Moxi GO II – System Check Bead – Verification Procedure

Materials required

- Moxi GO II (Orflo Cat #MXG102)
- Moxi GO Dragon 488nm – 525-45nm System Check Beads (Orflo Cat# MXA026)
- Moxi Flow System Check Beads (Orflo Cat# MXA016)
- Cassettes: CST (Orflo Cat# MXC040)

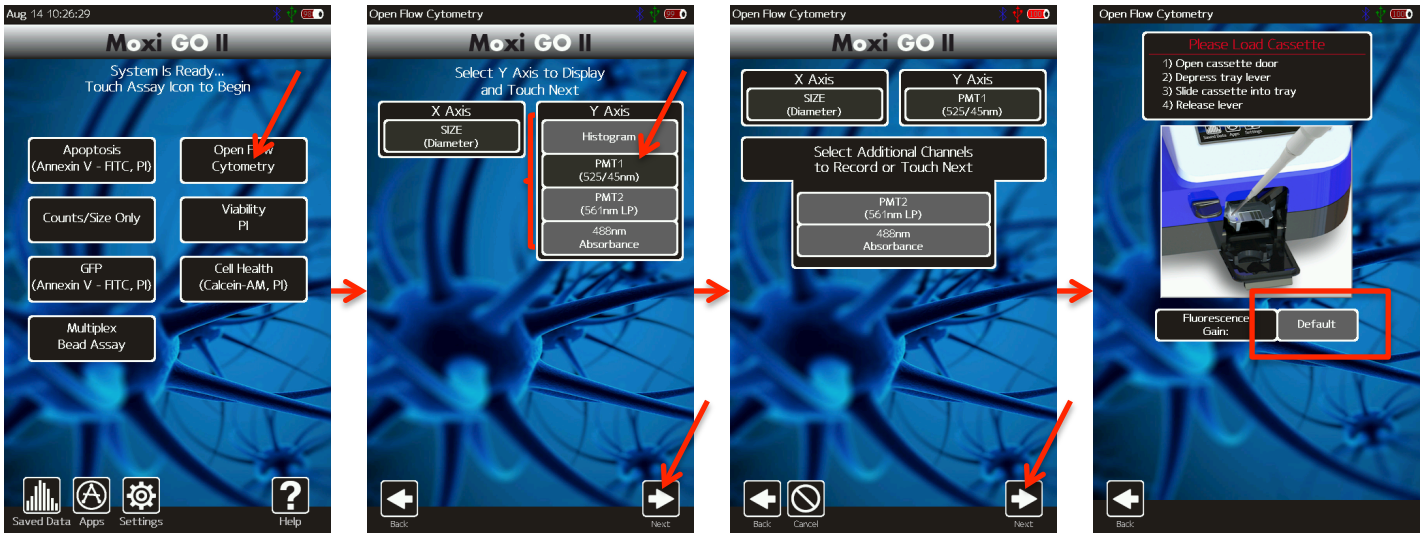
Pre-Preparation

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.

Running the Tests

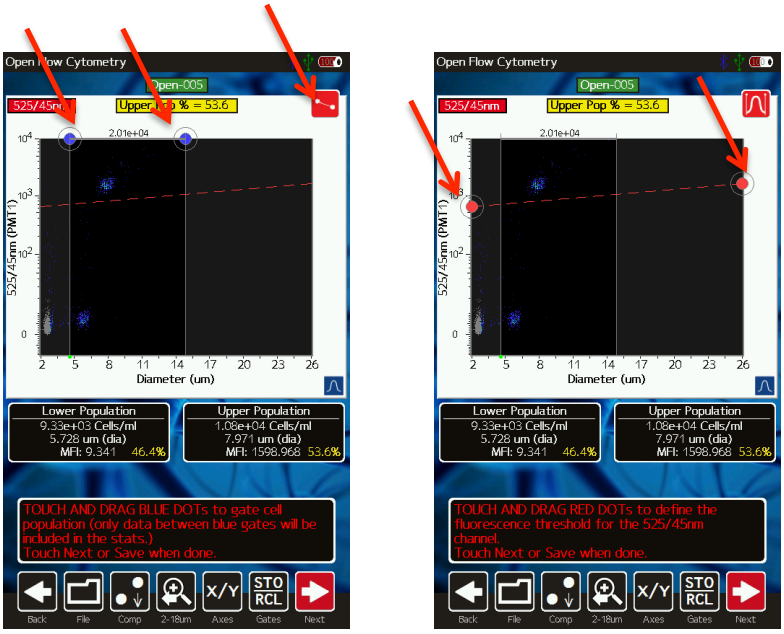
488nm Bead Test

1. Mix beads by slowly inverting the bottle 10x initially (3x between runs)
2. Turn Moxi GO II unit on and select "Open Flow Cytometry" App
3. Follow the steps below (see image as reference)
    a. Select PMT 1 "525/45nm" as the display axis and touch "Next"
    b. Touch "Next Again
    c. Make sure the Fluorescence Gain is set to "Default" (touch to change if necessary)

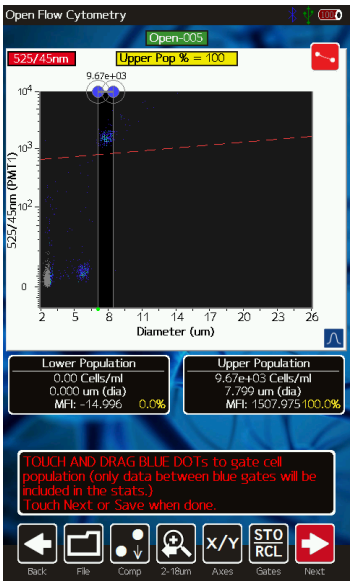


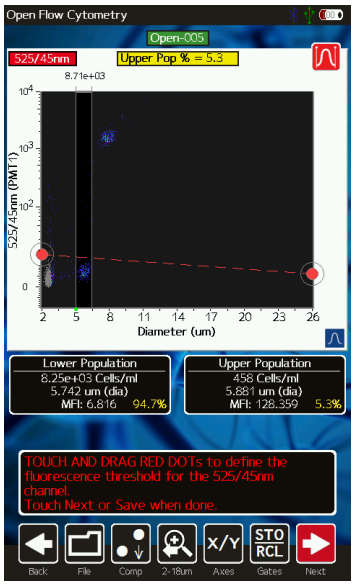
4. Insert a cassette and wait for the laser cassette alignment to complete.
5. When the alignment completes (and prompted "Enter the Sample" at the top left black bar), pippette 60µL bead sample into the loading well in one fluid motion.
6. Immediately close the door (try not to delay longer than a second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

488nm System Check Beads – Result Analysis



1. To get the concentration of each bead type:
    a. Adjust the size gates (blue vertical markers) to include both bead populations while leaving out the noise cluster (see image above)
    b. Toggle to enable fluorescent gating by touching the red gate icon (top right of scatter plot in the image to the right). Angle the fluorescent gate just below the upper (Bright) bead population (see images above)
2. To get the size diameter of each bead type:
    a. Adjust the size gates (blue vertical markers) ***tightly*** around each of the bead populations separately (see image below). ***Notes:***
        i. The blue gates should be placed tightly around the main cluster (do not include the tails to the right of the main cluster
        ii. The fluorescence gate should be placed tightly below (bright bead) or above (dark bead) the bead cluster for most accurate results.

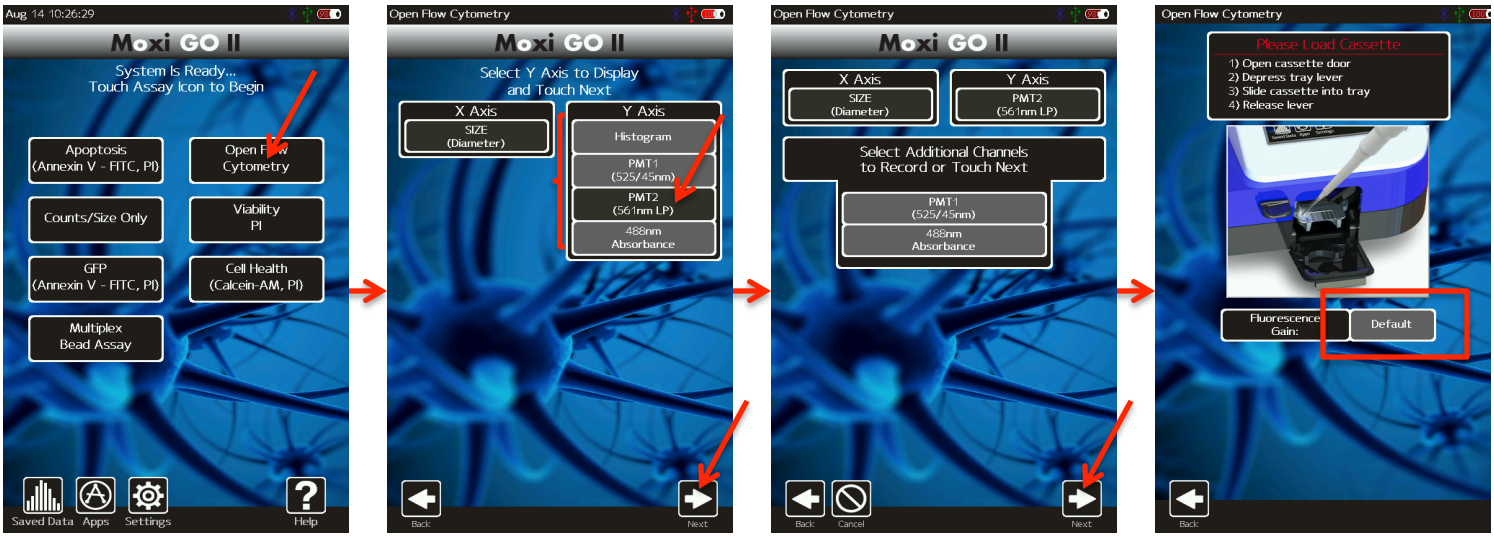




3. Verify the reported concentration and size to the expected values on the bottle. The margin of error should be 10% or less for both concentration and size. ***Note: For exact lot size and concentration data, please email tech_support@orflo.com with the bead lot number to obtain a COA for that lot.***

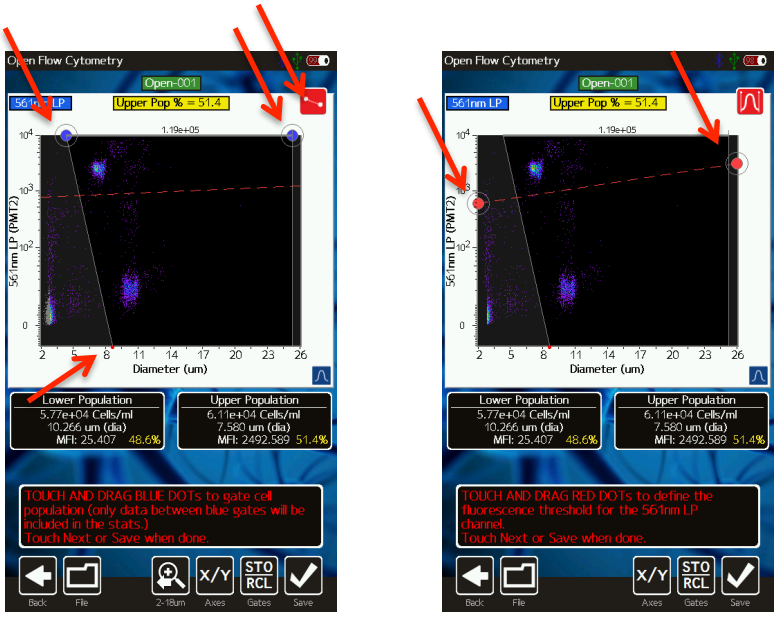
561nm/LP (Moxi Flow System Check Beads) Test

1. Mix beads by slowly inverting the bottle 10x initially (3x between runs)
2. Turn Moxi GO II unit on and select "Open Flow Cytometry" App
3. Follow the steps below (see image as reference)
    a. Select PMT 2 "561nm/LP" as the display axis and touch "Next"
    b. Touch "Next Again
    c. Make sure the Fluorescence Gain is set to "Default" (touch to change if necessary)

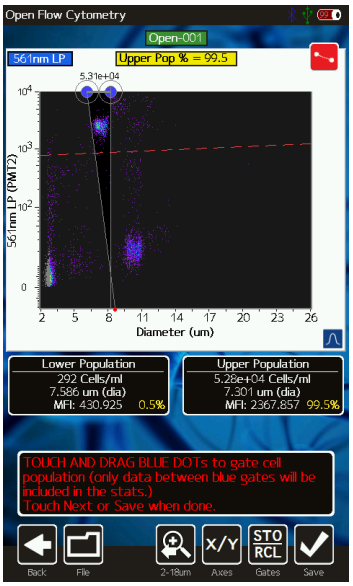


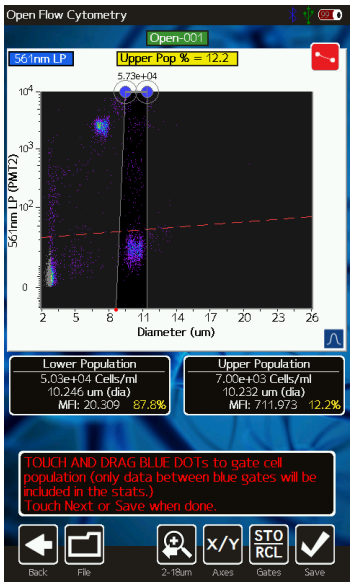
4. Insert a cassette and wait for the laser cassette alignment to complete.
5. When the alignment completes (and prompted "Enter the Sample" at the top left black bar), pippette 60µL bead sample into the loading well in one fluid motion.
6. Immediately close the door (try not to delay longer than a second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

561nm/LP (Moxi FLow) System Check Beads – Result Analysis



1. To get the concentration of each bead type:
    a. Adjust the size gates (blue vertical markers) to include both bead populations while leaving out the noise cluster (see image above)
    Note: The left marker can be angled by toggling the left gate pivot (touch the red or green dot at the bottom of the gate to toggle). To do this:
        1. *Move the left size/blue gate in-between the bright (7µm) and dark (10µm) bead clusters.*
        2. *Touch the green dot at the bottom of the left size gate to "lock" it (it will turn red)*
        3. *Touch and drag the blue dot at the top of the left size gate to the left, angling it past the bright (~7µm) bead population (example images to right).*
    b. Toggle to enable fluorescent gating by touching the red gate icon (top right of scatter plot in the image to the right). Angle the fluorescent gate just below the upper (Bright) bead population (see images above)
2. To get the size diameter of each bead type:
    a. Adjust the size gates (blue vertical markers) ***tightly*** around each of the bead populations separately (see image below). ***Notes:***
        i. The blue gates should be placed tightly around the main cluster (do not include the tails to the right of the main cluster
        ii. The fluorescence gate should be placed tightly below (bright bead) or above (dark bead) the bead cluster for most accurate results.





3. Verify the reported concentration and size to the expected values on the bottle. The margin of error should be 10% 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:

1. Temperature of the bead solution or environment can slightly affect the reported diameter. Bead solution and environment should be within 20-25°C.
2. Microbial contamination of the bead solution can also affect the reported diameter. Avoid contamination by using a clean pipette tip to aliquot the solution. Store the beads in 2-8°C when not in use.
3. Improper mixing can affect the reported concentration. Slow inversion of the bottle after sonicating and vortexing is key to ensuring single-bead suspension.