MATERIALS REQUIRED

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

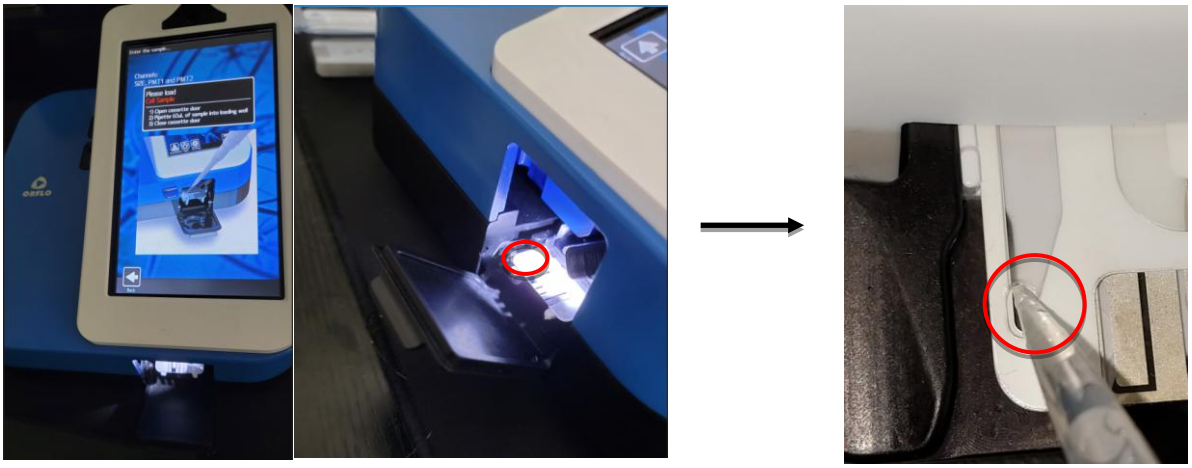
PREPARATION

1. Ultrasonicate both check bead vials 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).**

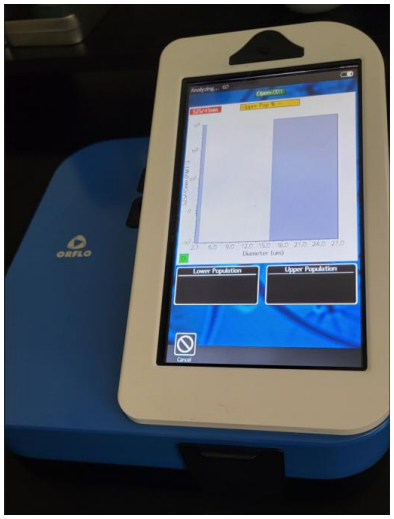
RUNNING THE TESTS

1. Mix the beads by slowly (~once every second) inverting the bottle 30x initially (5x between runs, if repeats are being performed)
2. While mixing the beads, turn the Moxi GO II unit on and select the correct App (**See below: "VALIDATION CHECK BEADS, GREEN CHANNEL (MXA026)" and "VALIDATION CHECK BEADS, ORANGE/RED CHANNEL (MXA028)" for correct App)**
3. Open the door fully, insert an unused cassette, close the door, and wait for the laser cassette alignment to complete.

4. 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 check bead sample into the loading well in one fluid motion.



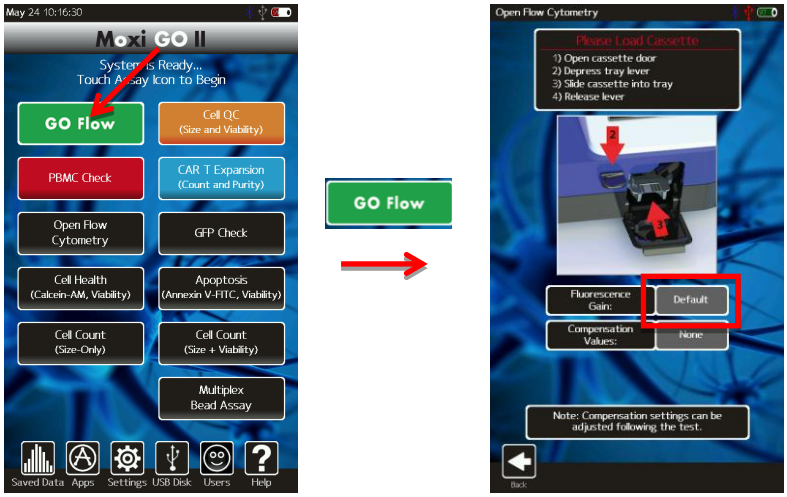
5. Immediately close the door (try not to delay longer than a second or two or the beads will begin to settle). Once the door is closed, the test will automatically run.



VALIDATION CHECK BEADS, GREEN CHANNEL (MXA026)

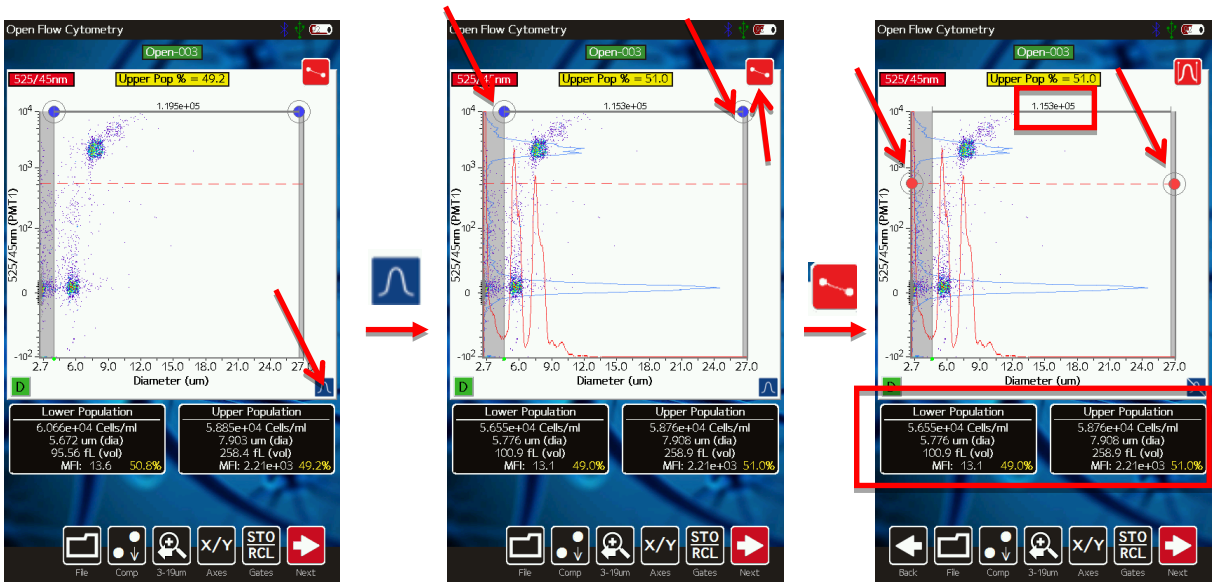
Running the Test

1. Mix beads by slowly inverting the vial (~once every second) 30x initially (5x between runs, if repeats are being performed)
2. **Touch "GoFlow" app (refer to images below)**
3. Make sure the Fluorescence Gain is set to **"Default"** (touch to change if necessary, see red box in middle image below)
4. **Run the sample test in accordance with the above "RUNNING THE TESTS" section**

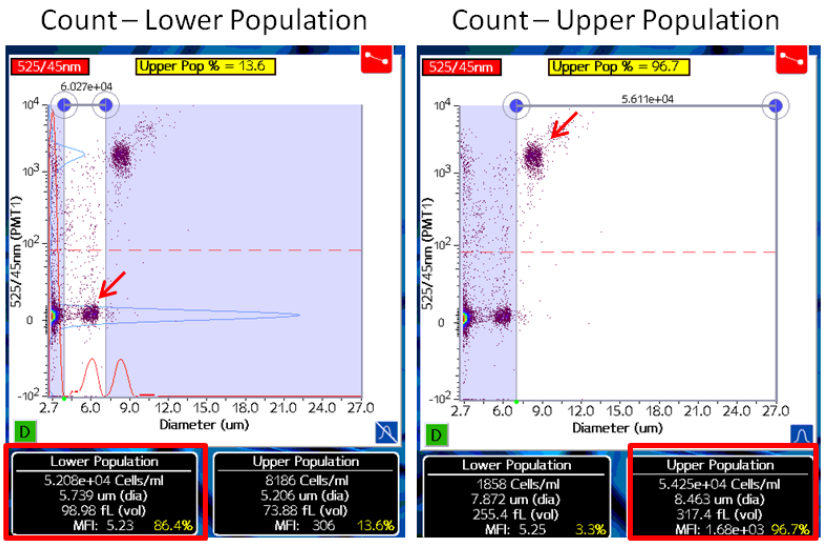


Analyzing the Results

5. The results will be displayed in a scatterplot similar to the results shown below. The data will be analyzed by adjusting both the x-axis and y-axis gates manually.
    a. 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. These histogram overlays will be used for proper gating.
    b. Adjust the size gates using the blue vertical markers (see image below/middle).
    c. Toggle the red button in the upper right of the scatterplot to enable fluorescent gating by touching the red gate icon (top right of scatter plot; see image below/right).



6. Follow the guidelines below for properly isolating and quantifying the two populations of beads present:



a. <u>Dark bead (non-fluorescent) population (image above/left):</u>
   i. Adjust the x-axis size gates such that they are just to the left and right of the first histogram peak (**left gate ~4µm, right gate ~7µm**)
   ii. Adjust the y-axis fluorescence gate such that it is directly above the first blue histogram peak; if this was previously done for the light bead population, leave the y-axis gates the same to ensure accurate results
   iii. Record all information displayed in the "Lower Population" box at the bottom of the screen (image above/left; highlighted in red)

iv. Store the x and y-axis gating information by pressing the "Gates" button and choosing one of the numbers on the top row in green. These gates can now be recalled for future validation tests by pressing the "Gates" button and clicking the corresponding number on the bottom row in red.

b. Light bead (fluorescent) population (image above/right):

i. Adjust the x-axis size gates such that they are just to the left of the second histogram peak and all the way to the far right side of the plot (**left gate ~7µm, right gate ~27µm**)

ii. Adjust the y-axis fluorescence gate such that it is directly above the first blue histogram peak; if this was previously done for the dark bead population, leave the y-axis gates the same to ensure accurate results

iii. Record all information displayed in the "Upper Population" box at the bottom of the screen (image above/right; highlighted in red)

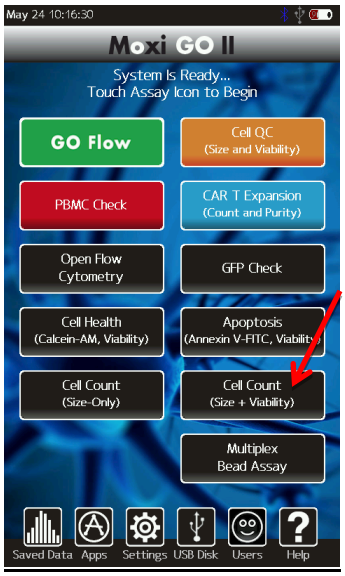
iv. Store the x and y-axis gating information by pressing the "Gates" button and choosing one of the numbers on the top row in green. These gates can now be recalled for future validation tests by pressing the "Gates" button and clicking the corresponding number on the bottom row in red.

7. Compare 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.

VALIDATION CHECK BEADS, ORANGE/RED CHANNEL (MXA028)

Running the Test

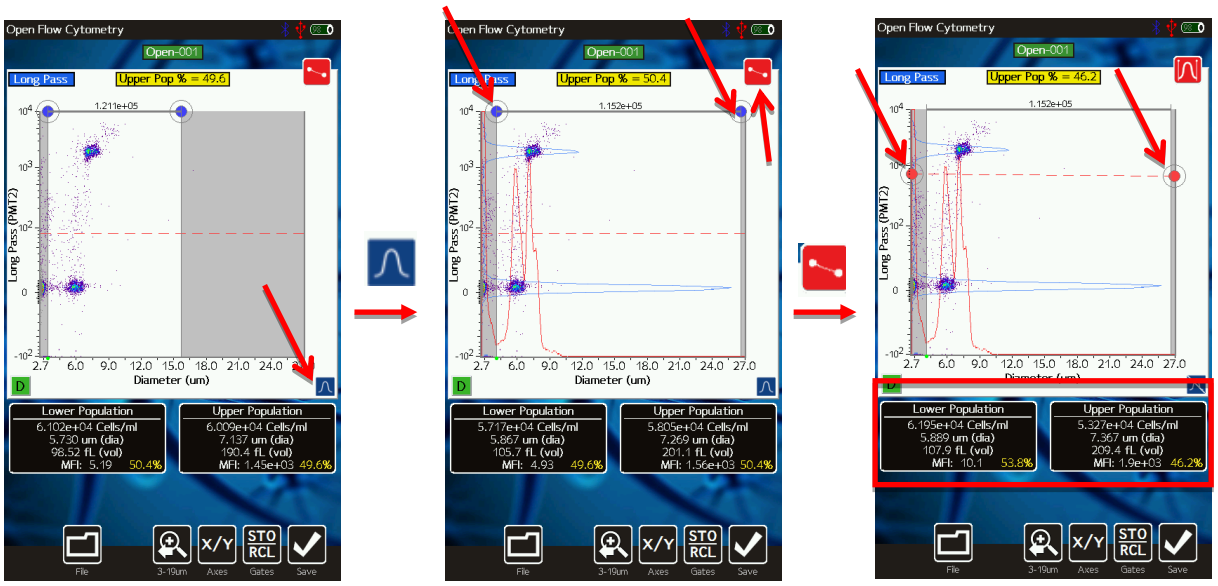
1. Mix beads by slowly inverting the vial (~once every second) 30x initially (5x between runs, if repeats are being performed).
2. **Touch "Cell Counts (Size + Viability)" app (refer to images below)**



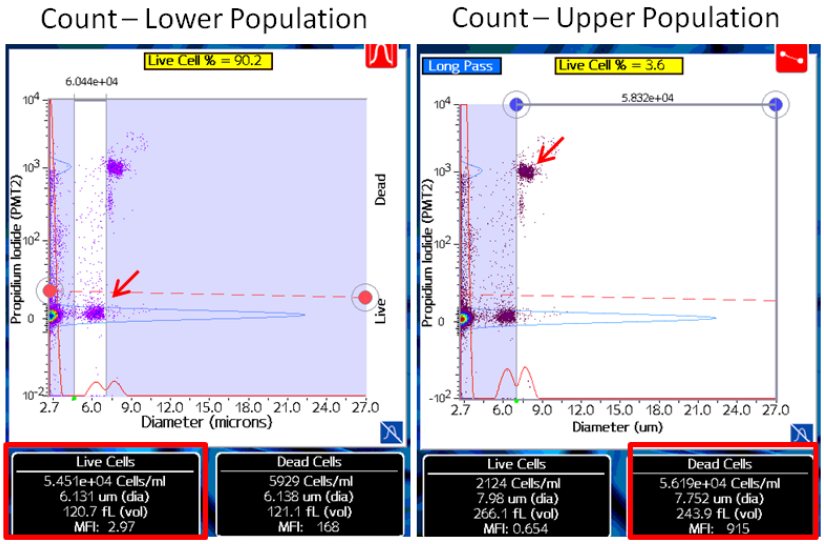
3. **Run the sample test in accordance with the above "RUNNING THE TESTS" section**

Analyzing the Results

4. The results will be displayed in a scatterplot similar to the results shown below. The data will be analyzed by adjusting both the x-axis and y-axis gates manually.
    a. 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. These histogram overlays will be invaluable for proper gating.
    b. Adjust the size gates using the blue vertical markers (see image below/middle).
    c. Toggle the red button in the upper right of the scatterplot to enable fluorescent gating by touching the red gate icon (top right of scatter plot in the image below/right).



5. Follow the guidelines below for properly isolating and quantifying the two populations of beads present:



a. <u>Dark bead (non-fluorescent) population (image above/left):</u>
   i. Adjust the x-axis size gates such that they are just to the left and right of the first histogram peak (**left gate ~5µm, right gate ~6.5µm**)
   ii. Adjust the y-axis fluorescence gate such that it is directly above the first blue histogram peak; if this was previously done for the light bead population, leave the y-axis gates the same to ensure accurate results
   iii. Record all information displayed in the "Live Cell" box at the bottom of the screen (image above/left; highlighted in red)

iv. Store the x and y-axis gating information by pressing the "Gates" button and choosing one of the numbers on the top row in green. These gates can now be recalled for future validation tests by pressing the "Gates" button and clicking the corresponding number on the bottom row in red.

b. Light bead (fluorescent) population (image above/right):

i. Adjust the x-axis size gates such that they are just to the left of the second histogram peak and all the way to the far right side of the plot (**left gate ~6.5µm, right gate ~27µm**)

ii. Adjust the y-axis fluorescence gate such that it is directly above the first blue histogram peak; if this was previously done for the dark bead population, leave the y-axis gates the same to ensure accurate results

iii. Record all information displayed in the "Dead Cell" box at the bottom of the screen (image above/right; highlighted in red)

iv. Store the x and y-axis gating information by pressing the "Gates" button and choosing one of the numbers on the top row in green. These gates can now be recalled for future validation tests by pressing the "Gates" button and clicking the corresponding number on the bottom row in red.

2. Compare 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.

FACTORS THAT CAN AFFECT RESULTS

1. The plastic beads can stick to the sidewalls of plastic containers. It is critical to ultrasonicate the beads, prior to first use, to disrupt that adhesion and ensure all beads are in solution.
2. 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.
3. 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.
4. 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.
5. 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 mixing of the vial 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
	2.3	• Updated ORFLO contact information
	2.4	• Updated overall protocol to improve test explanations and to be more comprehensive and useable for end users