Moxi GO 532™

USER GUIDE

For Research Use Only. Not For Use In Diagnostic or Therapeutic Procedures.

Contents

Introduction

The Moxi GO 532™ from Orflo Technologies is a benchtop instrument that utilizes a disposable thin-film cassette and a combination of a 532 nm laser with a 580/37nm – filtered photomultiplier tube (PMT), Coulter Principle-based cell measurements, and on-board software to provide easy-to- run applications and data analysis. The instrument relies on disposable cassettes for sample handling, which alleviates the need for flow cell cleaning and fluidics maintenance and the instrument is small enough to be portable between the lab bench and the hood.

The Moxi GO 532 enables highly accurate cell counts and quantitative assessments of cell viability, apoptosis, and immunolabeling. Data acquisition occurs within approximately 10 seconds per test. Test results are displayed in the form of histograms and scatter plots, and quantitatively show cell number, cell percentage(s) gated, and mean fluorescent intensity value(s) in a graphical display. The instrument can store up to 16 Mb of data and the data may be downloaded to a PC or Mac via USB connectivity.

IMPORTANT NOTE:

The Moxi GO 532™ is intended for research use only (RUO). Not for use in diagnostic procedures. It is not intended for use in medical diagnostic or therapeutic procedures, and it has not been approved or cleared by the United States Food and Drug Administration for such uses.

About the User Guide

The *Moxi GO 532™ User Guide* provides detailed information for operating, maintaining, and troubleshooting the Moxi GO 532, including the system, the Cassettes, and application workflows. This includes information on how to analyze test results using the onboard analysis features of the software.

The user guide does not include instructions for analyzing the provided/output flow cytometry standard (FCS, version 3.1) files offline in third party software.

Similarly, the user guide does not include detailed protocols for preparing samples prior to running them on the instrument. The results of the assays are dependent upon the proper use of reagents and the instrument. Please refer to the appropriate kit or reagent datasheet for specific instructions and limitations. Datasheets are shipped with the products or may also be found by searching www.orflo.com using the product number, and selecting the Data Sheets & Documentation section at the bottom of the web page.

For the latest information on optimized reagents and kits available for Moxi GO 532 applications, please check here: http://www.orflo.com.

To update the Moxi GO 532 firmware to the latest version, please visit http://www.orflo.com, and register your instrument by entering a valid email address and serial number. The registration key provided after registering will allow users access future firmware updates.

Conventions Used in the User Guide

Within this manual, the following conventions are used to call out certain points, and also to reminder users of areas of precaution for safety reasons.

WARNING   Alerts you to a situation that may cause injury to the user.

CAUTION   Alerts you to a situation that may cause damage to the system, loss of data, or incorrect results.

NOTE   Indicates additional related information that may be helpful to the user.

Safety Precautions

The Moxi GO 532™ has integrated safety features that are designed for the protection of the user. THE INSTRUMENT SHOULD BE USED ONLY BY PROPERLY QUALIFIED AND TRAINED LABORATORY PERSONNEL, AND SHOULD BE USED ONLY AS DIRECTED IN THIS GUIDE. PLEASE REVIEW AND UNDERSTAND ALL SAFETY INSTRUCTIONS BELOW, AND AS INDICATED THROUGHOUT THE USER GUIDE, BEFORE OPERATING THE SYSTEM.

Electrical Safety

WARNING   To avoid the danger of electric shock, including the possibility of permanent injury or death:

- Prior to use, verify that the USB cable and USB charging adapter are plugged securely into a properly grounded AC power outlet. Verify that the connection between the USB cable and the instrument is secure and the AC power in your location is within the specifications for the instrument (see page 44).
- Do not immerse the instrument, USB cable, or USB power adapter in liquid or allow liquid to enter the instrument.
- Do not attempt to disassemble or service the Moxi GO 532. The instrument has no user serviceable parts. **All service must be performed by ORFLO**, **or an approved ORFLO vendor**.

Laser Safety

WARNING The Moxi GO 532 is a Class 1 laser product in accordance with IEC 60825-1:2007. The system contains a Class IIIb laser that operates at 532 nm with a maximum output power of 60 mW. Direct exposure to laser radiation, even for a fraction of a second, can be harmful to skin, eyes, or other body parts, including posing a risk of burns and permanent vision impairment or blindness. The following guidelines should be followed to avoid exposure:

- An interlock on the door prevents the laser from operating when the door is open. Do not attempt to defeat the interlock and run a test with the door open.
- Do not operate the Moxi GO 532 if there is any damage to the instrument's enclosure.
- Do not attempt to disassemble or service the Moxi GO 532 to service or adjust the laser. The instrument has no user serviceable parts, and attempts to adjust or service the laser may result in hazardous radiation exposure. **All service must be performed by ORFLO**, **or an approved ORFLO vendor**.

Biological Safety

WARNING Biological samples have the potential to transmit harmful or fatal disease. The following guidelines should be followed to minimize the risk of exposure to biohazardous materials.

- Handle all biological samples as if they are capable of transmitting harmful or fatal infections.
- Wear appropriate personal protective equipment (PPE) including clothing, eyewear, and gloves.
- Do not pipette by mouth.
- Dispose of biological waste in accordance with all applicable local, state, and federal regulations.

Moxi GO 532™ System Overview

The Moxi GO 532™ Kit includes the Moxi GO 532 system, USB Cable, USB Power Adapter, one pack of 25 Cassettes, and a Quick Start Reference Guide. The following figure shows the main visible components of the Moxi GO 532 and the table provides a description of each component.

USB Cable Port (on

back surface)

Power/Reset Button

Cassette Tray Latch

Cassette Tray Door

Disposable Cassette

Component	Function
Touch Screen Display	Allows user to interface with the instrument by pressing icons and targets. Displays all information needed for operation and analysis of results.
Power/Reset Button	Turns instrument on and off, and resets the unit when pressed and held for >5 seconds.
Cassette Tray Door	The door is manually opened and closed by user to allow insertion of a cassette into cassette tray. Door must be closed prior to running a test.
Cassette Tray Latch	Manually depressed by user to open cassette tray and allow cassette insertion and removal. Release of lever, with cassette in place, latches cassette into position for acquisition
USB Cable Port	Connects instrument to USB cable.
Cassette	Disposable cassette used for loading samples. Each cassette contains two fill ports, one at each end, thereby allowing for two tests to be run per cassette.

Moxi GO 532™ Accessories

The Moxi GO 532™ uses a USB Cable and Power Adapter for power source. The Moxi GO 532 Kit will come with the power adapter required for your local region. For more information on the approved regional power adapters, please see Section 14, Ordering Information. Additional adapter types may be available for other regions, please contact technical support at ORFLO for more information.

Power Adapter (US style)

USB Cable

Part	Function
USB Cable	Connects instrument to Personal computer (PC or Mac) or power adapter
Power Adapter, US Style	Connects USB cable to a 110 VAC outlet

Getting Started

The Moxi GO 532™ is shipped in a condition ready for initial use; though we recommend checking for software updates and updating the Moxi GO 532 with the most current software version upon receipt. To begin, plug the USB Cable into the instrument at one end (mini-USB). Plug the opposite end of the USB Cable (standard USB) into the Power Adapter. Plug the Power Adapter into an AC outlet that matches the rating of the Power Adapter.

⚠️ **CAUTION**   Use only an ORFLO supplied USB Cable and Power Adapter. Use of other products may result in inaccurate test results or damage to the instrument, and will void warranty.

NOTE   Tests should only be performed with the instrument powered by an AC source.

Note: the device will power on and users can access files without attaching to a power outlet but for data collection the device must be plugged into an electrical outlet.

General Guidelines

The Moxi GO 532 is typically used with Type S+ (MXC030, MXC032) Cassettes. For larger particles, the MF-M (MXC021, MXC023) can be used. Refer to the Specifications section for information on the operating size and concentration ranges for the system.

The sample volume for a test should be 60 µL.

Cells should be suspended in 0.9% salt solution (e.g. PBS or equivalent) to ensure proper conductivity for unit operation and to ensure proper particle sizing.   Water, hypotonic, or hypertonic solutions are **not** acceptable diluents.

Cells need to be prepared as single-cell suspensions. Clusters/aggregates should be broken apart with mechanical trituration and/or protease dissociation (e.g., Accutase®). For stubbornly

aggregated samples, pre-filtration with a 40 μm cell strainer is recommended.

As the Moxi GO 532™ uses a 532 nm laser with 587/30 nm filter, dye/fluorophore selection should be based accordingly. The recommended fluorophores are propidium iodide (PI) for viability and phycoerythrin (PE) for immunolabeling and Apotosis assays.

For assays other than the "Open Flow Cytometry" ORFLO optimized and validated kits/reagents are strongly recommended. Please see (website link) for a list of currently available kits and validated antibody conjugates. For cell counting application, cells can be run without fluorochromes in the Open Flow Cytometry application.

Materials Required

- Cell or bead sample (diluted and dissociated, if necessary); 60 μL minimum.
- Type S+ (MXC030, MXC032) Cassettes (Recommended for most applications)
- MF-M (MXC021, MXC023) Cassettes (Large particles only)
- Pipette and appropriately sized pipette tips
- Cell staining/labeling reagents (test specific)

Using the Moxi GO 532

Press the Power Button to turn on the instrument. The Home Screen will become available.

Home (Start) Screen
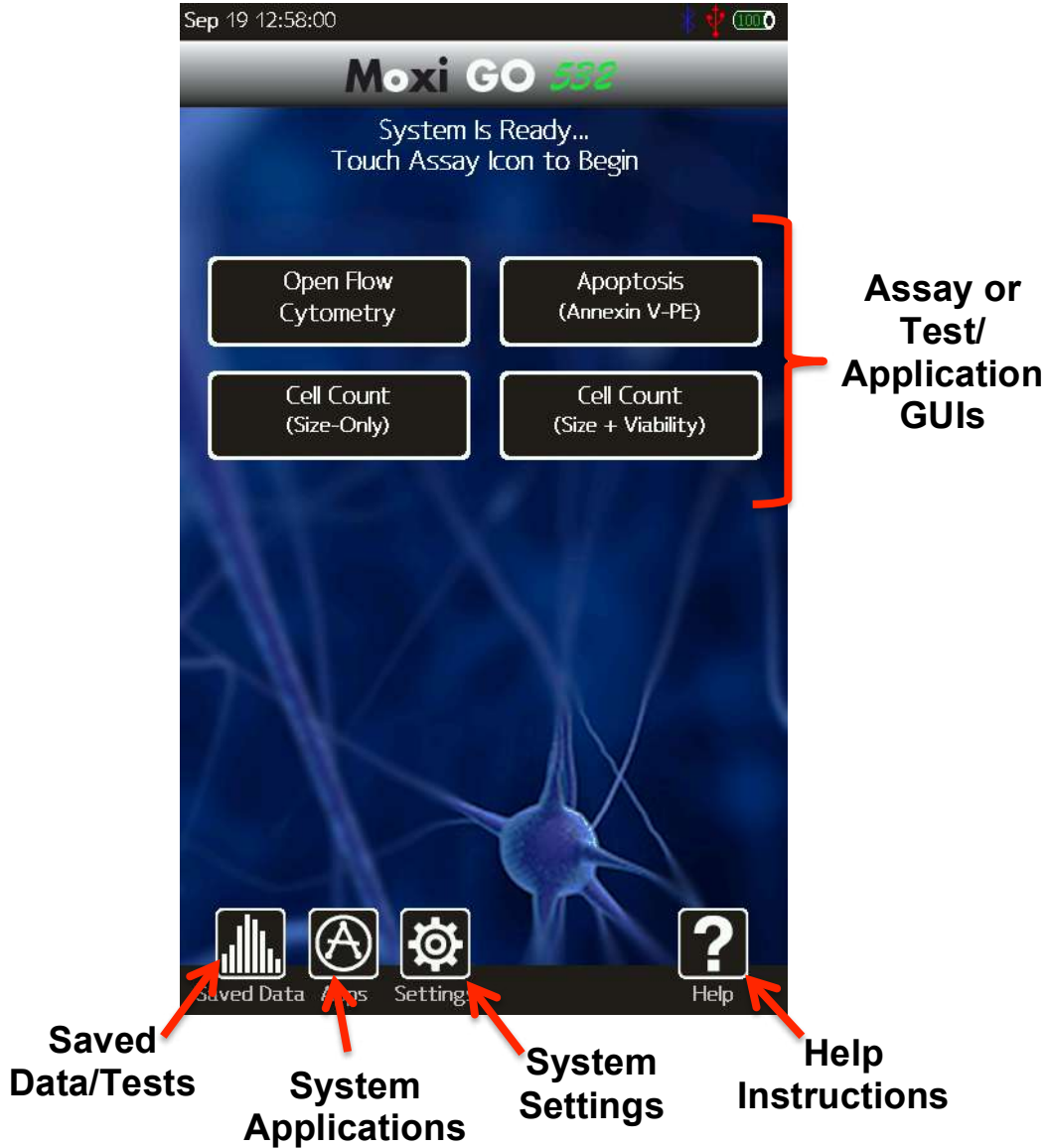
NOTE: The home page appearance may change as additional applications are launched and new GUIs (graphical user interface buttons) are added to the Moxi GO 532 software program.

Settings

Data and Time

Set the date and time by pressing the **Settings** icon on the main menu of the instrument. Then use the arrow keys and follow the instructions displayed on the screen.

Passcode Lock

The Moxi GO 532 can be setup to require a password login to use the instrument and access stored data. To set the Password lock, select the **Settings** icon and then use the left and right arrows on the screen to highlight the "Passcode Lock" field. Use the up or down arrows to change the "Passcode Lock" field value to "On" and select **Done**. The system will present a keyboard to enter the four-digit numeric passcode. On entering the passcode, the user will be required to "Re-enter Passcode" to prevent accidental entry of a wrong code. Upon successful re-entry of the passcode, the system will display a "lock enabled" message. The system will now require a passcode for access on initial startup and after entering sleep mode.

> NOTE: In order to turn the Passcode Lock off, the user will be required to enter the correct passcode. If you forget this passcode, the entire system will have to be reset and data could be lost. Keep the passcode recorded in a safe place to prevent loss of data.

Fluorescence Gain

The "Fluorescence Gain" field sets the level of fluorescence gain for the "Open Flow Cytometry" assay only. Four possible values (Very Low, *Low, Default, Medium, High*) can be entered here and will seed the value of the dropdown list on the "Open Flow Cytometry" start screen. Note: the user can also make a one-time (per-test) change to the fluorescent gain value of the "Open Flow Cytometry" by touching the value on the test start screen as described in Step 3 of the "Analyzing a Sample" | "Running a Test" section.

Auto Noise-Find

This field sets the behavior of the system with respect to identifying system noise during tests. There are two possible settings: *Yes* or *No*. If set to *Yes*, the system applies an algorithm to each test, after acquisition, to try to identify the noise (and small-particle "debris") in the sample. If set to *No*, the system will use a hard-coded values for the noise location for each test, capping the noise area to a region defined by events <2.9µm AND fluorescence levels <10mV.

Analyzing a Sample

Sample Preparation Considerations

There are four key considerations for preparing a sample for analysis with the Moxi GO 532™:

1. *Solution Conductivity* – Cells must be suspended in 0.9% salt solution (e.g. PBS or equivalent) for proper particle sizing and test function. Deviations of ~50% can be tolerated without sacrificing count performance. However, the reported particle diameter

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will vary accordingly (~ linear correlation between percent increase in measured volume and percent in solution impedance)

2. ***Particle Size and Concentration*** – For best results tests must be run within the size and concentration range specifications of the cassette, as specified below.
   a. Size Range:
      i. Type S+ Cassettes (MXC030, MXC032)
         - **MEAN** particle diameter: 3 – 20 µm (14 – 4189 fL volume)
         - Overall detection (display) range: 2.7 – 27µm (10.3 – 10306 fL)
      ii. MF-M Cassettes (MXC021, MXC023)
         - **MEAN** particle diameter: 4 – 25 µm (34 – 8181 fL volume)
         - Overall detection (display) range: 3 – 35µm (14 – 22449 fL)
   b. Concentration range:
      i. Type S+ Cassettes (MXC030, MXC032)
         - Counts: 1×10^4 – 1.75×10^6 total particles/ml
         - Optimum Fluorescence Sensitivity: 1×10^5 – 5×10^5 particles/ml
      ii. MF-M Cassettes (MXC021, MXC023)
         - Counts: 1×10^4 – 7.5×10^5 total particles/ml
         - Optimum Fluorescence Sensitivity: 1×10^5 – 4×10^5 particles/ml

Optimal concentration ranges up may vary slightly depending upon your cell or bead type.

3. ***Single Cell Suspensions*** – While the Type S+ and MF-M Cassettes do have a pre-filter for removing sporadic large particles, cell samples should be prepared as single cell suspensions to avoid clogging of cassettes. Clusters/aggregates can be broken apart with mechanical trituration and/or protease dissociation (e.g. Accutase®). Larger particles or stubbornly aggregated clusters can be removed with a 40 µm cell strainer. Standard centrifuge tube strainers can be used for larger volumes or a pipette-tip Flowmi™ strainer can be used for smaller volume filtering.

4. ***Fluorescent Labeling/Stains*** - The Moxi GO 532™ uses a 532 nm laser with 587/30nm filter, dye/fluorophore selection should be based accordingly. The recommended fluorophores are propidium iodide (PI) for viability and phycoerythrin (PE) for immunolabeling and Apotosis assays

For assays other than "Open Flow Cytometry" ORFLO optimized and validated kits/reagents and associated preparation protocols are strongly recommended. Please see (website link) for a list of currently available kits, validated antibody conjugates, and associated protocols.

For cell counting application, cells can be run without fluorochromes in the Open Flow Cytometry application.

Running a Test – General Instructions (All Tests)

1. Please follow sample preparation guidelines above ("Sample Preparation Considerations") in preparing the sample. Note: The minimum volume required for a test is 60 µL.

2. Turn the instrument on by pressing the power button and the **Home** screen will be displayed. Select a test type by pressing one of the icons displayed on the screen. **NOTE:** The instrument must be plugged in to an AC source, or an external USB battery pack, to run a test. Tests CAN NOT be run on internal battery power alone.

3. Basic test instructions are presented for all tests (example for "Cell Count (Size + Viability) Test" shown below/left). For the "Open Flow Cytometry" test, the additional option of setting the "Fluorescence Gain" is presented (image below/right). Touch the gray box (red arrow in image) to change this gain. Four options, in order of increasing gain for the fluorescence signal output, are possible: *Very Low, Low, Default, Medium, and High*.
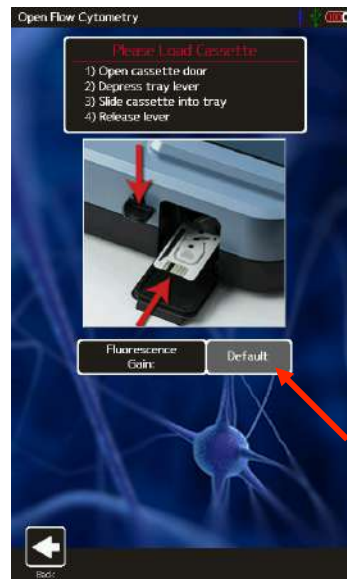
The initial value populated in this field is determined by the global "Fluorescence Gain" setting that can be set from the **Settings** icon off of the **Home** Screen.

4. Open the Front Door and depress the Cassette Tray Lever. (add better reference image with cassette being added, lever being pressed). Slide a test cassette, with ORFLO lettering-side up into the cassette tray (either end 1 or 2). Release the Cassette Tray Lever and close the Door. The system will automatically perform a calibration step as outlined in the images below. (This occurs for all tests except "Size Histogram") The automated sequence aligns the laser to the cassette flow cell ("Aligning the laser"), and prompts the user to open the Door Notes:

   a. System status messages are displayed in the black bar at the top of the screen.
   b. For the first test-run on the unit, following power up, an initial laser "cam align" is performed ("System Calibration" in image below/left). Following that initial test, only the standard laser alignment (images below/middle and below/right) are performed.
   c. Note: Should this result in an error make sure that your cassette is pushed into the sled completely such that it sits in an indentation.
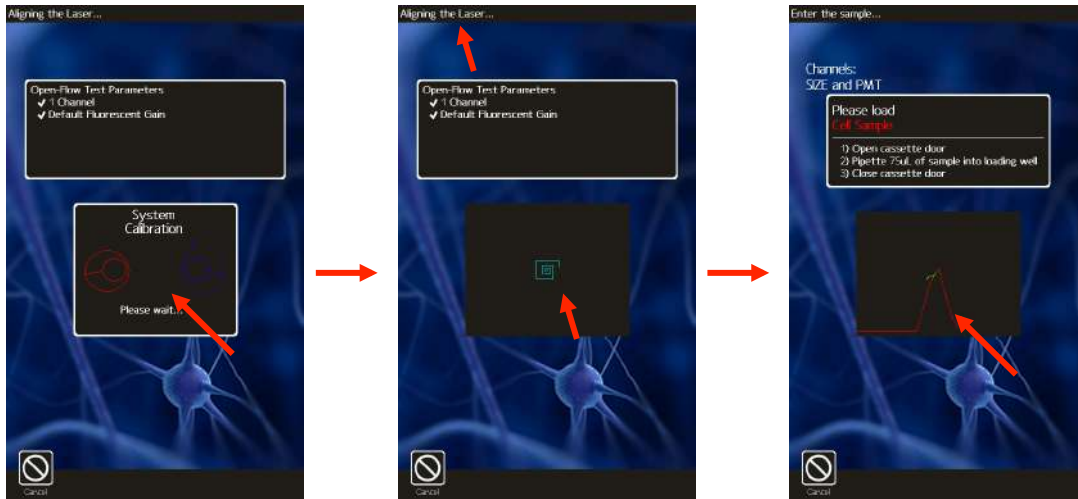
5. System messages at the top of the screen prompt the user to open the door and insert the cassette (image below/left). Once the cassette is loaded, pipette a 60 µL sample ("Enter the Sample", image below/middle) into the fill port of the cassette (either test 1 or test 2, depending on which end of the cassette was inserted into the instrument) and close the Door. The current disk usage space is also indicated at the bottom right of the screen at this point. *Note: The system provides the user a 60 second window to complete each step (opening the door, loading the sample, waiting for the fluid to reach the start electrode). If the time limit for any of the steps is exceeded, the test will automatically cancel and the user will be returned to the **Home** screen.*
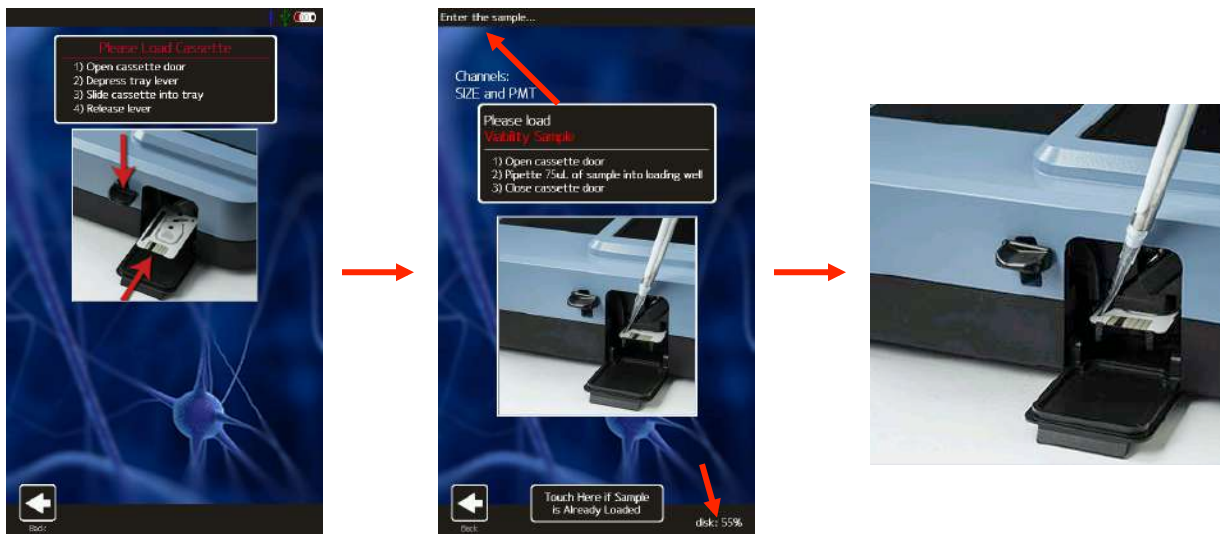
6. The instrument will begin the test. While the system waits for sample/fluid detection an "Analyzing…" message is indicated in top left of the screen a countdown until the test is auto-cancelled (auto cancel occurs after 60 seconds if no fluid is detected). Once the fluid is detected, the system message changes to "Counting…" and the results are dynamically populated on the scatter/dot plot. Results for most samples will be complete in approximately 10 seconds but may be longer for higher concentration, sticky, or aggregated samples.

Running an "Open Flow Cytometry" Test

The Open Flow Cytometry is meant to be a generic test assay that can be used to run any user sample, whether or not it has been prepared with reagents obtained from ORFLO. The requirement for labeling of the sample is that the fluorophore used is compatible with the 532 nm laser excitation and 587/30 nm emission filter. For non-ORFLO kits, it might be necessary to perform titration assays and/or adjustment of the Fluorescent Gain (Very Low, Low, Default, Medium, or High) to achieve desirable test results.

1. As with all assays, please follow sample preparation guidelines above ("Sample Preparation Considerations") in preparing the sample. Note: The minimum volume required for a test is 60 µL.
2. Select the "Open Flow Cytometry" assay from the **Home** screen (image below/left)
3. To adjust the Fluorescent gain for this test only (permanent changes to the fluorescent gain must be made in the global settings as described in the "Settings | Fluorescent Gain" section above) touch the gray box next to the "Fluorescent Gain" field (red arrow in image below/left).
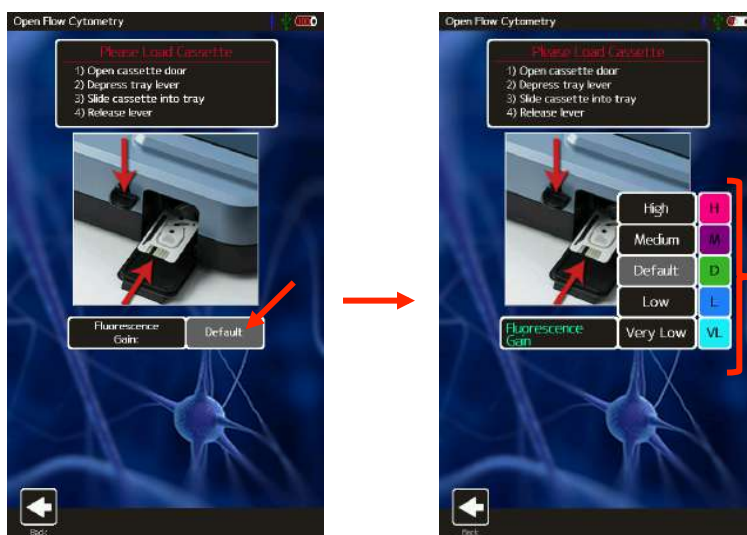
4. A pop-up menu will appear with the available options (image above/right). Touch the desired value to change the setting.
5. Select "Start" or insert a new cassette and close the door to begin the test (start the laser alignment). See "Running a Test - General Instructions" above for further detail.

Test Screen Outputs

There are two main test output formats: 1.) Fluorescence vs. Size scatter plots and 2.) Histograms (either size or fluorescence channel). Fully labeled images are provided below for each type of output. More detailed workflow instructions for each function are outlined in the "Managing the Data" section below.

Fluorescence vs Size Scatter Plot

Test Type

Test name
(touch to change)

Population %
(defined by red and blue gates)

Toggle between gates
Size (blue), fluorescence
(red), and noise (yellow)

Total size-gated counts
(between blue gates)

Left size marker adjust
(Drag to move)

Right size marker adjust
(Drag to move)

Fluorescence scale
("Logicle" scaling)

Fluorescence gate –
Defines upper (+) vs.
lower (-) populations

Touch/toggle dot to lock
In place for angling left
size gate

Test scatter/dot plot

Upper gated region
(above red line) results

Lower gated region
(below red line) results

File options
(print, save, delete)

Select axis display
(size histogram, fluorescence
histogram, or scatter plot)

Store and recall gate
Locations in memory

Histograms

Test Type

Test name
(touch to change)

Population %
(defined by red and blue gates)

Histogram bin counts

Lower (negative/low Fluorescence) cell population (green)

Lower gated region (below red line defined in PMT vs. size view) results

Upper gated region (above red line defined in PMT vs. size view)

Fluorescence scale PMT output in mv (logicle scaling)

Upper gated region (above red line) results

File options (print, save, delete)

Select axis display (size histogram, fluorescence histogram, or scatter plot)

Store and recall gate Locations in memory (pre-sets)

Managing the Data

Test results are presented to the user immediately following a test in the form of a scatter (dot) plot (below/left) for fluorescent tests or as a size-only histogram (below/right, "Size Histogram" test only).
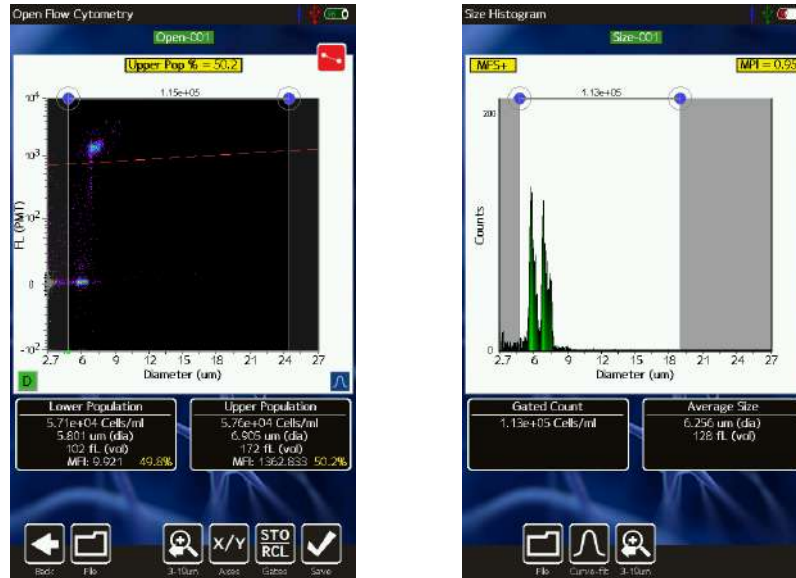
Saved tests can be accessed by selecting the **Saved Data** icon off of the **Home** screen (Image below left). Once selected, the user is presented with a list of saved tests, ordered by test date/time. *Note: the disk usage percentage is indicated in the upper right corner of this screen*. Selecting a thumbnail opens that test for editing, identical to the post-test editing, with the exception that the size (x) axis rescale capabilities are no longer available.

General Recommended Post-Test Workflow

The general workflow for post-test processing is shown below. The goal is to first set the size range for optimal size-resolution and histogram binning by re-scaling the x-axis to the optimal range. Next the gates are set to ensure that only the relevant points are saved (noise gates), that the only the cells of interest are including in the statistics (size gates), and that events are properly categorized based on fluorescence intensities (fluorescence gate).

Adjust the size (x) axis to optimize the size resolution.	Adjust the noise (yellow) gate to exclude unnecessary (noise/debris) points from analysis/saving.	Define the analysis region by adjusting the size (blue) markers.	Gate the test results based on fluorescent intensity (red markers).	Read results and perform file operations (e.g export screenshot, save, save gates)
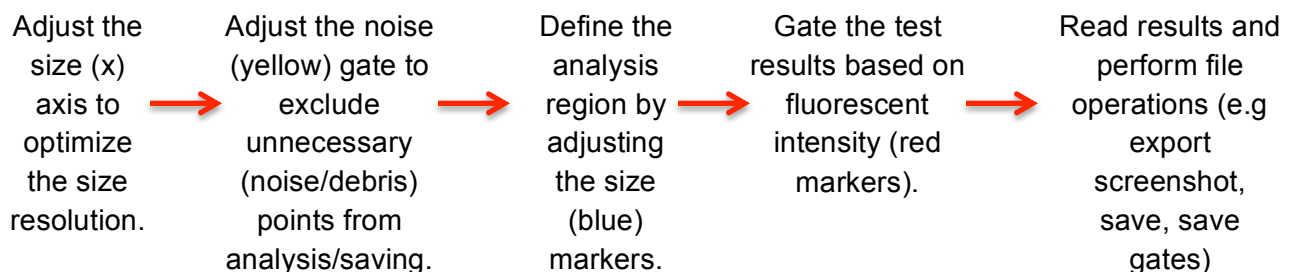
Re-scaling the X axis (size range)

Upon completion of a test (but prior to saving the test), the X axis scale can be adjusted, similar to a biexponential function in flow cytometry. In this function unnecessary decades for analysis are excluded to optimize the representation of the data. This is accomplished by selecting the re-scale button below the scatter plot (magnifying glass with arrow, see image sequence for a Type S+ cassette below). With each button press, the text under the button changes to indicate the scale range that will be displayed on the next button push. When the smallest size range (2.7-11µm) is reached, hitting the button returns the user to the initial, 2.7-27 µm, display range. The possible size scale ranges are:

- Type S+ Cassettes: **2.7-27 µm**, **2.7-19 µm** and **2.7-11 µm**. (Example sequence shown in the images below)
- MF-M Cassettes: **3-35 µm**, **3-27 µm, 3-19,** and **3-11 µm**.
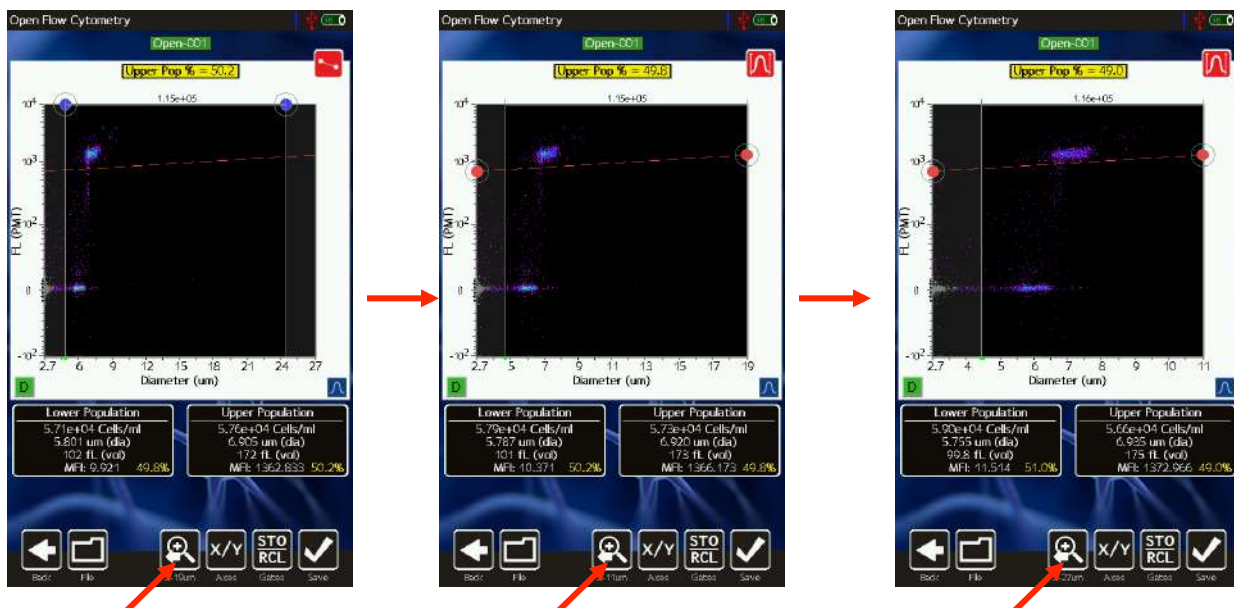
Rescaling the axis results in reprocessing of the raw data to ensure that resolution is not lost during the rescale process (1000 histogram bins). This allows (and is recommended for) users to achieve finer resolution of smaller particle populations and potentially increased separation between particle sub-populations. ***However, once a test has been saved, rescaling the x axis is not a possibility.***

Gating the data

Immediately following a test acquisition (excluding the "Size Only" test assay, which is described below), there are three gating modes/categories: 1.) Noise Gating, 2.) Size Gating and 3.) Fluorescence gating. The user can cycle through each of the modes by touching the red icon in the upper right of the scatter plot (see images below). Each time the red gate icon is touched, the system toggles through the gate modes, enabling a different set of adjustment markers. The yellow gate/dot allows the user to adjust the noise gate location to define the range of data

to be saved. The blue dots allow the user to identify the size range that will be used for generating the summary information (statistics) on the sample. The red dots allow the user to adjust the fluorescent threshold to determine which events will be included in the positive vs. negative designations for the display fluorescence channel. Each gating mode will be discussed in further detail in the corresponding sections below.
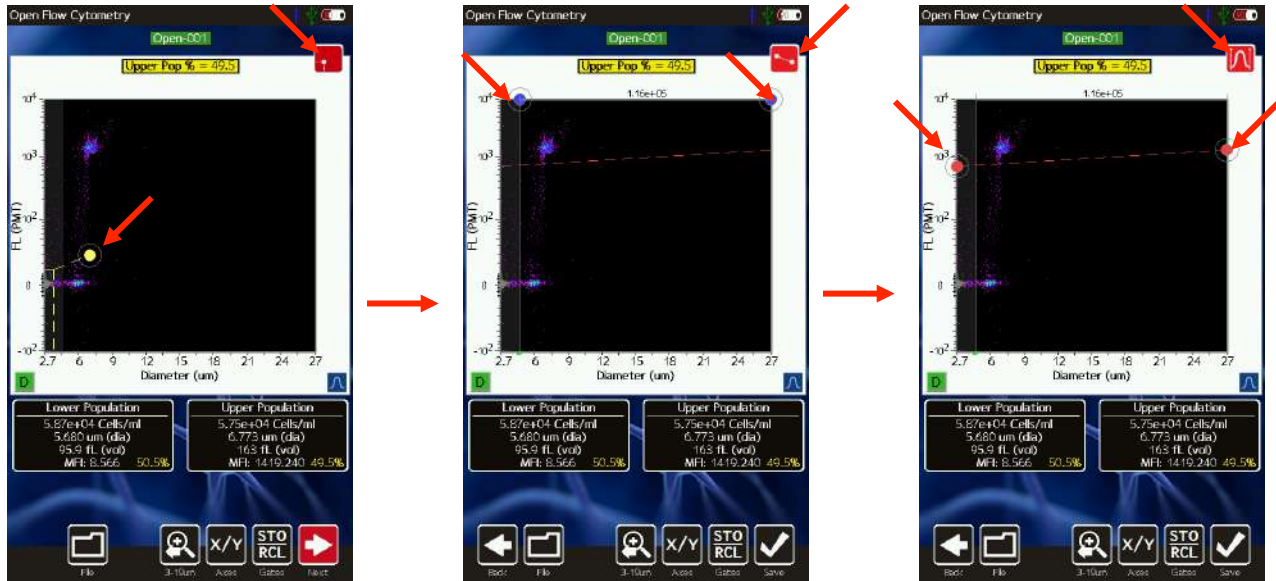
Size Gating of Scatter (Dot) Plots

The results of all fluorescent tests (all tests except for the "Size Histogram" test) are initially displayed as scatter plots with the size (blue) gates enabled (see image below, left). Gates can be adjusted by touching and dragging the blue markers around the edges of the scatter plot (left image below). Touching the marker at the base of the left gate toggles the marker from green (unlocked, images below/left and below/middle) to red (locked, image below/right). Touching the marker again will switch it back to the prior state. Moving the left cursor with base unlocked (green) results in the base moving synchronously/equivalently with the top left cursor (gate line is forced to be vertical). Moving the left cursor with the base marker locked (red) anchors the base point allowing the user to pivot the top point around it. This top cursor can even be dragged around the left edge of the scatter plot if needed. Events to the left of the left size gate and to the right of the right size gate are not included in the count totals or population percentages (e.g. Live Cell %). The total particle count in the size-gated region is displayed above the scatter plot, between the blue gating markers.
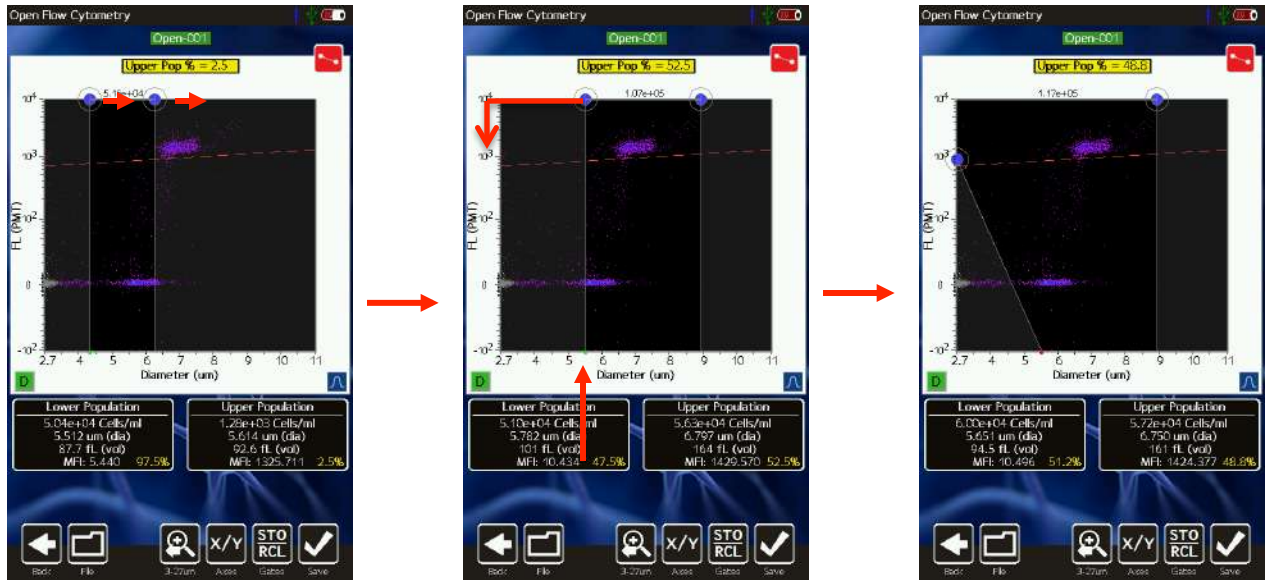
Fluorescent Gating of Scatter (Dot) Plots

Touching the red gate toggle marker when the size (blue) markers are currently enabled correspondingly disables the (blue) size gate markers and enables the (red) fluorescent gate markers. The user can then touch and drag the red circles to adjust the positioning of the fluorescent gating line. All events/dots that are located above the fluorescent gating line (and within the blue size gates) are included in the "Upper Population" statistics (images below, black box to the right underneath the scatter plot). Those events/dots below the line (and within the blue size gates) are included in the "Lower Population" statistics (images below, black box to the left underneath the scatter plot).
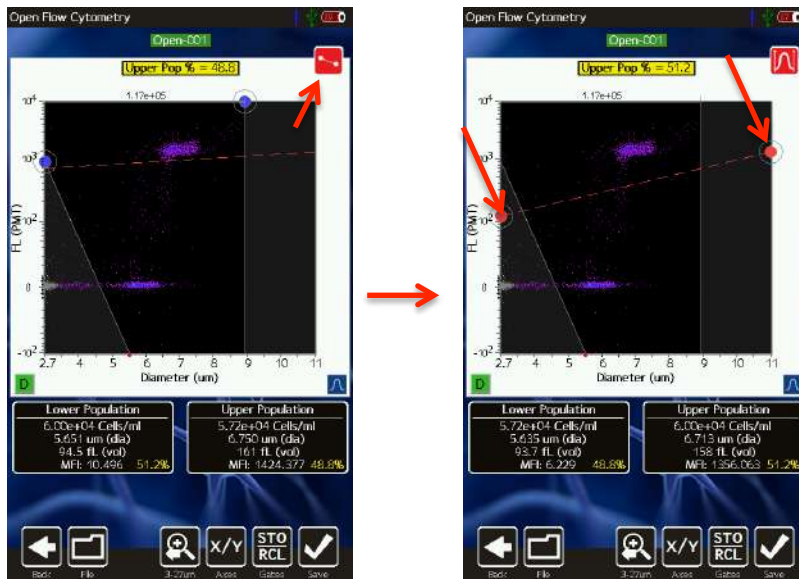
Noise Gating of Scatter (Dot) Plots

Touching the red gate toggle marker when the fluorescent (red) markers are currently enabled correspondingly disables the (red) fluorescence gate markers and enables the (yellow) noise gate markers. The user can then touch and drag the yellow circle (images below) to define the points that will be identified as noise. These noise points will be grayed out (image below/middle) and will not be saved with the test. Note: DATA GATED AS NOISE WILL NOT BE SAVED WITH YOUR FILE!! As an example, the image below/right shows the edited (saved) test for the noise setting defined in the image below/middle. These points are also not included in the population statistics (e.g. total counts, and "Upper Popul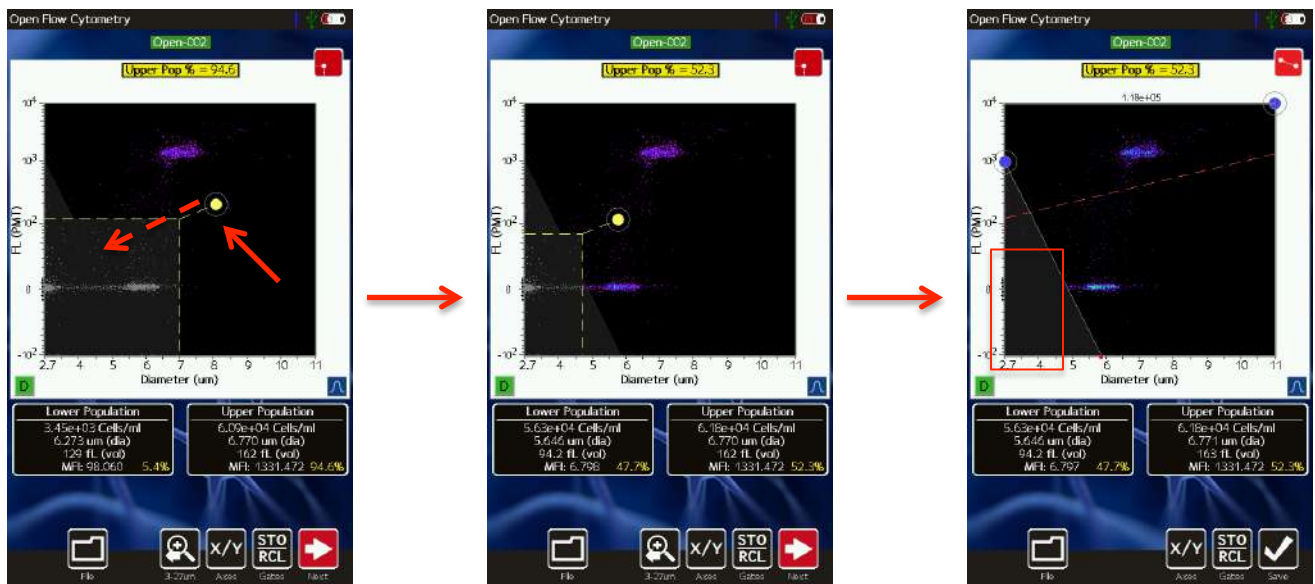ation" and "Lower Population" fields) even if the size gate includes part of the grayed out region. The primary purpose of the noise gate is to reduce the saved file size (by eliminating the quantity of saved events) to correspondingly increase the storage capacity of the disk in terms of number of tests and images that can be saved.

Store and Recall of Gate Settings

By default, the Moxi GO 532™ gates are "sticky" meaning that each new test defaults to the gate settings (size and fluorescence) of the prior test. The instrument also allows the user to store up to five gate locations in memory for recall. A given set of gate settings can be saved by hitting the **STO/RCL** button (arrow in image below, upper/left). Doing so brings up a list of five memory locations (numbered zero through four, see image below, upper/right). Memory locations that are already occupied are shown in red and empty locations are shown in black. Touching either red or black locations will result in that location being overwritten by the current fluorescent and size gate settings.

Gates can be recalled at any point (after a test or for a saved test) by selecting the same **STO/RCL** button. Available memory bank locations are listed in green (see image below, lower/right). Selecting one of the buttons will automatically apply the gate parameters that were saved at that current location. A full gating sequence is illustrated in the images below. In the upper/right image, the gates are saved into location two. The gates are correspondingly moved (image below, lower/left) and recalled (image below, lower/right). Recalling the gates, returns

the positioning to the locations as shown in the image at the upper/left. Note: Gates can only be recalled from the same graphical representation (histogram/dot blot) and the same parameters.
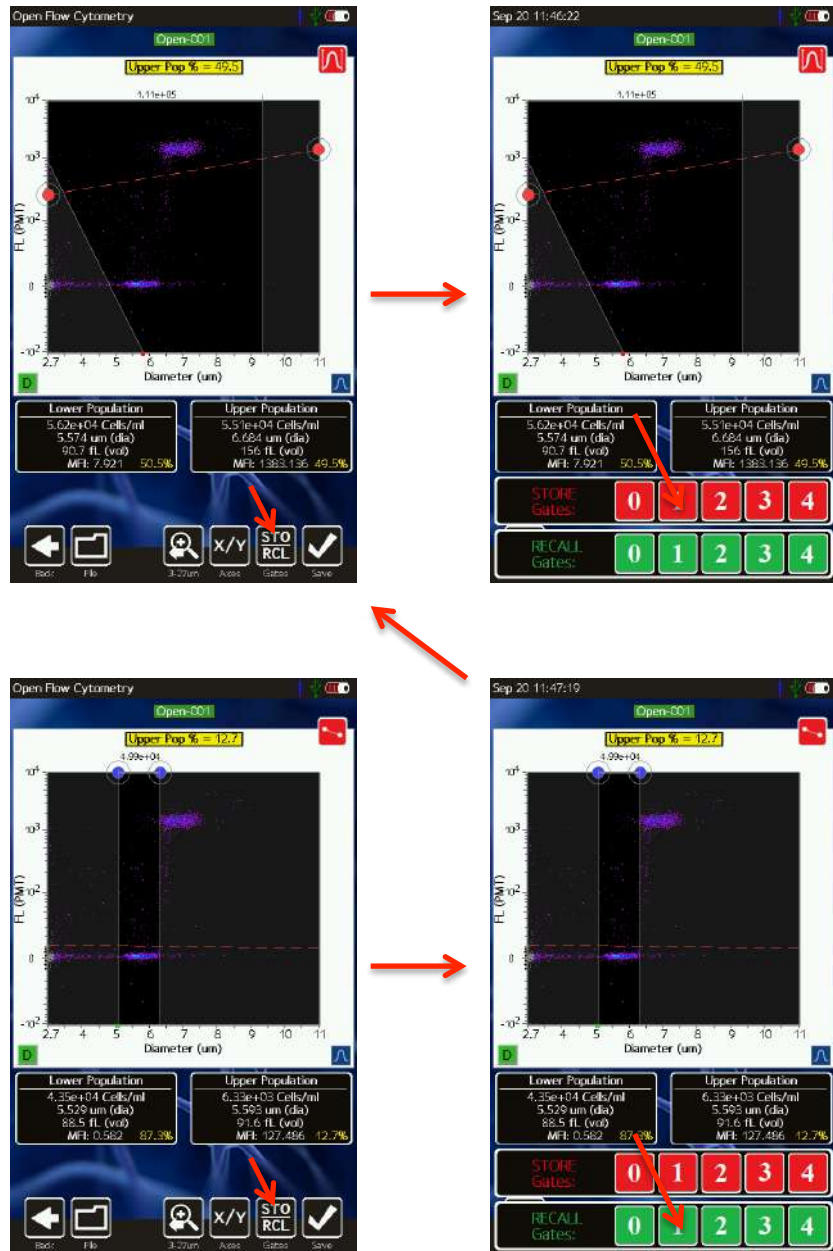
Histogram Overlays on Fluorescence vs Size Scatter Plots

Fluorescent and Size histograms overlays on the scatter/dot plot can be toggled on and off by pressing the blue histogram button at the bottom right of the scatter plot (see images below). With histogram overlays turned on, the fluorescent histogram appears as a blue histogram with a base on the left (fluorescent) axis. The size histogram is displayed as a red histogram with a base on the bottom (size) axis. In the example below, the yellow arrows point to the two size histogram (red) peaks and the blue arrows point to the two fluorescent histogram (blue) peaks.

The histograms can be rescaled by swiping your finger in the appropriate direction: Up-down for the size histogram and left-right for the fluorescent histogram.
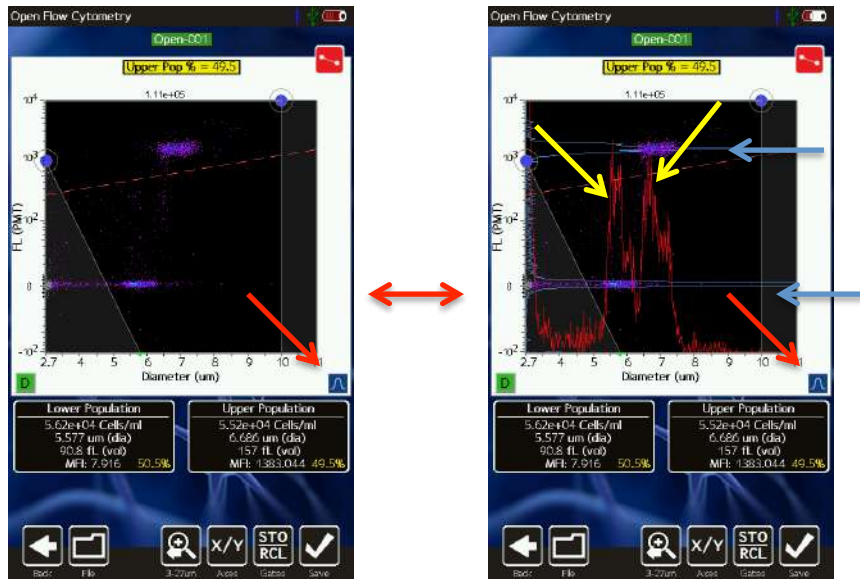
"Cell Count (Size-Only) Histogram" Assay

For samples that lack any staining or fluorescent label, quick counts and particle sizing information can be obtained by running a counts and size-only assay. As the laser is not used with this assay, time is saved as the system skips the laser/cassette alignment step. Size-only tests can be run by selecting the "Cell Count (Size-Only)" assay from the home screen (image below/left). During acquisition a size-based histogram is displayed as opposed to the traditional fluorescence vs. size scatter (dot) plot. Upon completion of the test, counts for the size histogram can be obtained by moving the blue gating markers to define the size region for the counts (image below/middle). Alternatively, for monodisperse mammalian cell samples, the user can have the system automatically identify the core Gaussian population by touching "Curve-fit" (image below/middle). The system automatically identifies the largest Gaussian population (e.g. image below/right) and will adjust for multiplex counts based on the mean size of that singlet population (e.g. particles that are twice the size will be counted as doublets, etc.). The user can switch back to Gating mode by touching the "Gating" icon (image bottom/right)
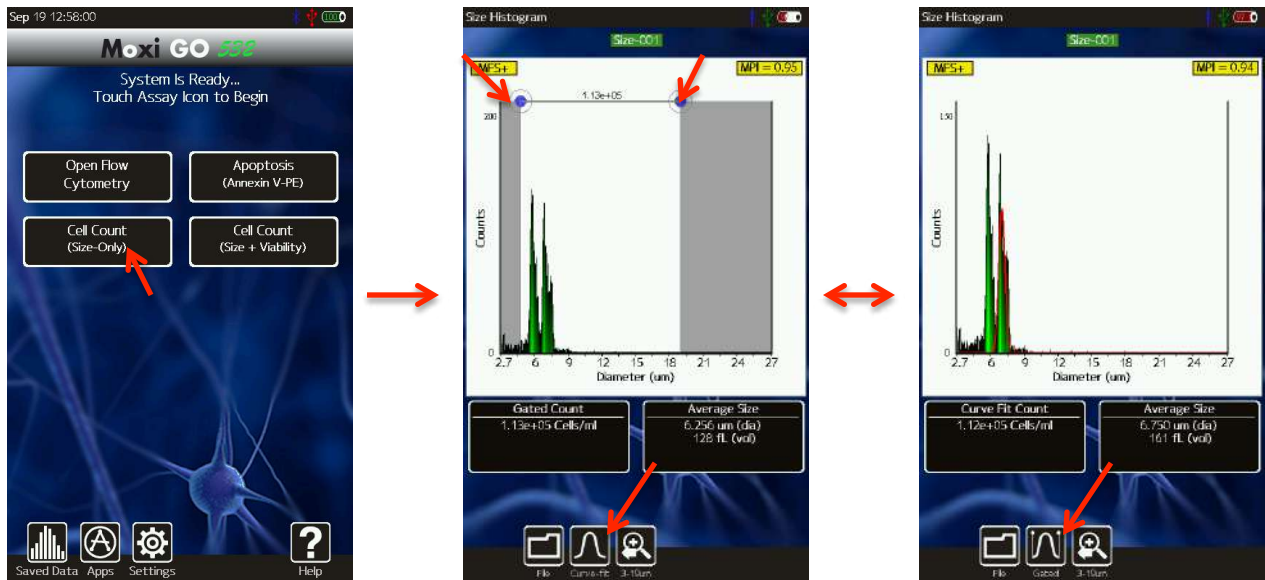
For smaller particle populations, improved size resolution can be achieved by rescaling the x-axis (as discussing in the above "Rescaling the X-axis" section) to 2.7-19 µm or 2.7-11 µm for Type S+ cassettes (or 3-27µm, 3-19µm, 3-11µm for MF-M cassettes). Rescaling doesn't just show a subset of the currently displayed histogram. Rather, system reprocesses the raw data wave to generate a 1000 bin histogram across the smaller size range, thereby improving the resolution.

The Counts (Y axis) scale can be adjusted swiping your finger up or down in the white histogram area. For low concentration cell samples, it is important to decrease the counts scale (up arrow) in order to properly view and gate the population.

As the lower display scale (2.7µm for Type S+ cassettes, 3µm for MF-M cassettes) is lower than the lower size detection limit of the system/cassettes, some inherent system noise is inevitably present. To help identify this noise, the counts in this region are automatically grayed out by the system.

In size-only mode, an MPI value is also provided in the yellow box at the top right of the display. This value is a measure of the purity of the sample by providing a ratio of the size-gated (or curve-fit) population to the total particle population (not including the automatically grayed out noise region). This metric has shown utility in tracking culture health by highlighting increases in small particle components that inevitably result from cell necrosis or microbial growth.

Changing Display Axis for Tests (PMT vs Size, Histogram Only Views)

The test output can be modified to display any recorded channel vs another channel or, individually, with a histogram-only view. To change the display axis, the **X/Y** button (image below, frame at the top/right) can be selected. When **X/Y** is pressed the available channels for each axis are displayed in the gray boxes at the top and to the right of the current scatter (image below, frame at top/middle) plot (or histogram). The user can select any combination of y-axis and x-axis values to generate the corresponding data display. The size axis can only be selected as the x-axis (can't be set as the y-axis). The display permuations are shown in the

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figure below, including:
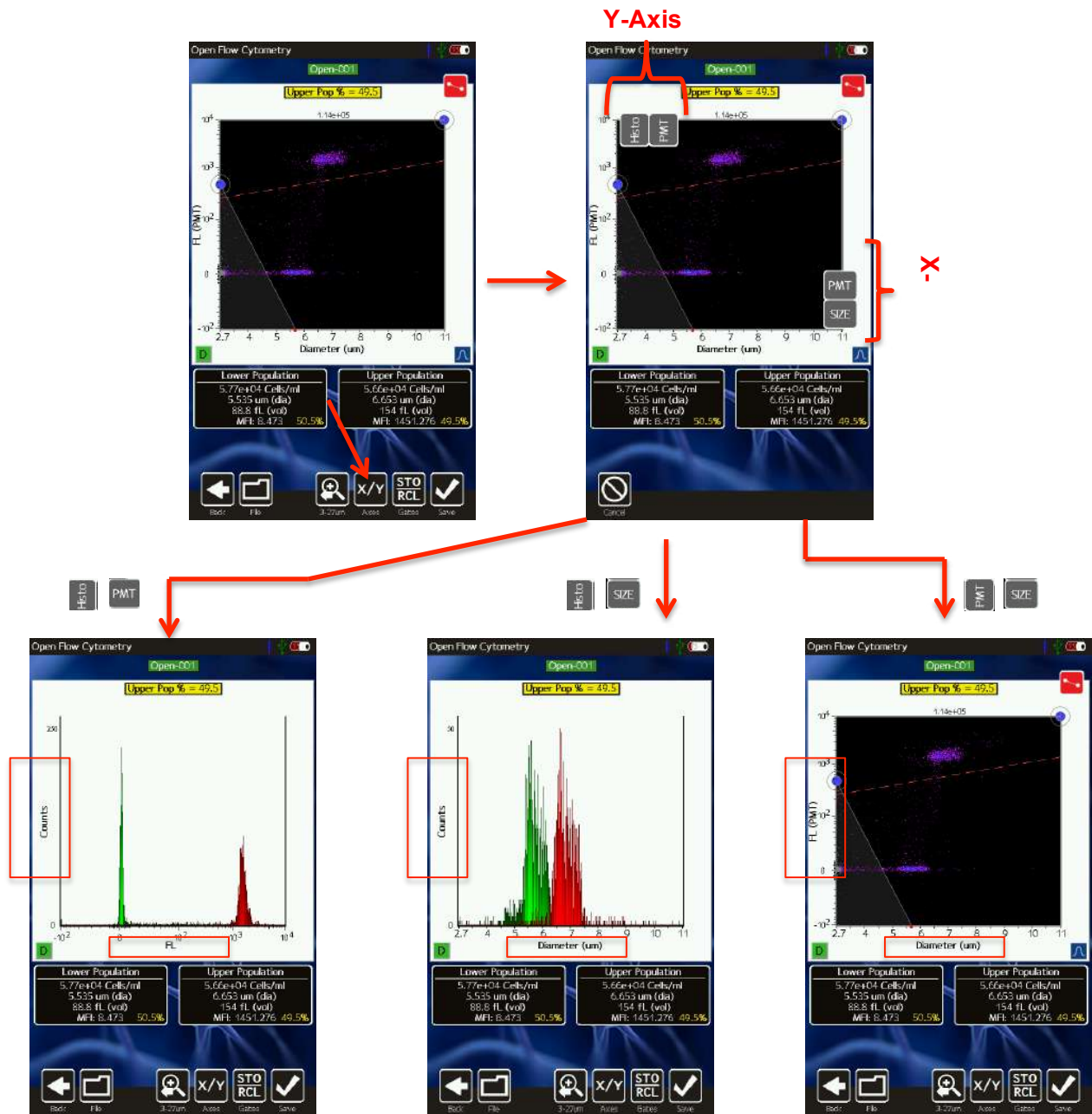
- 587/30nm fluorescence histogram (image above, bottom/right frame). Displayed by selecting "Histo" at the top and "PMT" at the right. Notes:
  - o For fluorescence histograms, the binned data is defined by the size-gated region in the corresponding fluorescence vs. size histogram (image above, frame in the top/left for this example).
  - o Bins are shaded red or green in the histogram as defined by whether the data falls above (red) or below (green, respectively) the fluorescent gate defined in the corresponding fluorescence vs. size histogram (image above, frame in the

top/left for this example)
- Size histogram (image above, bottom/middle frame). Displayed by selecting "Histo" at the top and "Size" at the right. Notes:
  - For size histograms, the binned data is defined by the size-gated region in the corresponding fluorescence vs. size histogram (image above, frame in the top/left for this example).
  - Bins are shaded red or green in the histogram as defined by whether the data falls above (red) or below (green, respectively) the fluorescent gate defined in the corresponding fluorescence vs. size histogram (image above, frame in the top/left for this example)
- 587/30nm fluorescence vs size scatter plot (image above, bottom/right frame). Displayed by selecting "PMT" at the top and "Size" at the right.

Concentration, Size, and Percentage Information

The Moxi GO 532™ provides particle concentration and precise sizing information for each gated region, total counts information, fluorescent percentages, and median fluorescent intensities (all where applicable based on the channels selected) for each test. Two example test view screens are shown below :Fluorescence vs. size scatter (dot) plot (below/left) and Single Channel Histogram Display (below/right)
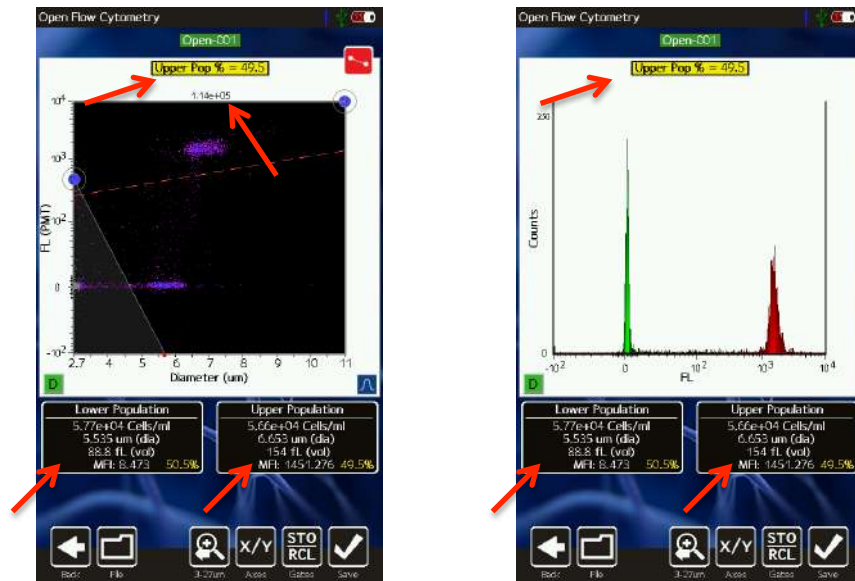
Fluorescence vs. size scatter (dot) plot

Image above/left: For this plot, statistics are only generated for the size-gated (between the blue markers), noise excluded (points that aren't grayed out following a test) region. The total concentration (provided as particles/ml) is listed above the scatter plot between the blue gate region (1.14 x 10^5 cells/ml in the example above/left). The two black boxes below the scatter plot provide the subset concentration (cells/ml), mean particle diameter (*Note: This is an "effective" diameter that is based on a precise volumetric measurement (Coulter Principle) combined with a spherical assumption.*) and median fluorescence intensity (MFI) values for the regions below ("Lower population") and above ("Upper Population") the red fluorescent gate (but

within the size-gated region).   The percentage of events that fall above/below the fluorescence (red) gate is displayed in the yellow box above the scatter plot.

Single Channel Histogram Plot

Image above/right: Only the points within the size-gated region for the fluorescence vs. size plot (e.g. PMT vs. size plot for the histogram shown above/left) are included in generating a histogram. This is deliberately done to avoid having noise contributions in the histogram. The red and green shading are determined by the positioning of the fluorescence (red) gate marker on that same fluorescence vs. size plot. The two black boxes below the histogram provide the subset concentration (cells/ml), mean particle diameter (*Note: This is an "effective" diameter that is based on a precise volumetric measurement (Coulter Principle) combined with a spherical assumption.)*, and median fluorescence intensity (MFI) values for the negative (green, "Lower Population") region and the positive (red, "Upper Population") region. *Note: For size-only tests, the size-region is determined by positioning of blue gate markers directly on the histogram (as described in the "Cell Count (Size-Only)" Assay section above) and the histogram is only shaded green.*   For size histograms (not shown) displayed for fluorescence tests, the size-gated region from fluorescence vs. size scatter plot (above/left) is used to determine which points are included in the histogram binning.

File/Test Operations

Test Naming and Renaming Tests

By default, tests are named with an automated prefix based on the test type ("Via" for Viability Counts, "Open" for Open Flow Cytometry, "Apop" for Apoptosis, and "Size" for Size Histogram). Files within test types are auto-enumerated by the system (e.g. "Via-001", "Via-002", …) with the lowest available number for that test type. Tests can be renamed at any point, immediately after the run or through editing a saved test. However, per the FCS 3.1 standard, the "test name" keyword is permanently fixed once the file has been saved. Changing the name of a saved test on the unit at that point updates the "File Name" only. Changing the name of a test prior to saving the test (immediately after acquisition), changes both the "File Name" and "Test Name" fields. To change the name on the Moxi GO 532™, touch the green test name box at the top of the scatter plot or histogram (see image below/left). The default name is presented and

can be deleted by hitting the **Clear** key or successive "Backspace" keys. Capital letters can be achieved by hitting the **Caps** button. Symbols and special characters can be accessed by hitting the **Punc** button. Select **Save** to assign the newly entered name to the test.
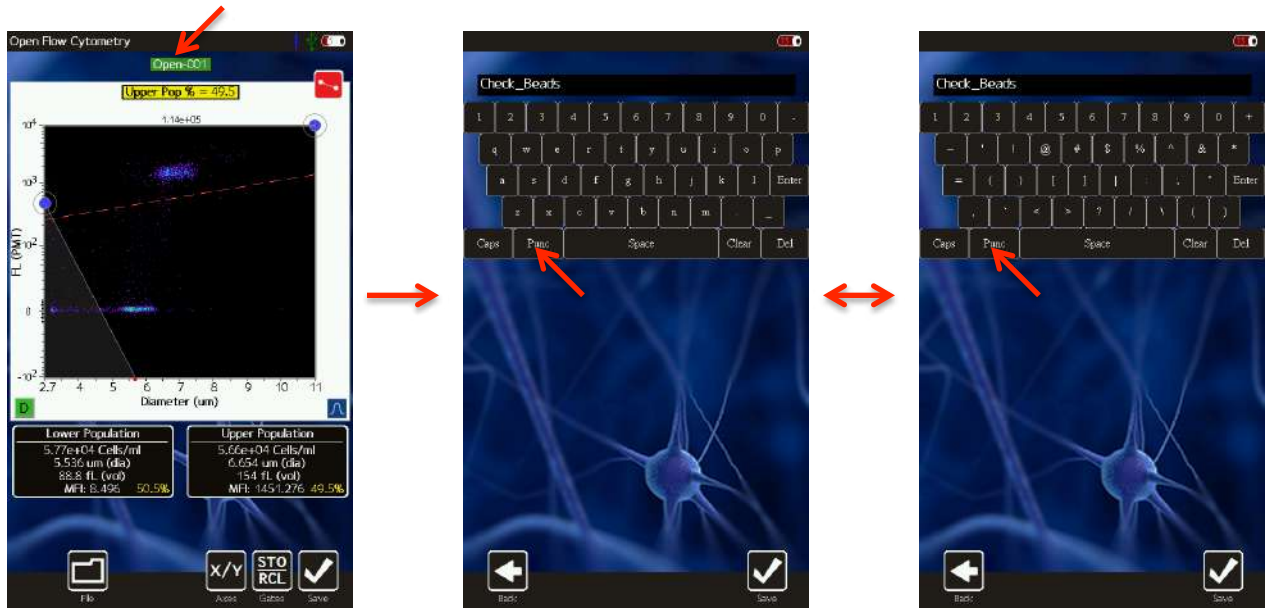
Saving and Deleting Data/Tests

Selecting the **File** button presents the user with the options to **Save** (immediately following a test or after changing gates on saved data. If saved data has been opened but not edited, this button will appear as **Done** instead), **Print** (see below), or **Delete** a test. Selecting Save results in the data being written out to the user disk in FCS 3.1 format for retrieval on a Mac/PC or for later retrieval from the **Saved Data** area (button off of the **Home** screen). After hitting **Save**, a blue status indicator bar is shown at the top of the screen to let the user know the status of the save procedure. Selecting **Delete** presents the user with a pop-up option of **Yes** or **No** to delete the test.

Selecting **Yes** will remove any history of the test. Selecting **No** will cancel the delete operation.

All saved tests can be deleted as a batch by selecting the **Delete All** button in the **Saved Data** area of the instrument or by checking tests in the **Saved Data** area and selecting the **Delete Selected** button.

Exporting Data Screenshots

Exact data screenshots can be generated from the instrument by selecting **File** | (see image sequence below) from the test screen. Selecting this option will save a bitmap file named after the test with a .bmp extension (in the example below, it will be saved as Check_Beads.bmp). *Note: Subsequent screenshot exports for the same test will overwrite any existing screenshot associated with that test.* The screenshot will be an exact pixel-by-pixel representation of the current screen display without the buttons at the bottom of the screen. These screenshots can then be copied to a computer following the instructions in the "Exporting Data" section of this User Guide.
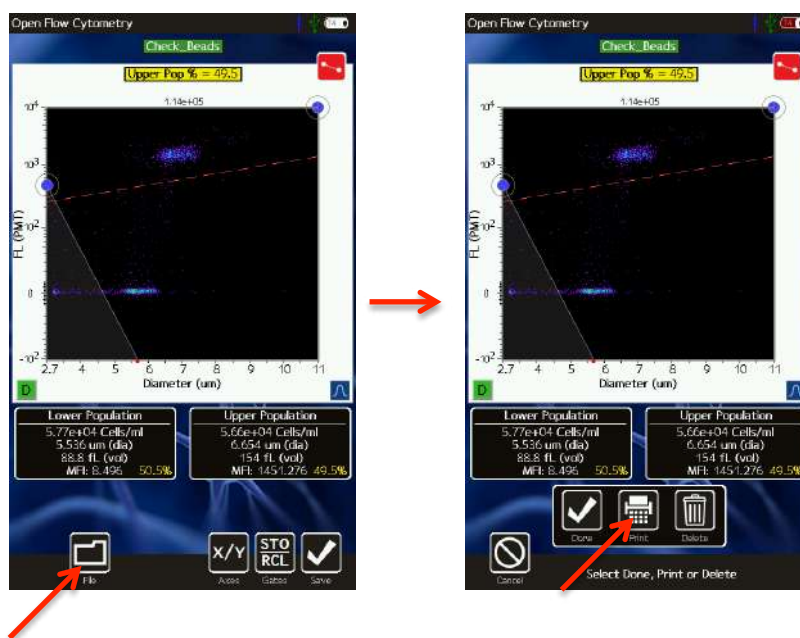
Editing Saved Tests

All saved tests can be accessed by touching the **Saved Tests** icon from the **Home** screen. Doing so provides a list of all files stored on the Moxi GO 532™. File types are color coded (image below) as follows:

- Test (FCS) files are displayed as green rows.
- Exported screenshots are displayed as dark gray rows
- Operating system files are displayed as black rows.
- Moxi GO 532 configuration files are displayed as light gray.

Tests can be sorted by either *Name*, *Date*, or *Type* (FCS, BMP, etc.) by touching the corresponding icon in the "Sort by" field at the bottom left of the screen (Image below,

top/middle, red box). Touching the same icon (e.g. *Name*) repeatedly reverses the sort order (ascending vs. descending).

Test files and screenshot files can be opened by simply touching the green (or dark gray row). An example is shown in the images below (frames from top/middle to bottom/left on touching the "Via-007" green row).

NOTE: Only ten file entries are displayed on the screen at a time. To see additional file listings, it is necessary to:
- **Scroll up/down (one file at a time) the list by touching the up/down arrows (image below, top/right, red bracket) to the right of the file list.**
- **Page up/down (ten files at a time) the list by sliding the up/down arrows (image below, top/right, red bracket) to the right of the file list.**

Files can be selected for batch operations by touching the corresponding white checkbox (or deselected by touching the red "X" checkbox). Once files have been selected, the **Compare/Overlay** (Future functionality) and **Delete Selected** button functionality is activated (image below, top/right to bottom/right frames).

Files can also be deleted in batch by file type. Simply select **Delete All** and a menu will be presented asking whether you want to delete **Bitmap Files**, **Test Files**, or **All Files** (image below, bottom/middle frame). Touch the desired button to complete the operation.

Compare/Overlay Saved Tests

Moxi GO 532 provides the functionality to directly compare two tests by overlaying the data from each test in a scatter plot or histogram view. The compare test functionality is accessible through the **Saved Data** option from the **Home** screen. To compare two tests, select the checkbox next to the two tests to be compared (example in image below top/left). Once a file has been selected, the **Compare/Overlay Tests** icon will appear in black. When two, comparable files have been selected, the **Compare/Overlay Tests** button will turn from black to green, indicating the compare option is viable. The criteria for comparing two tests is as follows:

- The tests need to be of the same assay type (e.g. Open Flow Cytometry, Viability, etc)
- Only two tests can be compared at a time.
- Open Flow tests need to have identical Fluorescent Gain Settings.
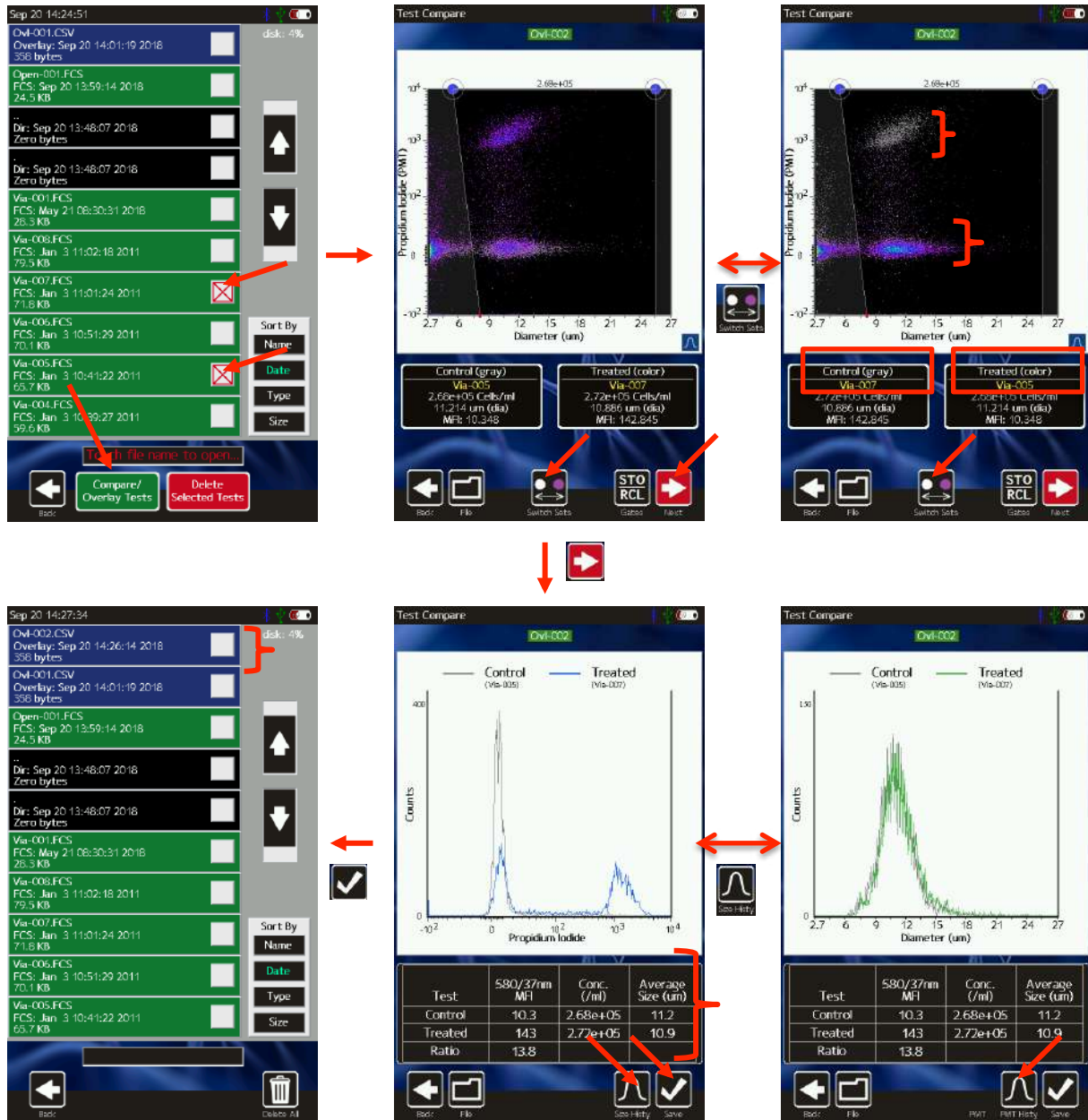
On selection the **Compare/Overlay Tests** icon, the system presents a scatter plot view of the two overlaid tests (image above, top/middle) with the older test presented as the "Control" population (gray dots) and the newer test presented as the "Treated" population (density colored dots). To switch the designations, touch the **Switch Sets** icon as shown in the images (image above,Top/middle to Top/Right, note the change in the cluster coloring as well as the test listings in the black boxes below the scatter plot). Adjustment of the size gate markers (left and right blue dots, image above, top/middle) determines the data points that will be used in the

comparison summary data and histogram overlays. Once that size region is properly defined, touching **Next** will display the fluorescence (PMT) histogram overlay (image above, bottom/middle) for the two data sets.   The fluorescence histogram for the designated "control" data is shown in gray and the fluorescence histogram for the designated "treated" data is shown in blue. It is also possible to view a size histogram overlay (image above, bottom/right) of the two sets by touching the **Size Histy** icon.   Summary data for the two sets is included in the table below the histogram. The overlay information (tests compared and gate locations) can be saved by touching the **Save** icon (or **File** | **Save** icons). The overlay is then available as a blue comma-separated-value (CSV) file entry in the **Saved Data** area (see image above, bottom/left, example Ovl-002.CSV).

Exporting Data – Connecting Via USB (USB on-the-go)

Each saved Moxi GO 532™ test is saved in the Flow Cytometry Standard (FCS) v3.1 format (International society for Advancement of Cytometry, http://isac-net.org) and can correspondingly be opened by any commercially available FCS v3.1 compatible software package (e.g. FlowJo 10.x). One FCS file is saved for each test and the files are named with the test name followed by a .fcs extension (e.g. Via-001.fcs). Quantitative fluorescence values (fluorescent assays) and size values (all tests) are saved within the file for each event. To access the files follow the instructions below:

1. Unplug the USB Cable from the Moxi GO 532™ Power Adaptor.

2. Plug the wide end (standard USB connector) of the USB Cable into your computer's USB port.

3. Plug the small end (mini-USB connector) of the cable into the back of the instrument.

4. Make sure the instrument is powered on and wait for it to appear as a disk (or flash) drive in your computer's file system (Windows Explorer for PC's or Finder for Mac). The Moxi GO 532 disk will be mounted to (appear as a drive on) the computer following completion of this file export process.

5. Open the Moxi GO 532 drive folder and copy the files to your computer (drag and drop or copy and paste)

6. When Complete, safe-eject the Moxi GO 532 drive from the computer.
   a. Windows: Click on the "Safely Remove Hardware" icon in the system tray (bottom right of Windows taskbar).   Select the option to eject the Moxi GO 532 disk.
   b. Mac: In the Mac Finder, click on the arrow to the right of the "Moxi Go 532" external drive that corresponds to the Moxi GO 532 disk.

Moxi GO 532™ Help and System Information

1. For help with operation of the Moxi GO 532™ and for unit-specific system information, press the **Help** icon on the **Home** screen. Visual instructions for inserting a cassette will be presented.
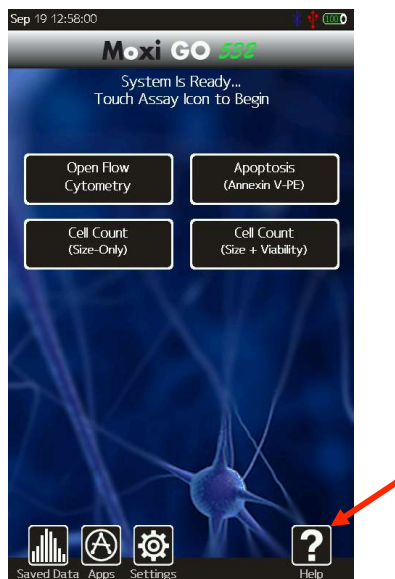
2. Press the **Next** icon to view the next visual instruction that demonstrates how to pipette a sample into the cassette.

3. Press the **Next** icon to proceed to the next instruction screen or press the **Previous** icon to return to the previous screen. **The last screen of the help menu provides information on the version of the Moxi GO 532™ OS being run on the unit, the build date, as well as the Bluetooth ID number (see image to the right). This information is required by ORFLO Technical Support when assisting users with troubleshooting issues.**

4. Press the **Done** icon to exit the help screens and return to the **Home** screen.

   For additional help, see the **Troubleshooting** table (page 27).

Instrument Firmware and Software Upgrades

This User Guide describes OS version 2.09 of the Moxi GO 532™ firmware. The firmware may be updated periodically. For information on the most up-to-date firmware, visit the ORFLO website at www.orflo.com. Register your instrument in order to receive notification about relevant firmware upgrades.

Updating the Firmware

The firmware can be updated through a USB connection. Note: For the latest upgrade information, files, and instructions, please visit the ORFLO website. Generic update instructions are provided below. For firmware updates, the user must connect to the computer's USB port via the provided USB cable.

1. Download and extract the firmware update files (provided as a .zip file). The number and file names can vary with each firmware update. Typical files included with the update include: MANIFEST.TXT, moxiprog.hex, runonce.sh, and KERNEL.COF. Note the location of the files for later use.
2. Turn off the instrument by pressing the power button.
3. Unplug the power adapter to expose the USB connector for the computer. Plug the Moxi GO 532 into the computer that has the downloaded firmware update files.
4. Next, the user needs to put the instrument into a firmware update mode. This can be done in either of the following two ways:
    a. With the unit powered off, quickly press the power/reset button five times (reminder: the unit needs to be in the off position to start this process). When successful, the screen will display with text under the ORFLO logo that indicates that the system has entered the "OS Loader" (see image to the right/top). If unsuccessful, turn the power off and retry.
    b. From the **Apps** screen (off of the **Home** screen) press the **Loader** app icon
5. The Moxi GO 532 will show up as an external drive on the computer (on Mac use the Finder, on PCs use the Windows Explorer to see it).
6. Drag all extracted files onto the Moxi GO 532 drive. The Moxi GO 532 screen will update to show a "USB File Transfer" message with an **OK** (see image right/bottom).
7. Once the file copy has completed, safe-eject the disk from your computer.
8. Touch the **OK** button to proceed. The unit will then load the new firmware (showing a status bar) and automatically power-off.
9. The firmware update is now complete. Unplug the unit from the computer and plug it

back into the AC adapter. The unit has been updated and is now ready for use.

Moxi GO 532 Apps

Moxi GO 532™ Applications can be entered by touching the **Apps** icon on the **Home** screen. Included applications in version 1.22 are:

- Clock

- Programmable timer (with sound notification)

- Loader – Puts unit is Firmware/OS loader mode

- Restart – Reboots the unit

- Off – powers the unit off.

Troubleshooting

Symptom	Cause	Corrective Action
Battery will not fully charge	Battery is faulty or has surpassed its service life	Return instrument for battery replacement
Fluid not pulling into cassette	Test type not selected	Select the assay/test to run on the **Home** screen
	Door not closed	Close door and select a test type
	Cassette manufacturing error	Try other side of cassette or a new cassette.
	Pump error	If the above steps don't resolve the issue, it is possible there is an error with the instrument pump. Contact ORFLO Technical Support.
Instrument stops responding	Internal firmware issue due to instrument malfunction or high level of external interference	Reset instrument by pressing and holding the power button for at least 5 seconds. If problem persists, return instrument for service.
No Clear Scatter (Dot) Plot clusters or "smearing" of scatter plot output	Cell/bead concentration too low	Try running the cell sample at a higher concentration.
	Sample Flow Issue	This is a situation that can potentially occur when running over-concentration, highly-aggregated, and/or "sticky" samples that result in clogging of the filters and/or aperture. This can result in stopped or hindered flow that affects the fluorescent and impedance signal. Dilute the sample further and try to further dissociate the sample with pipette trituration and/or protease (e.g. Accutase$^®$) treatment
	Low/protein expression	For poorly expressed proteins, surface labeling of the protein might not provide a sufficient fluorescent separation from the dark sample.
	No fluorophore present	Cell preparation protocol labeling step was omitted.
Questionable concentration	Concentration of cell/bead sample is too high or too low	Make sure concentration of cell or bead sample is within recommended guidelines. Refer to Specifications section.
	Wrong diluent	Cells should be suspended in 0.9% salt solution (e.g. PBS or equivalent) to ensure proper conductivity for unit operation and to ensure proper particle sizing. Water, hypotonic, or hypertonic solutions are **not** acceptable diluents.
	Cell clumping	Ensure the cells are in a single-cell suspension. Break clumps by pipetting up and down with a standard pipette. Protease treatment can also be used to dissociate clusters. ORFLO recommends Accutase$^®$ or equivalent.
	Improper Size Gating	Please refer to the "Managing the Data" or "Gating the Data" section for instructions on gating. The noise/debris cluster in the bottom left corner should be excluded from count analysis.

Questionable cell diameter	Wrong diluent	Cells should be suspended in 0.9% salt solution (e.g. PBS or equivalent) to ensure proper conductivity for unit operation and to ensure proper particle sizing. Water, hypotonic, or hypertonic solutions are **not** acceptable diluents.
	Cell clumping	Ensure the cells are in a single-cell suspension. Break clumps by pipetting up and down with a standard pipette. Protease treatment can also be used to dissociate clusters. ORFLO recommends Accutase® or equivalent.
	Improper gating	Size measurements are reported as the mean size for a gated range. If a cell population is not properly gated, contributions of other cell types, system noise, or debris particles can contribute to the mean size calculation.
No clear histogram peak on size histogram	Improper scaling	This is usually associated with very-low-concentration samples. For histogram overlay use finger swipes (left - right) for the fluorescence histogram and (top – bottom) for the size histogram. For size histograms (not overlaid), use the white (black box background) arrows to adjust the vertical scale to properly scale the population.
	Small Particles	A smaller particle population might have a left tail that extends into the system noise (below the specified range of the cassette). Re-scaling the x-axis (can only be done after a test, prior to saving) can increase the binning resolution around these populations, helping to better view the peak.
	Debris-filled sample or size-overlapping cell samples (polydisperse samples).	Overlapping size populations can obscure the downward slopes of cell peaks. Often debris populations can obscure the left side of a cell peak.
Overly Long Test Times	Insufficient fluid (<60 µL)	Adjust pipette to ensure sufficient volume for the test. For improperly calibrated pipettes, this might require a setting >60 µL
	Cells/beads too large	Make certain that the particle type is within the specified size range of the cassettes being used
	Highly/strongly aggregated cells clogging filter and blocking flow	Try breaking apart cells using pipette trituration and/or protease treatment.
Gray Screen – "Software Exception"	System firmware has detected a malfunction.	Please take a photo and send to techsupport@orflo.com. Reset the instrument by pressing and holding the power button for at least 5 seconds.
System shuts off during alignment/test	System is unplugged	Plug the system in to the AC wall adapter and re-try.

| Error Messages	Cause	Corrective Action
Alignment Failed: Cassette Aperture Not Found	Cassette error	Try the other side of the cassette or a new cassette
Calibration: Please remove the cassette	Cassette in tray on startup	Remove the cassette from the tray and tap "OK"
Error: The disk is full	Not enough space to save a test.	Delete files from the "Saved Tests"
Invalid or empty filename	Filename field is blank	Enter a valid filename.
No Suitable Fit	No clear Gaussian population in a Size Histogram	Revert to gating mode to obtain sizing and counts.
Gray Screen – "Software Exception"	System firmware has detected a malfunction.	Please take a photo and send to techsupport@orflo.com. Reset the instrument by pressing and holding the power button for at least 5 seconds.
	System settings error	Contact ORFLO Technical Support
Resource Error: Missing Resource	Missing System Files	Contact ORFLO Technical Support.
Sensor Error: This sensor is an unknown type	Cassette error	Try the other side of the cassette or a new cassette
	Sensor Contact error	If this affects multiple cassettes, please contact ORFLO Technical Support
Test Error: Fluid was not Detected	Sample not loaded or volume too small	Make sure sample is loaded and volume is at least 60 µL.
Test Error: Incomplete Test	Insufficient fluid (<60 µL)	Adjust pipette to ensure sufficient volume for the test. For improperly calibrated pipettes, this might require a setting >60 µL
	Cells/beads too large	Make certain that the particle type is within the specified size range of the cassettes being used
	Highly/strongly aggregated cells clogging filter and blocking flow	Try breaking apart cells using pipette trituration and/or protease treatment.
Test Error: Stop Detected before Start	Used cassette or cassette error.	Try the other side of the cassette or a new cassette
Used or Invalid cassette	Previously used sensor detected	Do not reuse sensors.
User/System Defaults loaded	New Firmware has been loaded	No problem, simply touch OK to acknowledge
	Instrument has detected corruption or new version of firmware and reset all settings to factory defaults	Contact ORFLO technical support
Warning: Low Battery	Battery needs to be recharged	Recharge instrument for a minimum of 4 hours and run tests with the instrument plugged into the AC adapter.
Warning: High Concentration	Particle count exceeds cassette recommended concentration range	Dilute sample (e.g. 10x) and re-run
Warning - Time/Date lost	Battery ran out of power and system clock reset	Charge the unit overnight and reset the Date/Time in the **Settings** section (icon off of **Home** screen)

Maintenance and Storage

Storage

Store the Moxi GO 532™ and Cassettes at room temperature in a dry environment. For best results, cassettes should be used within one year of purchase.

Avoid prolonged exposure to ultraviolet light as it may degrade the touch screen display or discolor the external surfaces of the instrument.

Charging the Battery

The Moxi GO 532™ contains a 3.7 V lithium ion battery that can be charged for approximately 500 cycles. The battery may be charged at any time in the discharge cycle and can be charged continuously without damage, using a PC/Mac computer or the power adapter. Attach the small connector of the USB cord to the Moxi GO 532™ and the larger connector on the opposite end of the cord to a USB port on a computer or an
Orflo power adapter. If using the power adapter, plug it into an AC outlet with an appropriate power rating. Charging time will vary but a complete charge is typically achieved in 4-8 hours. The charge indicator in the upper right hand corner of the display (see image below) will change from flashing red to solid green when the battery is fully charged.
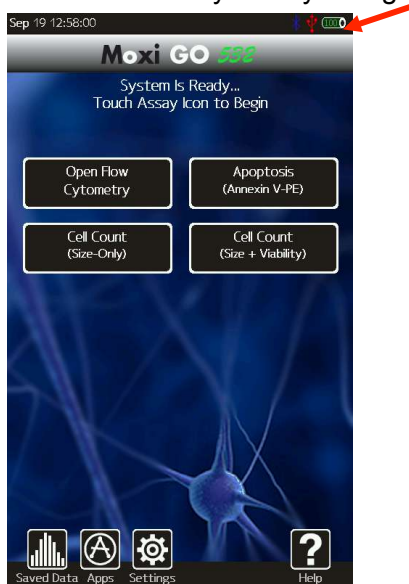
CAUTION: To prevent battery damage, use ONLY an ORFLO supplied power adapter

or the USB port of a computer.

The battery life is about 2 to 5 years depending on use. Lithium ion batteries discharge even if they are not in use. To prevent battery damage from self-discharge, charge the battery at least once every two months.

Cleaning/Sanitizing

The Moxi GO 532^{TM} is **NOT** autoclavable. Extreme heat will damage the battery, touch screen display, and other electronic components.

The external surfaces of the Moxi GO 532^{TM} body and touch screen display can be sanitized by wiping with a soft, nonabrasive cloth moistened with 70% isopropyl alcohol (IPA) or 70% ethanol. Do not clean the instrument with any more aggressive solutions.

⚠ **CAUTION:** When sanitizing, make certain that no liquid enters any internal cavities of the instrument. This could result in damage to the instrument.

Maintenance

There is no routine maintenance required for the Moxi GO 532^{TM}. In addition, there are no user serviceable parts. Instrument repairs must be carried out by authorized personnel only.

Specifications for the Moxi GO 532™ and MFS Cassettes

System Performance	
Detection Parameters	3 (One color fluorescence, cell count, cell size)
Excitation Laser Wavelength	532 nm
Number of PMTs	1
Optical Detection Range	587/30 nm LP (e.g PE or PI)
Particle Count/Size Detection	Impedimetric (Coulter Principle)
Display	480 x 800 pixels, color touchscreen
Resolution	1000 histogram bins
Data Storage	16 Mb (full histograms and scatter plot data)
Connectivity	USB on-the-go
Data Output	On-system data analysis with optional export of BMP files and FCS compatible raw data files *(FCS 3.1, compatible with FlowJo and other offline software analysis platforms)*
Measurement Modes	
Open Platform	Yes, Open Flow Application available allowing users to define their own protocols and analysis ** Requires fluorochrome compatible with above filter and laser*

Pre-Defined Assay Protocols
   Apoptosis (Annexin V-PE)
   Cell Count (Size+Viability)
   Cell Count (Size-Only)
	Yes. Follow Orflo Recommended protocols at http://www.orflo.com

Cassette Performance	
Compatible Sample Types	Cells (Adherent & Non-adherent primary cells or cell lines) and Beads
Number of tests per cassette	2
Sample Volume Required	60 µl minimum
Cell/Bead **MEAN** Size Range	
Type S+	
Diameter	3 – 20 µm
Volume	14 – 4189 fL
MF-M	
Diameter	4 – 25 μm
Volume	34 – 8181 fL
Overall Size Range	
Type S+	
Diameter	2.7 – 27µm
Volume	14 – 10306 fL
MF-M	
Diameter	3 – 35μm
Volume	14 – 22449 fL
Optimal Concentration for	
Cell/Bead Counting	
Type S+	$1 \times 10^4 - 1.75 \times 10^6$ cells/ml
MF-M	$1 \times 10^4 - 7.5 \times 10^5$ cells/ml
Fluorescence Detection	
Type S+	$1 \times 10^4 - 5 \times 10^5$ cells/ml
MF-M	$1 \times 10^4 - 4 \times 10^5$ cells/ml
Measurement Time	~ 10 seconds
Software	Includes an instrument-only FW/SW package that drives instrument performance and on-instrument data analysis
	Offline analysis requires FCS v3.1 compatible software package for viewing files (e.g., FlowJo X)
Physical Attributes	
Benchtop	Yes
Dimensions (HxWxD)	3.25 in x 9.3 in x 8.7 in / 8.3 cm x 22.1 cm x 23.6 cm
Weight	10 lbs / 4.54 kg

Electrical Specifications	
Internal Battery	Rechargeable 3.7V, 7500 mAh lithium ion
AC Power Adapters (US, EU, and UK types)	Input: 100-240 VAC (50/60 Hz), 0.2 A Output: 5 VDC, 1 A
Operating Environmental Conditions	
Temperature	15-30ºC (indoor use only)
Maximum Relative Humidity	20-80% (non-condensing)
Altitude	2000 meters (maximum)
Applicable Pollution Degree	Pollution Degree 2
Degree of Ingress Protection	IPX10

Ordering Information

This section lists catalog numbers for the Moxi GO 532™ and related products. You can purchase ORFLO products through a regional distributor or on-line at www.orflo.com. See the Technical Assistance section for information about contacting ORFLO.

Product Description	**Product Code**
Moxi GO 532™ Kit, US Version	ZF002
Moxi GO 532™ Kit, Base Unit Only, US Version	ZF001
Type S+ Cassettes (25 pack)	MXC030
Type S+ Cassettes (250 pack)	MXC032
MF-M Cassettes (25 pack)	MXC021
MF-M Cassettes (250 pack)	MXC023
Moxi GO 532 USB Cable and Adapter	MXA120

Technical Service

For technical service, contact ORFLO Technologies at 855-879-6694, option 1 or email us at tech_support@orflo.com for technical questions or info@orflo.com for sales questions.

Warranty

What is Covered By Your Warranty

ORFLO Technologies warrants its products will meet their applicable specifications when used in accordance with their published instructions.* The ORFLO product warranty is a "bumper to bumper" warranty on your ORFLO automated cell analysis product for the term of the warranty. This includes all electrical parts, mechanical parts, batteries, displays, materials and workmanship. Our warranty also covers the materials and workmanship of our cassettes and accessory products.

* Please ensure that you have the latest version of the product user guides. The most current version are found on our website at www.orflo.com

How Long is Your Warranty

Your ORFLO products are covered for a period of one (1) year from the original date of purchase from ORFLO or an approved sales distributor.* You may purchase, for an additional fee, an extended warranty for the Moxi GO 532 for an additional one (1) or two (2) years from the anniversary of the date of purchase. The extended warranty extends the duration of your warranty coverage from the date of original purchase from ORFLO or an approved sales distributor, but does not alter, change, or otherwise add to the coverage described here.

* In accordance with EU requirements, our standard product warranties remain in effect for two (2) years in EU countries.

What is Not Covered by Your Warranty

The following exclusions apply to your ORFLO warranty:

- Damage resulting from misuse, abuse, or accidents
- Damage resulting from failure to follow the proper operating instructions contained in the User Guide
- Damage resulting from failure to use a ORFLO-provided USB cable and power adapter.
- Damage resulting from unauthorized, non-ORFLO repair or improper tampering with the product
- Damage resulting from fire, flood, or "Acts of God" beyond the control of ORFLO

What To Do If You Think You Have a Warranty Issue

Please contact ORFLO promptly at 855-TRY-MOXI (855-879-6694), select option 1 or send an email to tech_support@orflo.com to describe the issue you are experiencing. Please be prepared to supply your name, contact information, the serial number of your product, and the original purchase date of the unit.

If your issue remains unresolved after consulting with one of our technicians, ORFLO will provide a Returned Material Authorization (RMA) number and you will be asked to ship your product back to us at:

ORFLO Technologies
Attn: Warranty Returns (RMA#: XXXX)
260 Northwood Way
Ketchum, ID 83340

An ORFLO technician will promptly examine your product to determine if the issue is covered under the ORFLO warranty. If it is, we will decide to either repair it or replace it at no cost to you, including shipping. Repair or replacement will be done as expediently as possible to ensure minimum disruption to your laboratory.

Express or Implied Warranties and Other Legal Stuff

ORFLO Technologies makes no other warranty, express or implied. There is no warranty or merchantability or fitness for a particular purpose. The warranty provided herein and the data, specifications, and descriptions of ORFLO products appearing in ORFLO's product literature may not be altered except by expressed written agreement signed by an officer of ORFLO Technologies. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon. ORFLO Technologies shall not be liable for consequential, incidental, special or other damages resulting from economic loss or property damage sustained by any customer from the use of its products.

Applicable Patents

The following core patents apply to the Moxi GO 532 and cassettes. This is not intended to be an all-encompassing list of related patents.

- U.S. Patent # 7,515,268
- U.S. Patent # 7,520,164
- U.S. Patent # 8,082,603
- U.S. Patent # 8,171,778
- U.S. Patent # 8,182,635
- U.S. Patent # 8,608,891
- U.S. Patent # 8,735,853
- U.S. Patent # 8,804,105
- U.S. Patent # 9,293,311