads, Green Channel (Orflo Cat# MXA026-3)
- System Check Beads, Orange/Red Channel (Orflo Cat# MXA028-3)
- Cassettes: Type S+ (Orflo Cat# MXC030-3)

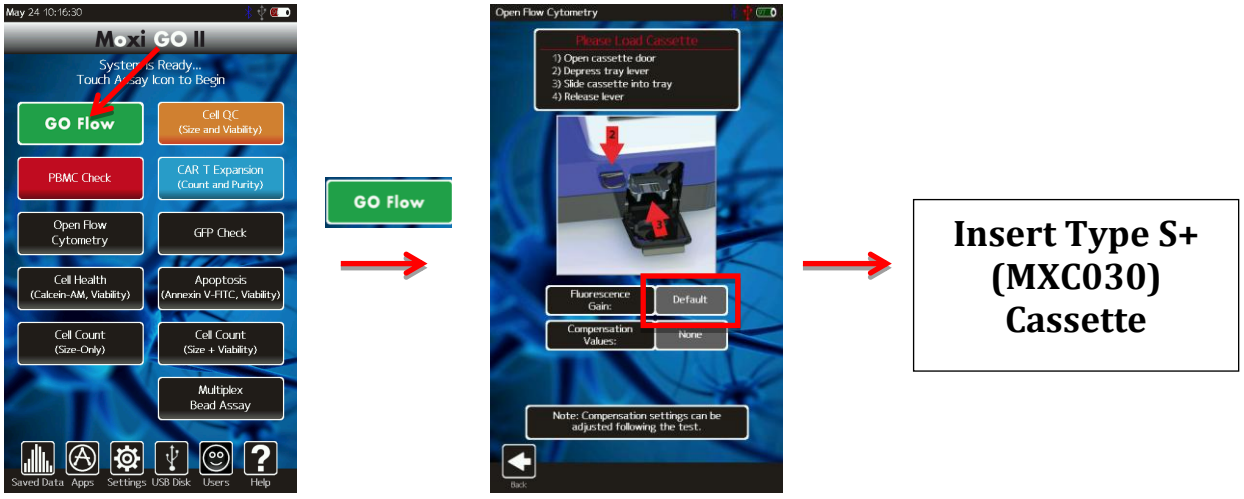
PREPARATION

1. Ultrasonicate Both Check beads for 60 seconds at room temperature (22.5°C)
2. Vortex the beads at the highest speed setting for 30 seconds.
3. Make sure to install the 561nm/LP filter in the PMT2 slot (back slot on top of the Moxi GO II).

SYSTEM CHECK BEADS, GREEN CHANNEL (MXA026)

Running the Test

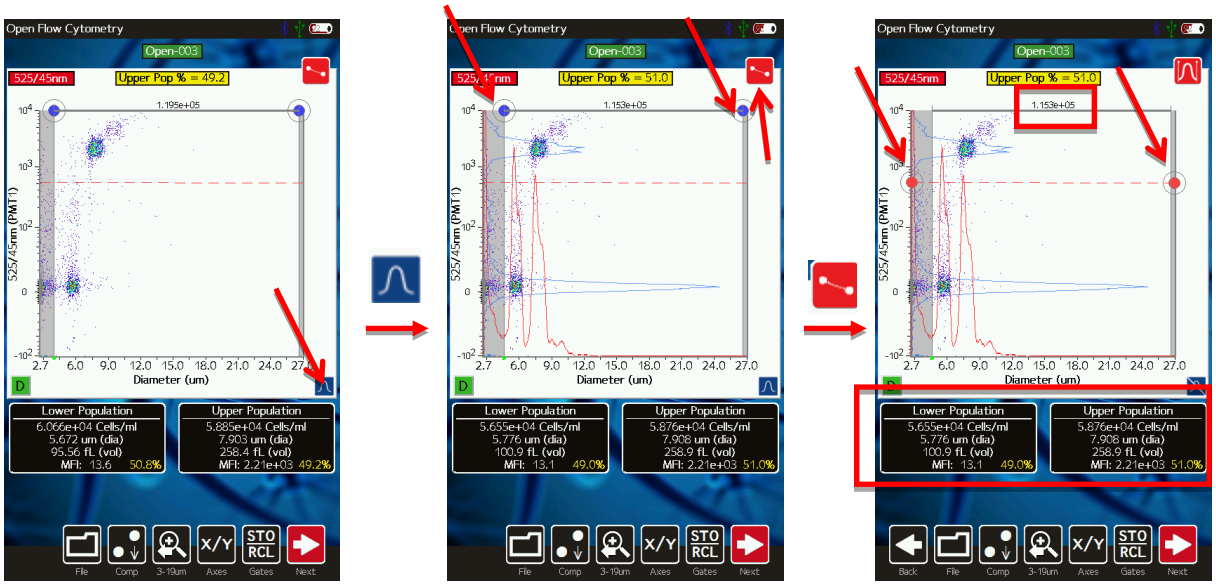
1. Mix beads by slowly inverting (~once every second) the bottle 30x initially (5x between runs, if repeats are being performed)
2. Touch "Go Flow" app (refer to images below)
3. Make sure the Fluorescence Gain is set to "Default" (touch to change if necessary)
4. Make sure the Fluorescence Gain is set to "Default" (touch to change if necessary, see red box in middle image below)
5. Insert a cassette and wait for the laser cassette alignment to complete.



6. When the alignment completes (and prompted "Enter the Sample" at the top left black bar), pipette 60µL bead sample into the loading well in one fluid motion.
7. Immediately close the door (try not to delay longer than a second or two or the beads will begin to settle) and the test will automatically run.

Analyzing the Results

8. (Refer to images below) Touch the blue histogram overlay icon at the bottom right of plot, to overlay the size (red, x- axis) and fluorescence (blue, y-axis) histograms
9. Adjust the size gates (blue vertical markers) to place it in the "valley" (minima) of the red histogram, to the left of the bead peaks (see image below/middle).
10. Toggle to enable fluorescent gating by touching the red gate icon (top right of scatter plot in the image below/middle). Place the fluorescent gate just below the upper (Bright) bead population (below the upper peak on the blue histogram, see image below/right).

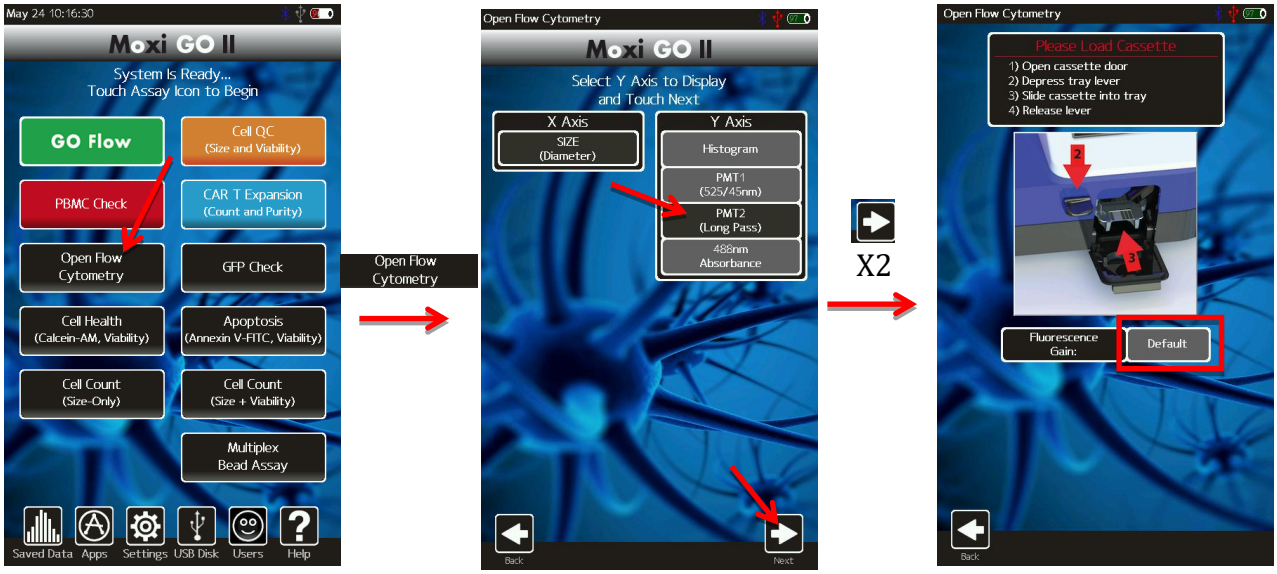


11. With this gating, you can record all relevant stats
    a. The total concentration is listed at the top of the scatter plot, between the blue/size gates.
    b. Dark Bead concentration and size is listed in the "Lower Population" black box to the lower/left of the scatter plot.
    c. Bright Bead concentration and size is listed in the "Upper Population" black box to the lower/right of the scatter plot.
12. Verify the reported concentration and size to the expected values on the bottle. The margin of error should be 15% or less for both concentration and size. ***Note: For exact lot size and concentration data, please email tech_support@orflo.com with the bead lot number to obtain a COA for that lot.***

SYSTEM CHECK BEADS, ORANGE/RED CHANNEL (MXA028)

Running the Test

1. Mix beads by slowly inverting (~once every second) the bottle 30x initially (5x between runs, if repeats are being performed).
2. Touch "Open Flow" app (refer to images below)
3. Touch the "PMT2 (Long Pass) box on the right side of the screen (image below/middle)
4. Make sure the Fluorescence Gain is set to "Default" (touch to change if necessary, see red box in right image below)

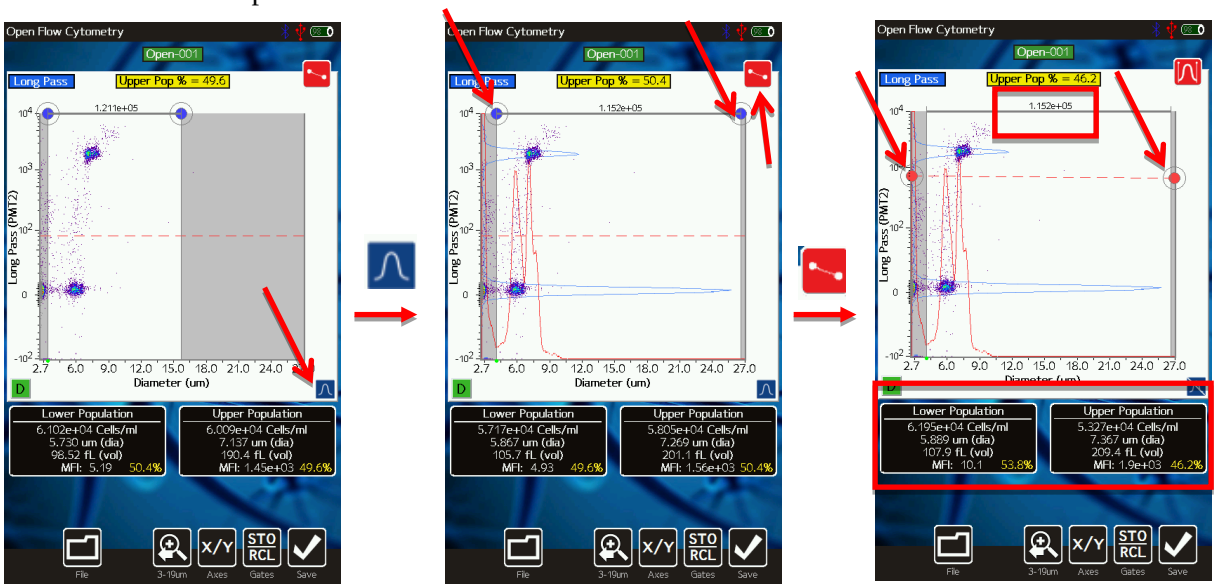


5. Insert a cassette and wait for the laser cassette alignment to complete.
6. When the alignment completes (and prompted "Enter the Sample" at the top left black bar), pipette 60µL bead sample into the loading well in one fluid motion.
7. Immediately close the door (try not to delay longer than a second or two or the beads will begin to settle) and the test will automatically run.

Analyzing the Results

8. (Refer to images below) Touch the blue histogram overlay icon at the bottom right of plot, to overlay the size (red, x- axis) and fluorescence (blue, y-axis) histograms
9. Adjust the size gates (blue vertical markers) to place it in the "valley" (minima) of the red histogram, to the left of the bead peaks (see image below/middle).
10. Toggle to enable fluorescent gating by touching the red gate icon (top right of scatter plot in the image below/middle). Place the fluorescent gate just below the upper (Bright) bead population (below the upper peak on the blue histogram, see image below/right).
11. With this gating, you can record all relevant stats
    d. The total concentration is listed at the top of the scatter plot, between the blue/size gates.

e. Dark Bead concentration and size is listed in the "Lower Population" black box to the lower/left of the scatter plot.
f. Bright Bead concentration and size is listed in the "Upper Population" black box to the lower/right of the scatter plot.



12. Verify the reported concentration and size to the expected values on the bottle. The margin of error should be 15% or less for both concentration and size. ***Note: For exact lot size and concentration data, please email tech_support@orflo.com with the bead lot number to obtain a COA for that lot.***

Factors that can affect the results:

- The plastic beads can stick to the sidewalls of the plastic containers. It is critical to ultrasonicate the beads, prior to first use, to disrupt that adhesion and ensure all beads are in solution.
- If the beads freeze, they will irreversibly aggregate resulting in lower measured concentrations. Any bead vial that has a noticeable solid mass in it (feel it while mixing) or has been confirmed to have frozen (fully or partially) should be discarded.
- Temperature of the bead solution or environment can slightly affect reported bead diameter. The values are inversely correlated (temperatures > 22.5˚C will progressively lower the reported bead volume/diameter). Bead solution and environment should be within 20-25˚C.
- Microbial contamination of the bead solution can also affect the reported diameter. Avoid contamination by handling the beads aseptically whenever possible (e.g., using a clean pipette to aliquot the solution). Store the beads at 2-8˚C when not in use.
- Improper mixing can affect the reported concentration. Sonicating does not evenly disperse the beads; it only detaches from the walls of the bottle. Slow inversion of the bottle after sonicating is key to ensuring a homogenous suspension.

Document #	Revision #	Reason
MG2.SYS.007	002	Updated company information from Orflo to Gemini Bio Simplified protocol instructions, updated system photos, revised CV acceptance criteria
	2.1	• Added 30x inversion mixing requirement (that had been overlooked in the 2.0 revision) as step 1 in “running a test” • Added Ultrasonication (bullet 1) to “Factors that can affect results” • Added bead freezing (bullet 2) to “Factors that can affect results • Removed the “avoid warming in a water bath” consideration, when discussing microbial contamination (bullet 4) • Adjusted fonts for consistency
	2.2	• Changed logo from Gemini Bio to ORFLO • Changed contact footer info to ORFLO (from Gemini Bio) • Updated part numbers to kits