




Personal
Cell Analysis

Moxi GO II™

USER GUIDE



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Introduction

The Moxi GO II™ from ORFLO Technologies is a benchtop instrument that utilizes a disposable thin-film cassette and a combination of a 488 nm laser, two photomultiplier tubes (525/45 nm filter with either a 561nm LP filter or 646nm/LP filter, user-swappable), 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. The instrument is small enough to be portable between the lab bench and the hood. A touchscreen interface and simplified GUI allows for ease of operation without extensive training.

The Moxi GO II enables highly accurate cell counts, cell sizing, quantitative assessments of cell viability, apoptosis, immunophenotyping, and GFP checks for a broad range of cellular analysis. Data acquisition occurs within approximately 5-10 seconds per test. The instrument can store up to 4Gb of data. Data is provided in the industry-standard Flow Cytometry Standard (FCS v3.1) format as well as user-generated bitmap screenshots. Data can be downloaded to a PC or Mac via USB connectivity.

IMPORTANT NOTE:


The Moxi GO II™ is intended for research use only. 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 II™ User Guide* provides detailed information for operating, maintaining, and troubleshooting the Moxi GO II, 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 the Protocols section (<http://www.orflo.com/v/vspfiles/pages/protocols.html>) of the ORFLO website for more details on sample preparation.

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To update the Moxi GO II 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 remind 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 II™ 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. IT 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.
- 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 II. Aside from swapping of filters and replacement of cassette tray inserts, the instrument has no user

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serviceable parts. **All service must be performed by ORFLO, or an approved ORFLO vendor.**

Laser Safety


 **WARNING** The Moxi GO II is a Class 1 laser product in accordance with IEC 60825-1:2007. The system contains a Class IIIb laser that operates at 488 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 II if there is any damage to the instrument's enclosure.
- Do not attempt to disassemble or service the Moxi GO II 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

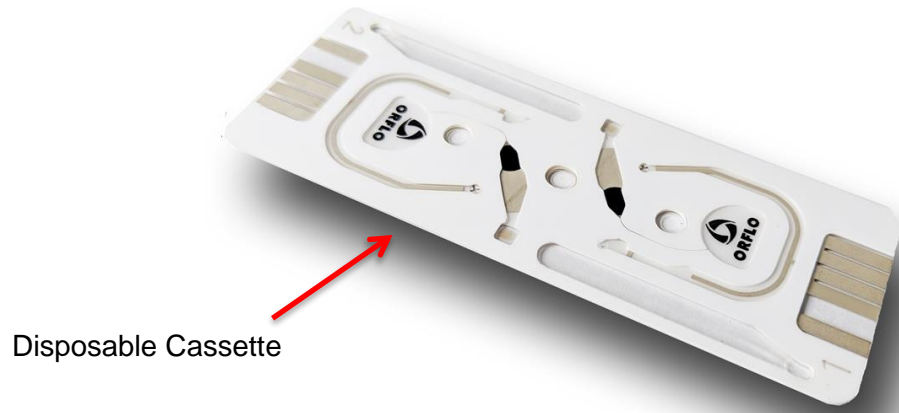
 **WARNING** Biological samples have the potential to transmit harmful or fatal disease. The following guidelines should be followed to minimize the risk of exposure to biohazardous materials.


- 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.

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Moxi GO II™ System Overview

The Moxi GO II™ Kit includes the Moxi GO II system, USB Cable (mini USB B to USB A 2.0), USB Power Adapter, one pack of 25 Type S+ (MXC030) Cassettes, the extra 646nm/LP filter, a spare cassette tray insert, and a Quick Start Reference Guide. The following figure shows the main visible components of the Moxi GO II and the table provides a description of each component.



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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 ~10 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 Lever	(Older models only). For the newer systems (no cassette tray lever), the tray automatically depresses when the cassette tray door is opened. For older models, the tray is 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.
Swappable Optical Modules	Custom optical detection filters for testing with various dyes/fluorophores.
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 II™ Accessories

The Moxi GO II™ uses a USB Cable and Power Adapter for power source. The Moxi GO II 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.

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Power Adapter (US style)



USB Cable



Replacement Cassette Tray Insert



Part

Function

Power Adapter, US Style

Connects USB cable to a 110 VAC outlet

USB Cable

Connects instrument to Personal computer (PC or Mac) or power adapter

Cassette Tray Insert

Extra tray insert can be user-installed in cassette tray when adhesive build-up interferes with insertion/removal of cassettes.


Getting Started

The Moxi GO II™ is shipped in a condition ready for initial use; though we recommend checking for software updates and updating the Moxi GO II 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.

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NOTES

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

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 II is used with Type S+ or MF-M Cassettes. 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 - 75 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 (e.g. pipetting vigorously) and/or protease dissociation (e.g., Accutase® or Accumax®). For stubbornly aggregated samples, pre-filtration with a 40 µm cell strainer is recommended.

As the Moxi GO II™ uses a 488 nm laser with 525/45 nm and either (561nm/LP or 646nm/LP, user-swappable) detection filters, dye/fluorophore selection should be based accordingly. A few examples for each include:

PMT Filter	Compatible Fluorophore Examples (not complete list)
525/45 nm	FITC, Alexa Fluor® 488, BB515, GFP, Calcein, Acridine Orange
561nm/LP	PE, PI, DsRed
646nm/LP	PI


For assays other than “Open Flow Cytometry” and “GO Flow”, using ORFLO optimized and validated protocols are strongly recommended. Please see (<http://www.orflo.com/v/vspfiles/pages/protocols.html>) for a list of currently available protocols.

For cell counting application, cells can be run without fluorochromes using any of the assays.

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Additional Materials Required

- Cell or bead sample (diluted and dissociated, if necessary); 60 µL minimum.
- Type S+ (MXC030, recommended for almost all assay) or MF-M (MXC010) Cassettes
- Pipette (typically P100 or P200) and appropriately sized pipette tips
- Cell staining/labeling reagents (test specific, see guidelines in “Sample Preparation”)

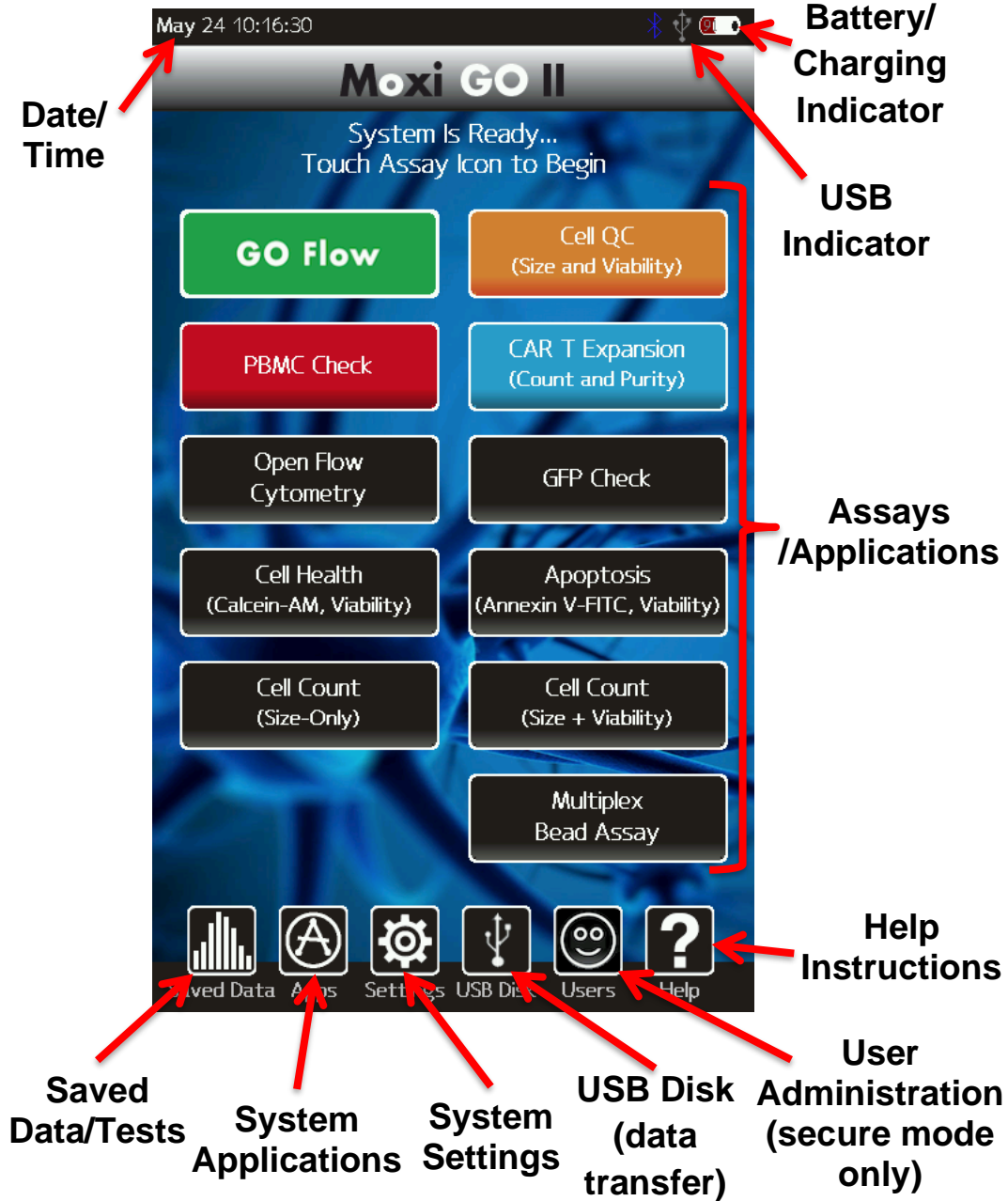
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Using the Moxi GO II™

Press the Power Button to turn on the instrument. The Home Screen will become available. The image below identifies the key items on the Home Screen.

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Home (Start) Screen




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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 II software program.

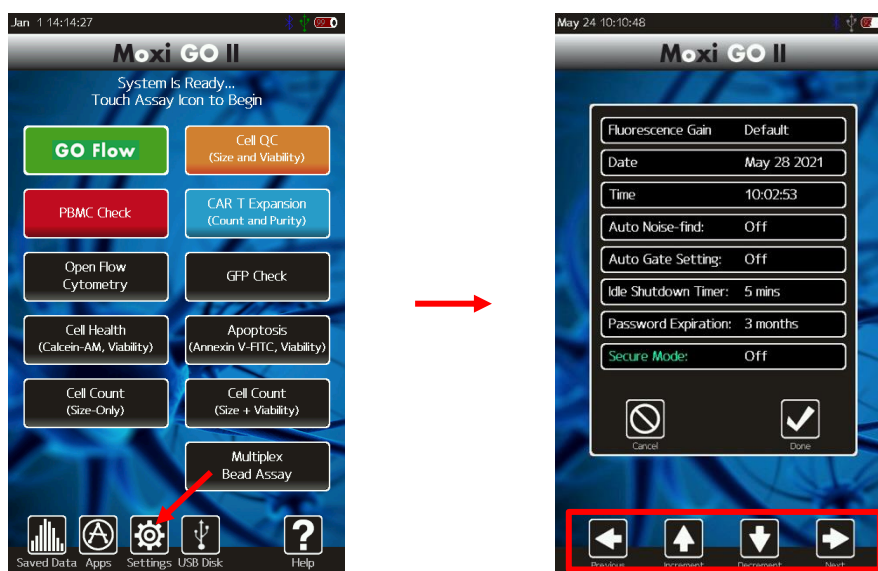
Depending on the initial purchased configuration of the Moxi GO II system, certain assays/applications may be disabled (examples in images below). These assay can be enabled by contacting ORFLO (info@orflo.com, 855-879-6694, option 2).



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Settings

Access to settings is available to any operator when Secure Mode is Off. When Secure Mode is on (see 21 CFR Part 11 Operation section, below), only system administrators can change system settings, with the exception of Fluorescence Gain which is still accessible by all users.




Fluorescence Gain

The “Fluorescence Gain” field sets the level of fluorescence gain for the “Open Flow Cytometry” and “GO Flow” assays only. Five 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” and “GO Flow” start screens. To change the values simply tap on the current value and the system will toggle the gains with each tap. Note: the user can also make a one-time (per-test) change to the fluorescent gain value of the “Open Flow Cytometry” and “GO Flow” assays by touching the value on the test start screen as described in “Running a Test” “Open Flow Cytometry Test” section (step 6).

Date and Time

Set the date and time by pressing the **Settings** icon on the main menu of the instrument. Then touch the Date or Time field. Use the up/down arrow keys to change the values.

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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 $<2.9\mu\text{m}$ AND fluorescence levels $<10\text{mV}$. ***With the v2.10 (or later) release of the firmware, it is recommend that this setting is left in the “Off” setting.***

Auto Gate settings

The user is given control in setting the system auto-gating to one of three settings:

1. ***Off*** – the system will not auto-gate any tests.
2. ***Viability Assays*** – the system will auto-gate the Viability tests only: Cell QC, PBMC Check, CAR T Expansion and Cell Count (Size + Viability).
3. ***All Assays*** – the system will auto-gate the first PMT vs. Size display output for all tests.

To toggle the auto-gate value, simply tap on the current setting for that field.

Idle Shutdown Timer


Time period for system to automatically go to sleep (and log out the user if Secure Mode is On). Tap field to toggle values. Possible values are : 5min, 15min, 30min, 45min, 60min, 2hrs.

Password Expiration

Time period for system to auto-expire a User’s password. Once the password expires, the system will force the user to update it prior to logging in. Tap field to toggle values. Possible values are 1, 3, 6, 9, and 12 months.

Secure Mode

This field turns on/off the 21 CFR, Part 11 functionality for the system. See the 21 CFR Part 11 section of the User Guide.

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Sample Preparation Considerations

There are several key considerations for preparing a sample for analysis with the Moxi GO II™:

1. **Solution Conductivity** – As the system utilizes an electrical measurement for counting cells, sizing cells, and metering the fluid volume, cells need to be suspended in a conductive media. The target solution conductivity would be 0.9% salt solution (e.g. PBS or equivalent). Deviations of +/- ~50% conductivity can be tolerated by the system. Most medias that are isotonic to PBS will work.
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+ cassette: 3 - 27 µm diameter (14 – 10,306 fL volume)
 - ii. MF-M cassette: 4 - 35 µm diameter (14 – 22,449 fL volume)
 - b. Concentration range:
 - i. Type S+ Cassettes:
 1. Counts: 1×10^4 – 1.75×10^6 total particles/ml
 2. Optimum Fluorescence Sensitivity: $< 5 \times 10^5$ particles/ml
 - ii. MF-M Cassettes:
 1. Counts: 1×10^4 – 7.5×10^5 total particles/ml
 2. Optimum Fluorescence Sensitivity: $< 3 \times 10^5$ 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® or Accumax®). 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 II™ uses a 488 nm laser for excitation with 525/45 nm and either 561 nm/LP or 646nm/LP (second filter is user-swappable) detection filters. Dye and fluorophore selections should be based accordingly. Note: When using PI for viability, it is recommended to use 2µg/ml PI if the 561nm/LP filter is installed in the PMT2 (back) slot or 5µg/ml PI if the 646nm/LP filter is installed in the PMT2 slot.

PMT Filter	Compatible Fluorophore Examples (not complete list)
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525/45 nm	FITC, BB515 [®] , Alexa Fluor [®] 488, GFP, Calcein, Acridine Orange
561nm/LP	PE, PI, DsRed
646nm/LP	PI

- 5. Post-Incubation Washes for fluorescent labels** – To optimize sensitivity of the fluorescent signal, it is recommended (for most samples) that the sample be washed 2x (minimum) with a minimum of 1.5ml staining buffer or PBS to remove the excess (free) antibody.
- 6. Red Blood Cell (RBC) containing samples** – For samples with high levels of RBC's (e.g. peripheral blood), it is necessary to eliminate/reduce the level of RBC's in order to prevent those cells from "masking" the cells of interest. The preferred approaches to purification of a sample are density-gradient centrifugation (e.g. Ficoll-Paque[®]), magnetic bead separation, or "pre-plating" (culturing of adherent cells). RBC lysis approaches can be applied but users should be aware that those approaches have limited efficiency and can still leave a significant number of intact RBC's behind.

For assays other than "Open Flow Cytometry" and "GO Flow", using ORFLO optimized and validated protocols are strongly recommended. Please see website (<http://www.orflo.com/v/vspfiles/pages/protocols.html>) for a list of currently available protocols.

For cell counting application, cells can be run using any of the assays.

Running a Test

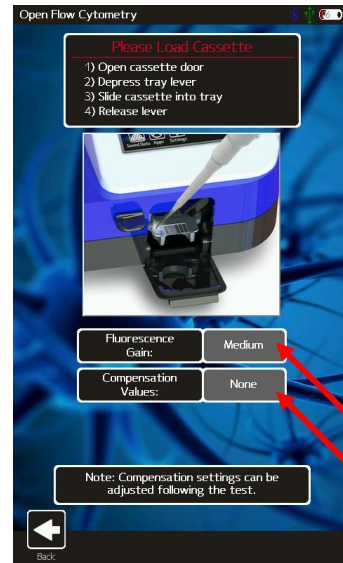
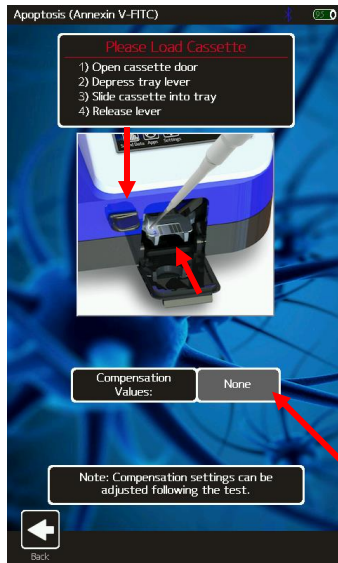
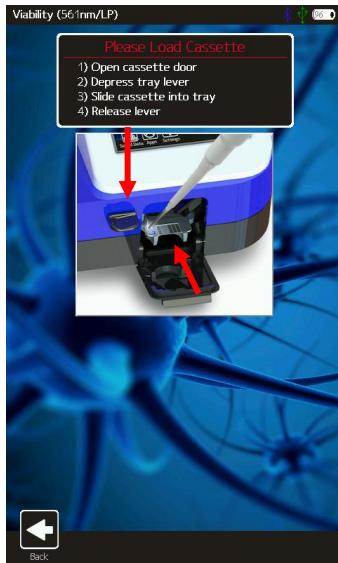
General Instructions (All Tests except "Cell QC", "Open Flow" and "Multiplex Bead Assay")

- Please follow sample preparation guidelines above ("Sample Preparation Considerations") in preparing the sample. Note: The minimum volume required for a test is 60 µL.
- 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 to run a test. Tests **CAN NOT** be run on internal battery power alone or computer USB power.



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- Basic test instructions are presented for all tests (example for “Viability Test” shown below/left). For two PMT tests, a compensation menu (image below/middle and below/right) is presented to provide the option to select a previously-used (“Use Prior”) compensation setting. For the “Open Flow Cytometry” and “GO Flow” tests, the additional option of setting the “Fluorescence Gain” (on a per-test basis) is presented (image below/right). Touch the gray box (red arrow in image) to change this gain. Five options, in order of increasing gain for the fluorescence signal output, are possible: *Very*

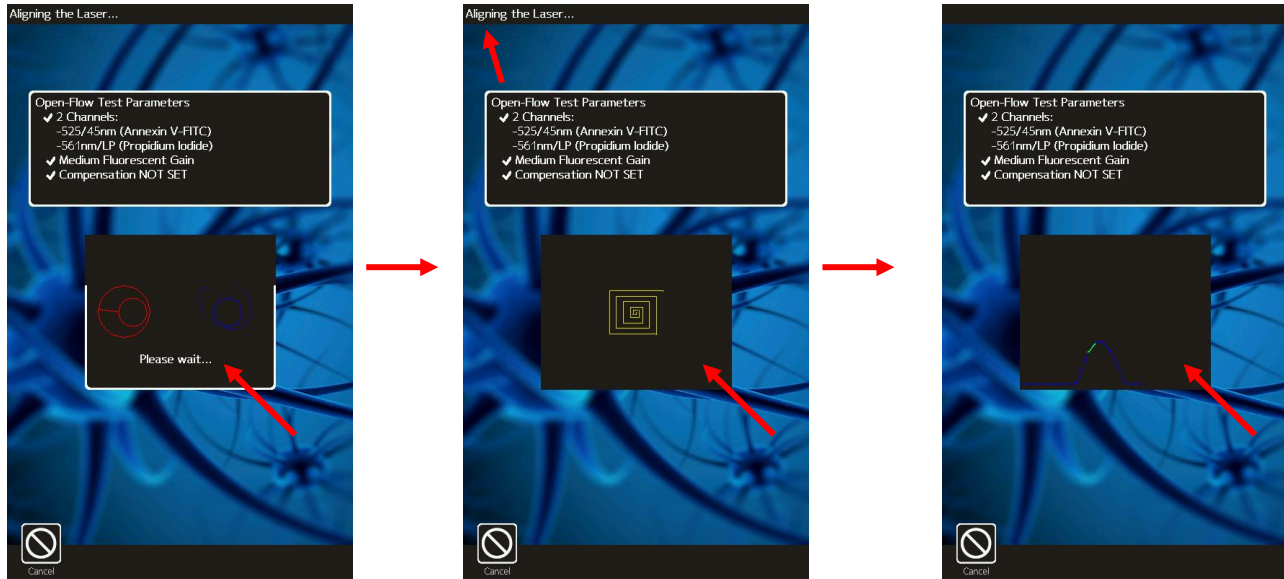


Low, Low, Default, Medium, and High. The initial value populated in this field is determined by the global “Fluorescence Gain” setting that can be set from the **Settings** icon off of the **Home** Screen.

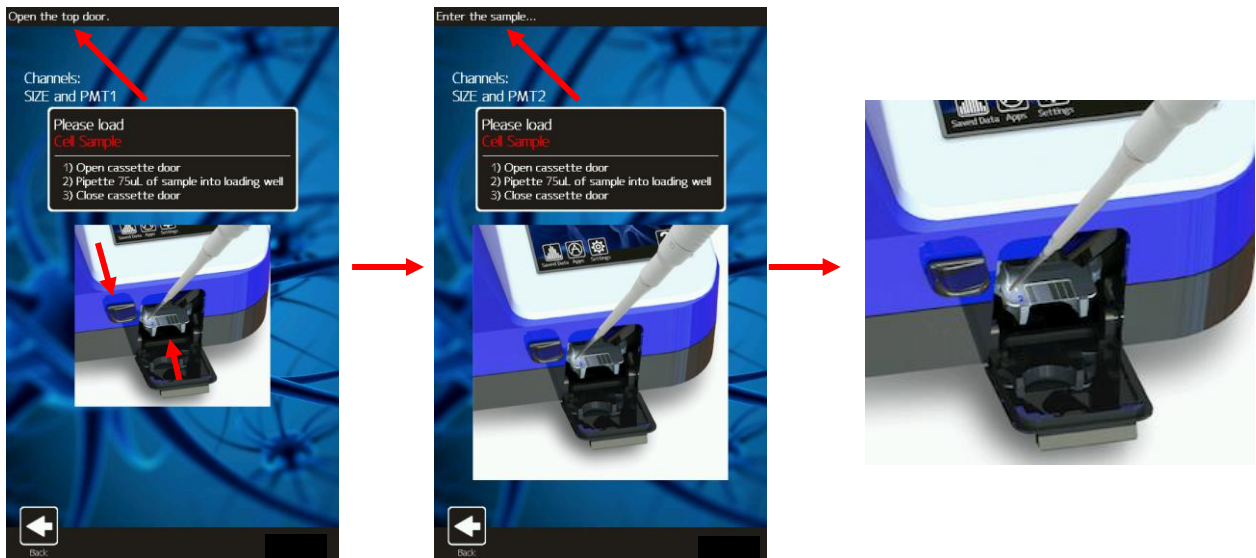
- **Inserting a Cassette:** Open the Front Door and depress the Cassette Tray Lever (image below). Slide a test cassette, with ORFLO lettering-side up into the cassette tray (either end 1 or 2). Release the Cassette Tray Lever and close the Door. *Note: If the cassette had been previously used, the system will generate a “Used or Invalid Cassette” error message.*



- **System Calibration:** The system will automatically perform a calibration step as outlined in the images below. 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 (see image below/left). Following that initial test, only the standard laser alignment (images below/middle and below/right) are performed.
 - c. Should the alignment result in an error make sure that your cassette is pushed into the sled. And, make sure the cassette is not inserted at an angle.



- Loading a sample:** System messages at the top of the screen prompt the user to “Open the Door” (image below/left) and pipette a 60 – 75 μ L 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.



Notes:




- To load the sample, place the pipette tip directly in the loading oval (touching the cassette with the tip is perfectly fine and recommended).
- It is best to place the pipette facing straight into the system (not angled left or right) and a ~45degree angle (up/down, see image below)



- Dispense the fluid in one, smooth motion as you would normally during pipetting. The system does apply a slight vacuum to pull the fluid it, but it is not recommended to try to “time the load” to the draw of the fluid. It is normal/expected to have a bead of fluid form over the entry well. That fluid will get pulled in eventually.
- 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.
- 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. **Please refer to the “Test Results – Analyzing/Gating Data” section for details on gating and interpreting the data output for all tests.**

“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 488nm

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laser excitation and 525/45nm and/or either 561nm/LP or 646nm/LP (depending on which the user has installed) filter sets. For non-ORFLO kits, it might be necessary to perform titration assays and/or adjust 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. The “X-axis” is always populated with Cell size as its selection; however multiple additional parameters can be selected under the “Y-axis” column. Select the first channel to record from the “Y-axis” column (image below/middle) and select “Next”.

Notes:

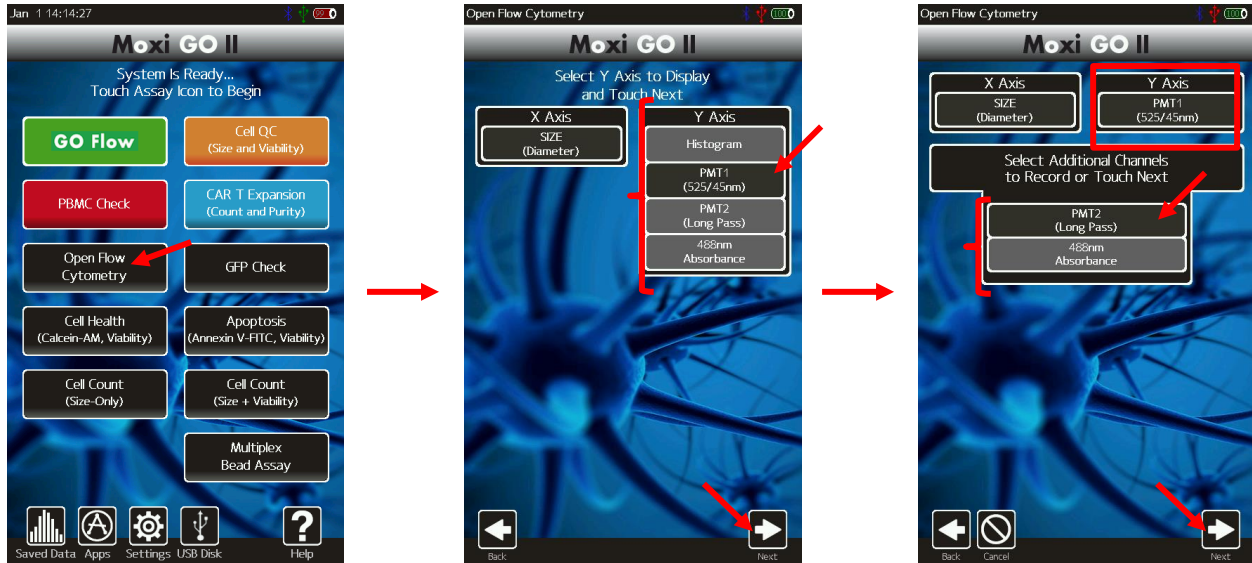
- a. *This is the channel that will initially be displayed during test acquisition.*
 - b. *Only one value from that column can be selected.*
 - c. *If “Histogram” is chosen, the system will perform a “Size Only” counts and sizing assay. For that configuration, no laser alignment will be performed, as the laser is not used.*
4. Select any additional channels to record (image below/right) and select “Next.”

Notes:

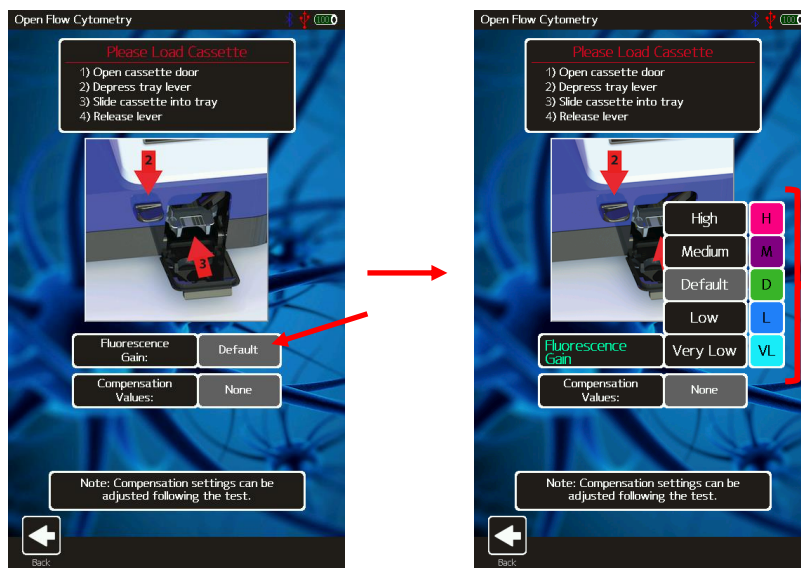
- a. *Any combination (none, only one, or both) of the channels can be selected for recording.*
- b. *These channels will not be displayed during sample acquisition but can be viewed immediately following a test by changing the display axis. See section below “Changing Display Axis”*
- c. *If “Histogram” was selected on the prior screen, this screen will be skipped.*




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5. 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). Note: Due to the innate nature of how data is collected, selecting a gain change will affect both PMT detectors.
6. A pop-up menu will appear with the available options (image below/right). Touch the desired value to change the setting.

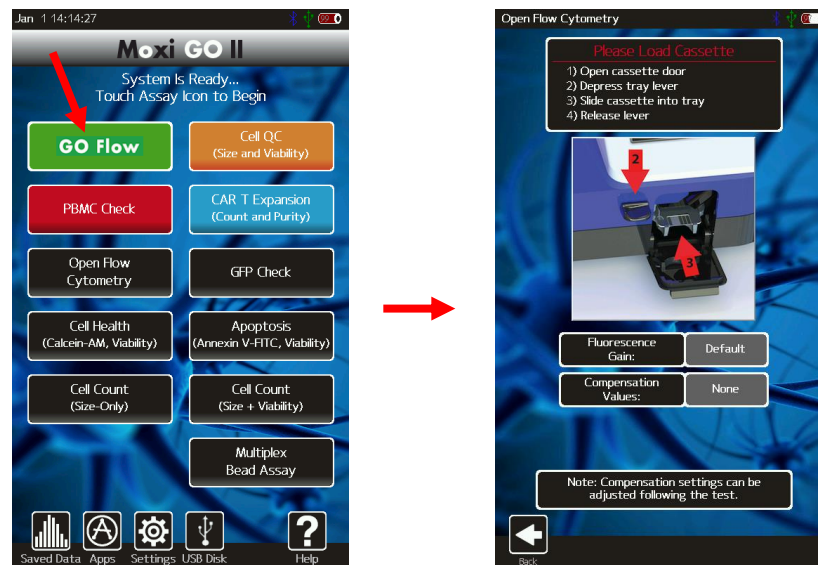


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7. Select “Start” or insert a new cassette and close the door to begin the test (start the laser alignment). See “Running a Test - General Instructions” above for further detail.


“GO Flow” Tests (Simplified version of “Open Flow”)

The “GO Flow” app is meant to simplify, expedite, the “Open Flow Cytometry” selection process by automatically selecting the PMT1 (525/45nm) channel as the “Display” channel and the PMT2 (“Long Pass”, either 561nm/LP or 646nm/LP) as a recorded channel. The only remaining selection options are the “Fluorescence Gain” per-test adjustment drop-box and the “Compensation Values” adjustment drop-box (see below). GO Flow tests are still saved with the “Open-“ file naming notations.



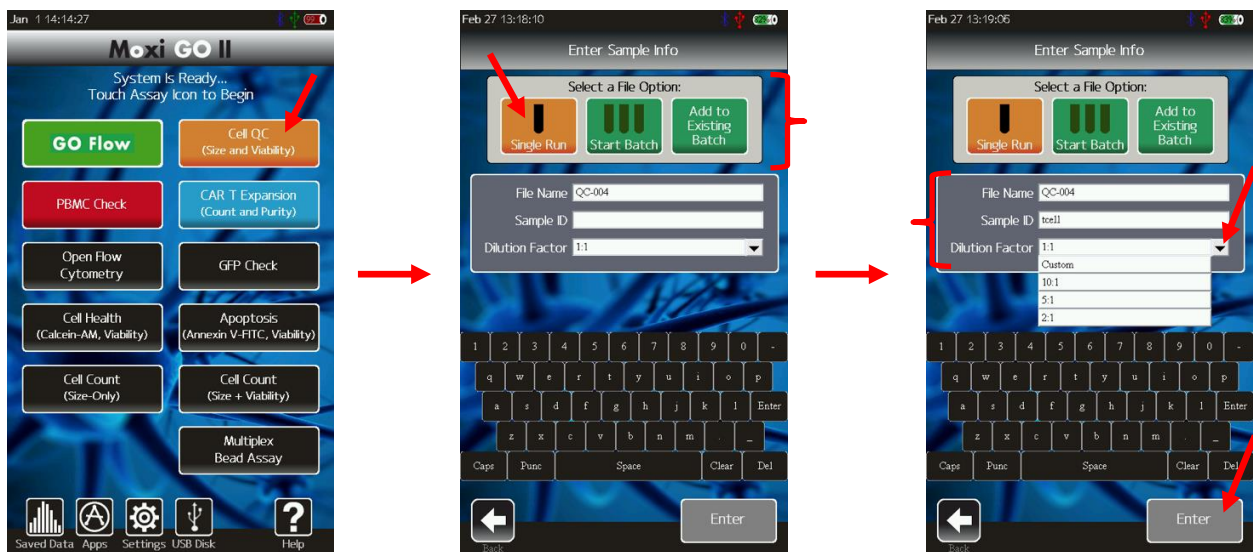
“Cell QC” Test

The “Cell QC” app is designed to provide simplified analysis of cell viability samples either one test at a time or in batch mode (rapid acquisition of multiple samples of the same cell type, using consistent gating). The initial display output of the Cell QC app is a simplified, list of the sample statistics, based on the system’s auto-gating of the sample. The batch mode, in-addition to providing for rapid acquisition of data, provides the same simplified output for each test, along with a final summary .csv (e.g. Excel) output file with the summary data for all tests.


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Touching the “Cell QC” icon on the Home screen brings up the Cell QC “Enter Sample Info” screen. The first selection option is the “File Option”. There are three file options: 1.) “Single Run” (run a single test). 2.) “Start a Batch” (run a series of samples of the same type in rapid succession) 3.) “Add to Existing Batch” (continue running a series of samples in batch mode, appending to an existing batch run). The selected option is display in orange. The other options in green.

Cell QC – Single Run

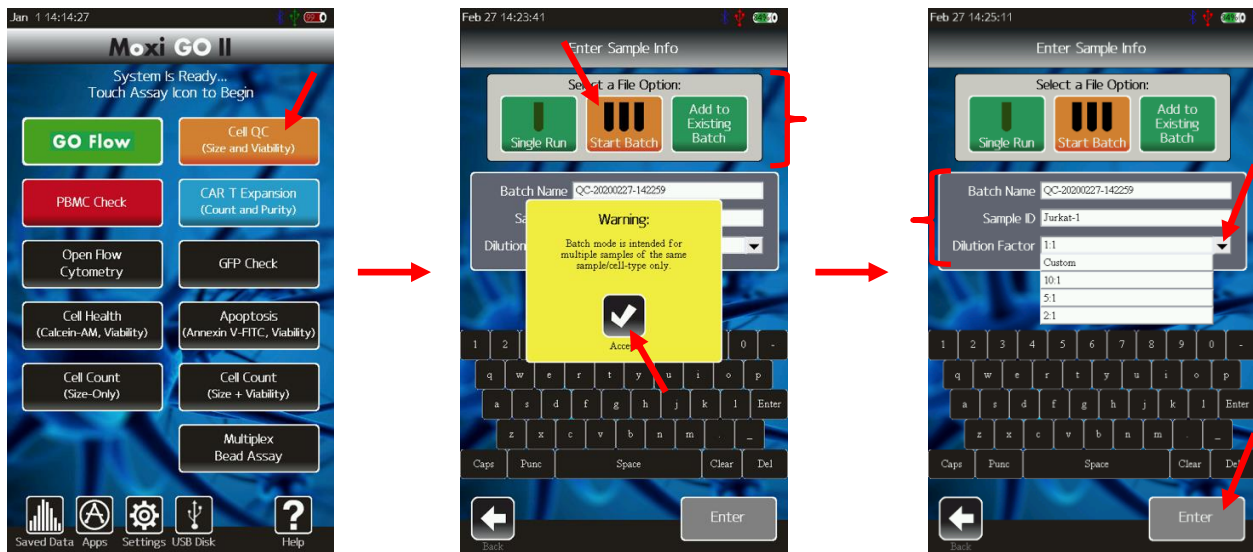


By default, the system sets the “File Option” as a “Single Run” (single test, see image above/middle). Below the file option selection, there are three test parameters that the user can set: 1.) “File Name” 2.) “Sample ID” and 3.) “Dilution Factor”. The system defaults the test to have a name of “QC-XXX” where XXX is a sequential number starting at “000” and incrementing with each test run. The system will auto-enumerate that number with the lowest available number (not already used by a saved test on the disk). This value also provides the test file name (with an .fcs extension, e.g. “QC-004.fcs”). This file name field can be changed by touching in the box and using the keyboard below to type a new name. *Notes:* 1.) *Capital letters can be typed by hitting the “CAPS” key to change the keyboard to capital letters.* 2.) *Additional punctuation/symbols are available by touching “Punc”.* The “Sample ID” field can be used as general text description of the sample being run. This field can be changed by touching in the box and using the keyboard below to type a description. The “Dilution Factor” field is a drop-down list with the options of entering “1:1” (no dilution), “1:2” (2x dilution), “1:5” (5x dilution), and “1:10” (10x dilution). **Note: the value selected here will automatically be applied to the test count output to adjust the measured counts for the dilution level,**


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providing the true (dilution adjusted) initial starting concentration. The value is set by touching the box (image below/right) and touching one of the dropdown entries. The “Enter” button can then be touched to start the test.

Cell QC – Start Batch

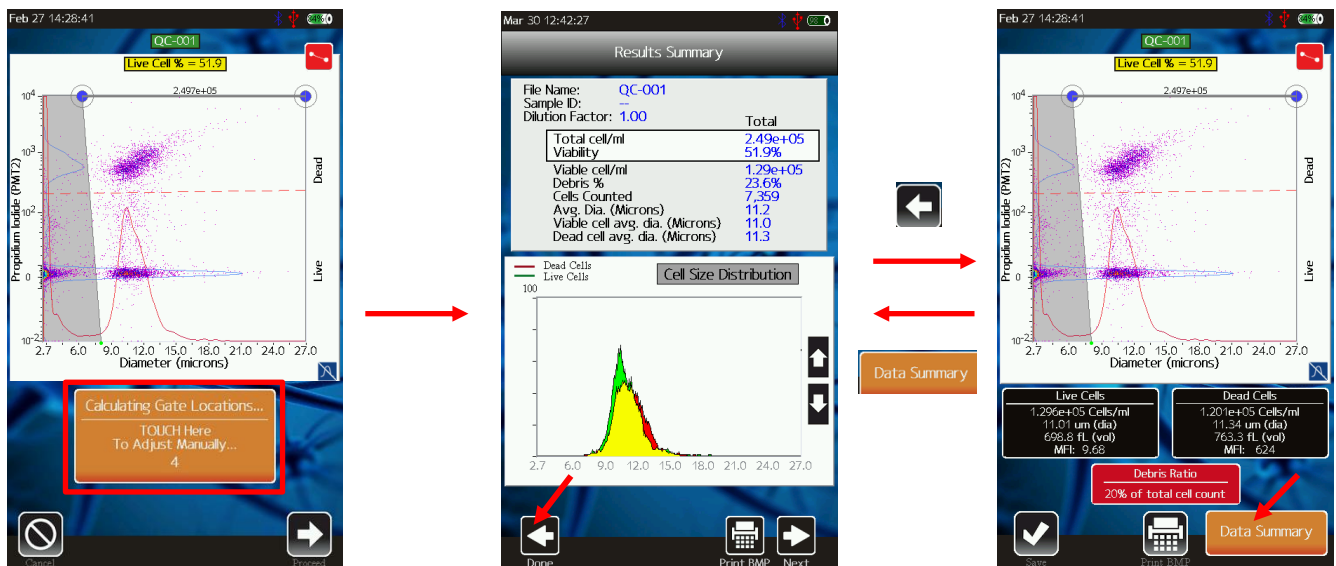


Selecting the “Start Batch” file option will prompt a warning that this mode is only meant for samples of the same cell type (see image above/middle). The reason for this is that, following the first test (which will be auto-gated by the system, with the option to manually adjust the gates), the subsequent tests will be run with the same gate settings as the first. This is intentionally implemented for consistency in running sample batches. With batch testing, there are three test parameters that the user can set: 1.) “Batch Name” 2.) “Sample ID” and 3.) “Dilution Factor”. The system defaults the batch name to have the format of “QC-YYYYMMDD-HHMMSS” (where YYYY is the year, MM is the numeric month, DD is the day, and HHMMSS is the time). This naming is implemented to capture both the acquisition time and date, as well as to ensure uniqueness in the naming. When running a batch, a sub-folder with the “Batch Name” is created in the “Test-Files” data directory on disk. The individual tests in the batch will be saved to that sub-folder with the naming format of “QC-XXX.fcs” where XXX is a sequential number starting at “000” and incrementing with each test run in the batch. The “Sample ID” field can be used as general text description of the sample/test being run. This field can be changed by touching in the box – and using the keyboard below to type a description. The Sample ID field can be changed for each/any test in the batch. *Notes:* 1.) *Capital letters can be typed by hitting the “CAPS” key to change the keyboard to capital letters.* 2.) *Additional punctuation/symbols are available by touching “Punc”.* The “Dilution Factor” field is a drop-down list with the options


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of entering “1:1” (no dilution), “1:2” (2x dilution), “1:5” (5x dilution), and “1:10” (10x dilution). **Note: the value selected here will automatically be applied to all tests’ count outputs to adjust the measured counts for the dilution level, providing the true (dilution adjusted) initial starting concentration.** The value is set by touching the box (image below/right) and touching one of the dropdown entries. The “Enter” button can then be touched to start the test/batch. For the first test after the system is powered-up, the system will perform the laser-homing routine (as described in the laser/cassette alignment section). For every subsequent test/cassette side, the system will perform a spiral find and laser sweep to find align the laser ideally to the cassette detection zone (see laser/cassette alignment section).

Cell QC - Initial Test Output (both Single Run and Batch)



An example of the Cell QC output is shown above. Initially (during and right after acquisition) the user is shown the raw scatter plot data (image above/left) and given the option (by touching the orange rectangle) to manually adjust the system’s auto-gate placement. Touching the orange rectangle will bring the user the scatter plot gating screen (image above/right). If the user does not touch anything, the simplified “Results Summary” screen (image above/middle) is displayed. This initial screen is designed to provide a simple representation of all the cell/sample data based on the system auto-gating of the PMT2 (Propidium Iodide, Viability) vs Size scatter plot (more detail below) to identify the core cell population (by size) and determine the viability (by PI fluorescence). The user is provided the opportunity to review the system gate placement by touching the “Back” button. Touching that button brings up the standard PMT2 (Propidium Iodide) vs. Size scatter plot (example in image above/right). Gates can be adjusted

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by touching and dragging the markers (blue/size, red/fluorescence, yellow/noise) as described in the “PMT vs. Size Gating” section of this User Guide. It is recommended that users verify that gate for the initial test/run with any new sample/cell types. Once verified, the user can simply refer to the simplified test output screen. The user can return to the simplified “Results Summary” screen by touching the orange “Data Summary” button under the scatter plot.

The “Results Summary” screen has the test information at the very top. If this test is part of a batch, the “Batch Name” will be added to that summary (as shown above). The Dilution Factor, selected by the user during test configuration, is automatically applied to the concentration data provided below. *Some notes on the data:*


- *The key information, dilution-adjusted concentration and viability, are highlighted in the white box.*
- *The “Debris %” is calculated as the particle counts in the range from 3µm (minimum detectable particle size) to the left size gate (start of the cell region).*
- *The “Cell Counted” is the absolute number of cells in the size-gated region, based on the 22.6µL metered test volume.*
- *The histogram shows the live cell population in green, the dead cell population in red, and the overlapping regions in yellow.*

The up/down arrows on the histogram can be used to resize the histogram y-axis (scaling). The “Print BMP” button will save a Bitmap (image, .bmp) file to the Moxi GO II data disk (in the main “Test-Files” folder for single runs or the batch folder for batch runs). That image will be an exact representation of the “Results Summary” screen.

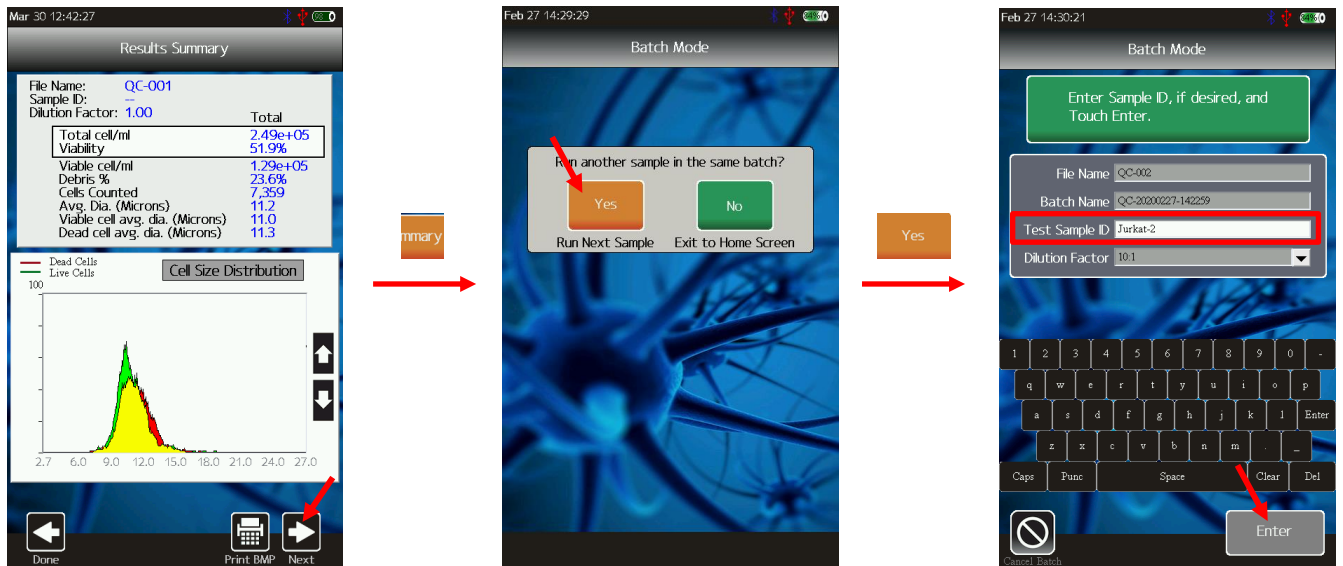
Touching the “Save” button for a Single Run Cell QC test, will save the data (.FCS format) and will return the system to the Home Screen. For Batch Runs, the system will proceed to walk the user through loading more samples as described below in the “Cell QC – Test Output – Continuing Batch Testing” section.

Cell QC – Test Output – Continuing Batch Testing


Touching the “Save” button for a Batch Run will save the latest data/test (.FCS format) and result in a user prompt as to whether the user wants to continue running tests in the batch. As long as the user touches “Yes” after each test, tests/files can be added to the batch indefinitely. Touching “No” will end the batch acquisition and return to the Home screen. *Note: There is always an option to “Add to Existing Batch” (see “Cell QC - Add to Existing Batch” section below) to continue adding tests to an existing batch (e.g. if a batch is accidentally ended prematurely).* Once the “Yes” button is selected, the user will have the option to type/enter a

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“Test Sample ID” for the next test/sample in the batch using the keyboard at the bottom. *Notes:* 1.) Capital letters can be typed by hitting the “CAPS” key to change the keyboard to capital letters. 2.) Additional punctuation/symbols are available by touching “Punc”. The Test Sample ID value is optional. It can be used as a unique identifier for each sample in the batch and will be populated in the final “Batch Summary.csv” (batch summary table) that is generated for each batch.




Following completion of a batch, the user can exit the batch processing by touching the green “No” icon (image above/middle) to exit back to the home screen.

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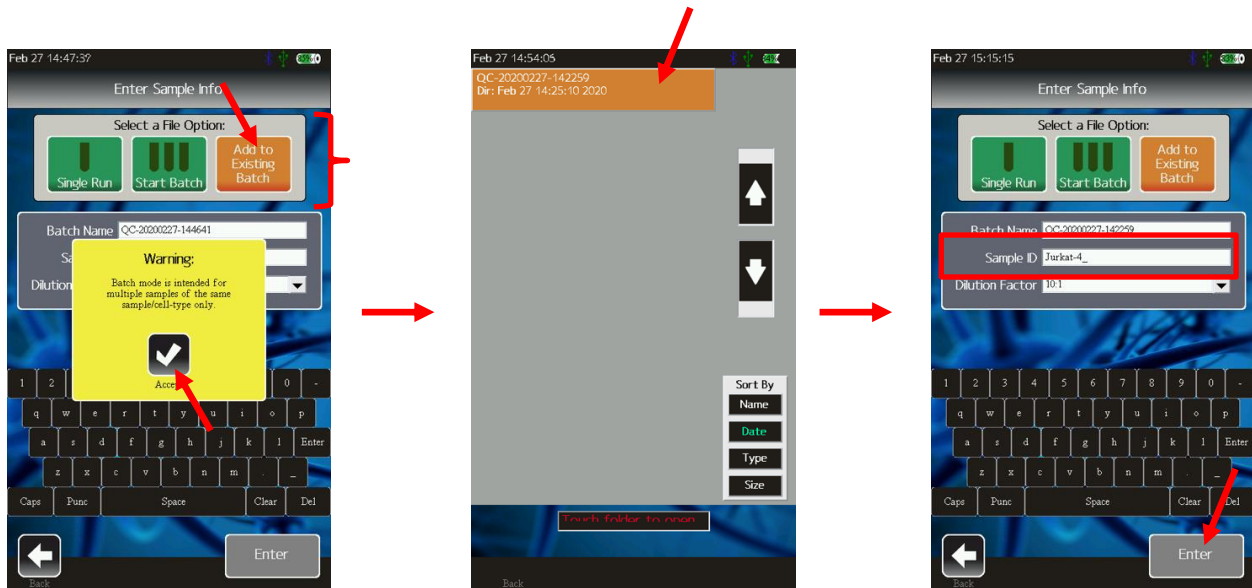
Cell QC – Batch Run – Saved Data and .CSV Summary File

	A	B	C	D	E	F	G	H	I
1	Batch Name	QC-20200227-142259							
2	System:	Moxi-Go II - SN 0080254429B8							
3	Firmware:	OS 2.10 - Feb 26 2020							
4	Assay Type:	Cell Counts and Viability							
5									
6						Dilution Adjusted - Results			
7						Total Cells		Debris (3um to left gate)	
8	File Name	Date	Time	Sample ID	Dilution	Conc. (/ml)	% Viable	Conc. (/ml)	%
9	QC-001	27-Feb-2020	14:26:30	Jurkat-1	10:1	2.50E+05	51.9	6.13E+04	19.7
10	QC-002	27-Feb-2020	14:30:43	Jurkat-2	10:1	2.43E+05	51.7	6.00E+04	19.8
11	QC-003	27-Feb-2020	14:36:16	Jurkat-3	10:1	2.35E+05	50.1	7.26E+04	23.6
12	QC-004	27-Feb-2020	15:17:37	Jurkat-4	10:1	2.45E+05	49.3	6.67E+04	21.4
13	QC-005	27-Feb-2020	15:19:43	Jurkat-5	10:1	2.48E+05	50.1	7.72E+04	23.7
14									
15									

The batch information is all contained in a sub-folder (under the “Test-Files” main folder) of the Moxi GO II μ SD data disk. Within that folder, all the test FCS (.fcs) files are saved, any generated screenshot files, and the batch summary file “Batch_summary.csv” file. An example screenshot of a Batch_summary.csv file (opened in Microsoft Excel in this case) is shown above. The summary file contains the full batch information along with a row entry for each test run in the batch. In that row, values for file name, test date, test acquisition time, sample ID (optional, entered by the user), dilution level, total cell concentration (dilution adjusted), percent viable cells, debris concentration (particles in the range of 3 μ m to the left cell gate), and debris percentage (as a function of the total cell concentration) are listed.

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Cell QC – “Add to Existing Batch”




To append data to existing batch, the user can simply select the “Add to Existing Batch” icon (see above/left) at the first “Cell QC” configuration screen. The system will provide the standard warning (yellow pop-up, see above/left) that batch mode is meant for samples of the same type. Touch “Accept,” and a list of available batches will be displayed (see above/middle). There will be an orange rectangle/entry for every batch on disk. Touch the desired batch (orange box) and the system will load the batch information. At that point, the sample ID can be updated using the displayed keyboard. *Notes: 1.) Capital letters can be typed by hitting the “CAPS” key to change the keyboard to capital letters. 2.) Additional punctuation/symbols are available by touching “Punc”.* Touching enter will start the test, beginning with the laser/cassette alignment zone (see laser/cassette alignment section).

Multiplex Bead Assay

Overview – Current State

The Moxi GO II has an assay/app that was designed for providing **relative** (comparison of MFI levels) protein expression (e.g. cytokine) levels for two lysed cellular samples. **At this point, the kits for that application are not yet developed by ORFLO.** To use the system in this way, users would need to perform some protocol/kit development on their end to get that functionality up-and-running for their application. The general principle is to immobilize capture antibodies on functionalized beads using an amine or carboxyl coupling approaches. Coupling kits and functionalized (either carboxyl or amine) beads can be sourced through several vendors (e.g. Bangs Labs and Spherotech). The system would use distinct bead sizes to

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categorize or map the beads the particular analyte of interest with a maximum “plex” capability of 4 beads (sizes). The bead sizes would need to be in the 3.5µm to 10µm range (ideally, but could possible extend higher). The user would then assign a different capture antibody to each bead size for immobilization (mapping the analyte type to a particular bead size). The bead sizes do need to be sufficiently spaced (e.g. 4µm, 5.5µm, 6.5µm, 9µm) for unique size discrimination (no size overlap in the size distributions) so that each unique protein (specific to the antibody) could be detected (has its own size “channel” on the scatter plot). As a starting point, the Bangs Labs beads (<https://www.bangslabs.com/products/polystyrene-microspheres/functionalized-polystyrene>) in the 4µm, 5µm, 6µm, and 7µm bead sizes should work. Beyond that, the approach would use the classic “sandwich assay” methodology in which one would incubate the functionalized beads with your lysed sample, wash the sample, apply a conjugated (biotinylated) secondary antibody specific to each protein target, wash the sample, and then add a PE-Streptavidin reporter. Note: PE is the required reporter for the system. Typically, these steps would be performed on vacuum-filter plates or with magnetic bead systems.

As the assay is a relative quantification assay, a minimum of two tests would need to be run. The first test would always be a “reference” or “control” sample to which all future “unknown” samples would be compared. The Multiplex Bead Assay app is designed to walk the user through the running of a reference sample. Once the reference data has been recorded, any number of a samples can be compared to it.

When developing a custom multiplex assay, it is necessary to contact ORFLO Technical Support (tech_support@orflo.com, 855-879-6694) to get a firmware file/setting to correspond to the number of bead categories, target bead sizes, and expected bead concentration.

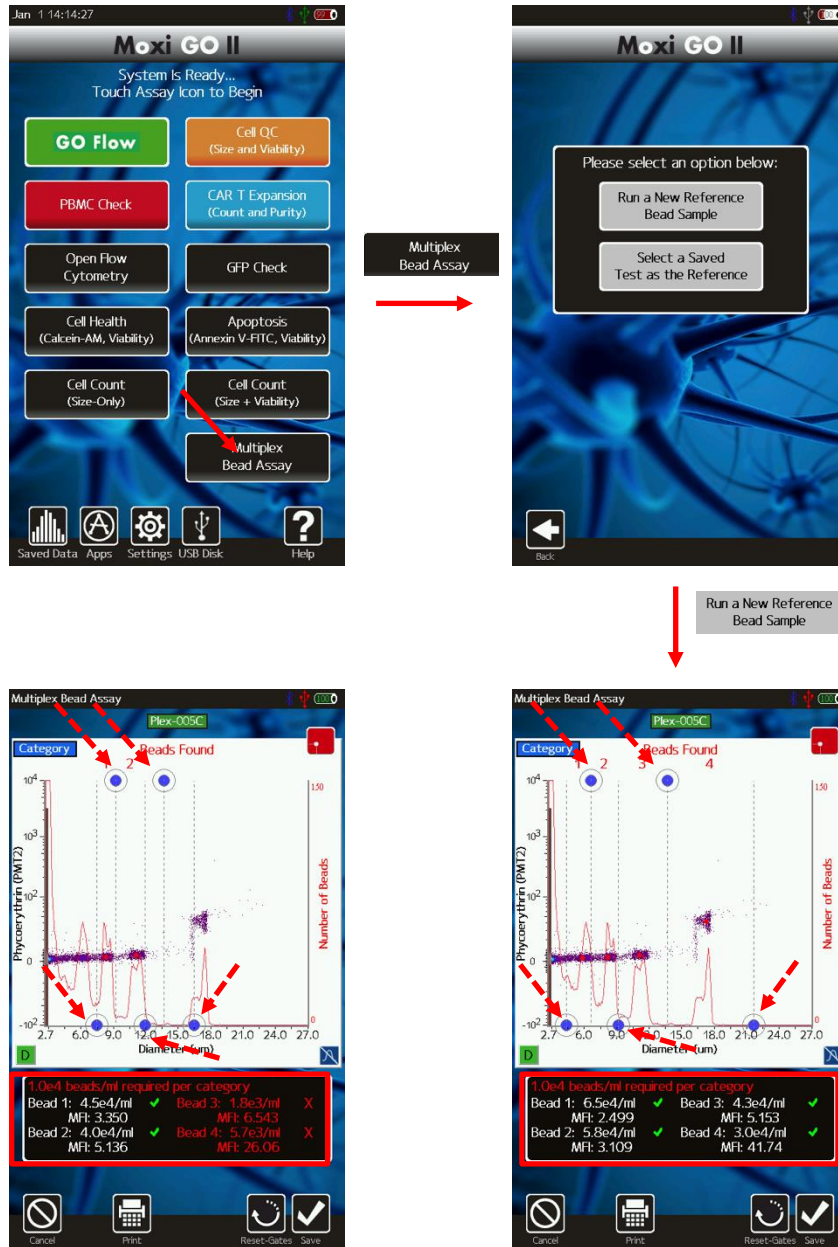
Running a Multiplex Reference Sample


As mentioned above, the first step in running a Multiplex Assay is running a reference sample for all other samples to be compared against. The general workflow for running a reference sample is shown below. After selecting the “Multiplex Bead Assay” test (image below – top/left), the user is presented with the option to “Run a Reference Bead Sample” (Image below – top/right). Touching that icon walks the user through the standard assay (See “Running a Test – General Instructions”). An example output for a Multiplex bead reference test is shown in the image below – bottom/right. The blue size/categorization markers are pre-set based on a system configuration file (in this example, beads are expected at 6µm, 8µm, 11µm and 16µm). If the correct bead sizes are present, the system should auto-identify the clusters in region (refer to image below - bottom/right) by:

1. Putting a red number at the top of the charge under “Beads Found.”
2. Marking the centroid of the bead cluster with a red dot.



- List the bead concentration, MFI level for the bead in that lane/category/size-region, and a green check mark in the black box below the scatter plot




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For a reference bead set, the user has the option of adjusting the size markers so that new lanes are demarcated for identifying the beads. In doing so, the system will automatically update all three of the above indicators. An example for moving the columns in a way that removes beads from falling into the detection lanes is shown in the image above – bottom/left. Note (for that screenshot) that there are only two red-dotted clusters, only two beads numbers at the top of the graph, and two of the (four) expected beads are listed in red with a red x (below) indicating they were not found. The main criteria for the system not finding beads is the concentration (particle count) in the defined size lane/category.

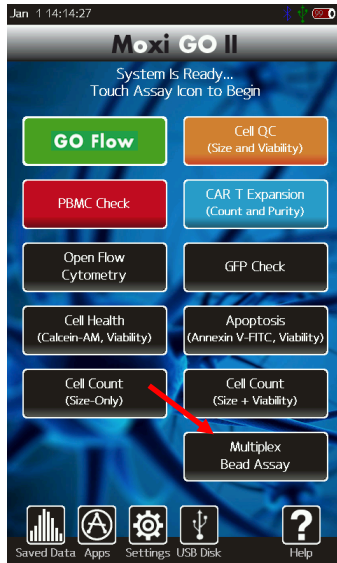
Following the reference bead run and gate adjustment (if necessary), the user can hit “Save.” At that point, the size lanes for that reference test are locked-in. Any test compared to it will use those set the size ranges for determination of beads for those sets (no adjustment is possible on the tests to be compared). Hitting “Save” will prompt the user to remove the old cassette and load a new one. At that point, the test is saved in the same FCS format as all other tests, with the naming syntax “Plex-XXXC.fcs” where XXX is an auto-enumerated value, starting at “001”. The “C” is used to designate the test as a “reference” multiplex test. Once saved, a new test will automatically start (beginning with the laser alignment) with the expectation that an Unknown sample will be loaded to compare to the Reference sample. The process of running unknown sample/tests will repeat until the user cancels out of the cycle, following any given test. For details on the output of unknown samples, please refer to the section below.

Running a Multiplex Unknown Sample

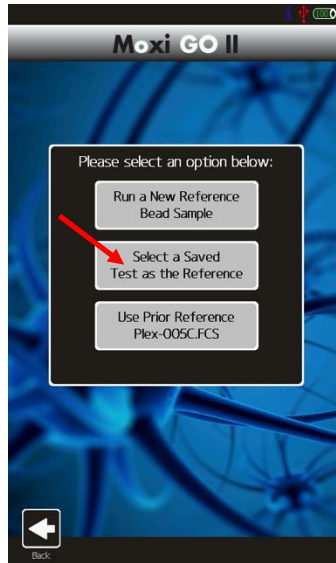
The sequence from running an unknown sample is shown in the image below with the Home Screen as a starting point. *Note: If the user is continuing from a “Run a Reference Sample” section, the sequence will pick up in the second row, first image.* After touching the “Multiplex Bead Assay”, the user has the option to use a “Saved Test as the Reference” or, if a reference had already been run since the last power-on of the system, to “Use the Prior Reference”. Selecting the “Saved Test” will bring up a file listing with only the saved Multiplex Assay References tests showing (see image below - top/right. Touching a saved test (or starting with the “Use Prior” earlier) walks the user through the standard assay (See “Running a Test – General Instructions”) with the user loading the unknown sample. After the test, the output will look like the image below – bottom/middle (as an example output) with a table showing the bead number (column 1), MFI of the reference bead in that size range/column/category (column 2), the MFI of the unknown bead (just ran/measured, column 3), and a ratio of the two (column 4). The system can be set to automatically highlight rows (in yellow) at an certain level of significance (e.g. if the ration is >100, not shown). From that screen, the user has the option of hitting “back” button to see the raw data (see image below – bottom/left). But, as the unknown

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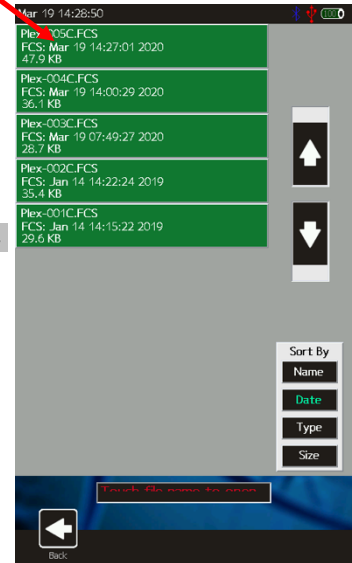
data is being compared to a reference set, the gates cannot be altered. By touching the bead number icon, a histogram overlay is generated showing a histogram of the reporter fluorescence (mV) with both the reference bead output and the unknown bead output displayed (for that size/column/category only). Touching “Save” will save the file in the same FCS format as all other tests, with the naming syntax “Plex-XXX.fcs” where XXX is an auto-enumerated value, starting at “001”. Once the unknown file is saved, the system also automatically generates an “MPX-XXX.CSV” (where XXX is an auto-enumerated number starting at 001) that allows the rapid viewing of the comparison data for those two sets (reference bead and that particular unknown).



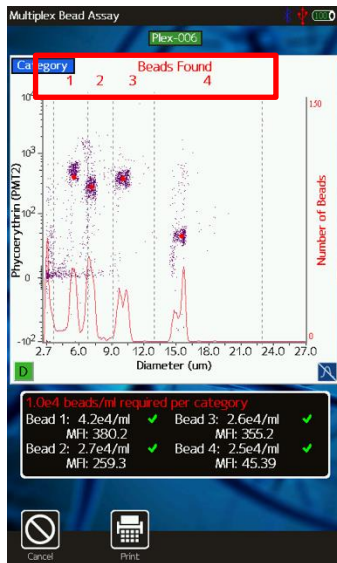
Multiplex Bead Assay



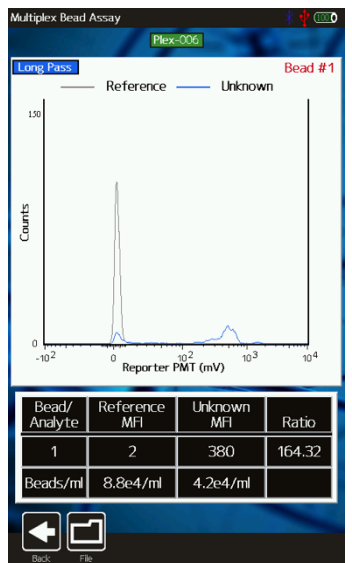
Select a Saved Test as the Reference



Sample Load and Test




Bead	Reference MFI	Unknown MFI	Ratio
1	2	380	164.32
2	3	259	79.77
3	5	355	69.05
4	42	45	1.09



Locating all Unknown tests linked to a "Reference" sample

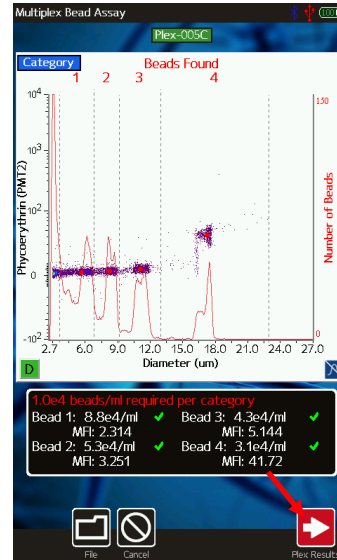
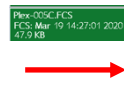
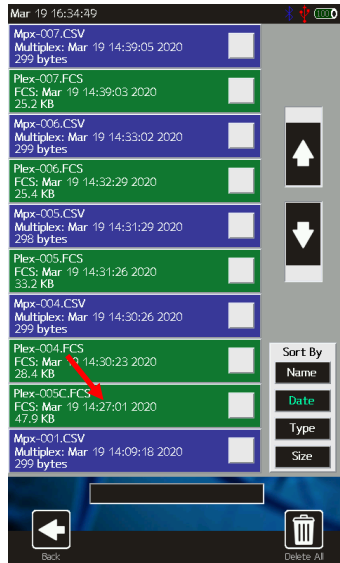
As shown in the sequence below, the full list of unknown multiplex sample/tests that are linked to a particular reference multiplex tests can be accessed from the "Saved Data" area. After

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selecting “Saved Data” from the Home screen, the user can tap one of the “Plex-XXXX.FCS” files to open a particular reference test (Image below – above/left). Opening the file shows the Scatter plot with the gated bead lanes. At that point, touching the “Next” icon (image below – above/right) will bring up a list (image below – bottom/right) of multiplex unknown files that are linked with that reference file. Touching any one of those files will bring up the data table comparison (image below – bottom/left) for those files.



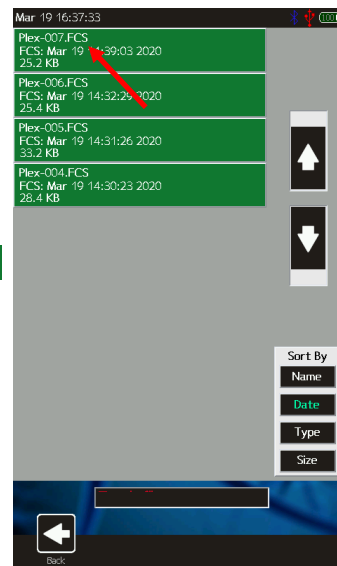
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Mar 19 16:38:22

Bead	(Plex-005C) Reference MFI	(Plex-007) Unknown MFI	Ratio
1	2	384	165.88
2	3	243	74.68
3	5	351	68.29
4	42	44	1.05

Back File Done



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Test Results

Recorded Channels

The Moxi GO II system has the capability of recording the following channels of data for each sample:


- Particle Size
- PMT1 Fluorescence (525/45nm filtered, e.g. FITC, Alexa 488, GFP, Calcein, et.c)
- PMT2 Fluorescence (561nm/LP filtered, e.g. PE, or 646nm/LP filtered, e.g. PI)
- Forward Extinction (“Absorbance”)

Data Display Formats – General Output Types

Certain tests (PBMC Check, CAR T Expansion, and Cell QC) provide simplified data outputs, presenting just the essential test information based on auto-gating algorithms implemented by the system. For more detail on interpreting/reading those test outputs, please refer to the appropriate section for that test in this User Guide. Other tests, will immediately display the raw data for the user view to directly. This data may-or-may-not be auto-gated as well, depending on the system “Auto Gate Setting” setting (see “settings” section). *Note: All test results are ultimately based on the gating of the raw data displays. Even with the simplified tests, the user always has the option to view/review the raw data and adjust the gates if needed.*


The raw data can be displayed in three main formats: 1.) Histograms (one channel display – any channel) – Values for each event are binned across a 1000 point histogram and displayed. 2) Fluorescence vs. Size scatter plots (all fluorescent assays) – For each event, the recorded PMT millivolt level (range 0-10,000mv, corresponding to fluorescence intensity on that channel) is plotted against the recorded cell size (effective diameter in microns). This forms a 2D scatter/dot plot 3.) Fluorescence vs. Fluorescence Scatter plots (two fluorescence channel assays only). On these plots the recorded PMT millivolt level (range 0-10,000mv, corresponding to fluorescence intensity on that channel) on one PMT channel is plotted against the other PMT channel (or Absorbance).

Detailed labeling for each of the three types of display are provided on the three pages/images below. After that, an explanation of how to read the key statistics (e.g. counts, sizes, and percentages) from those data displays is provided.

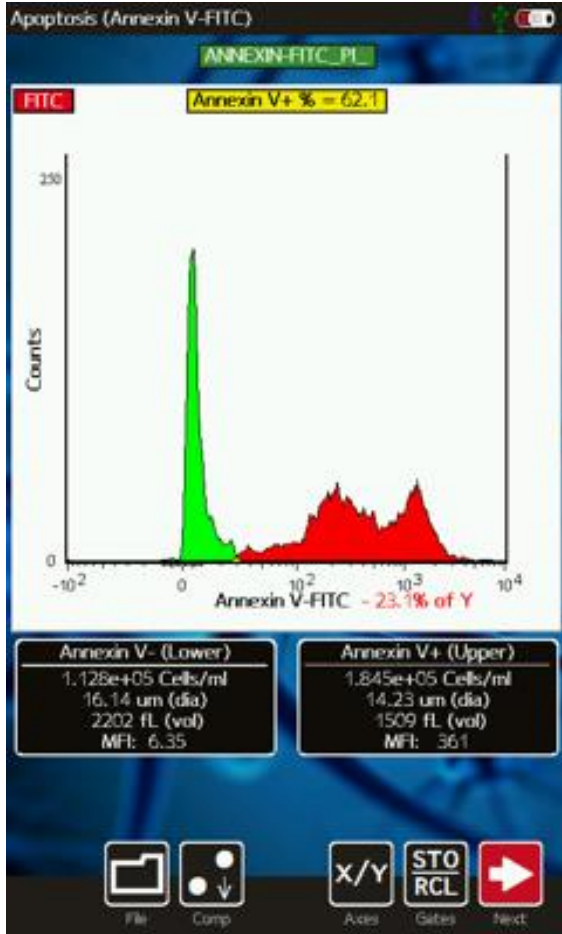
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Fluorescence vs Size Scatter Plot

Test type	Test name (touch to change)	Population % (defined red and blue gates)
Displayed fluorescence channel		Toggle between gates:
Total size-gated counts		(red), and noise (yellow)
Left size marker adjust		Right size marker adjust
Fluorescence scale (mV)		Fluorescence gate – lower(-) populations
Touch/toggle dot to lock angling left size gate		Test scatter/dot plot
Lower gated (below red statistics/results)		Histogram
File options		Upper gated region statistics/results
	PMT spillover settings	Next button to progress through screen workflow
	Select axis/	Store & recall gate (pre-sets)

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Histograms

Test type	Test name (touch to change)	Population % (defined by red and blue gates)
<p>Displayed histogram Channel</p> <p>Histogram bin counts</p> <p>Lower (negative/low Fluorescence) cell pop (green)</p> <p>Lower/Upper overlap Region (yellow)</p> <p>Lower gated (below red line defined in PMT vs size view) population results</p> <p>File options (print, save, delete)</p>	 <p>Annexin V- (Lower) 1.128e+05 Cells/ml 16.14 um (dia) 2202 fL (vol) MFI: 6.35</p> <p>Annexin V+ (Upper) 1.845e+05 Cells/ml 14.23 um (dia) 1509 fL (vol) MFI: 361</p> <p>File Comp Axes Gates Next</p>	<p>Swipe up/down in Histogram area to change y-axis scale</p> <p>Upper gated (above red Line defined in PMT vs Size view) pop results</p> <p>Fluorescence scale (PMT output in mV (with logicle scaling) - red text shows compensation values</p> <p>Upper gated region (Above red line) pop results</p> <p>Next button to progress through screen workflow</p> <p>Store Gate locations in memory (pre-sets)</p>
	<p>PMT compensation Settings</p> <p>Select axis/channel display</p>	

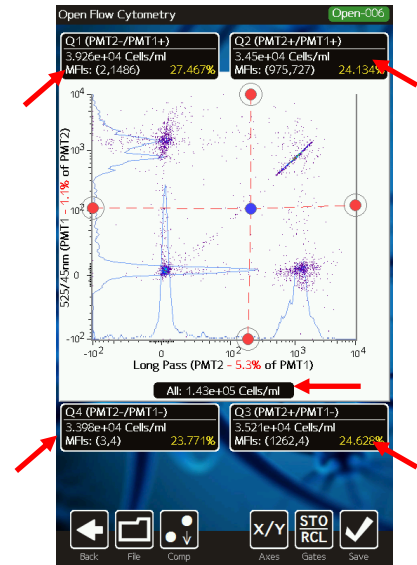
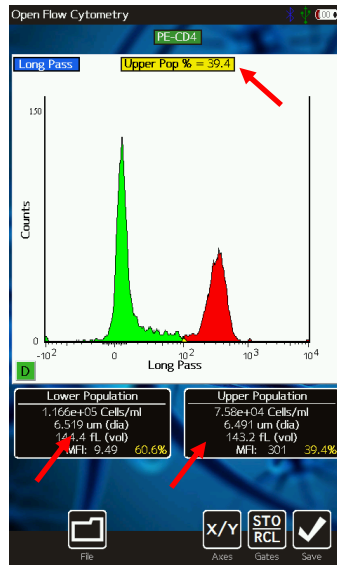
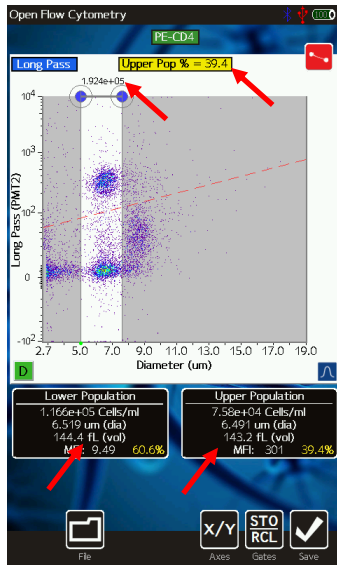
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Fluorescence vs. Fluorescence Scatter Plot

Test Type	Test name (touch to change)
<p>Quadrant 1 (Upper/Left) Concentration, MFIs are (x-axis, y-axis)</p>	<p>Quadrant 2 (Upper/right) Concentration, MFIs</p>
<p>Scatter/dot plots (points displayed set by size region of PMT vs size display)</p>	<p>Touch/drag to move gate</p>
<p>Fluorescence Level Of PMT (mV) for each channel/axis</p>	<p>Tap blue maker and then touch anywhere around Blue marker (Drag to Move center of marker)</p>
<p>Quadrant 4 (Lower/left) Concentration, MFIs are (x-axis, y-axis)</p>	<p>Total cell count</p> <p>Quadrant 3 (Lower/right) Concentration, MFIs</p>
<p>File options (print, save, Delete)</p>	<p>Save test button</p>
<p>Back to last (PMT vs Size view)</p>	<p>Store gate locations in memory</p>
<p>PMT Compensation settings</p>	<p>Summary Table (some tests)</p>
	<p>Select axis/ channel to display</p>



Reading Raw Data Output (Conc., Size, MFI, and %)




The Moxi GO II™ 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. Three example test view screens are shown above (left to right: Fluorescence vs. size scatter (dot) plot, Single Channel Histogram Display, Fluorescence vs. Fluorescence Scatter (Dot) plot).

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 (1.924×10^5 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.*), particle volume (Coulter-Principle measured), 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 (e.g. 39.4% in this example).

Single Channel Histogram Plot

Image above/middle: An example of the on-unit histogram display for the PMT2 (Long Pass channel) Only the points within the size-gated region for the corresponding fluorescence

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channel vs. size plot (e.g. Long Pass (PMT2), image above right) 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 (image above/left) with the red representing the fluorescence+ points (above the red line) and the green representing the fluorescence- (below the red line) points. Any areas of overlap in the histogram (between red and green populations) are shaded yellow. *Note: The overlap is a natural consequence of converting the 2D scatter plot data to a 1D Histogram.* 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.*), particle volume (directly measured by the Coulter Principle approach), and median fluorescence intensity (MFI) values for the negative (green, “Lower Population”) region and the positive (red, “Upper Population”) region. size-only tests, the size-region is determined by positioning of blue gate markers directly on the histogram (as described in the “Size Histogram” Assay section above) and the histogram is only shaded green. For size histograms displayed for fluorescence tests, the size-gated region of the primary (display channel) fluorescence vs. size scatter plot is used to determine which points are included in the histogram binning.


Fluorescence vs. Fluorescence Scatter (Dot) Plot

Image above/right: Only the points within the size-gated region for the **LAST VIEWED** fluorescence (PMT) channel vs. size scatter plot are included in the fluorescence vs. fluorescence display. This is deliberately implemented so that just the cell (debris and noise excluded) population can be analyzed. The scatter is visually divided into four quadrants by the positioning of the gates. For each quadrant, a black box containing the quadrant statistics is placed adjacent to the quadrant. Included in the box are particle concentration (cells/ml), MFI’s – displayed as (x-axis MFI, y-axis MFI), and percentage of total counts (in yellow). The total counts for all four quadrants is provided in the black box at the bottom of the scatter plot.

Simplified Output and Auto-Gating Overview

These three main outputs listed above are the main way raw data is viewed and analyzed. Ultimately, the data needs to be “gated” to properly identify sub-populations by size and fluorescence intensities. The system has a couple of built-in algorithm for “auto-gating” populations. For size-only (no fluorescence) outputs, the system offers a curve-fitting algorithm that automatically identifies a core Gaussian cell population, allowing for automated gating/counts of monodisperse (single cell type) samples.

The system can also auto-gate any PMT vs size display to identify both cell populations (i.e. size gating) and fluorescence positive and negative (e.g. live/dead) populations. For the Cell QC, CAR-T, and PBMC assay, this algorithm is automatically implemented to provide a simplified, quick-read output to end-users. Even with system auto-gating or curve-fitting, users

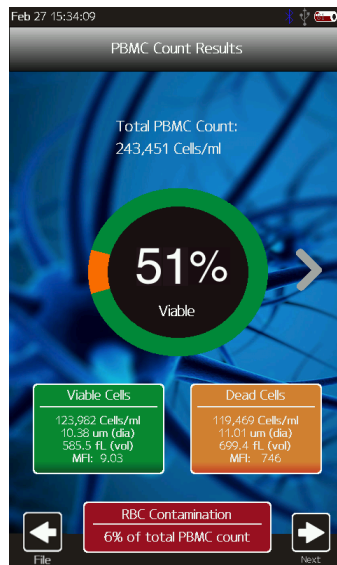
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always have the ability to view the gated histograms and scatter plot to critically analyze the gates and adjust them as needed.


The three core, two-channel cell health assays also have a simplified data output/representation of the PMT vs PMT views that shows a Table and Chart view of the four quadrant data (PMT1+/PMT2-, PMT1+/PMT2+, PMT1-/PMT2+, PMT1-,PMT2-).

All data outputs are discussed in detail below first for test-specific and simplified outputs followed by general gating instructions (for the three main data formats) in the “Manual Gating” section. The general gating instructions are applicable to all assays.

“PBMC Check” Output



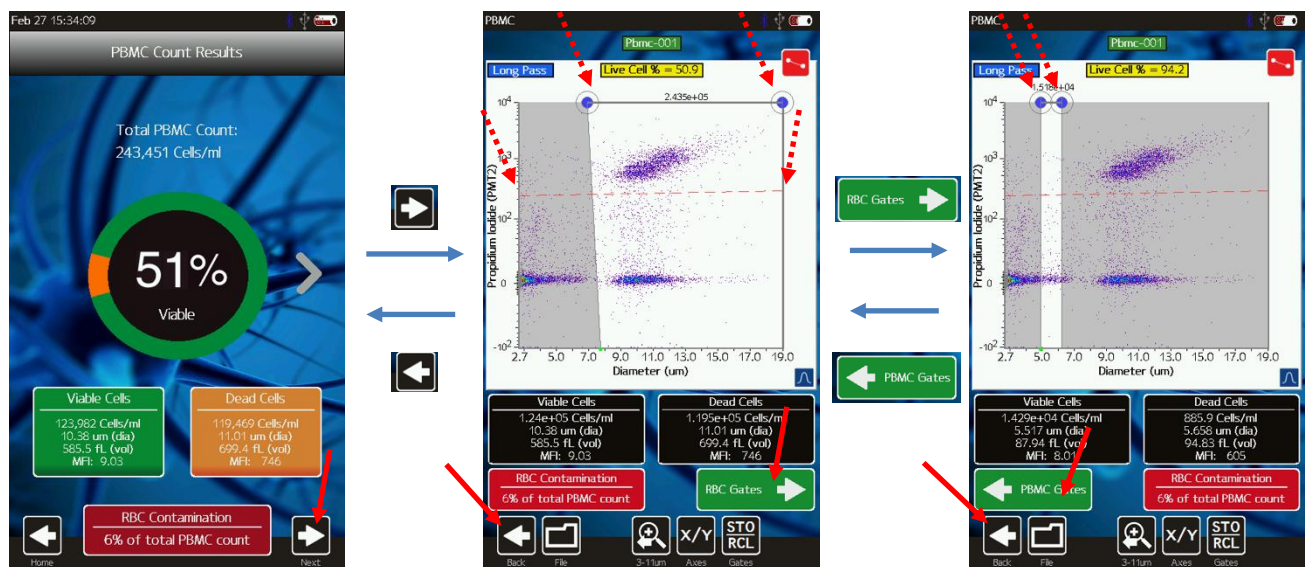
The initial, simplified output of the PMBC test is shown above. This output is based on the system’s auto-gating of the PMT2 (Propidium Iodide, Viability) vs. Size scatter plot (more detail below) to identify the core PBMC population (by size) and determine the viability (by PI fluorescence). The core information is provided at the top with total PBMC count (e.g. 243,451 cells/ml in the image above) and Viability (e.g. 51%, image above). Further detail on the statistics (concentration, cell diameter, cell volume, and median fluorescence intensity) are provided below for both viable (green box, image above) and dead cell (orange box, image above) populations. Finally, the system calculates the degree of red blood cell (RBC) contamination (red box, image above). This value is calculated as the cell counts in the range of 5µm to 6.2µm, corresponding to the region where RBC’s would be expected. The user has

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the option to adjust that gate/region depending on the profile of the RBC contamination is presented as the (total RBC counts / total PBMC counts) * 100%. As the RBC contamination are presented as a ratio/percentage relative to the PBMC counts, the number can exceed 100%.

The data scatter plots/histograms and associated system-set gates can be viewed and adjusted by the user. It is recommended, particularly for the first test in a sequence of experiments, that these gates be checked by the user. The scatter plot is accessed by touching the “Next” icon (image below/right).

The user can save, print a screenshot (bitmap), or delete the test by using the “File button at the bottom left. Touching the button brings up the three options.



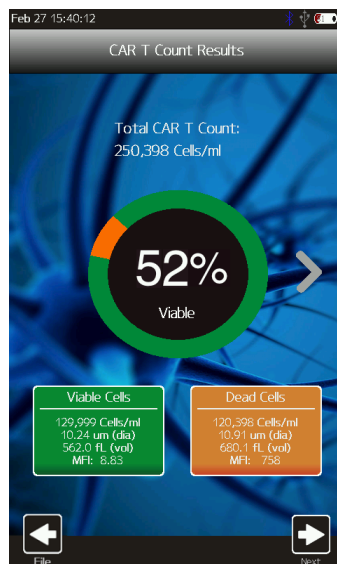
The PMT2 (Propidium Iodide, viability) vs size scatter plot is display (above/middle). The placement of the size gates (blue markers, vertical lines, two top dashed red arrows in image above/middle) and fluorescence gate (horizontal red line, two lower dashed red arrows in image above/middle). The gates, size scale, and axis display can all be adjusted as described in the “Manual Gating – PMT vs Size Gating” section. Briefly, gates are moved by touching and dragging the markers (blue for size gates, red for fluorescence gate, yellow for noise gate). The gate mode (size, fluorescence, noise) can be toggled by touching the red icon at the top/right of the plot. The RBC gates, used for the RBC contamination calculation, can also be accessed by touching the green “RBC Gates” icon (above/middle). The blue size gate positions (dashed red arrows, image above/right) are displayed. The range of the RBC gate can be adjusted by

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touching and dragging the blue icon (more detail in the “Manual Gating – PMT vs Size Gating” section).

“CAR-T Expansion” Output

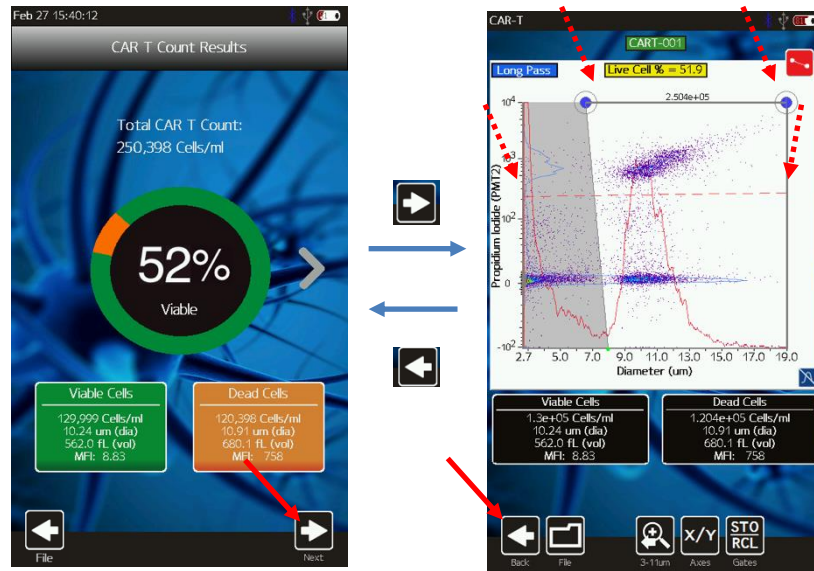
The initial, simplified output of the CAR-T test is shown below. This output is based on the system’s auto-gating of the PMT2 (Propidium Iodide, Viability) vs Size scatter plot (more detail below) to identify the core CAR-T population (by size) and determine the viability (by PI fluorescence). The core information is provided at the top with total PBMC count (e.g. 250,398 cells/ml in the image below) and Viability (e.g. 52%, image below). Further detail on the statistics (concentration, cell diameter, cell volume, and median fluorescence intensity) are provided below for both viable (green box, image above) and dead cell (orange box, image above) populations. The user can save, print a screenshot (bitmap), or delete the test by using the “File button at the bottom left. Touching the button brings up the three options.



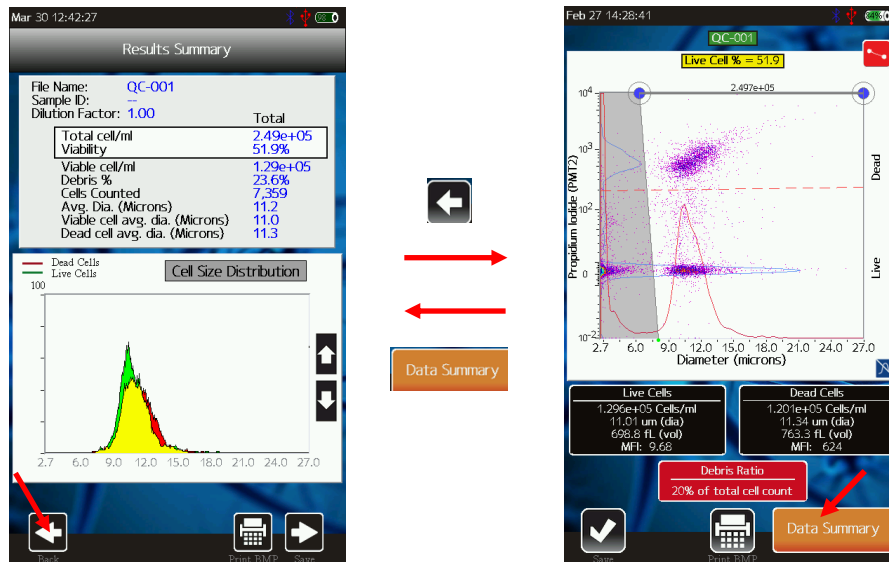
The data scatter plots/histograms and associated system-set gates can be viewed and adjusted by the user. It is recommended, particularly for the first test in a sequence of experiments, that these gates be checked by the user. The scatter plot is accessed by touching the “Next” icon (image below/right). The PMT2 (Propidium Iodide, viability) vs size scatter plot is display (above/middle). The placement of the size gates (blue markers, vertical lines, two top dashed red arrows in image above/middle) and fluorescence gate (horizontal red line, two lower dashed


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red arrows in image above/middle). The gates, size scale, and axis display can all be adjusted as described in the “Manual Gating – PMT vs Size Gating” section. Briefly, gates are moved by touching and dragging the markers (blue for size gates, red for fluorescence gate, yellow for noise gate). The gate mode (size, fluorescence, noise) can be toggled by touching the red icon at the top/right of the plot.



“Cell QC” – Output (both Single Run and Batch)



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An example of the Initial Cell QC “Results Summary” is shown in the image above/left. This initial screen is designed to provide a simple representation of all the cell/sample data based on the system auto-gating of the PMT2 (Propidium Iodide, Viability) vs Size scatter plot (more detail below) to identify the core cell population (by size) and determine the viability (by PI fluorescence). The user is provided the opportunity to review the system gate placement by touching the “Back” button. Touching that button brings up the standard PMT2 (Propidium Iodide) vs. Size scatter plot (example above/right). Gates can be adjusted by touching and dragging the markers (blue/size, red/fluorescence, yellow/noise) as described in the “PMT vs. Size Gating” section of this User Guide. It is recommended that users verify that gate for the initial test/run with any new sample/cell types. Once verified, the user can simply refer to the simplified test output screen. The user can return to the simplified output/summary screen by touching the orange “Data Summary” button under the scatter plot.

The “Results Summary” screen has the test information at the very top. If this test is part of a batch, the “Batch Name” will be added to that summary (as shown above). The Dilution Factor, selected by the user during test configuration, is automatically applied to the concentration data provided below. *Some notes on the data:*

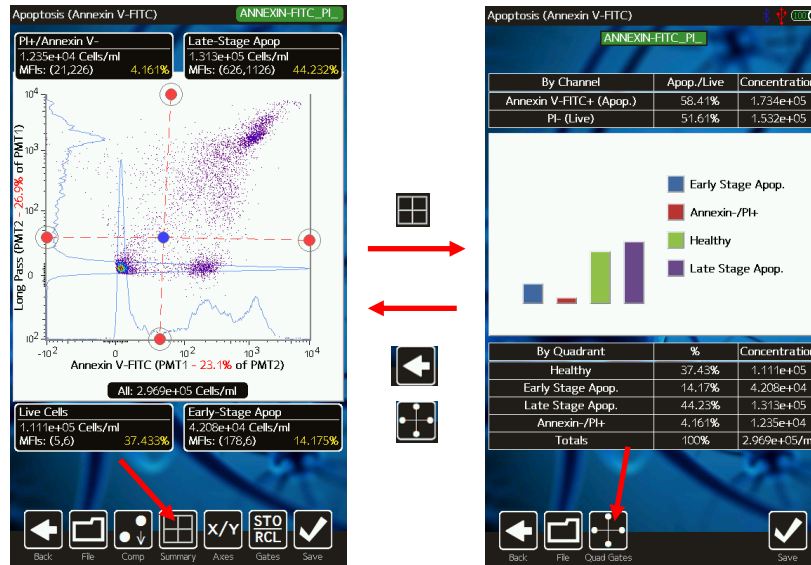
- *The key information, dilution-adjusted concentration and viability, are highlighted in the white box.*
- *The “Debris %” is calculated as the particle counts in the range from 3µm (minimum detectable particle size) to the left size gate (start of the cell region).*
- *The “Cell Counted” is the absolute number of cells in the size-gated region, based on the 22.6µL metered test volume.*
- *The histogram shows the live cell population in green, the dead cell population in red, and the overlapping regions in yellow.*

The up/down arrows on the histogram can be used to resize the histogram y-axis (scaling). The “Print BMP” button will save a Bitmap (image, .bmp) file to the Moxi GO II data disk (in the main “Test-Files” folder for single runs or the batch folder for batch runs). That image will be an exact representation of the “Results Summary” screen.

Touching the “Save” button for a Single Run Cell QC test, will save the data (.FCS format) and will return the system to the Home Screen. For Batch Runs, the system will proceed to walk the user through loading more samples as described below in the “Cell QC – Test Output – Continuing Batch Testing” section.

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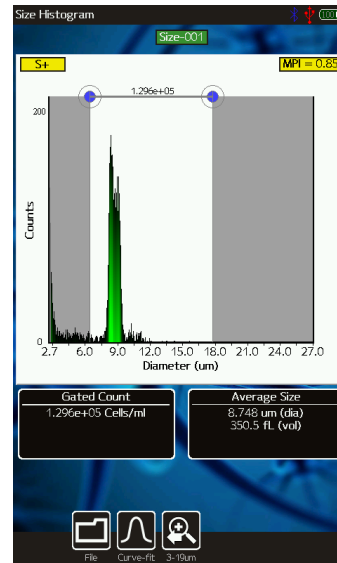
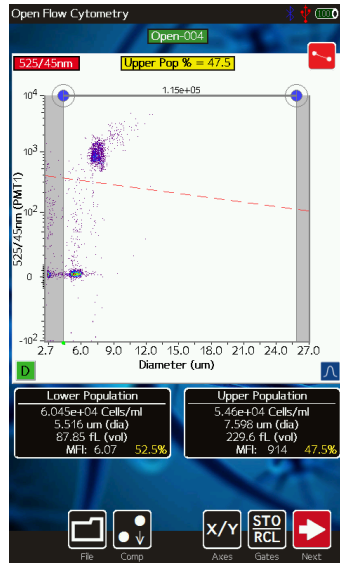
Quadrant – Data Summary Table Output (Apoptosis, GFP, Cell Health)



For the Apoptosis, GFP, and Cell Health tests, the system provides an alternative representation of the quadrant (PMT vs PMT gated) data. Following gating of the PMT vs PMT, quadrant view (example in image above/left for an apoptosis assay), the user has the option of touching the “Summary” button. This button brings up a table and bar chart output (example in image above/right for the same apoptosis assay). That table provides the per-channel fluorescence summary data (above the bar chart) as well as the four quadrant data summaries. The labels are automatically provided, appropriate to the particular assay being run.

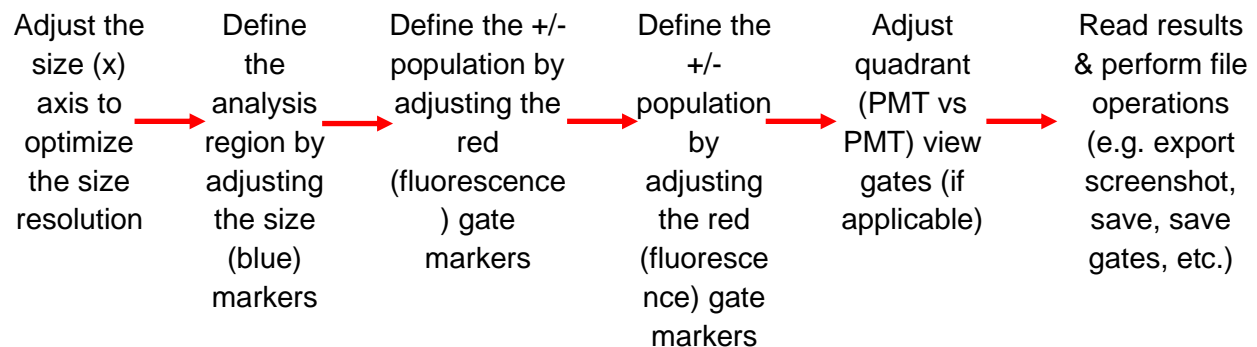
Analyzing/Gating the Data

For tests other than the PBMC, CAR-T, and Cell QC tests, the 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). *Note: Details on the PBMC, CAR-T, and Cell QC tests test outputs can be accessed as described in their respective sections of the User Guide.* Depending on the global setting of the “Auto Gate Setting” value (see “Settings” section for details on changing values), the system may initially auto-gate the data. Whether or not the data is auto-gated, the user will still have the option to adjust/fine-tune the gates manually.




Fluorescence data is presented using Logicle scaling. Logicle scaling is similar to biexponential scaling in flow cytometry. It is implemented to allow for the presentation of negative data (that might result from compensation or baseline averaging) and to better represent the variance of the data by compressing the data region around zero.

(General) Recommended Post-Test Workflow



The general workflow for post-test processing is shown above. 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 analysis region is set by adjusting the blue size gates. Following that, the red fluorescence gate can be adjusted to distinguish the fluorescence positive vs. fluorescence negative populations on the initial PMT vs. size view. If two fluorescence channels are recorded, the quadrant (PMT vs PMT) gating can then be set to identify the four categories of

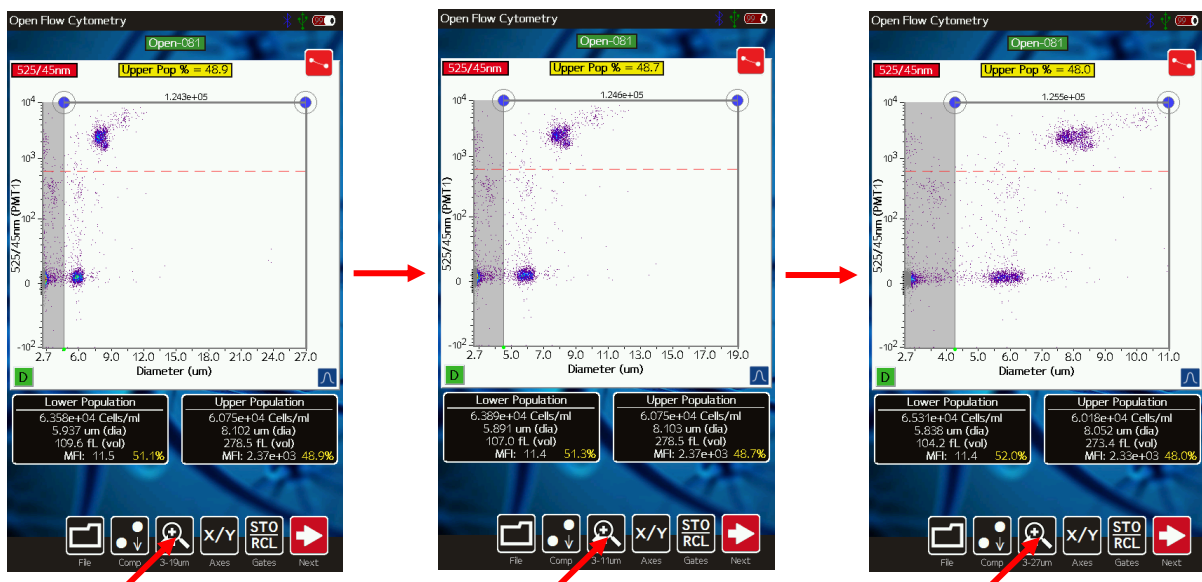
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fluorescence (PMT1+/PMT2-, PMT1+/PMT2+, PMT1-/PMT2+, PMT1-/PMT2-) are properly identified. Finally, the file operations are performed to record/log the data.

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. This is accomplished by selecting the re-scale button below the scatter plot (magnifying glass with arrow, see image sequence for a Moxi GO II™ 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.
- MF-M Cassettes: 3.0 - 35 µm, 3.0 – 27µm, 3.0 - 19 µm and 3.0 - 11 µm.



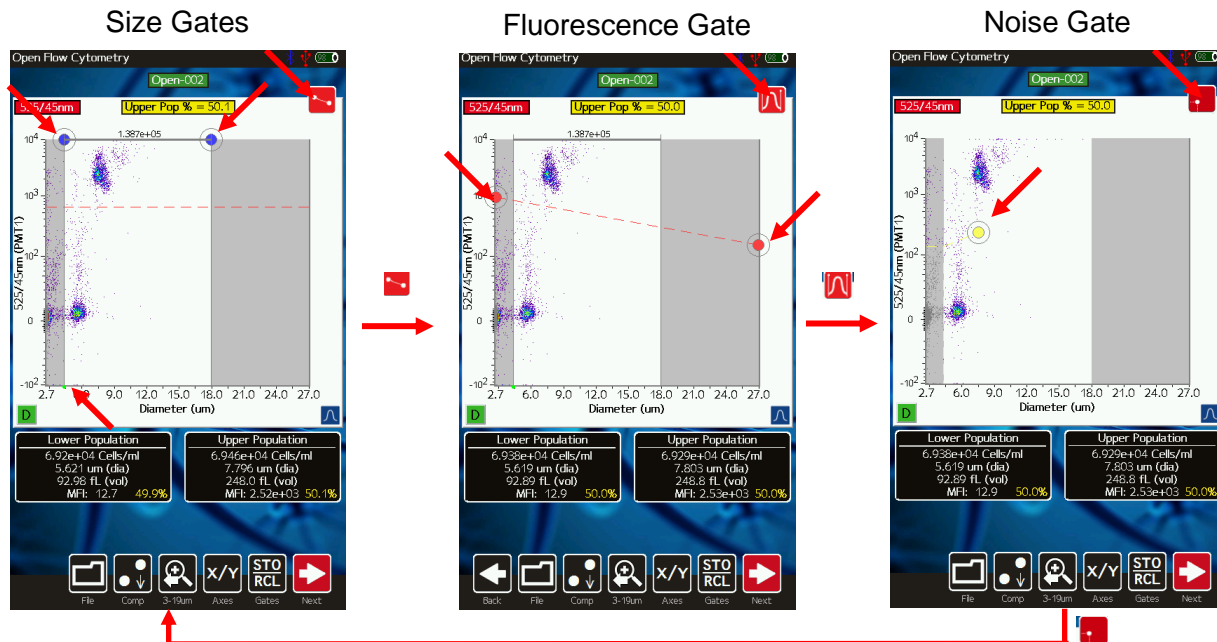
Rescaling the axis results in reprocessing of the raw data to ensure that resolution is not lost during the rescale process (1000 histogram bins). This allows (and is recommended for) users to achieve finer resolution of smaller particle populations and potentially increased separation between particle sub-populations. **However, once a test has been saved, rescaling the x axis is not a possibility.** Note: Any events that are off-scale (i.e. larger than the maximum displayed scale) are not included in the statistics.



Gating the data

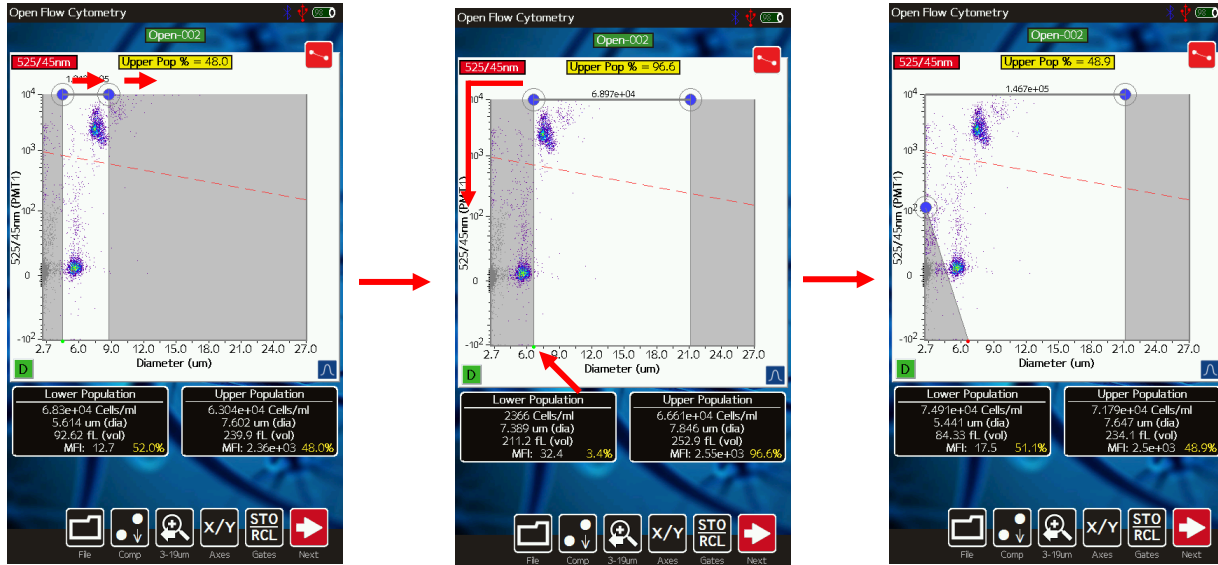
Overview

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 (or more specifically, to demarcate data to be excluded from being 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. **Please note that using “histogram overlays” of the scatter plot can greatly facilitate the placement of the gates (refer to section on “Histogram Overlays on Fluorescence vs. Size Scatter Plots”).**





Size Gating of Scatter (Dot) Plots



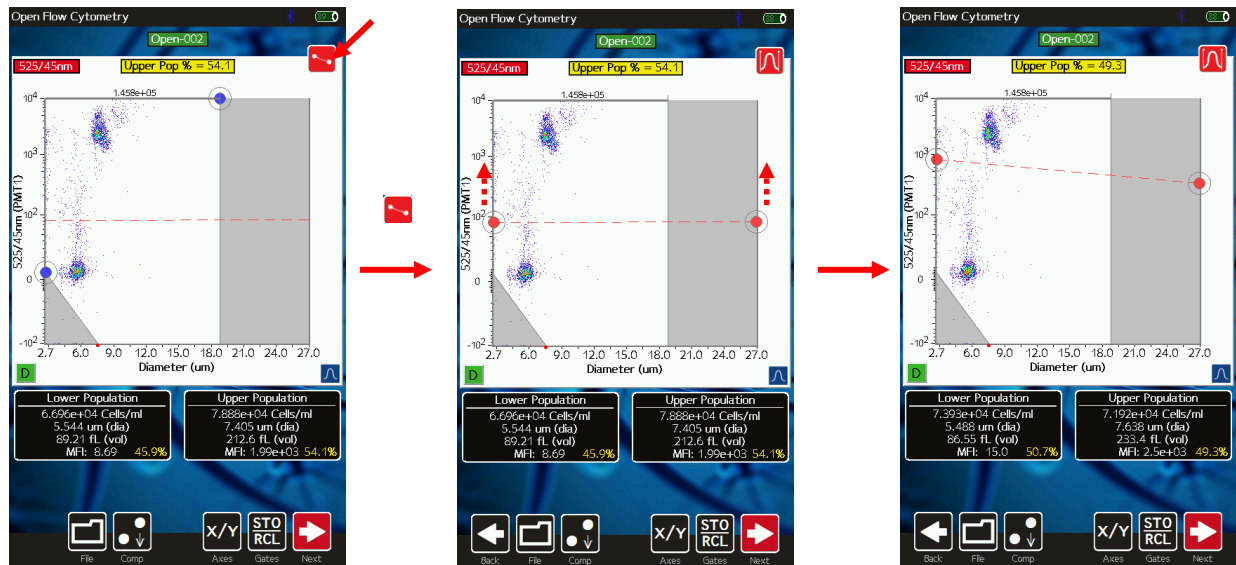
The results of all fluorescent tests (all tests except for the “Size Histogram” test) are initially displayed as scatter plots with the size (blue) gates enabled (see image above, left). Gates can be adjusted by touching and dragging the blue markers around the edges of the scatter plot (above). Touching the marker at the base of the left gate toggles the marker from green (unlocked, images above/left and above/middle) to red (locked, image above/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 (e.g. image above/right). 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.

The general recommended placement is to place the left gate in the gap or “valley” between the noise/debris counts (at the bottom left of the scatter plot) and the cell population. If there his “valley” can be easily visualized by turning on the histogram overlays (blue button at the bottom right of the scatter plot). The right gate is typically placed to the far right of the x-axis scale for monodisperse (single cell type) populations. For poly-disperse populations, the right gate can be placed in the valley between the populations (use histogram overlays to help identify precise location).



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).

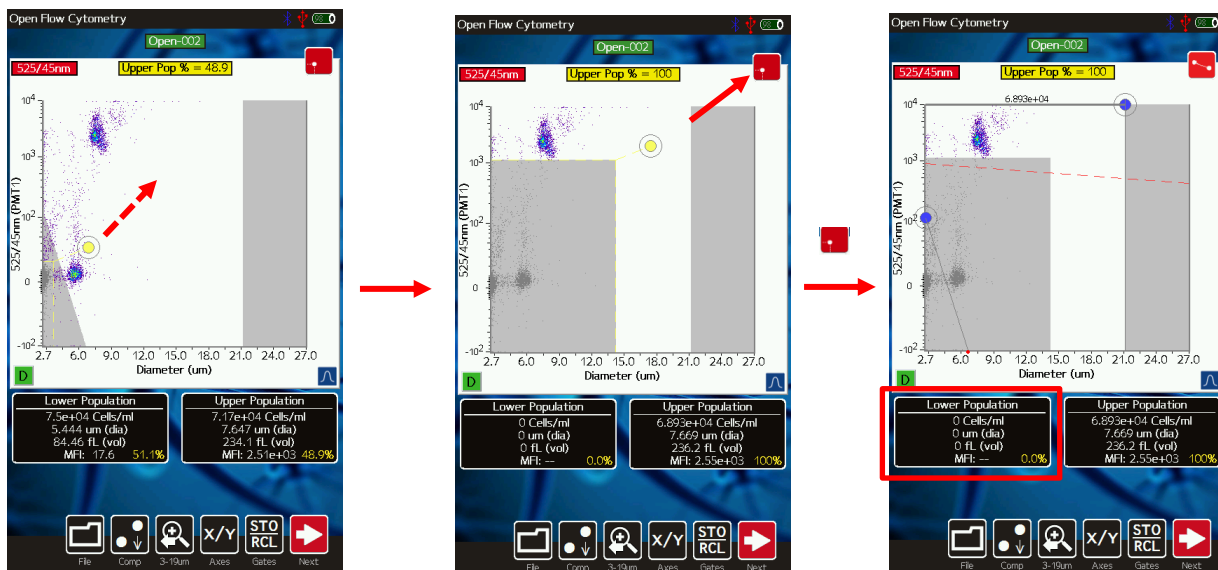


The general recommended placement is to place the fluorescence gate in the midpoint between the dark (non-fluorescent or noise) clusters and the bright (fluorescent) cluster. Often, this placement can be facilitated by turning on the histogram overlays (blue button at the bottom right of the scatter plot) and viewing the peaks. The red fluorescent gate can then be put in the valley of that distribution. If multiple fluorescent peaks are not present, the recommendation is to place the fluorescence gate at the top/tail of the noise cluster. Gate placement can also be facilitated by running control (negative or positive) tests, setting the gates (above or below the control, respectively), storing the gates locations (see section on storing/recalling gates), and then recalling the gates for the standard, labeled sample.



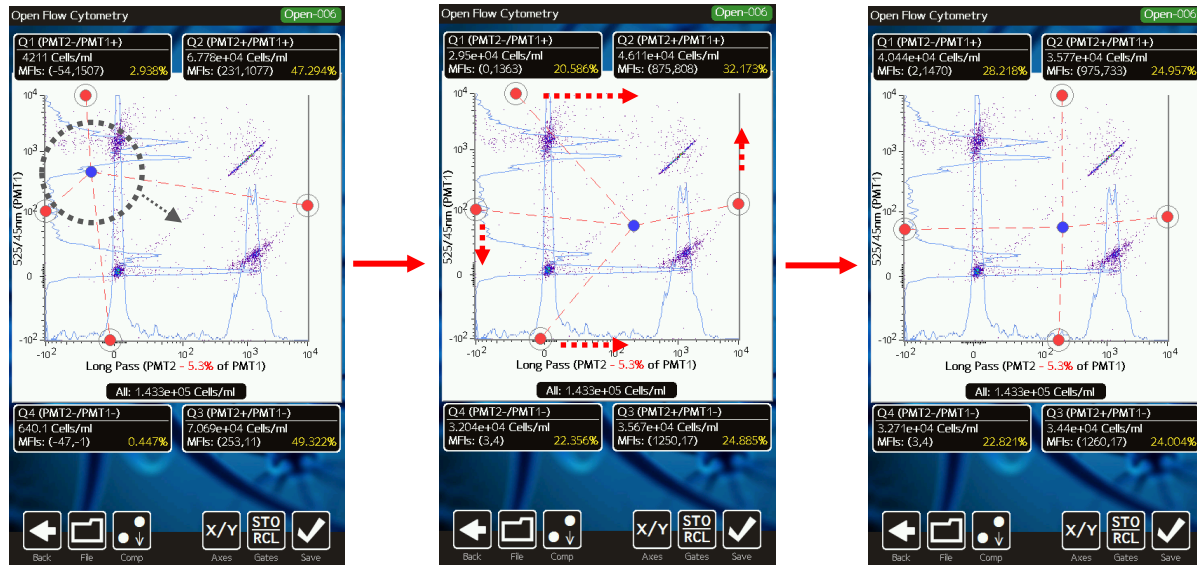
Noise Gating of Scatter (Dot) Plots

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. *Note: The noise gate is only available after a test is run, before it is saved. It is not available for saved data.* 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!!** Additionally, data in the noise region will not be included in the stats (counts, sizes, percentages, etc). For example, referring to the image below/right, note that the lower bead cluster (~6µm), while in the size region and below the red fluorescence gate line, is not included in the Lower Population counts (i.e. those counts are all zero). This is because that cluster was included in the noise region during noise gating (below/middle). The primary purpose of the noise gate was 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. This was more significant with the v2.09 and earlier releases of the firmware that were limited to 16Mb storage (vs. 4Gb+ with v2.10 or later firmware). For almost all tests, it is sufficient to leave this gate, unadjusted, at the system-specified noise exclusion setting.






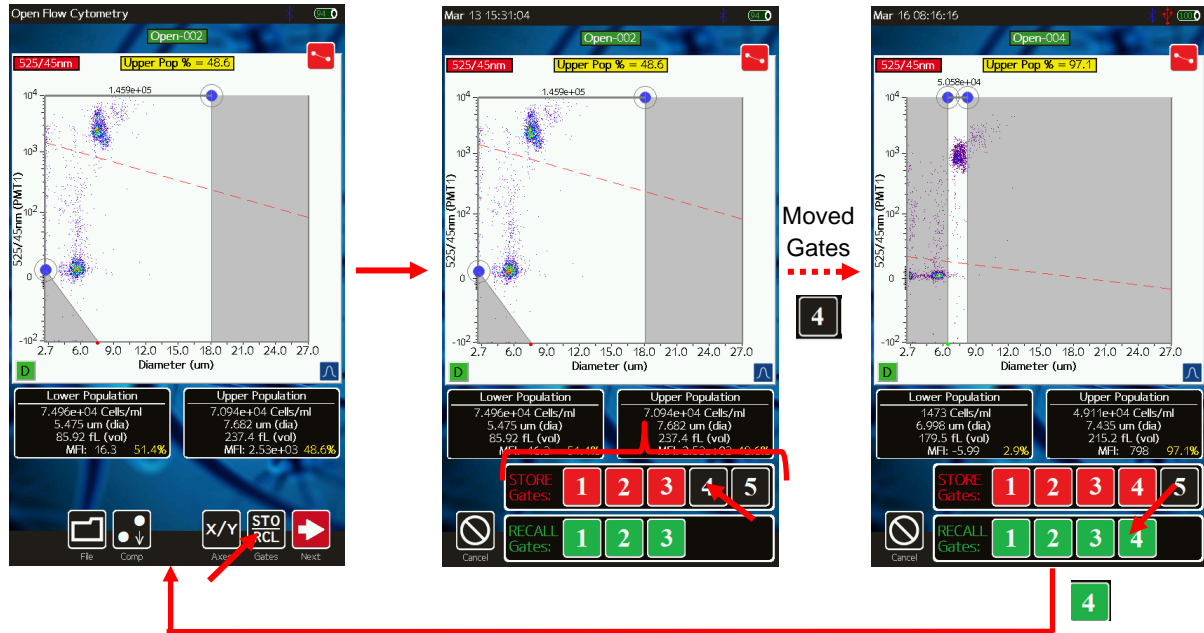
“Web” or “Quadrant” Gating – Fluorescence vs. Fluorescence Scatter Plots



In analyzing tests with two recorded fluorescent channels, it is usually desirable to plot one fluorescent channel vs. the other fluorescent channel to identify the four possible quadrants of data (as defined by the permutations of positive and negative for each channel: PMT1⁺/PMT2⁻, PMT1⁺/PMT2⁺, PMT1⁻/PMT2⁺, PMT1⁻/PMT2⁻). The gating for fluorescence vs. fluorescence (PMT vs PMT) displays is presented with a quadrant or “Web” gating interface (see images above). The first step is to define the center point of the quadrants using the blue dot. This dot can be adjusted by touching (anywhere in the general vicinity, e.g. the region defined by the yellow circle in the image above/left) and dragging the marker to the desired location. Touching in the vicinity of the marker (but not on the marker) prevents the positioning of the marker from being obscured by your finger. Each red/edge marker can then be touched and dragged (image above/middle) to achieve the optimal quadrant gating (image above/right). Summary data/statistics for each quadrant are displayed in the black boxes adjacent to the quadrant. The total count for all four quadrants is listed in the black box below the scatter plot.


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Store and Recall of Gate Settings



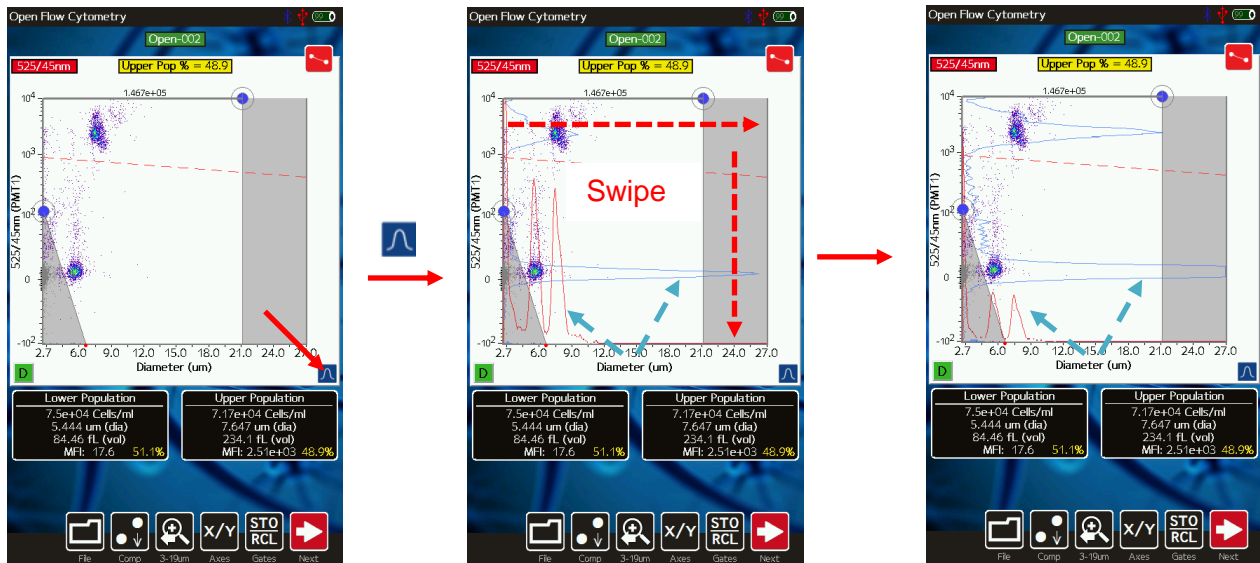
If the system auto-gating is not enabled for a particular test, the Moxi GO II™ gates are then “sticky” by default. “Sticky” means that each new test defaults to the gate settings (size and fluorescence) of the prior test. The instrument also allows the user to store up to five gate locations in memory for recall. A given set of gate settings can be saved by hitting the **STO/RCL** button (arrow in image below/left). Doing so brings up a list of five memory locations (numbered one through five, see image above/middle). 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 above/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 image above. In the above/middle image, the gates are saved into location one. The gates are correspondingly moved and recalled (image above/right). Recalling the gates, returns the positioning to the locations as shown in the image at the above/left. *Note: Gates can only be recalled from the same graphical representation (histogram/dot plot) and the same parameters.*

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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 (blue dashed lines point to the histograms in the images below). The size histogram is displayed as a red histogram with a base on the bottom (size) axis. 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. The image below/right shows the effect of making broad left-to-right swipes as well as top-to-bottom swipes in the scatter plot region of the middle image below. The result is that the fluorescence histogram scale is decreased (increasing histogram height) and the size histogram scale is increased (shrinking the size histogram).



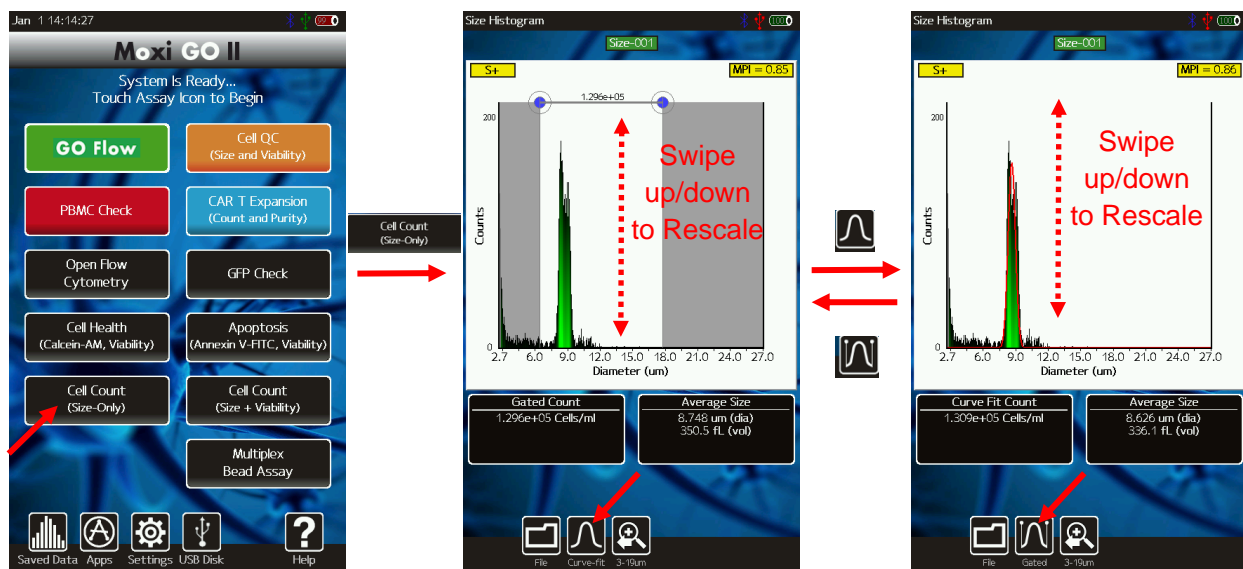
"Size Histogram" Assay

For samples that lack any staining or fluorescent label, quick counts and particle sizing information can be obtained by running a "Size Histogram" 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 "Cell Counts (Size Only)" assay. 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). For monodisperse cell populations, the system can also be set to curve-fit the data automatically. When curve-fitting, the system looks to find the core, singlet Gaussian population. That population is curve-

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fit automatically. The system uses the mean of the Gaussian to determine (and report) the singlet cell size/volume (black box in the 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 2-17 μm or 2-9 μm . 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.

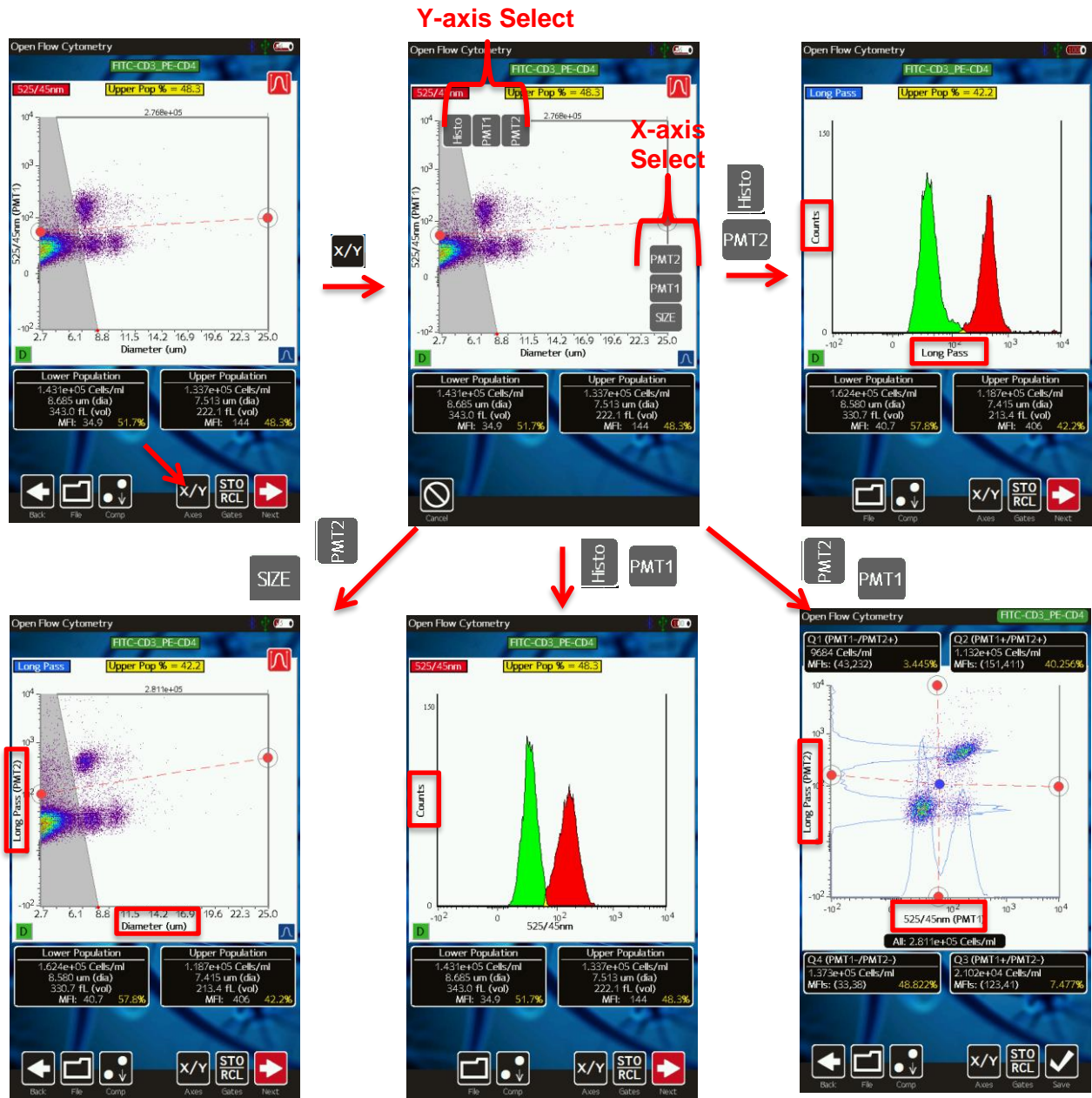



The Counts (Y axis) scale can be adjusted swiping your finger up or down in the white histogram area. For low concentration cell samples, it is important to decrease the counts scale (swipe from the bottom to top) in order to properly view and gate the population.

As the lower display scale (2.7 μm for Type S+ 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, “Web” or Quadrant Views)



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The test output can be modified to display any recorded channel vs another channel or, individually, with a histogram-only view. The 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. Some examples are shown in the above figure (but not all possible permutations) including:

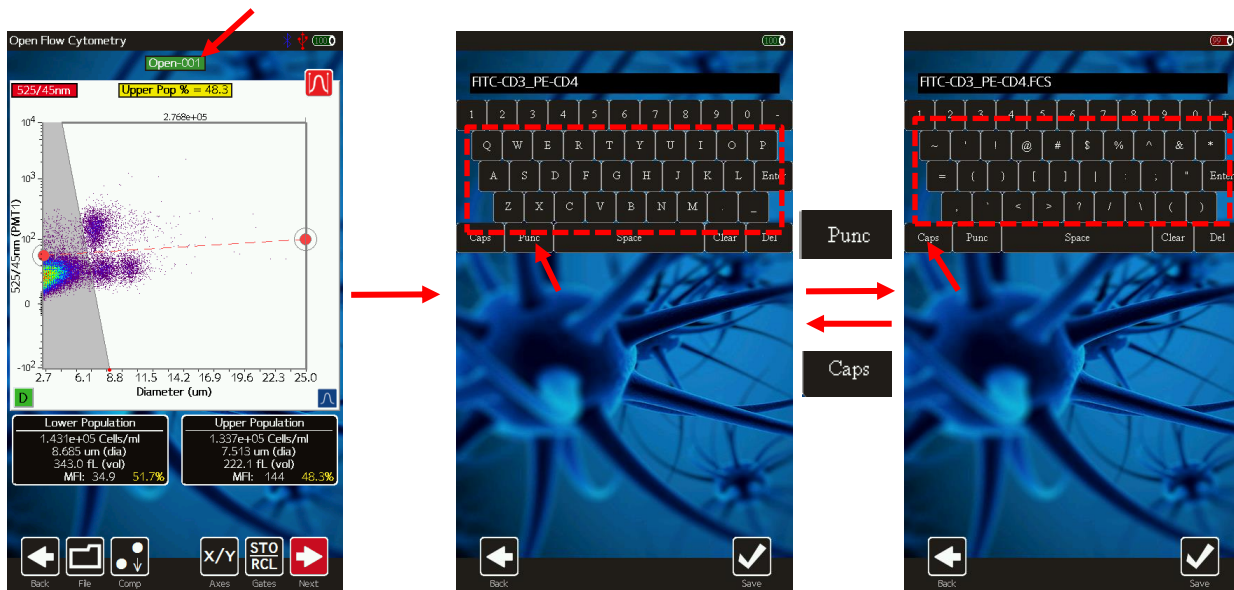
- Long Pass (PMT2) fluorescence histogram (image above, top/right frame). Displayed by selecting “Histo” at the top and “PMT2” at the right. *Notes:*
 - *For fluorescence histograms, the binned data is defined by the size gated region in the corresponding fluorescence vs. size histogram (image above, frame in the top/middle for this example).*
 - *Bins are shaded red or green in the histogram as defined by whether the data falls above or below, respectively, the fluorescent gate defined in the corresponding fluorescence vs. size histogram (image above, frame in the top/middle for this example)*
 - *The fluorescence channel being displayed is identified by the blue box at the top left of the histogram (image above, top/right frame).*
- 561nm/LP fluorescence vs size scatter plot (image above, bottom/left frame). Displayed by selecting “PMT2” at the top and “Size” at the right. Note: The fluorescence channel being displayed is identified by the red box at the top left of the scatter plot.
- 525/45nm fluorescence histogram (image above, bottom/middle frame). Displayed by selecting “Histo” at the top and “PMT1” at the right. *Notes:*
 - *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 bottom/left for this example).*
 - *Bins are shaded red or green in the histogram as defined by whether the data falls above or below, respectively, the fluorescent gate defined in the corresponding fluorescence vs. size histogram (image above, frame in the bottom/left for this example).*
 - *The fluorescence channel being displayed is identified by the blue box at the top left of the histogram (image above, bottom middle frame).*
- 561nm/LP fluorescence vs 525/45 nm fluorescence (image above, bottom/right frame). Displayed by selecting “PMT2” at the top and “PMT1” at the right. Note: By selecting a PMT vs PMT (fluorescence vs fluorescence) display, the system automatically implements the “web” or quadrant gating view.

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File/Test Operations

Test Naming and Renaming Tests


By default, tests are named with an automated prefix based on the test type (“GFP” for GFP Check, “Via” for Cell Counts (Size + Viability), “Open” for Open Flow Cytometry and GO Flow, “PBMC” for PBMC Check, “CAR” for CAR-T Expansion, “QC” for Cell QC, “Apop” for Apoptosis, “Hlth” for Cell Health, “Plex” for multiplex tests, 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 II™, 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



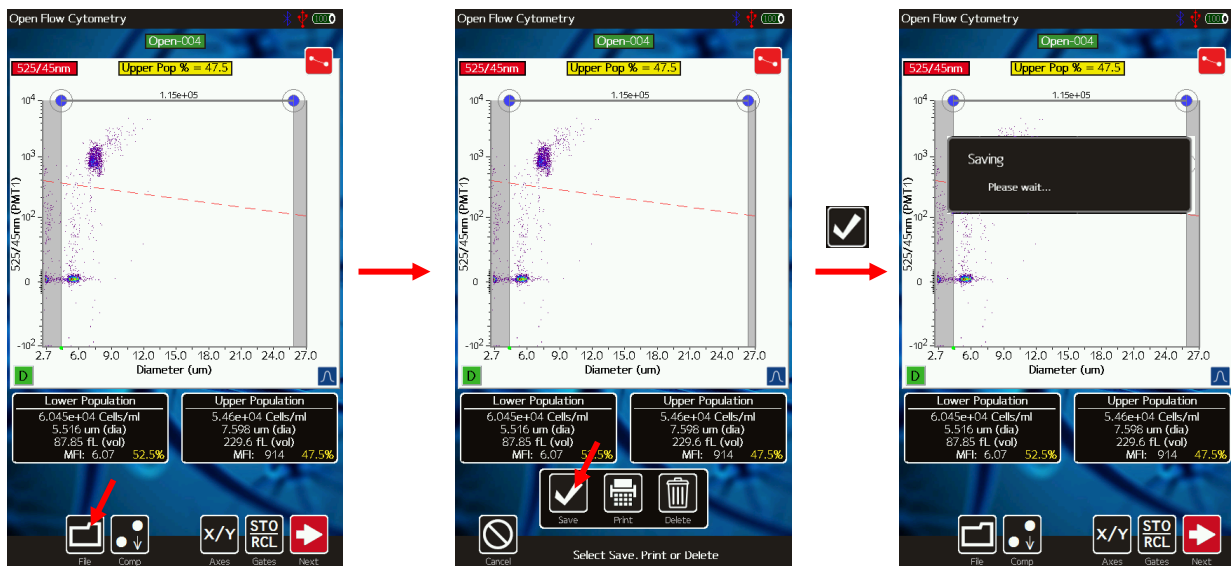
can be deleted by hitting the **Clear** key or successive “Backspace” keys. Capital letters can be achieved by hitting the **Caps** button. Symbols and special characters can be accessed by hitting the **Punc** button. Select **Save** to assign the newly entered name to the test.

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

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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 | Print | Export Screenshot** (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 Open-011.bmp). *Note: Subsequent screenshot exports for the same test will overwrite any existing screenshot associated with that test. The exception to that is the Cell QC assay. When saving the raw data, scatter plot view for those tests, a “_scatter.bmp” extension is used so that it does not overwrite any screenshot of the simplified view.* 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.

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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 II™. File types are color coded (image below) as follows:

- Test (FCS) files are displayed as green entries.
- QC Batch Directories (and user created directories) are shown as orange entries
- Exported screenshots are displayed as dark gray rows
- Operating system files are displayed as black rows.
- Special .csv “linkage” files (files that correlate two tests for quick reference/loading, e.g. for Overlays or Multiplex comparisons) are shown in blue
- Moxi GO II 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. 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 image above (frames from top/middle to bottom/left on touching the “QC-001” green row).

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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 to the right of the file list.**
- **Page up/down (ten files at a time) the list by sliding the up/down arrows 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 frame).


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/right frame). Touch the desired button to complete the operation. When deleting files, the file status/operation is shown in the upper black box (system will scroll through files being deleted). The touchscreen will be unresponsive until that operation is completed.

Compare/Overlay Saved Tests

The Moxi GO II 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 the identical number of recorded channels (525nm/45nm, 561nm/LP, Absorbance)
- The x-axis size scale (rescale level) needs to be identical

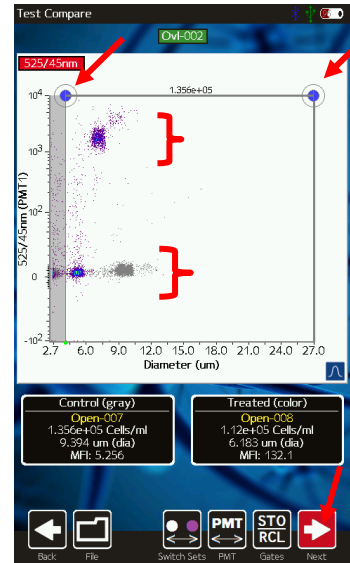
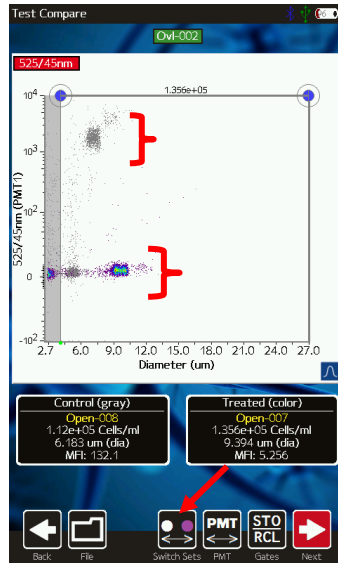
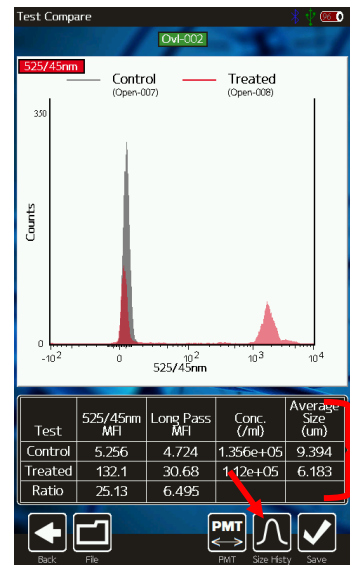
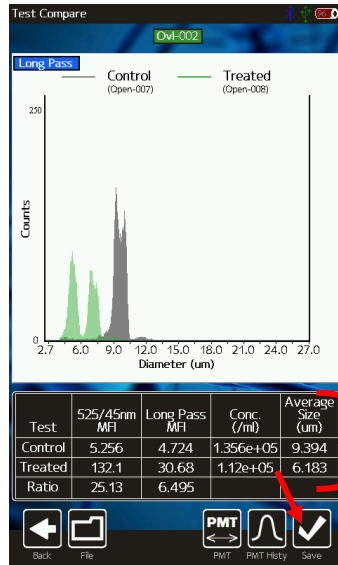
On selection the **Compare/Overlay Tests** icon, the system presents a scatter plot view of the two overlaid tests (image below top/middle) with the older test presented as the “Control” population (gray dots) and the newer test presented as the “Treated” population (density colored dots). To switch the designations, touch the **Switch Sets** icon as shown in the images (Image below - top/middle to Top/Right). To change the PMT2 display (y-axis), the user can simply touch the **PMT** icon. Adjustment of the size gate markers (left and right blue dots) 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 histogram overlay (image below bottom/right) of the two data sets for the displayed PMT channel (525/45nm


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PMT1 in this case). The fluorescence histogram for the designated “control” data is shown in gray and the fluorescence histogram for the designated “treated” data is shown in red. To view the corresponding overlay for the other PMT (if the channel was recorded), simply select the **PMT** icon. It is also possible to view a size histogram overlay 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).



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Compensation (of Spillover)

Overview/Background


There is an expected spectral overlap when using PMT filter detection in 525/45nm and 561nm/LP for particular fluorophores. Depending on the fluorophores used in a test, and their relative intensities, the fluorescence signal of a given fluorophore might “spillover” from its primary fluorescent channel/PMT into the secondary fluorescent channel/PMT. In some cases, the spillover can interfere with the interpretation of the data. The result of this expected “spillover” is the data of a single color control runs in a diagonal at increasing MFIs in another PMT channel (See Below). In those cases, it may be necessary to “compensate” the data to remove that spillover effect. Compensation is performed, as is traditional, by subtracting a percentage of the primary flow signal magnitude for a given event from the corresponding value on the secondary channel. Due to the nature of the filter selection and fluorophore emission properties, in almost all instances on the Moxi GO II, the spillover will be from the Green (525/45nm) channel into the Red (561nm/LP) channel. The 646nm/LP filter selection for the red (PMT2) channel would be expected to minimize this spillover as its edge is set past the emission tail of most green dyes. However, it is still in a range that can pick up propidium iodide (PI, viability dye) emission. In this regard, it is ideal for use when using a PI counterstain. However, the 646nm/LP filter is pushed too far out for Phycoerythrin (PE) detection. So, when using PE in dual-marker immunolabeling, the 561nm/LP filter must be used.

Moxi GO II™ Compensation

General Workflow

To compensate spillover for any experiment, it is first necessary to create single-stain controls for any fluorophore that needs spillover compensation. **Compensation needs to be performed using a SINGLE-STAIN positive control. It is important not to use a dual-stained sample as we need certainty that any signal on the spillover channel is a result of spillover, not a fluorophore that is intended for that channel.** As mentioned above, by nature of the emission profiles of fluorophores, almost all spillover on the Moxi GO II is from the Green Channel (525/45nm) into the orange/red (561nm/LP) channel. However, there may be cases when an Orange/Red dye backspills. In those cases, a single stain control would need to be prepared for each dye. The general workflow would then be:

1. Run a negative control (unlabeled) sample (if you expect the single-stain control sample will be 100% positive/fluorescent). These samples will follow the “Load Control Set” compensation approach described in the section below.


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2. Run each single stain control on the Moxi GO II using the same assay that you intend to use for the overall experiment.
3. PRIOR TO SAVING the single-stain control, adjust the compensation using the approaches described below.
4. Save the data.
5. When running future tests, verify the compensation setting is toggled to “Use Prior Value” (other option is “None”). The system should automatically set this for you to ensure that the established compensation value is applied to all future tests in the experiment (until system power-off). *Note: If you power off the system, you will likely need to reset the compensation value. In those cases, because the compensation values have already been established, there is no need to run the single stain controls. You can just run the next sample, touch compensation, and manually adjust the values before saving (see below for more detailed instructions on adjusting the compensation level)*

Standard – Single Test - Compensation

On the Moxi GO II, data can be compensated by selecting the **Comp** icon at the bottom of a new or saved test (image below – top/left). *Note: As would be expected, the compensation icon is only available for tests in which multiple fluorescent channels have been recorded.* Selecting the compensation icon will load the quadrant-format scatter plot display of fluorescence vs. fluorescence (image below top/middle and image below top/right). *Notes:*

- *The only points displayed on this view are the points that were size-gated (between blue markers) on the PMT vs size graph (image below – top/left).*
- *In the vast majority of cases, spillover will occur from the green dye (PMT1) to the orange/red channel (PMT2).*
- *There is rarely to compensate for “backspill” from an orange/red dye (PMT2) into the Green (PMT1) channel.*
- *It may be necessary to select the Switch Axis button depending on the channel to be compensated. The spillover channel (almost always PMT2) should always be set to the y-axis. In the example below, PMT1 is originally on the y-axis (image below – top/middle). To the switch axis button is selected to change/swap the axis (image below - top/right) so that the FITC fluorophore (525/45nm PMT1, switched to x-axis) spillover into the 561nm/LP channel (switched to y-axis) can be performed (image below – top/right).*


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- *Initially, instructional text is displayed in red (over the scatter plot) and in yellow (on the scatter plot). Once the gates are moved or compensation is adjusted, that text will disappear until the user touches Clear (to remove changes) or Switch Axis.*

When spillover occurs, there is a characteristic narrow slop to the data points on the graph (images below – top/right and bottom/left). This occurs because of the direct correlation of a fluorescent event on the green channel to a recorded spillover event on the red channel (i.e. a



percentage of every green event is registered on the red channel). To proceed with compensation, use the red and blue gate markers to position the control population/cluster into the bottom-left corner of the quadrant plot and the spillover population/cluster into the bottom right corner of the quadrant plot (as shown in the image below – bottom/left). Note: If there is no dark/control cluster on the displayed test, it is possible to use a negative control test as the control. Please see the “Compensation with Negative Control - “Load Control Set” section

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below) Once the Control and Spillover populations have been properly gated into the desired quadrants, the user can begin compensation. The yellow “ Δ MFI” readout provides the relative difference in the y-axis median fluorescence intensity (MFI) levels for the two clusters (i.e. - 82.95mv in this case, image below – bottom/left). To compensate the data, it is necessary to reduce this number (to as close to zero as possible) to eliminate the effects of the spillover. Touching “Auto Adjust” will have the system automatically calculate and apply a compensation percentage (image below – bottom/right red arrow and box).

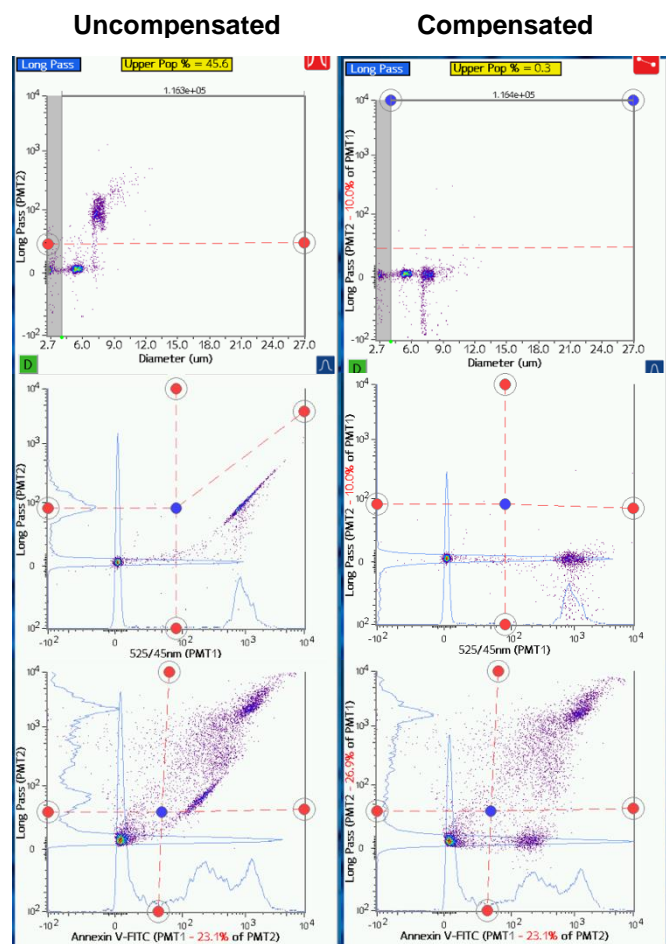
The compensation value can be fine-tuned (or manually adjusted) by touching the arrows (red bracket in image above – below/right) as follows:


- Double arrows – change the compensation percentage up/down by 1%
- Single Arrows – change the compensation up/down by 0.1%



Note: The system waits for a break in the tapping of the up/down arrows before applying the new value (refreshing) the scatter plot data on the screen. This allows the user to tap the arrows multiple times to quickly adjust the value without having to wait for a refresh with each press. The amount of compensation (as a percentage of the corresponding signal on the primary PMT, e.g. 525/45nm in this case, image above - below/right) is displayed in the white box (e.g. 10.0% in this example). The scatter plot is also updated to show the compensation effect with the value shown in red text after the axis label (see red box in image above – bottom/right). Also note the relative shift in the spillover cluster position (as well as the yellow box Δ MFI value) in the images above for uncompensated (image above - below/left, 0% compensation) to the fully (561nm/LP channel) compensated data (image above - below/right, 10.0% compensation). The characteristic spillover slant has been eliminated and the height of the spillover cluster (on the y-axis) is visually the same as the control cluster. This can also be confirmed by the updated Δ MFI value of (-0.23mV, essentially no difference in PMT2 fluorescence).

The image to the right shows compensation examples for data. The left column represents the initial, uncompensated data. The right column represents the result of compensation. The first and second rows show the bead compensation example first on the PMT2 (spillover channel) vs size view (row 1) and then on the PMT2 (spillover channel) vs PMT1 quadrant view. In both cases, you can see from the right column that the signal on the spillover channel (PMT2) is reduced to being equivalent to the dark bead cluster (it's at the same height on the PMT2 scale as the dark cluster). The third row shows an example of compensation for a dual-label (FITC-Annexin V and Propidium Iodide) Jurkat Apoptosis test. Note that the applied compensation only affects the cluster in the lower/right, which corresponds the FITC-



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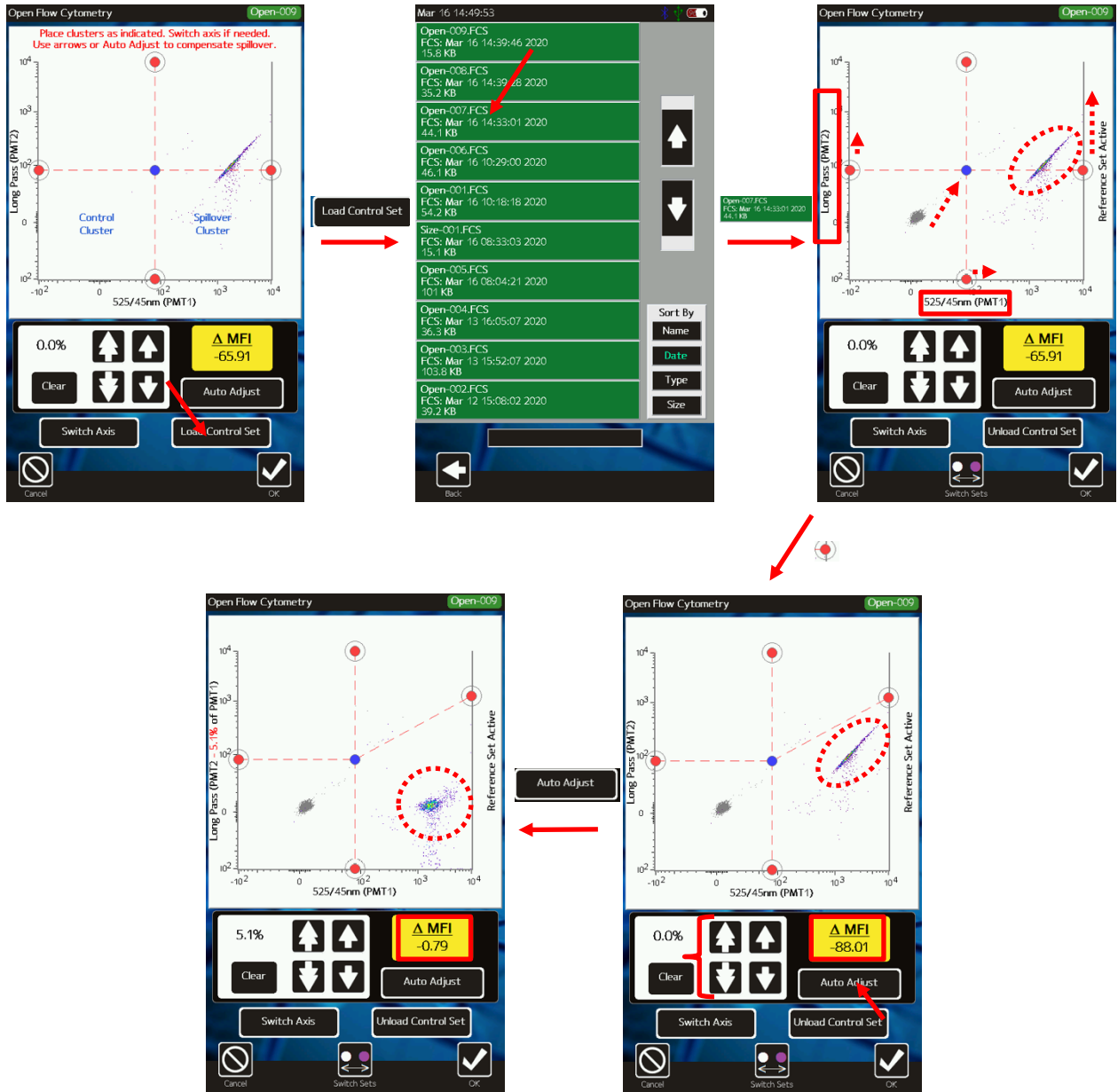
AnnexinV+/PI- population. For Annexin/PI assays, by nature, whenever a cell is PI+ (membrane is permeable), it is also Annexin V+. So this population does exhibit a slight upward slant due to the correlation of Annexin and PI staining (and due to DNA content differences as well). However, it does not exhibit the same “artificial” narrowness of the spillover populations.

Compensation with Negative Control (Load Control Set)

For many single-stain positive controls, there is no fluorescence-negative (dark) population to use to level the spillover cluster with compensation. In those cases, the user needs to first run a negative control test. That test can subsequently be used to compensate the single stain positive control sample by using the on-board “Load Control Set” compensation functionality. The workflow for that functionality is shown below. Looking at the image below, the screenshot at the top/left picks up from the point when the single-stain control test has completed acquiring data, and the user touches the “Comp” button to begin the compensation process. In this case, you can see that there is no “dark” population (i.e. all the beads are fluorescent). And, you can see the characteristic tight-slant of that population, an indication of the spillover effect. In this case, the user can touch the “Load Control Set” button to select a previously-run negative control data set to use. The system will display the full “Saved Data” list (image below – top/middle) for the user to touch/select that set. Once selected, the system will load that data and superimpose it (in gray) on the single-stain positive control scatter plot (see image below – top/right). As before (see instructions above), the spillover channel (in most cases PMT2) should be displayed on the y-axis. If it is not, the user can touch “Switch Axis”. At this point, the compensation can proceed as described above. The user needs to adjust the gates by touch/dragging the red and blue dots to get the spillover cluster in the bottom/right quadrant and the control cluster in the bottom/left quadrant (as shown in the image below – bottom/right screenshot). The yellow box at this point shows the MFI (mv) difference between the single-stain positive control (points in the bottom/right quadrant) and the control cluster (points in the bottom/left cluster). At this point, the user can touch “Auto Adjust” to have the system automatically calculate an applied compensation level. The user can also use the up/down arrows to manually adjust or fine-tune the system calculated compensation level. *Note: The single arrows adjust the compensation level by 0.1% and the double-arrows by 1.0%. Rapidly tapping the arrows will register multiple increment changes PRIOR to refreshing the screen.* When complete, the spillover cluster should be positioned at the same height (on the y-axis) as the control cluster and the delta MFI should be close to zero (see image below – bottom/left, try to minimize value to an absolute value <5mV).



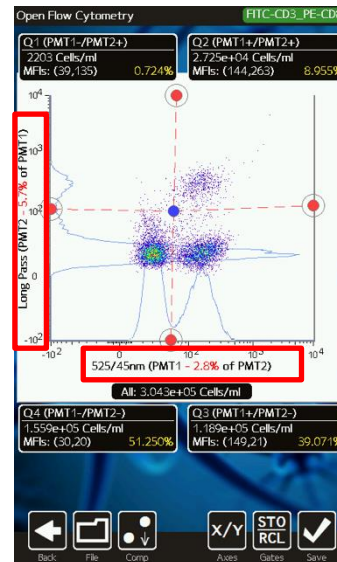
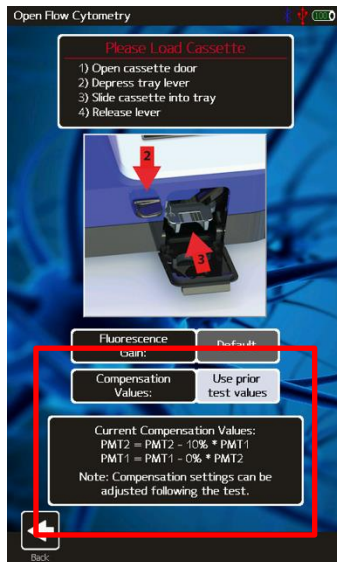
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Compensation – Future Tests

For compensated data sets, the level of compensation is always (all views, including histograms) displayed on the fluorescent axis label in red text. An example is shown in the image below/right for a fluorescence vs. fluorescence scatter plot view. The last, saved compensation levels for any particular assay type (e.g. Open Flow Cytometry/GO Flow, Cell Health, GFP Check, etc.) are also stored by the system. The user can then automatically apply these values (without have to manually re-adjust the compensation) to future tests by toggling the “Compensation Values” option on the test configuration screen from “None” to “Use prior test values.” (example is shown in the image below/right for the Open Flow Cytometry assay)



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

If **Secure Mode** is turned off, any operator can follow the instructions below to access data. However, if **Secure Mode**, is enabled, only user logins with Administrator privileges will be able to perform the steps below. Each saved Moxi GO II™ 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 or FCSEXPRESS 7). 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. For systems in **Secure Mode**, additional image and log files are stored (see 21 CFR, Part 11 section below for details) To access the files follow the instructions below:



Connect the Moxi GO II™ to a Computer

- i. Unplug the USB Cable from the Moxi GO II™ Power Adaptor.
- ii. Plug the wide end (standard USB connector) of the USB Cable into your computer's USB port.
- iii. Plug the small end (mini-USB connector) of the cable into the back of the instrument.
- iv. Power on the Moxi GO II (if not already on).



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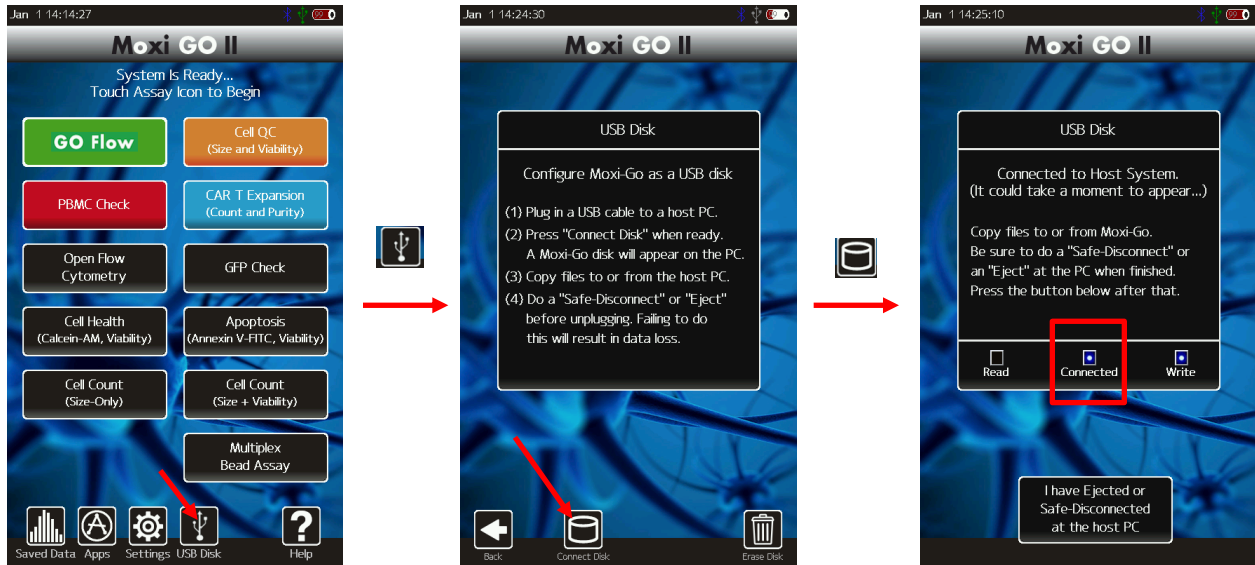
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Put the Moxi GO II™ in USB Connect mode




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- i. Touch the “USB Disk” icon (image above/left)
- ii. Touch the “Connect Disk” icon (image above/middle)
- iii. Wait for the “Connected” icon to light up (image above/right). *Notes:*
 - *It may take a couple of minutes to light up.*
 - *Once that disk is connected, the Moxi GO II disk will appear as an external drive on your computer. On Mac’s, the drive will appear in the Mac Finder under “Devices” as “Moxi Go2 SD”. On Windows computers, it will appear in the File Explorer as a flash drive (e.g. “E:”)*
 - *If the Moxi GO II is not physically connected to the computer, you will receive a message “USB Connection: Please connect a USB cable to a PC host first”*
- i. Once connected, the computer will have full control over the Moxi GO II files. This allows for:
 - Copying or cut-and-paste of files from the Moxi GO II to a computer
 - Deleting files from the Moxi GO II system
 - Adding older FCS data to the Moxi GO II system by copying them to the “Test-Files” directory (for data generated outside **Secure Mode**) or in the Users/UserId folder (for **Secure Mode** data)
 - Renaming of test file names

*Note: The data and image files are located in the “Test-Files” sub-directory for data generated with **Secure Mode** off. When **Secure Mode** is on, files are stored in the Users/UserId (where UserId corresponds to the userid of the person generating the data) folders. There is one FCS file per test. The Cell QC batch runs are saved in sub-folders (named with the batch name) under the Test-Files directory.*


Restoring Data to the system

Any data copied from the Moxi GO II system to a PC can be restored to the system when the system is connected as a USB disk (described above). However, when in Secure Mode, if any modifications were made to the files, the system will not open them as the checksum would be invalidated (security feature).

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Safely disconnect Moxi GO II™ from the Computer

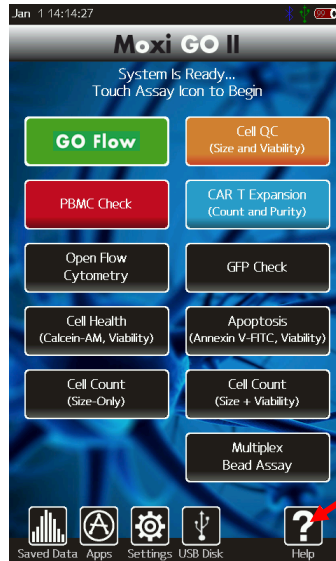


- i. Safe-eject the Moxi GO II drive from the computer.
 - Windows: Click on the “Safely Remove Hardware” icon  in the system tray (bottom right of Windows taskbar). Select the option to eject the Moxi GO II disk.
 - Mac: In the Mac Finder, click on the arrow to the right of the “Moxi Go2 SD” external drive that corresponds to the Moxi GO II™ disk.
- ii. Wait for Moxi GO II™ to recognize the lost connection. The “Connected” light will go dark (images above/ left to above/middle).
- iii. Touch the “I have Ejected or Safe-Disconnected at the host PC” button. The system will return to the Home Screen (image above/right) once complete. *Note: If the disk hasn't been ejected/safe-disconnected, an error will pop-up (“USB Connection: Please eject the disk first”). If that shows, retry ejecting the disk on the PC.*

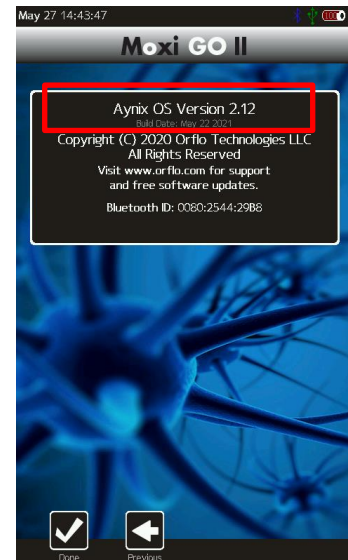
Moxi GO II™ Help and System Information

1. For help with operation of the Moxi GO II™ and for unit-specific system information, press the **Help** icon on the **Home** screen. Visual instructions for inserting a cassette will be presented.

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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 II™ 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.



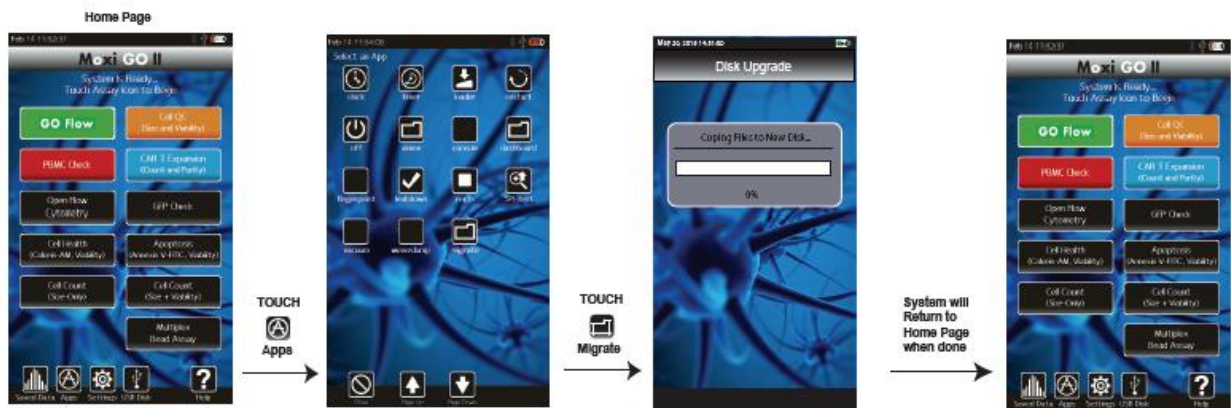
For additional help, see the **Troubleshooting** table (page 27).


Moxi GO II™ Apps

Moxi GO II™ Applications can be entered by touching the **Apps** icon on the **Home** screen. Included applications in version 2.12 are:

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- Clock
- Timer (with sound notification)
- Loader – Puts unit in Firmware/OS loader mode
- Restart – Reboots the unit
- Off – powers the unit off.
- Migrate – Used to migrate pre-v2.10 firmware data from old (16Mb) flash disk to the new (4Gb) μ SD disk. This should be done immediately following the firmware update. The general sequence is shown below.



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21 CFR Part 11 Operation (“Secure Mode”)

Overview

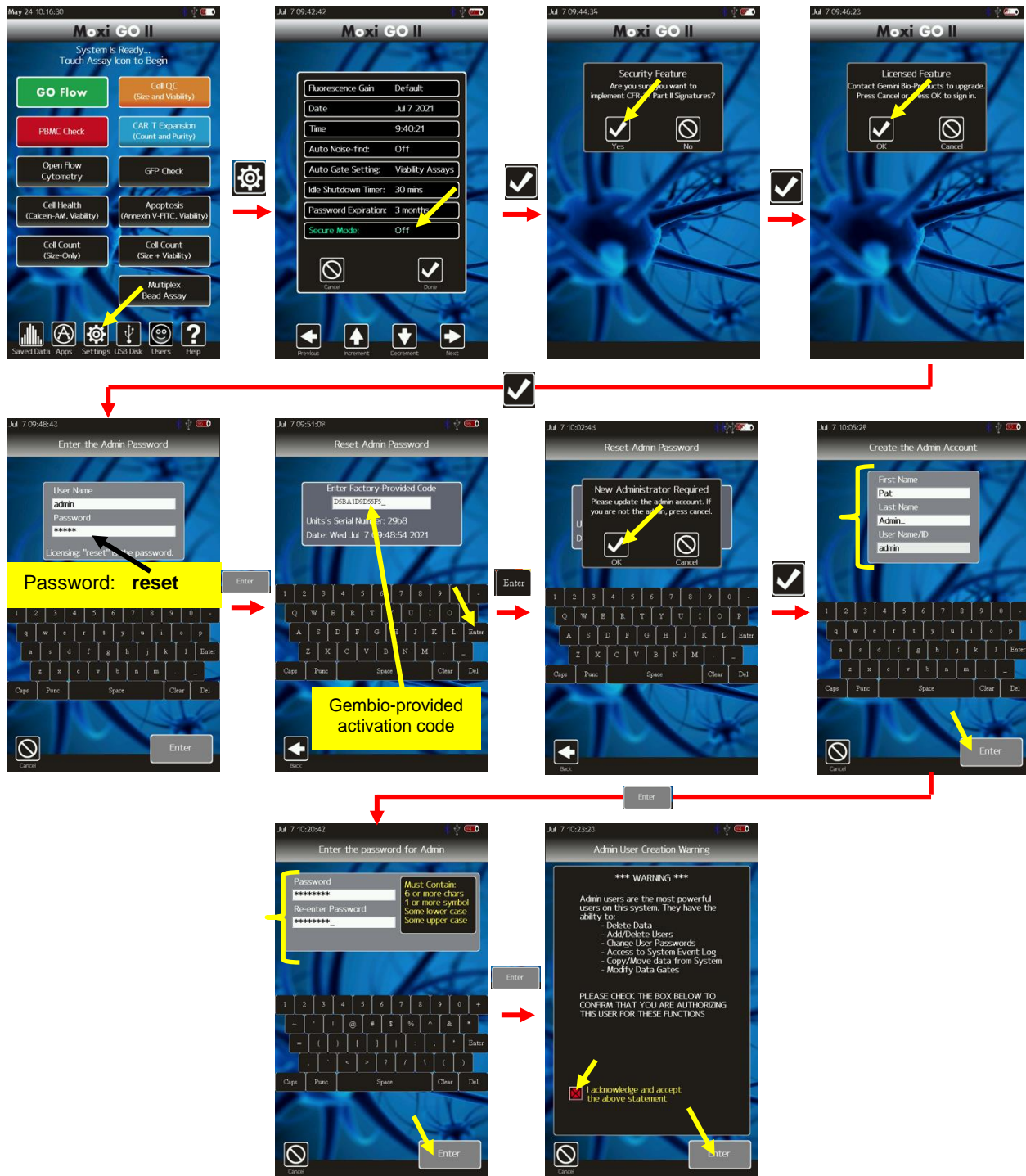
With v2.12 of the Moxi GO II firmware, the system can be set to operate in a 21 CFR, Part 11 compliant manner. When this “Secure Mode” is entered, the v2.12 firmware release implements features that greatly facilitates companies’ compliance with the FDA’s Electronic Code of Federal Regulations, Title 21, Part 11 (21 CFR, Part 11) specifications for electronic records and signatures. Compliance with 21 CFR, Part 11 is a critical requirement for companies in industries, such as pharmaceutical and medical device manufacturing, that are regulated by the FDA. Specifically, the Moxi GO II 21 CFR Part 11 v2.12 firmware features include: unique user logins, user system access control, electronic signatures, detailed system event logging, controlled access to data modification and backup, and automated logging of data generation and change events.


Notes:

- *The Moxi GO II software does not ensure 21 CFR, Part 11 compliance, by itself, as proper procedures, training, and internal controls (e.g cGMP/GLP) are also required by companies in conjunction with the firmware in order to be 21 CFR, Part 11 compliant.*
- ***The Moxi GO II remains a research use only (RUO) instrument, not for use in clinical diagnostic procedures***

Activating Secure Mode (21 CFR, Part 11 compliant mode)

The 21 CFR Part 11-compliant mode is enabled by going to the system **Settings** (off of the **Home** screen) and toggling (touch the “Off” entry or use the arrow keys as the bottom to navigate to it and change it) the “Secure Mode” field from “No” to “Yes”. This will initiate the process of activating the CFR firmware and setting up the initial system Administrator. After confirming the intent to activate, the user will be prompted for an admin login. To activate the software, type “reset” in the password field (without the quotes) to get to the license key entry. Once the CFR module/upgrade is purchased from ORFLO Sales (info@orflo.com, 855-879-6694), the activation key can be obtained from technical support (tech_support@orflo.com, 855-879-6694). It is necessary to provide ORFLO technical support the system full date/time and Bluetooth ID for activation. Following acceptance of the license code, the system will walk through the setup of the initial Administrator. The person chosen for this role should be carefully selected by the organization as this admin has the full security access to the system



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(see Roles section below). The system will walk this new admin user through the process of entering their full name and password, as shown in the images below. The process of doing, as indicated by the final acceptance screen, is the process of setting up the electronic signature for that user.

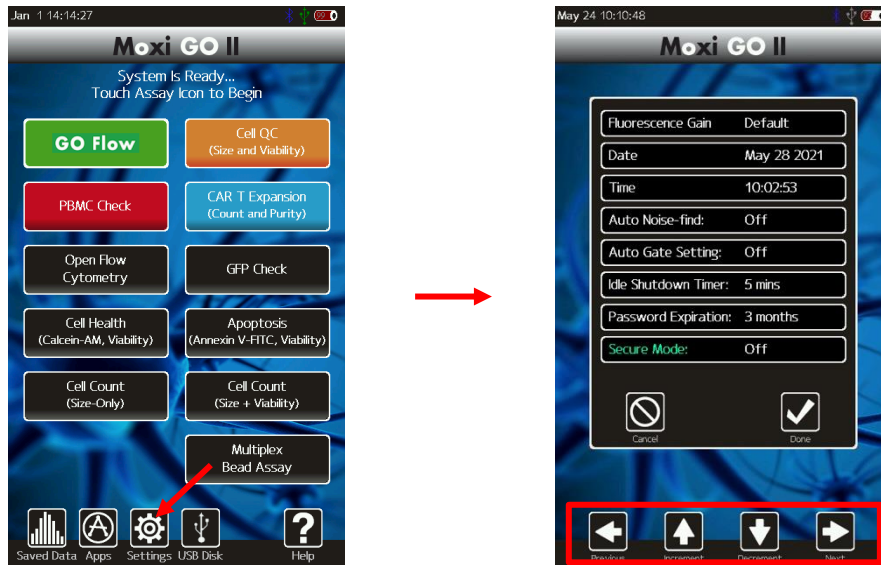
Notes:

- 1.) *The user can use the pop-up keyboard to enter all necessary information.*
- 2.) *Capital letters can be typed by hitting the “CAPS” key to change the keyboard to capital letters.*
- 3.) *Additional punctuation/symbols are available by touching “Punc”.*
- 4.) *Criteria for passwords include:*
 - *At least six characters long*
 - *Must include upper case character*
 - *Must include lower case character*
 - *Must include a symbol*

Secure Mode – Key Settings

Once **Secure Mode** is activated, only system administrators can alter system settings. For this reason, it is important to edit these settings up-front, to make sure they are set properly. Settings can be accessed from the **Home** screen by touching the **Settings** icon (see image below). Details on all the system settings can be found in the above “Settings” section. Three new settings were added as part of the v2.12 firmware release: “Idle Shutdown Timer”, “Password Expiration”, and “Secure Mode.”

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Descriptions of those key settings are described below.

Idle Shutdown Timer

Time period for system to automatically go to sleep (and log out the user if Secure Mode is On). Tap field to toggle values. Possible values are : 5min, 15min, 30min, 45min, 60min, 2hrs.

Password Expiration


Time period for system to auto-expire a user’s password. Once the password expires, the system will force the user to update it prior to logging in. Tap field to toggle values. Possible values are 1, 3, 6, 9, and 12 months.

Secure Mode

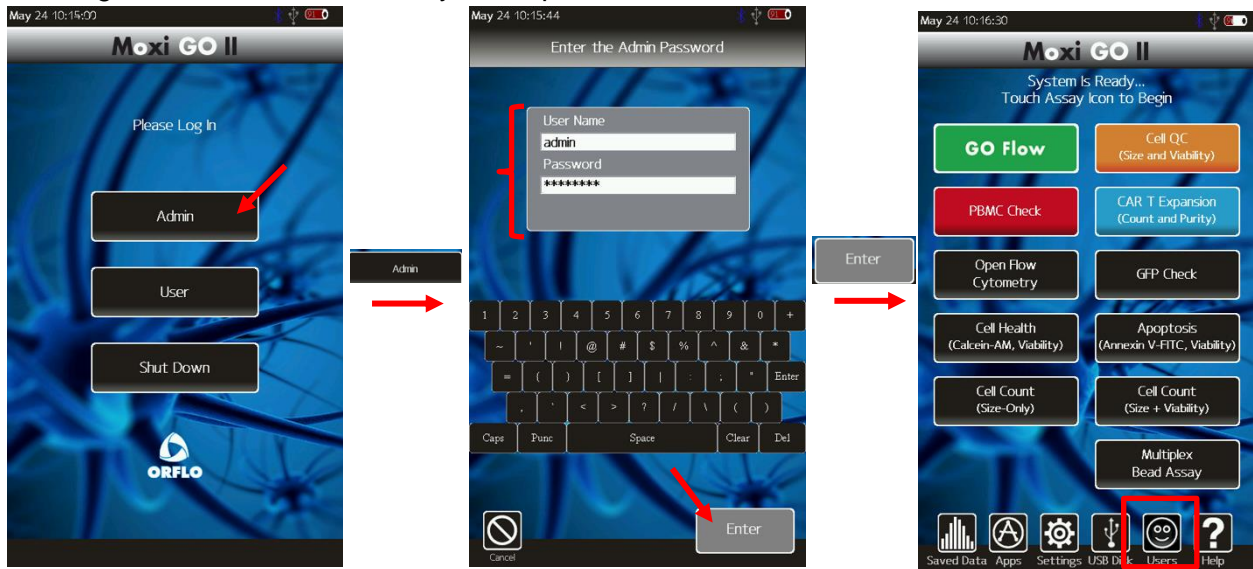
This field turns on/off the 21 CFR, Part 11 functionality for the system

System Login

Once **Secure Mode** is activated, all users of the system need to login with a Unique User ID and Password. Following the initial admin user setup, that user will be required to login for the first time. Following that, the admin user can create additional users as described below. Note that only the initial “admin” user can login by touching the “Admin” button. That user can also

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login through the “User” section. With **Secure Mode** activated, a new Users button shows, allowing for administration of all system operators.



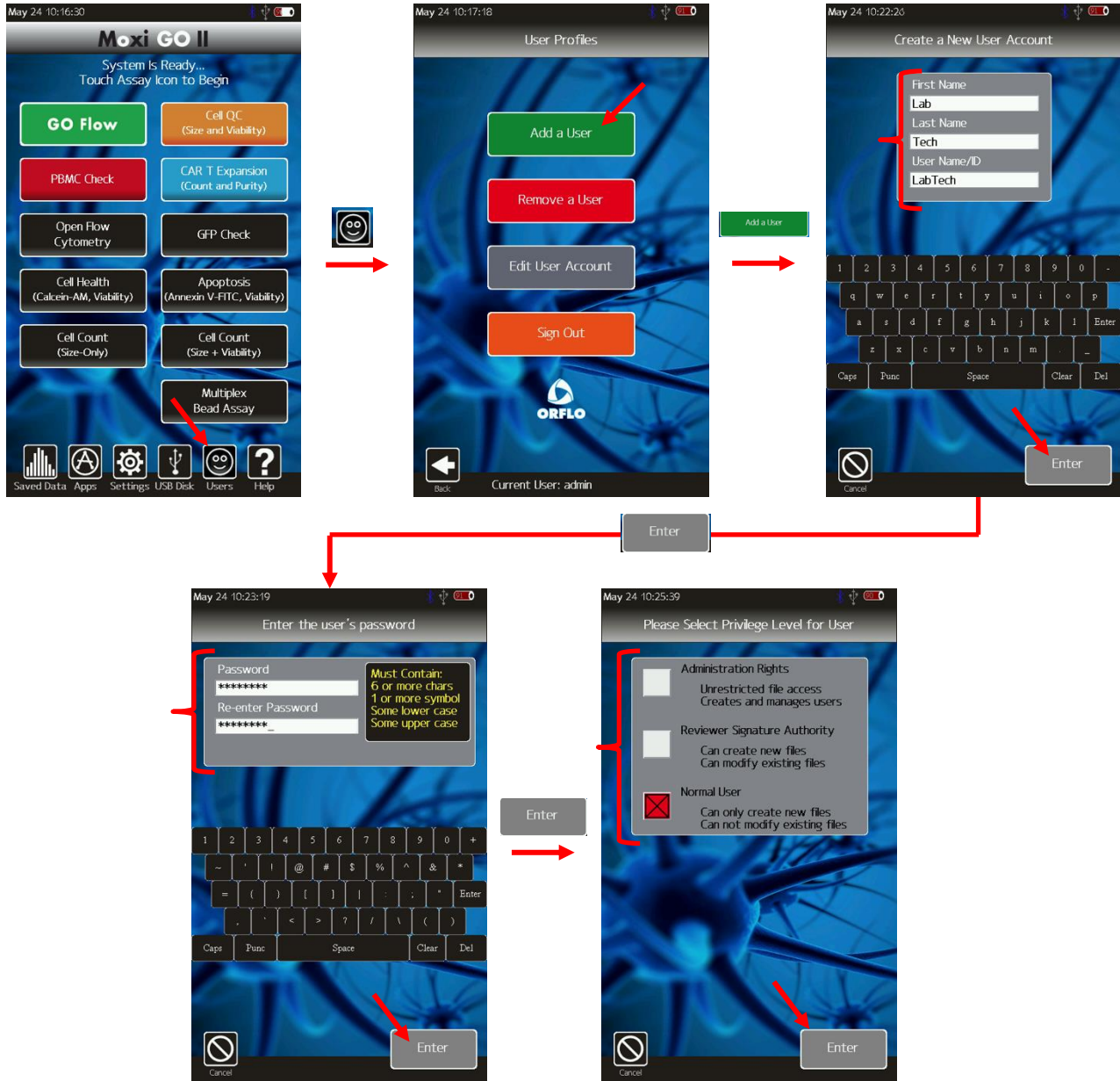
User Administration

Adding a User

When **Secure Mode** is active, a new **Users** button will be visible. Users can be added by touching the **Users** button, select “Add a User”, and following the workflow shown below. The administrator will be prompted to enter the full user name, a User ID, a password, and the role that the user will be assigned. Please note it is important to read the section below on “User Roles” so that the security implications being assigned to each User is understood. It is a requirement of the system that every User ID/Password combination is unique for each system user. That requirement will be enforced. Criteria for passwords include:


- *At least six characters long*
- *Must include upper case character*
- *Must include lower case character*
- *Must include a symbol*

When setting up each and every user, it is important to emphasize to that user that every entry of the User ID and Password combination, during system operation, constitutes a “signature” event. That “signature” should be viewed equivalents to physically signing a legal document.




User Roles

The Moxi GO II firmware classifies users into three separate roles: Normal Users (“Users”), Reviewers, and Administrators (“Admins”). Details on the privileges associated with each role are outlined below. It is critical that organizations carefully consider the role for which each

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system operator will be assigned. For this reason, the system only allows administrators to add users. And, any the Reviewer and Administrator roles are provided reminder messages of the privileges being assigned, when they are created. The privileges associated with each role are:

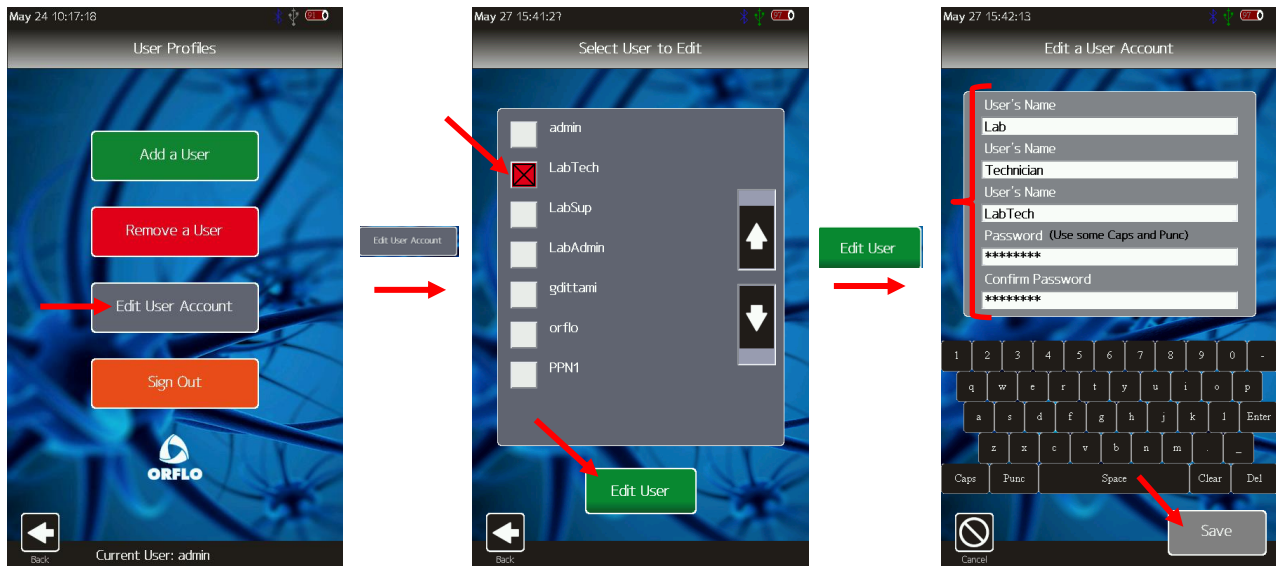
- **Normal Users (“Users”)**
 - System login
 - Run Tests
 - Select any assay
 - Load sample and view data
 - Adjust gates prior to saving
 - Assign a file name prior to saving
 - Store/Recall Gates
 - Compensate Data
 - Print Bitmaps of tests
 - Save Data (with Signature)
 - View Saved Data
 - View any saved data that they created
 - Print bitmaps of that data
 - Adjust gates on the data (adjustments can’t be saved without approval of a reviewer)
 - Adjust Fluorescent Gain Setting for tests
- **Reviewers**
 - All privileges associated with **Normal Users**
 - Ability to modify the gates on saved/edited data (requires signature)
 - Ability to approve modification of gates (by another user) for saved data.
 - Ability to navigate to other Users directories to view/edit data.
- **Administrators**
 - All privileges associated with **Normal Users** and **Reviewers**
 - Add/Delete and Modify Users
 - Change all System Settings
 - Date
 - Time
 - Auto Noise-Find
 - Auto Gate Setting
 - Idle Shutdown Timer
 - Password Expiration period
 - Secure mode On/Off Toggle
 - Delete saved data

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- Format the data disk
- Mount/connect system as an external disk to a computer via USB, allowing access to:
 - Transfer/delete all users data
 - Transfer/delete system log files
 - Transfer/Delete BMP files


Modifying Users

Only users with “Administrator” privileges can edit other user accounts. Administrators can change the User’s Name (first and last), User ID, and password. Should a user lose a password or need to change it for any other reason (e.g. password has been compromised), an Administrator would need to use this functionality to change it. The sequence for editing a user account is shown below. The Administrator would access the “Edit User Account” area (image below/left) by touching **Users** off of the **Home** screen. The Administrator would then check the box next to the user to be edited and touch “Edit User” (Image below/middle). Finally the users information would be displayed (image below/right). The Administrator can touch the field to be edited and use the on-screen keyboard to make the necessary changes. Note: to change a User’s role, it is necessary to delete user (see “Deleting Users” section below) and re-Add the User with the new role.

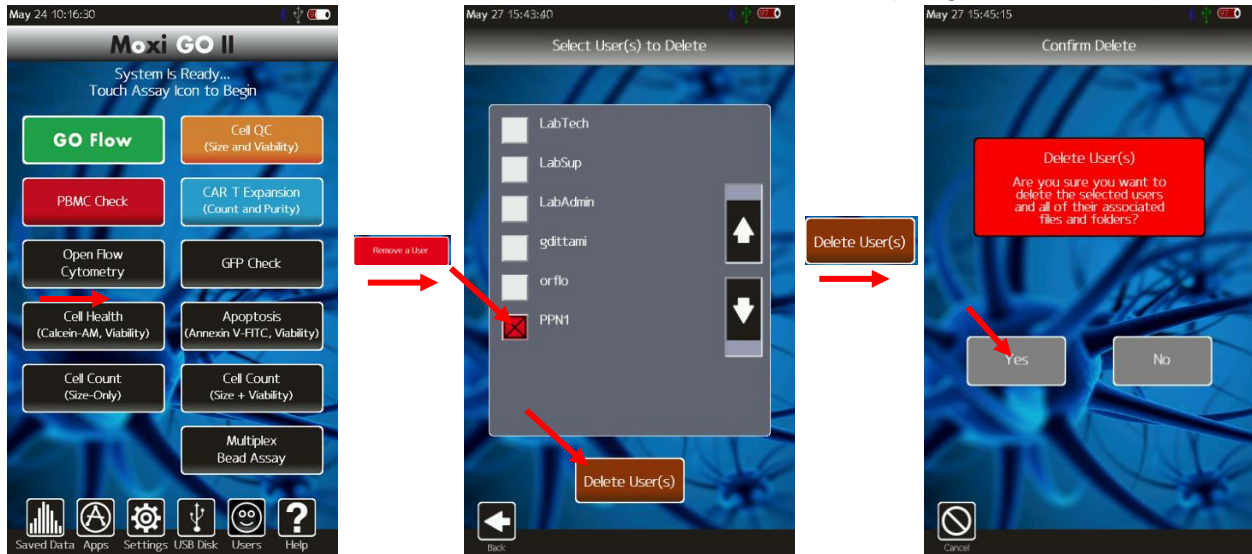


Deleting Users

Only users with “Administrator” privileges can delete other user accounts. The Administrator would access the Remove a User” area (image below/left) by touching **Users** off of the **Home**

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screen. The Administrator would then check the user to be deleted (image below/middle).



Finally, confirmation is required (image above/right) to complete the removal of the user.

Running Tests – Secure Mode

This section describes the specific features associated with running tests in Secure Mode. For more detailed test instructions, please refer to the appropriate test (“Running a Test” and “Analyzing/Gating the Data”) instructions above.

Saving Data

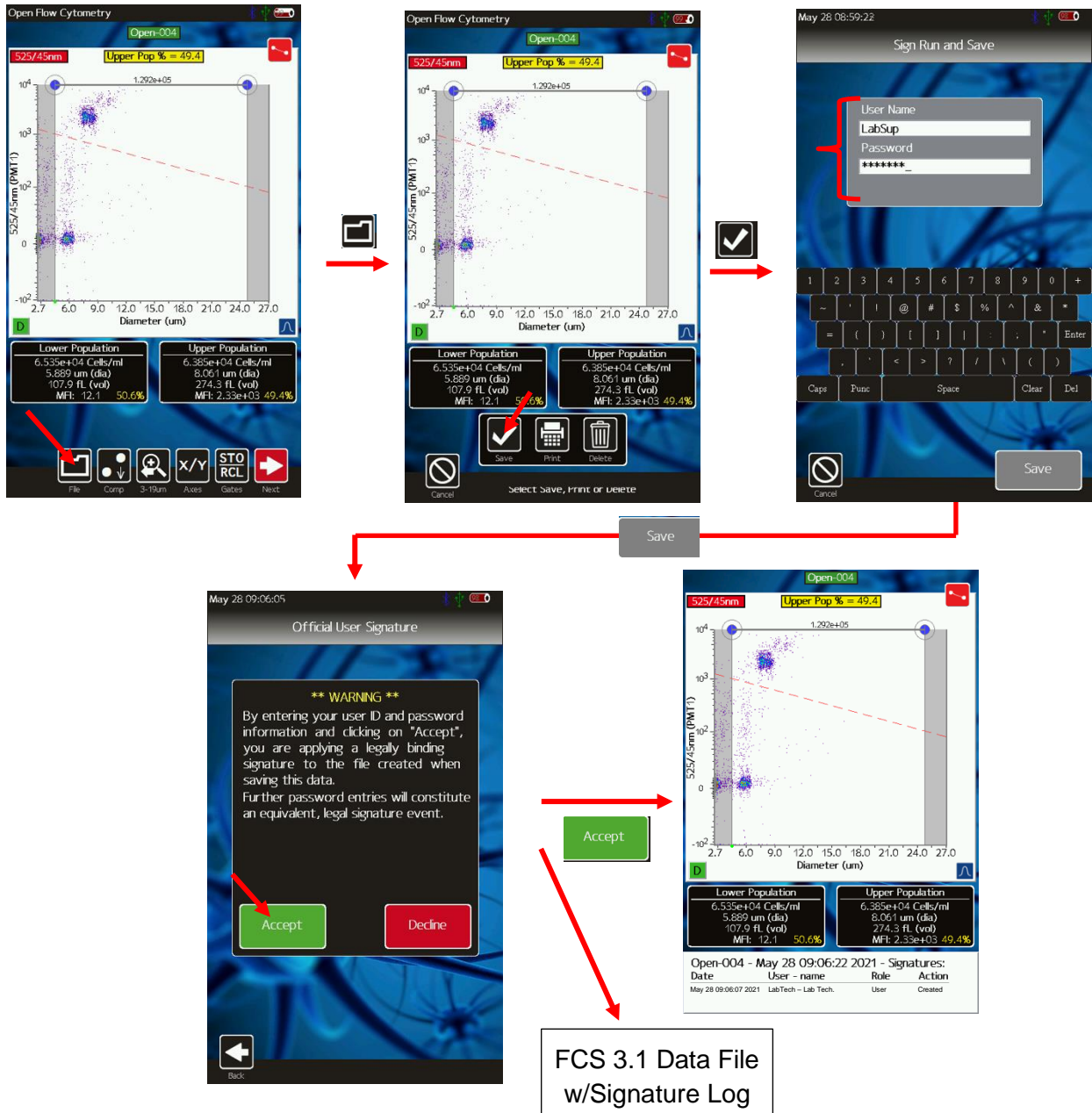
All established system users have permission to generate data. Once the test has completed, the User has the option to adjust the data gates (see “Analyzing/Gating the Data” section). However, once saved and signed (User Id/password entered), the gate locations can only be modified by a Reviewer or Administrator.


Electronic Signature

When the user saves the data, a dialog will be presented, requiring the user to digitally sign the data. Per the 21 CFR Part 11 specifications, the initial data save for every session (user login) requires the entry of both the User ID and Password. The user will be reminded, by a system screen (see image sequence below, bottom/left) that entering a user ID and password constitutes an electronic signature of the data. Any subsequent signature events require just



the user's password. Signatures apply to both newly generated data as well as saved data modification.



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File Save Formats

When in **Secure Mode**, the data is automatically saved in two formats: FCS (data) and BMP (image) for every save event.

FCS data


First, a flow cytometry standard (FCS 3.1) file is generated that contains the test information, binary data, and log events for the data set. The log events contains a detailed list of all actions performed on the file. Initially, this would include the creation event and marker locations upon saving. For data edits, the user that modified the file, the approval of the edits (Reviewer/Admin), and the updated gate locations are all added to the FCS event log. The FCS file is generated with a checksum to prevent any tampering with, or modification of, the data file outside of the system. FCS data can be pushed back to the system by an Administrator, using the USB disk connect functionality. However, if the data were changed in any way, the system will not open the data (will generate a “checksum error”).

BMP File

The system also automatically generates a new/separate image (BMP) file of the current data view when saving. All files will have an initial BMP file generated when the data is first saved. An example image is shown in the image sequence above, with the last image representing the auto-generated BMP. Any subsequent changes to the file will generate a new BMP view of the data to ensure thorough logging of the changes in a human-readable format.

Notes:

- *The BMP data view is the current data screen being viewed. E.g. If the User is viewing a PMT vs PMT view of the data, that will be the data stored in the BMP file.*
- *The BMP file will always be freshly generated, regardless of whether the user had already generated a screenshot of their own using the system functionality.*
- *BMP files are named with the following format following naming format “TestName YYYY-MM-DD HH-MM-SS.csv” where the following parameters correspond to the creating date/time of the log file:*
 - *TestName is the name of the test (e.g. Open-002)*
 - *YYYY is the year*
 - *MM is the month*
 - *DD is the day*
 - *HH is the hour*
 - *MM is the minute*

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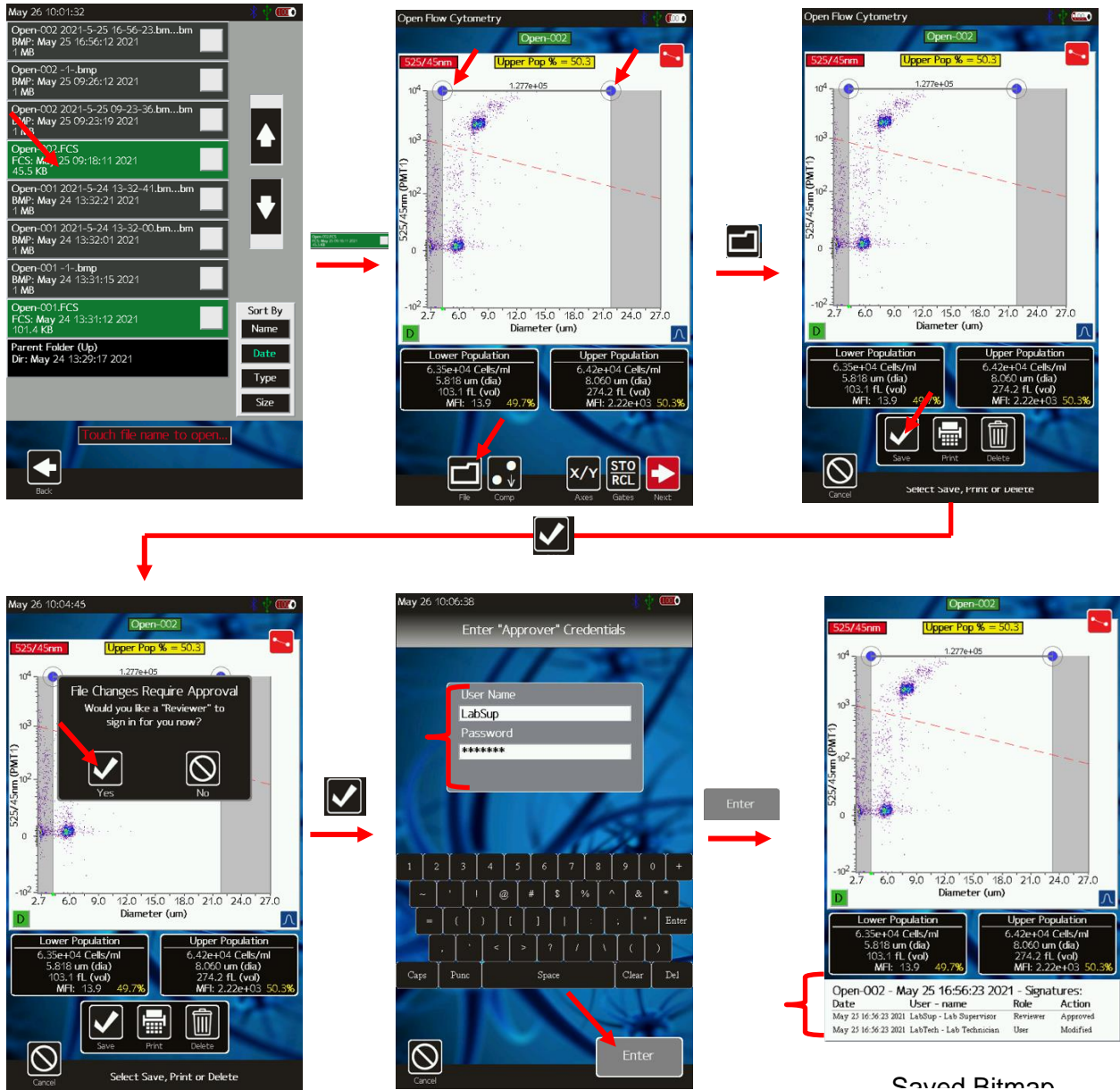
- *SS is the second.*

The BMP file provides a human-readable, unalterable snapshot of the data/results at that point in time for record-keeping purposes. At the bottom of the BMP, the responsible user's name, current action is listed to identify the last/current electronic signature event.

Reviewing Data – Secure Mode


The ability to review data has been enabled for system operators with the “Reviewer” or “Admin” role. The purpose of this function is to verify that the initial data had been properly gated prior to saving. Modification of the gates does not change the underlying data in any manner. However, as the gate placements can change, the result can change. For this reason:

- Reviewer and Administrator privileges should only be assigned to trusted expert users.
- All changes require an electronic signature by the reviewer (or administrator). Normal Users are not authorized to save changes.
- All modification events are logged in detail:
 - A system log event is generated.
 - The FCS file is updated with the modification and approval signatures and new gate positioning information.
- A new BMP snapshot of the data is saved. The BMP file provides a human-readable record of both the updated result and the reviewer's electronic signature.



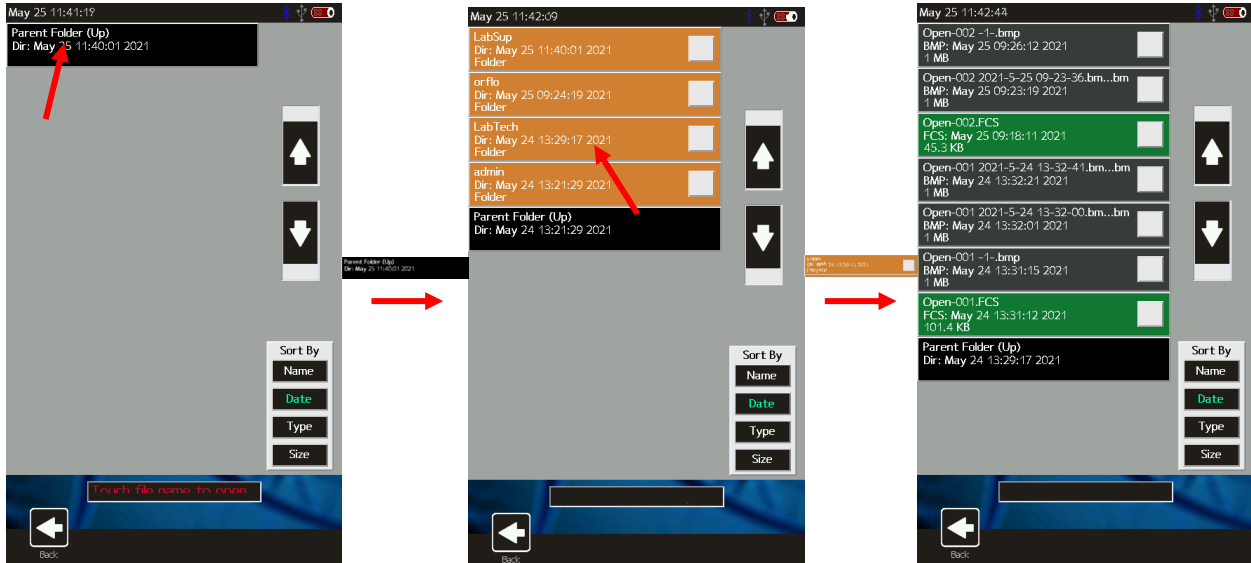
Saved Bitmap

Reviews can be initiated by Normal Users. The Normal User can enter the Saved Data section, Open a file, adjust a gate, and save the data. However, in order to save the data, they would need a Reviewer or Admin to approve the change. This review workflow is shown in the image sequence above. The general editing is the same as without Secure Mode. However, once the image is saved, the system walks the user through (bottom row of images) getting a Reviewer-


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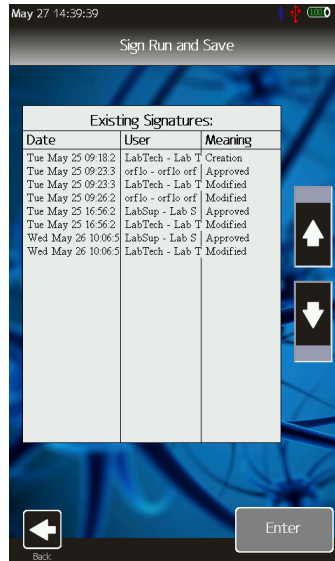
level user’s approval. Following the entry of the reviewer’s credentials/signature, the system will save both the updated FCS data file (with a log of the change and signature) as well as a bitmap image (image bottom/right) with a snapshot of the data, the signature of the user that modified the data, and the Reviewer’s signature event.

Alternatively, Reviewers and Admin can go to specific user directories to review the data directly. The workflow for reviewing data is shown in the image sequence below. To start, the Reviewer enters the **Saved Data** section where their data is stored. To get to the other users’ data directories, they can touch the black “Parent Folder (Up)” entry. The directory view is updated to show a listing of all the users’ directories, with folder names corresponding to the users User ID’s. Selecting a user folder will then list all the data generated by that user.



The Reviewer can open any data file by touching the associated entry. Changes to the file will still require a Reviewer or Admin signature. Prior to signing, the reviewer will be presented with the full log history of file events (example below).

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Following the save, the system will fully log changes in both the FCS data file as well as with a bitmap image with a snapshot of the data, the signature of the user that modified the data, and the Reviewer's signature event.

System Event Logging – Secure Mode

To comply with 21 CFR Part 11 requirements, the system automatically logs all relevant user actions in a system level event log. The event logs are stored in the “admin” directory (“/Users/admin” folder, image bottom/left shows example on Mac OS X) on the Moxi GO II disk when connected by an Administrator.



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	A	B	C	D	E	F	G
1	Moxi-Go System Log						
2	Time-stamp	User Name/	Event	Parameter			
3	Mon May 24	admin - Pat	/ Sign In	Admin			
4	Mon May 24	admin - Pat	/ Add a User	LabTech			
5	Mon May 24	admin - Pat	/ Add a User	LabSup			
6	Mon May 24	admin - Pat	/ Add a User	LabAdmin			
7	Mon May 24	admin - Pat	/ Add a User	gdittami			
8	Mon May 24	admin - Pat	/ Add a User	orflo			
9	Mon May 24	admin - Pat	/ Add a User	PPN1			
10	Mon May 24	admin - Pat	/ Sign Out	Admin			
11	Mon May 24	LabTech - La	/ Sign In	User			
12	Mon May 24	LabTech - La	Created	"cx=187	cy=175	wl=175	wr=175 wt=1
13	Mon May 24	orflo - orflo	Approved	Open-001.FCS			
14	Mon May 24	LabTech - La	Modified	"cx=187	cy=175	wl=175	wr=175 wt=1
15	Mon May 24	orflo - orflo	Approved	Open-001.FCS			
16	Mon May 24	LabTech - La	Modified	"cx=187	cy=175	wl=175	wr=175 wt=1
17	Mon May 24	LabTech - La	/ Sign Out	User			
18	Mon May 24	admin - Pat	/ Sign In	Admin			
19	Mon May 24	admin - Pat	/ Edit User	LabTech			
20	Mon May 24	admin - Pat	/ USB Connect	Privileged			
21	Mon May 24	admin - Pat	/ USB Disconn	Normal			

Log File Naming

Log files are generated on a weekly basis with the following naming format “SysLog-YYYYMMDD-HHMMSS.csv” where the following parameters correspond to the creating date/time of the log file:


- YYYY is the year
- MM is the month
- DD is the day
- HH is the hour
- MM is the minute
- SS is the second.

Log files are only accessible by system administrators. It is recommended that the log files are periodically (e.g. weekly) removed (transferred and deleted) from the system to secure external storage. It is incumbent on the organization to ensure the security of the log files following removal from the system. That security should include:

- Limiting access to authorized users.
- Preventing modification of the files.
- Backing up the data.

Logged System Events

All events are logged with the date/time of the event, the user id, user first name, the user last name, and the event type (see below for event types logged). There is also a general

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“Parameter” field that holds event-specific information such as the Role of the person responsible for the event or the updated gate locations for data events (red arrow in image above/right). The following event types are logged in the Moxi GO II system logs.

- Logins
 - Sign-in
 - Sign-out
 - Failed login event
- User Administration
 - Add a User
 - Edit User (modify a user)
- Password Change
- File checksum failure
- USB connect events
- Test/Data
 - Creation
 - Modification with Approval
 - Deletion
- System Power-on
- System Power-down.

Backing up / Transferring Data – *Secure Mode*


Only users with Administrator privileges can access data on the Moxi GO II once **Secure Mode** has been enabled. To transfer data, Administrators will need to use the power cable to connect their PC or Laptop (USB A connector) to the system (mini-USB connector). The user can select the **USB disk** button and “Connect Disk” button to connect the system to PC or laptop. (Note: For full details on connecting the system, see the “Exporting Data – Connecting Via USB” section above). Once connected, the system will appear as an external disk (i.e. flash drive) with two possible directories: “Test-Files” and “Users.”

Test-Files Directory

The “Test-Files” directory contains all data (FCS), image (BMP), and test-related .csv files for any tests generated in the non-secure mode of the system (i.e. **Secure Mode** = Off).

Users Directory

The “Users” directory contains all the data generated on the system with the Secure Mode turned on. For every system user (that has generated data), there is a sub-directory corresponding to the users’ user id. That directory will contain the data and images generated

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by the user. The “admin” user directory, representing the initial Administrator created by the system, also contains the system log files.

It is recommended that all files are periodically (e.g. weekly) removed (transferred and deleted) from the system to secure external storage. It is incumbent on the organization to ensure the security of the files following removal from the system. That security should include:

- Limiting access to authorized users.
- Preventing modification of the files.
- Backing up the data.

The Moxi GO II system assists in maintaining the security of the files by:

- Limiting USB access to administrators only
- Checking for externally modified data files, using file checksums
- Generating image snapshots, with electronic signatures, of every data change as a secondary, human-readable, unalterable log of the data at a given timepoint.

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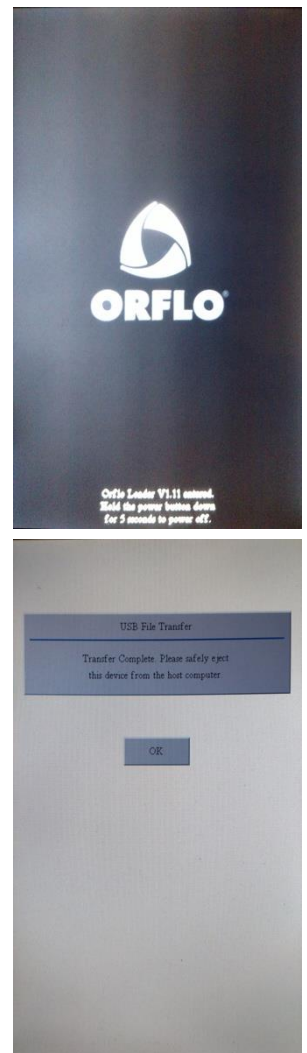
Instrument Firmware and Software Upgrades


Requirements:

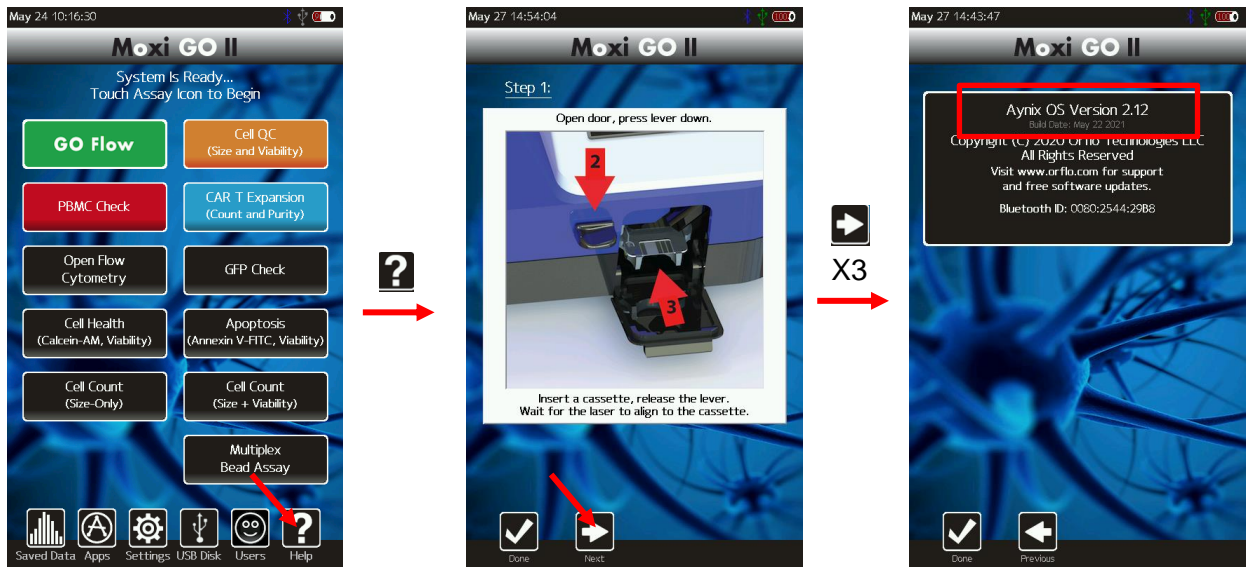
These instructions apply to updating the Moxi GO II OS/Firmware using a computer with a USB port (any OS). Please contact ORFLO Technical Support (Tech_support@orflo.com, 855-879-6694, option 1) for any technical inquiries assistance.

Upgrade Instructions:

1. Download the latest firmware file (e.g. “Moxi_GO-II_firmware_v2.12.zip”) for the Moxi GO II from the “Instrument Software” section of <http://www.orflo.com/v/vspfiles/pages/resources.html>. If you haven’t already registered your system, a brief registration process is required (click “register”).
2. Unzip the files on the computer to be used for updating your GO.
3. Plug the Moxi GO II into your computer (Mac or PC) using the provided USB cable
4. Starting with the system powered off, place unit into “Firmware Update” (“ORFLO OS Loader”) mode by pressing (and releasing) the start button quickly until the “Loader” text appears as the bottom (typically takes 8 quick button presses, stop pressing when the text appears. The loader mode is shown in the image to the right/top.
5. The Moxi GO II will appear as an external drive on the computer (look for it in the Windows Explorer as “External Disk”). Drag all of the extracted files (not the folder, copy all the files at once) onto this drive (or copy and paste them) onto the newly mounted Moxi GO drive.
6. **WAIT FOR ALL FILES TO FINISH COPYING BEFORE PROCEEDING (do not touch the Moxi GO II screen).**
7. Safe-eject the Moxi GO II drive from your computer.
8. The Moxi GO II screen will have changed to the “USB File Transfer” screen. Touch the “OK” button to start (image on right/bottom). The unit will begin updating each file onto the system and will automatically shut off upon completion. DO NOT attempt to turn off the system prior to completion. If you have any issues, please contact ORFLO Technical Support at Tech_support@orflo.com or via phone at 855-879-6694.
9. At this point, the new firmware should be properly installed. Touch the “Help” Icon and press “Next” three times to confirm the OS version (see image below).
10. To migrate older data (that was on the 16Mb flash disk, when upgrading from v2.09 or earlier) to the new 4Gb μSD disk, follow the “Migrate and USB Quick Start Rev 1.0.pdf” guide instructions.



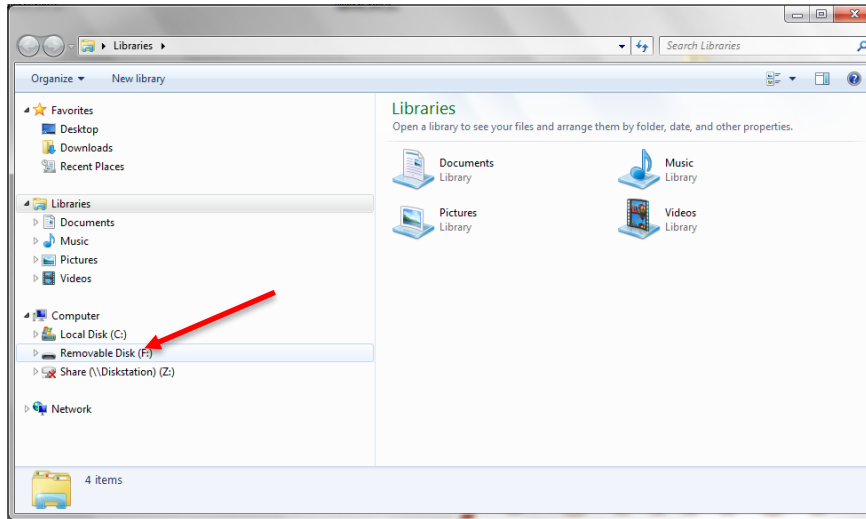
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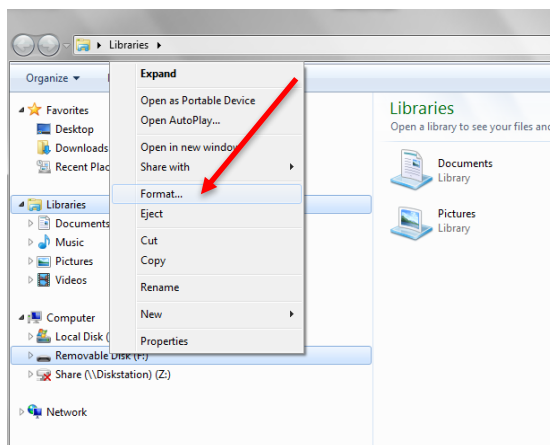
DATA (μSD) DISK FORMAT (STRONGLY RECOMMENDED)

For optimal performance (i.e. file/image save speed), it is strongly recommended that you format the Moxi GO II Data (μSD) Disk following the firmware installation. Formatting the disk requires connecting the system to Windows laptop. Briefly:

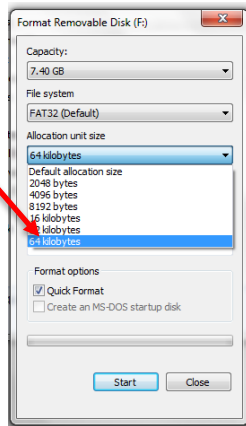
1. Disconnect the system power cable from the AC (wall) adapter and plug that USB 2.0 end into a Windows computer
2. Turn on the Moxi GO II system
3. If secure mode is turned on, login as an administrator.
4. Touch “USB Disk”
5. Touch “Connect”
6. Wait for the system to appear as an external drive on your Windows machine. You will see it in your Windows/File Explorer under “Computer” (Example below)



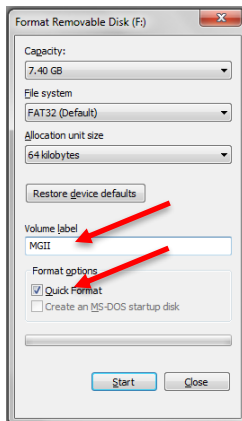
- 7. **BACKUP/COPY** all the data on the disk to the computer. **THIS IS CRITICAL AS THE NEXT STEPS WILL REMOVE ALL DATA FROM THE DEVICE**
- 8. Right click on that drive/device to bring up the options. Select "Format"




- 9. Select the **MAXIMUM** available allocation size (either 32kB or 64kB)



10. Make sure “Quick Format” is checked and type a volume name (e.g. “MGII”)



- 11. Select “Start”. The format should take less than a minute.
- 12. When complete, safe-eject the disk from your computer.
- 13. Reboot the Moxi GO II. The system is ready for use. Note: At this point, you can reconnect the Moxi GO II to the computer to copy the backed up data back to it.

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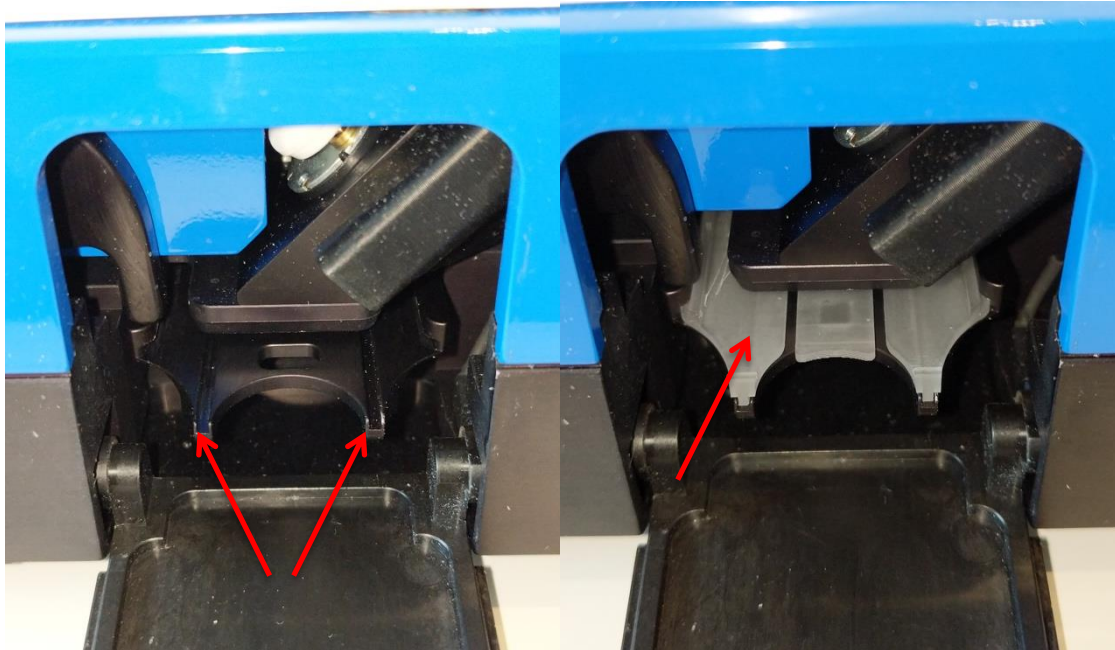
Moxi GO II – Tray insert replacement procedure

Background

A new cassette tray design has been created for the Moxi GO II to allow users to address the occasional need to clean the system cassette tray of adhesive build-up. The adhesive build-up can eventually lead to increased resistance to the insertion and removal of cassettes from the system. For customers with the initial cassette tray design, the update would require sending the system into ORFLO Headquarters for cleaning/replacement. The new tray insert allows users to simply remove a tray insert (that has the adhesive build-up), replacing it with a new insert.

System Requirements and Ordering Information

The new cassette tray design, that accepts the disposable inserts, must first be installed by ORFLO Technical Support. To modify a system with the older cassette tray, please contact ORFLO Technical Support 855-879-6694, Option 1 or Tech_support@orflo.com to have a return material authorization (RMA) issued and the upgrade to be scheduled. Visual verification of the base tray installation can be made by noting the white/natural plastic insert (red arrow in image below/right) or (if no insert installed), the two grooves on the black tray (red arrows in image below/left)



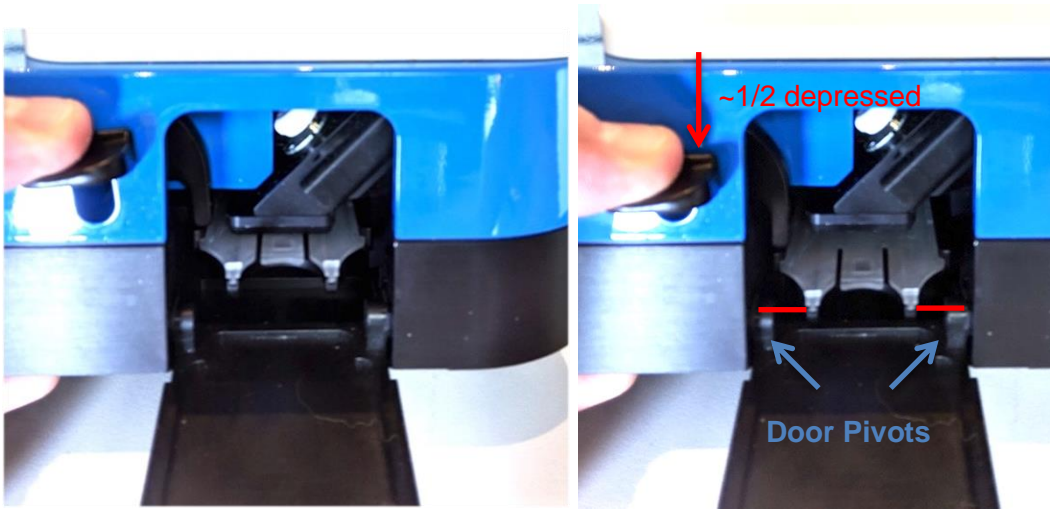
- Complimentary replacement inserts (up to 5 per instrument, per year) will be provided for systems under warranty.
- Additional inserts, or inserts for non-warranty systems, may be purchased using product code MXA090.


Removing an existing insert

IMPORTANT: Make sure to unplug Moxi GO II USB/Power cable from system before starting this procedure. Leaving it in place can result in damage to both the cable and the system when the system is being tilted back (connectors can break).

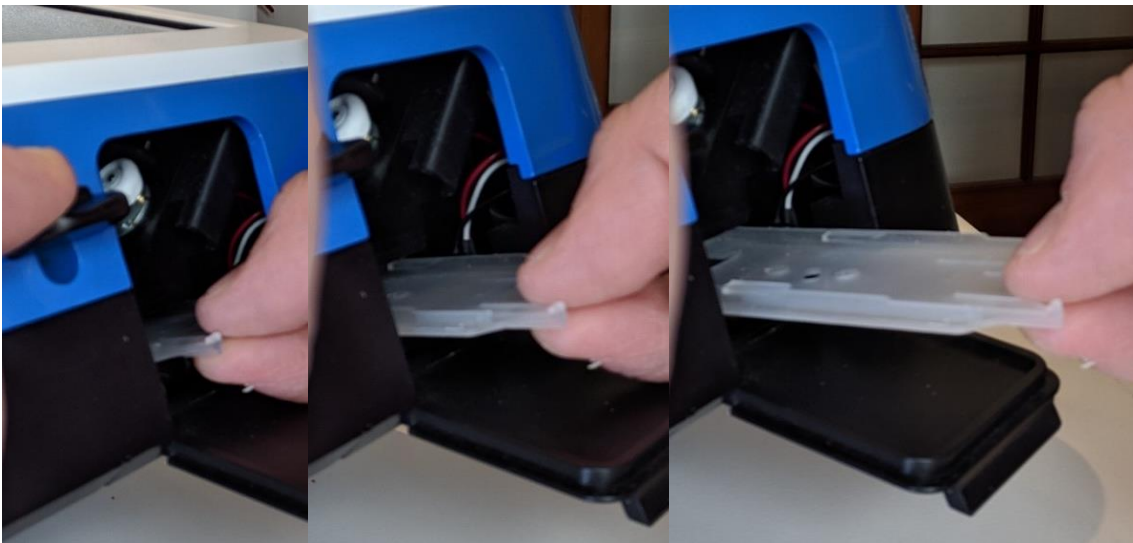
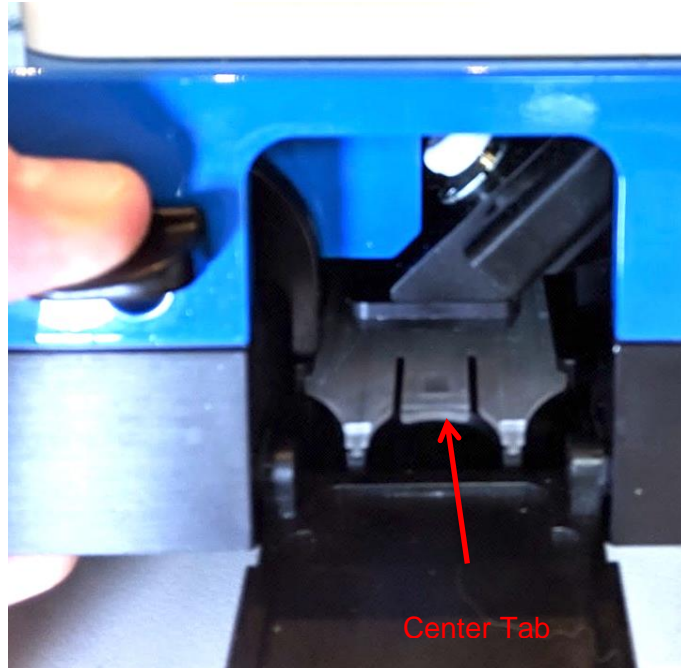


1. With left hand, push down on tray lever until the bottom of the white/natural tray insert is (roughly) just above the top of the door pivots (see images below). The tray lever will be roughly halfway depressed at that point.



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2. ***(While keeping the tray lever position with your left hand)*** Using right hand thumb and forefinger, gently lift up on the center tab (red arrow in images below) of the tray insert while pulling out to slide the insert out. Pull insert all the way out. Once out, the tray lever can be released. (Refer to images below) *Note: If significant friction is encountered when pulling on the insert, try slightly raising/lowering the lever while gently pulling on the insert.*



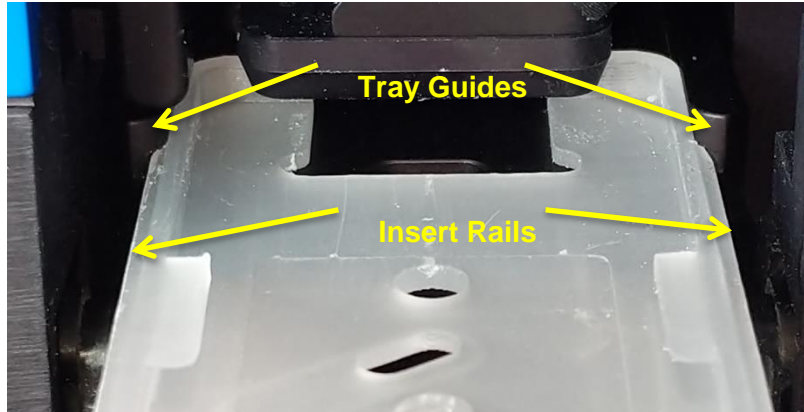
Installing an insert

IMPORTANT: Make sure to unplug Moxi GO II USB/Power cable from system before starting this procedure. Leaving it in place can result in damage to both the cable and the system when the system is being tilted back (connectors can break).

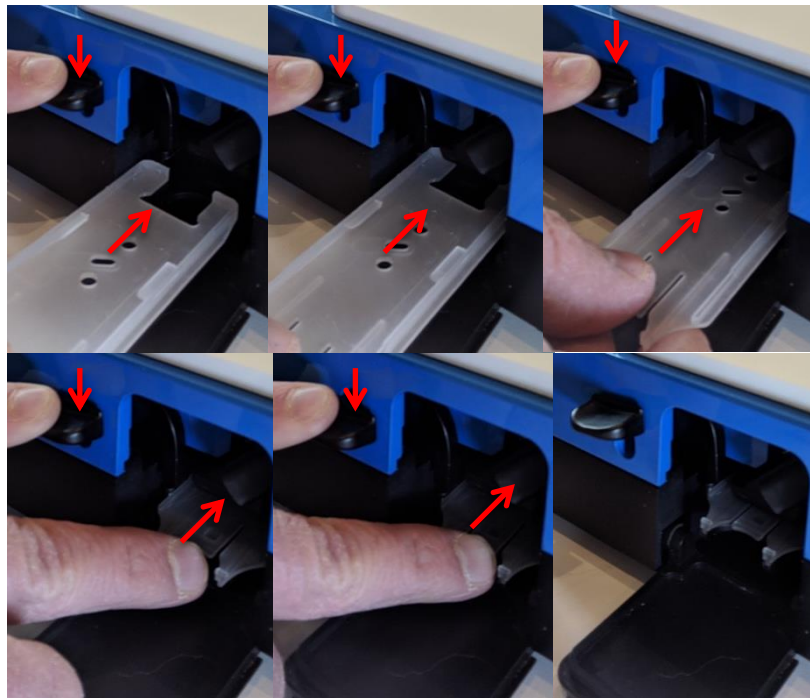


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1. Using your left hand, push slightly down on the tray lever (NOT ALL THE WAY, ~1/3 depressed) for the cassette tray.
2. Place back of the insert to the tray and align the insert rails with the tray guides (see image below)

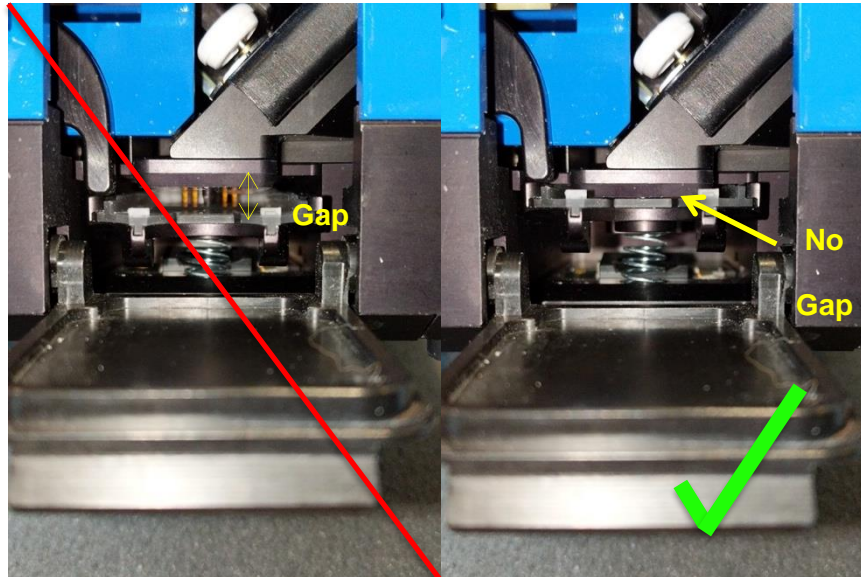


3. While continuing to hold the tray lever depressed, push the cassette insert into the system. *Note: If significant resistance is encountered, try slightly raising/lowering the tray lever while gently pushing on the insert.*





3. Once the insert is all the way in, release the tray lever. If insert is properly installed, the tray should close all the way and the insert should be flush with the black top mount in the system (see image below/right). If the tray doesn't close and there is a gap (see image below/left), push gently on the tray lever and try to push the insert further in.



4. To double-check insert is locked in: Press down on tray lever (~1/2 way) and pull on one of the two front tabs of the insert. The insert should not slide out. If it does, try to push it in further.



Troubleshooting

Symptom	Cause	Corrective Action
Battery will not fully charge	Battery gauge has reset or battery is faulty or has surpassed its service life	Try charging the unit overnight (minimum, over a weekend is recommended). Make sure battery is plugged into an AC adapter, not a USB port. Battery charges fastest when powered on. Contact technical support.
Boot Failure	Issue with system disk.	Contact Tech_support@orflo.com.
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 inserted crooked	Make sure cassette is laying flat in the tray. The tray should close fully (and lever return to the fully up position) when properly inserted. Try re-inserting the cassette.
	Cassette manufacturing error	Try other side of cassette or a new cassette.
Pump error	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.
	Internal firmware issue due to instrument malfunction or high level of external interference	Reset instrument by pressing and holding the power button for at 10 seconds. System should shut-off on release of the button. Try powering on the system after that. If problem persists, return instrument for service.
Instrument stops responding	Cell/bead concentration too low	Try running the cell sample at a higher concentration.
	Sample Flow Issue	<p>This is a situation that can potentially occur when running:</p> <ul style="list-style-type: none"> Over-concentration samples. Highly-aggregated samples (e.g. samples with large cell clusters) "Sticky" samples (e.g. samples with high levels of lysed DNA) that result in clogging of the filters and/or aperture. Samples diluted in media with surfactant (e.g. Triton or Tween) that can result in foaming/bubbles. <p>All the above can result in stopped or hindered flow that affects the fluorescent and impedance signal. Steps to address include:</p> <ul style="list-style-type: none"> Dilute the sample further (in media that is free of surfactant) to reduce the cell concentration. Wash sample (to get rid of surfactant) Break apart clusters by using an appropriate dissociation
No Clear Scatter (Dot) Plot clusters or "smearing" of scatter plot output		



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		reagent/protease (e.g. Accutase or Accumax) with pipette trituration.
No Fluorescence separation/detection	Low/protein expression	For poorly expressed proteins, surface labeling of the protein might not provide a sufficient fluorescent separation from the dark sample.
	Incorrect fluorophore was used.	Please check compatibility of the fluorophore with the system specifications (488nm laser excitation, 525/45nm filtered emission or 561nm/LP filtered emission)
	Use of Phycoerythrin (PE) with 646nm/LP filter in PMT2	Replace back filter with 561nm/LP filter. PE is not detectable when using the 646nm/LP filter
	Incorrect antibody used.	Make sure the proper antibody clone and target species is selected for the cells being used.
	Fluorescence background is too high.	Make sure to wash the sample 2x (in a minimum of 1.5ml of media) to eliminate residual/free fluorophore.
	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.



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	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 cell cluster (or 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.
Blue Screen – “Software Exception”	System firmware has detected a malfunction.	Please take a photo and send to tech_support@orflo.com. Reset the instrument by pressing and holding the power button for at least 5 seconds. When plugging the system into a computer to access the data, the system may report a crash log has been generated. Email that file “OS_Syslog.txt” to tech_support@orflo.com



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System shuts off during alignment/test	System is unplugged	Plug the system in to the AC wall adapter (NOT A COMPUTER USB PORT) and re-try. Make sure the battery is fully charged. If the battery is not fully charged, keep the system plugged into the wall outlet, make sure it is powered on, and charge it overnight
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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". Selecting "Delete All" and "Bitmaps Only" can be a method to quickly clear space on your disk without removing the data files. The Bitmap files are the largest files on the disk so, if there are a lot of them, this option will be particularly effective.
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.
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. Please be prepared to provide the cassette type and lot number.
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.



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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 overnight while powered on and plugged into the wall outlet. After that, make sure to run future 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)

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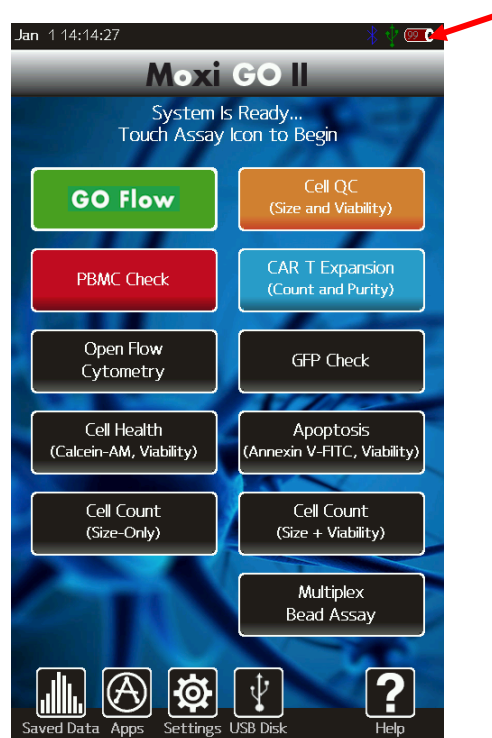
Maintenance and Storage


Storage

Store the Moxi GO II™ 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.

Battery/Charging



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The Moxi GO II™ contains a 3.7 V, 7500mAh lithium ion battery. The battery is incorporated in the system only to buffer the power surges that occur during the activation of the laser and PMT's and to ensure that a temporary unplugging of the system does not result in a power-off. ***The system is NOT intended to be operated under battery power. And, while the system does draw some charge from computer USB ports, it is not meant to be operated while plugged into one because of the lower current output of those ports.*** Attach the mini-USB B connector of the USB cord to the Moxi GO II™ and the larger connector on the opposite end (USB A 2.0) 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. There is a USB

indicator  that appears when the system plugs in. That indicator is red colored when plugged into the AC adapter, green when plugged into a computer USB or USB hub, and gray when it is trying to determine the type of charging source. The battery indicator  will turn red and scroll if the system is charging. If the system is fully charged, the battery indicator will remain solid green, even when plugged in. When unplugged, the level of fill of the battery icon will be proportional to level of charge of the battery (e.g. a 50% charged system will show a half-filled battery icon when unplugged). The battery may be charged at any time in the discharge cycle and can be charged continuously without damage, using the power adapter. The system charges fastest when it is in a powered-on state. Once the battery has reached full charge, it will automatically shut off. Charging time will vary but charging a completely drained battery will take >24 hours.




CAUTION: To prevent battery damage, use ONLY an ORFLO supplied power adapter or the USB port of a computer (for data transfer, not for charging).

The battery life is about five (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 II™ is **NOT** autoclavable. Extreme heat will damage the battery, touch screen display, and other electronic components.

The external surfaces of the Moxi GO II™ 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.


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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 II™. In addition, there are no user serviceable parts with the exception of the ability to swap out the tray insert (see instructions above). Instrument repairs must be carried out by authorized personnel only.

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Specifications for the Moxi GO II™ and Cassettes

System Performance Detection Parameters Excitation Laser Wavelength Number of PMTs Optical Detection Range Particle Count/Size Detection Display Resolution Data Storage Connectivity Data Output	5 (2-color fluorescence, cell count, cell size, forward extinction) 488 nm 2 PMT1: 525/45 nm (e.g FITC, GFP, Alexa 488) PMT2: User-swappable, either 561 nm LP (e.g PE) or 646nm/LP (PI, avoids need for compensation) Impedimetric (Coulter Principle) 480 x 800 pixels, color touchscreen 1000 histogram bins 4Gb USB on-the-go On-system data analysis with optional export of BMP files and FCS compatible raw data files <i>(FCS 3.1, compatible with FlowJo and other offline software analysis platforms)</i>
Measurement Modes Open Platform Assays/Protocols	Yes, Open Flow / GO Flow Applications available allowing users to define their own protocols and analysis <i>* Requires fluorochromes compatible with above filters</i> Please refer to the “Resources” “Protocols” section of www.orflo.com for published protocols. For questions regarding assays/protocols that are not listed on that web site, please direct inquiries to tech_support@orflo.com , including as much detail as possible on the intended application, cell type, fluorophore, etc.
Cassette Performance Compatible Sample Types Number of tests per cassette Sample Volume Required Particle/Cell Size Range	Cells (Adherent & Non-adherent primary cells or cell lines) and Beads 2 60 µl minimum



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Type S+ cassette MF-M cassette Concentration range Type S+ cassette Counts Optimal fluorescence Type S+ cassette Counts Optimal fluorescence Measurement Time	3-27µm diameter (14 – 10,306 fL volume) 4-35µm diameter (14 – 22,449 fL volume) 1 x 10 ⁴ – 1.75 x 10 ⁶ cells/ml < 5 x 10 ⁵ cells/ml 1 x 10 ⁴ – 7.5 x 10 ⁵ cells/ml < 3 x 10 ⁵ cells/ml ~ 5-10 seconds
Software	Includes a (pre-loaded) instrument-only FW/SW package that drives instrument performance and on-instrument data analysis. Periodic software updates are made available via www.orflo.com . These updates are offered both to fix bugs as well as to enable new functionality. <i>Offline analysis requires FCS v3.1 compatible software package for viewing files (e.g., FlowJo X or FCSExpress 7)</i>
Physical Attributes Benchtop Dimensions (HxWxD) Weight	Yes 5.8 in x 9.3 in x 8.7 in / 14.8 cm x 22.1 cm x 23.6 cm 10 lbs / 4.54 kg
Electrical Specifications Internal Battery AC Power Adapters (US, EU, and UK types)	Rechargeable 3.7V, 7500 mAh lithium ion Input: 100-240 VAC (50/60 Hz), 0.2 A Output: 5 VDC, 1 A
Operating Environmental Conditions Temperature Maximum Relative Humidity Altitude Applicable Pollution Degree Degree of Ingress Protection	15-30°C (indoor use only) 20-80% (non-condensing) 2000 meters (maximum) Pollution Degree 2 IPX10
Cassette Handling Storage	Cassettes will last indefinitely when handled/stored properly (no cigarette smoke, no exposure to extreme


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	temperature cycles, general storage temperature of 15-30C). The cassettes can be handled/touched anywhere on the cassette, with the exception that we recommend minimizing contact in the black regions towards the center of the cassette as those are the optical regions.
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Ordering Information

This section lists catalog numbers for the Moxi GO II™ 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.

<u>Product Description</u>	<u>Product Code</u>
Moxi GO II™ Kit, US Version	MXG102
Moxi GO II™ Kit, Base Unit Only, US Version	MXG402
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 II USB Cable and Adapter	MXA120
Moxi GO II 488 Optical Filter Module, 525/45	MOG003
Moxi GO II 488 Optical Filter Module, 561/LP	MOG004
Moxi GO II 488 Optical Filter Module, 646/LP	MOG005
Cassette Tray Insert	MXA090

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Technical and Customer Service

For technical service, customer service, including sales, pricing, and order information, contact ORFLO Technologies at 855-879-6694 or email us at tech_support@orflo.com.

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 II 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 an 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) or send an email to tech_support@orflo.com to describe the issue you are experiencing. Please be prepared to

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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 our repair facility.

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 II 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



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MXG.SYS.003	1.0	Initial release
	1.2	<ul style="list-style-type: none"> • Added “Not for used in Diagnostic procedures language. • Update for swappable filters • Sample volume recommendation from 75 to 60-75µl • Fluorophores for 650nm/LP filter • License level information – Grayed out apps • GO Flow Test Instructions • Product code and accessories update
	2.10	<ul style="list-style-type: none"> • Added Gemini Bio logo/information • HomeScreen Image updates • System image update • Cassette Update • Accessories update for tray insert • Licensing level updates (2 levels to 3 levels) • PBMC test instructions • CAR-T test instructions • Cell QC test instructions • Addition of “Very Low” fluorescent gain • Updates to data storage (flash to µSD) • Setting screen – touch to change values • Auto-Gate settings • Dynamic aperture impedance measurement/normalization • Improved sample prep recommendations • Improved sample loading instructions • Multiplex Bead assay instructions • Update images to reflect increased data display area and white background • Modified Data Analysis to include simplified output vs raw output • Update x-axis size range to 2.7µm min (from 3µm)



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		<ul style="list-style-type: none">• Improved spillover/compensation instructions, including “load control set”• New USB connect instructions• Migrate App instructions for migrating data from Flash drive (pre 2.10) to μSD (2.10)• Add Tray Replacement Procedure• Corrected/clarified troubleshooting table• Updates to battery information section• Specifications table update
	2.12	<ul style="list-style-type: none">• 21-CFR Release Functionality
	2.13	<ul style="list-style-type: none">• Removed Gemini Bio branding/Information

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