Aurora™ User's Guide

FCC Information
WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

NOTICE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, can cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense.

Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Réglement sur le matériel brouilleur du Canada.

CDRH Information
This equipment complies with CDRH Class I requirements.

Regulatory Information
For Research Use Only. Not for use in diagnostic or therapeutic procedures.

History

Revision	Date	Change
52-70001-0A	10/2017	Initial release

Contents

1

Introduction

About this Guide

This manual provides information on the Aurora flow cytometer, daily workflow, SpectroFlo™ software features, cytometer specifications, and instrument maintenance. It also includes troubleshooting tips and service information.

Safety

Safety Symbols

The Aurora is intended for research use only. Not for diagnostic or therapeutic procedures.

The following table lists symbols used throughout this guide.

Symbol	Meaning
	Caution: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death
	Risk of electric shock
	Biological risk

General Safety

- Do not place any object on top of the instrument.
- Before turning on the cytometer, visually inspect all containers. Wear the recommended protective laboratory attire such as protective gloves, eyewear, and lab coat.
- Purge the sheath filter if air bubbles are visible in the sheath filter, or if the plenum or sheath container have run dry.
- Fill the sheath container as needed. Never use tap water as sheath solution.
- Do not run bleach or detergent through the sheath filter. It is difficult to remove cleaning solutions from the sheath filter.

- Check the cytometer periodically for fluid leaks or crimped lines. If evidence of a leak is detected, contact Cytek Technical Support immediately. Do not attempt to repair the instrument.
- When performing daily QC, always select the correct bead lot number.

Electrical Safety

- Do not place liquids on top of the instrument. Any spill into the ventilation openings could cause electrical shock or damage to the instrument.

Biological Safety

- Empty the waste container when filling the sheath container or as needed to prevent leakage. Take care to avoid damaging the fluid level sensor in the waste tank.
- Biological samples are potentially dangerous and/or life threatening. Adhere to proper handling procedures for samples and reagents. Wear appropriate laboratory attire such as protective gloves, eyewear, and lab coat.
- Any instrument surface in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning the instrument or replacing parts.
- Concentrations of sodium hypochlorite higher than 10%, as well as other cleaning agents can damage the instrument.

Technical Support

For instrument support within the US, call 1-877-92-CYTEK. Visit our website, www.cytekbio.com, for up-to-date contact information.

When contacting Cytek, have the following information available:

- Serial number
- Any error messages
- Details of recent performance

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Overview

Aurora System

The Aurora system consists of the Aurora flow cytometer and a computer workstation running SpectroFlo™ software for acquisition and analysis. The system also includes SpectroFlo QC beads.

The cytometer is an air-cooled, compact benchtop instrument. It is equipped with three lasers and up to 48 detection channels for fluorescence, and up to two detection channels for scatter (FSC and violet laser SSC). Sheath and waste fluids are contained in either 4-L tanks or 20-L cubitainers. During operation the software notifies you when the fluid levels are getting high or low. The pressurized fluidics system includes a plenum for storing sheath, allowing you to fill and empty the fluids during operation.

The workstation is a dedicated USB-compatible PC with monitor, keyboard, and mouse. It runs Microsoft® Windows® 10 Pro 64-bit operating system, which is required for SpectroFlo software.

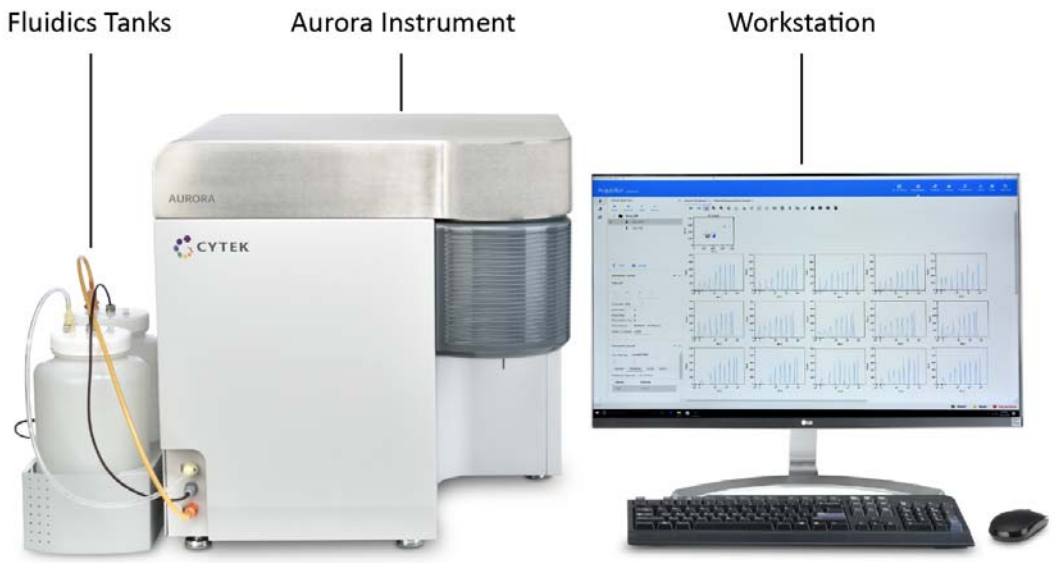


Figure 1. Aurora System

Cytometer Overview

The Aurora spectral flow cytometer is an air-cooled, multi-laser, compact benchtop flow cytometer. It is equipped with three lasers and up to 48 detection channels for fluorescence and up to two detection channels for scatter (FSC [forward scatter] and violet laser SSC [side scatter]). Solid-state lasers transmit light through a flow cell where particles in suspension are focused, single file for interrogation by the laser. Proprietary, high-sensitivity, 16-channel semiconductor detector arrays are equipped to capture the emission spectra of dyes that emit in the 400 to 900-nm wavelength range. The resulting fluorescence and scatter are then collected and converted into electronic signals. On-board electronics convert these signals into digital data that can be acquired and recorded on the workstation.

The cytometer power button is located on the left side of the upper panel of the cytometer (Figure 2). When the cytometer is powered on, the power button is illuminated.

The front panel opens on its hinges to the right to reveal the fluidics system. The top cover opens to reveal the optics.

Front of Cytometer

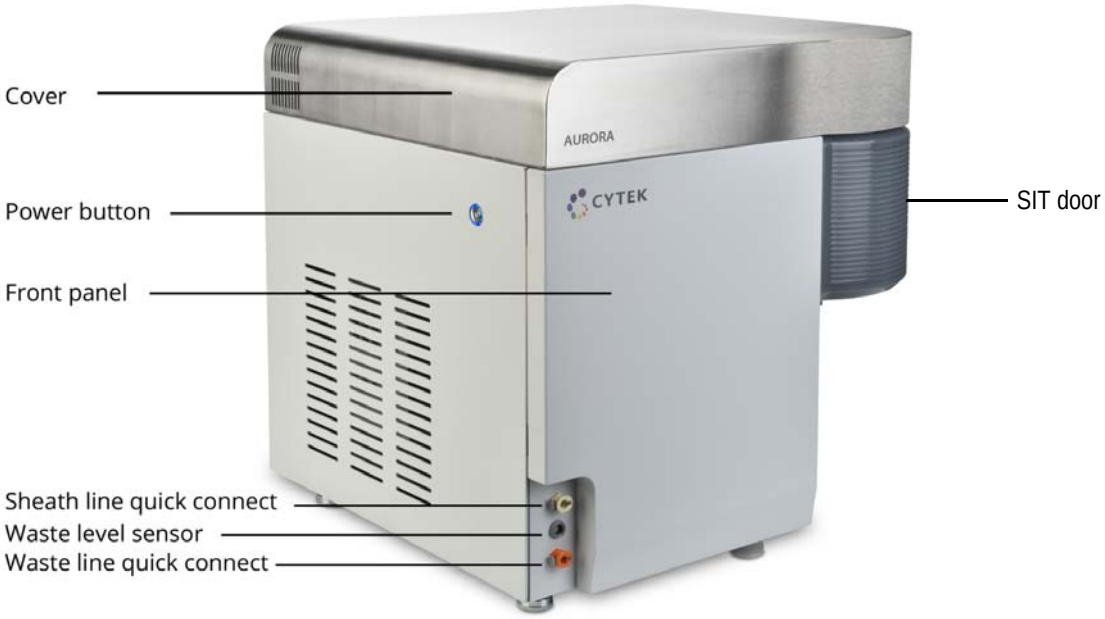


Figure 2. Aurora front and left side panels

Do not place any object on top of the instrument.

Do not place liquids on top of the instrument. Fluid leaking into the cytometer could cause electrical shock or damage to the instrument.

Back of Cytometer

Allow 12.7 cm (5 in) between the back of the cytometer and the wall for proper ventilation.

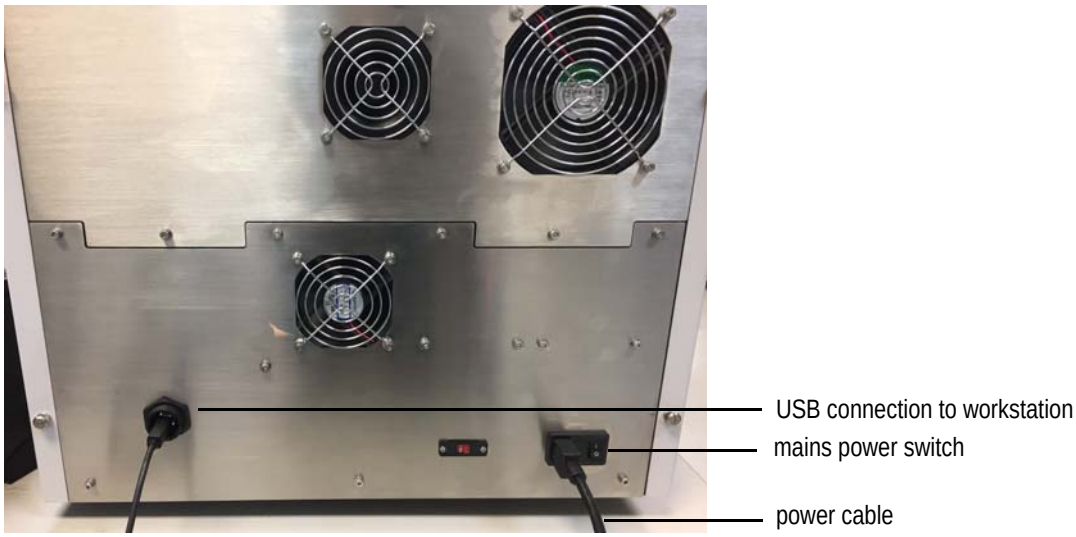


Figure 3. Back of cytometer

Fluidics

Sample Injection Port/Sample Injection Tube

Sample, contained in a standard 12 x 75-mm tube, enters the cytometer through the sample injection tube (SIT) that is contained within the sample injection port (SIP) [Figure 4]. The sample tube snaps into place under the SIP requiring no additional tube retention support. The SIT extends from the SIP during acquisition and retracts when the cytometer is not acquiring.

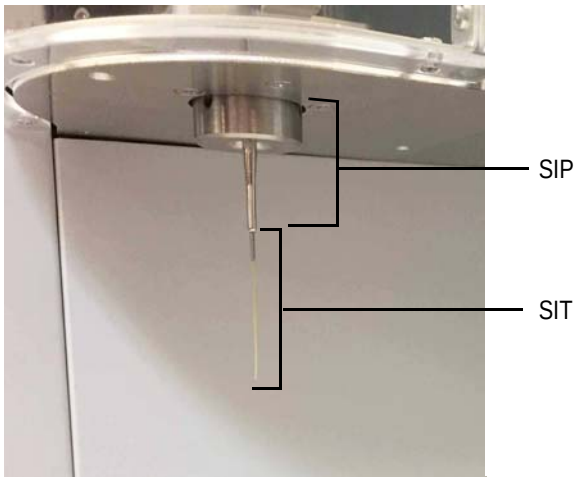


Figure 4. Sample injection port and sample injection tube

Fluid Containers

The Aurora draws sheath solution directly from a 20-L sheath cubitainer or the 4-L sheath tank provided by Cytek. It expels waste into an empty 20-L cubitainer or the 4-L waste tank provided by Cytek.

The fluidics tanks are contained in a holding reservoir located on the left side of the cytometer (Figure 5). The 4-L tank with the transparent fluidic line is for sheath solution. The 4-L tank with the orange fluidic line is for waste.

Figure 5. Aurora fluidics bottles and front panel

Fluid Flow

The Aurora fluidics is driven by vacuum. An accumulator vessel is the source of vacuum for the system. Sheath solution is drawn into and stored in the sheath plenum before passing through a sheath filter where debris and contaminants are removed. Before reaching the flow cell, the sheath stream passes through a degasser, which removes air bubbles. After passing the laser interrogation point, the combination of sheath solution and sample travels to the waste container.

Sheath and waste fluid levels are monitored by sensors. The waste level sensor is located underneath the waste tank cap. The sheath level sensor is located underneath the sheath plenum cap. Both sensors are monitored by the software.

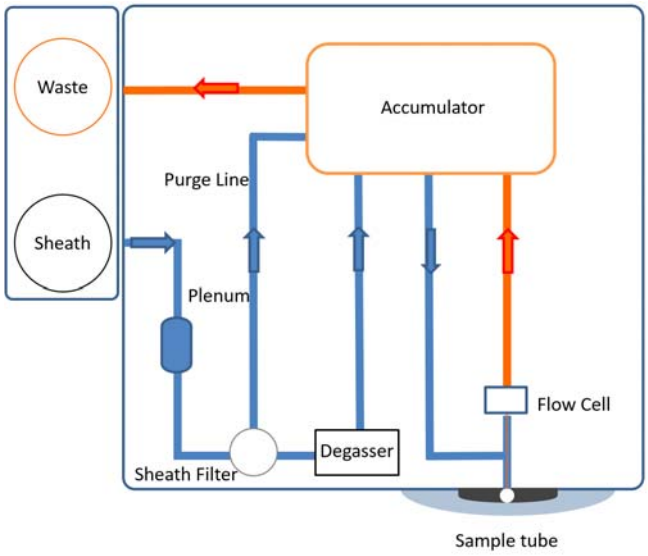


Figure 6. Aurora fluidics overview

Fluidics Components

The following figure shows the fluidics components.

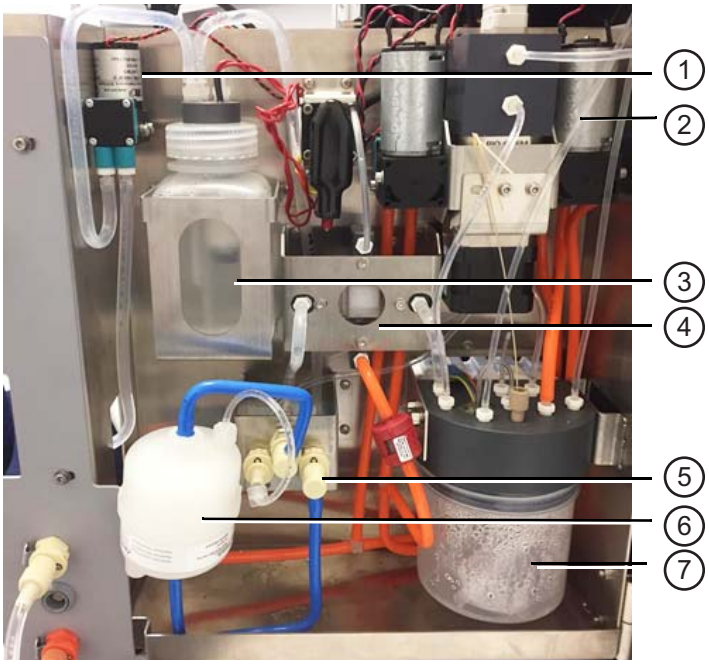


Figure 7. Fluidics components (inside fluidics compartment)

The following table describes the fluidics components.

No.	Component	Description
1	Plenum pump	Pulls sheath from the sheath tank to fill the plenum
2	Vacuum pump	Maintains the vacuum in the accumulator
3	Plenum	Storage vessel for sheath fluid before it flows to the sheath filter
4	Degasser	Removes air bubbles from the sheath fluid
5	Sheath filter quick connects (x3)	Sheath filter fluid input, fluid output, and vent line quick-connects
6	Sheath filter	Filters debris and particles from the sheath fluid
7	Accumulator	Vacuum source for the fluidics system

Optics

Unlike conventional flow cytometers that direct specific bandwidths of fluorescence light into discrete detectors or photomultiplier tubes (PMTs), the Aurora uses a solid-state, multi-channel, narrow-beam detector array for each laser. Each array can be configured with up to 16 detectors that are used to capture a part of the emission spectrum from each particle passing through the laser beam. The detector channels from all three lasers are used to capture the entire emission spectra from each fluorescent-labeled particle. Spectral deconvolution (unmixing) algorithms calculate the contribution of the known individual fluorophore's spectra to the total collected signal.

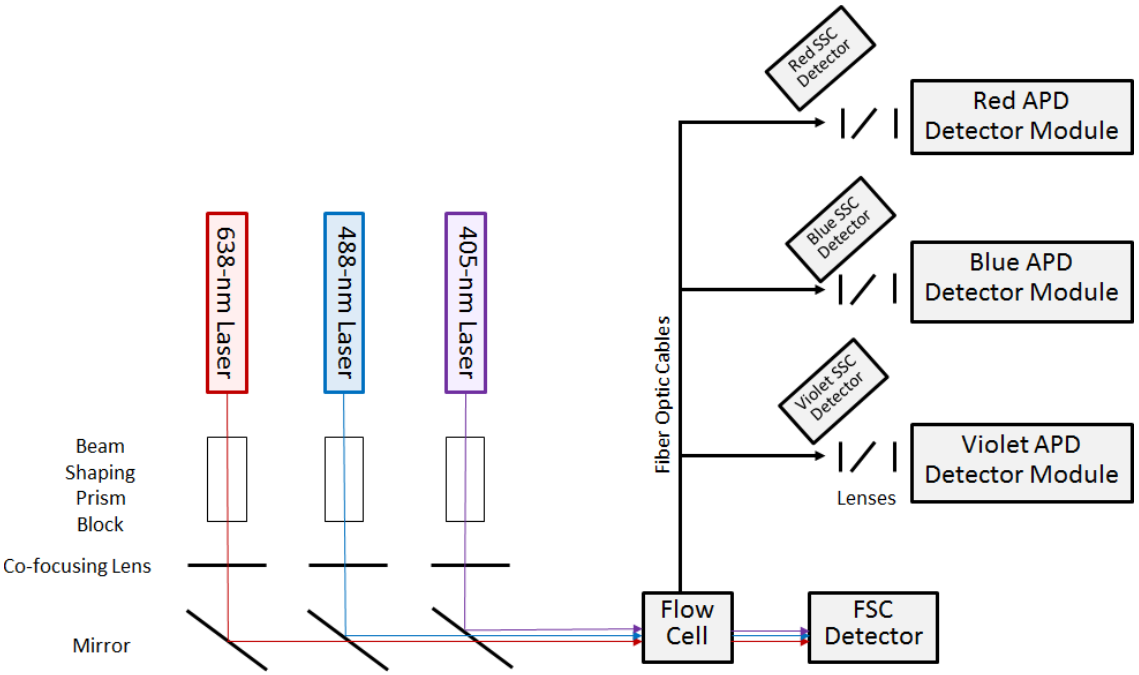


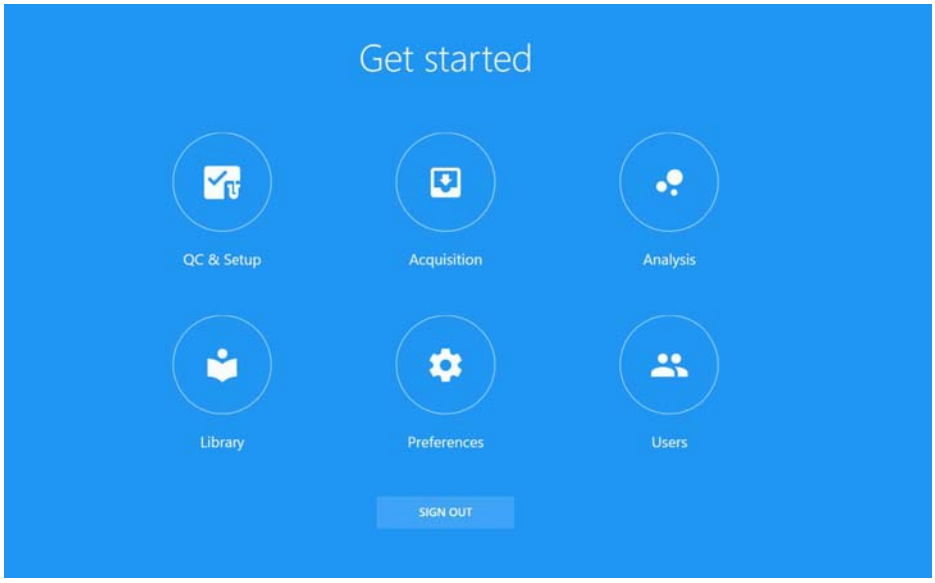
Figure 8. Optical schematic

The default optical configuration has 16 channels for detection off the violet laser, 14 channels off the blue laser, and 8 channels off the red laser. Detectors are referred to as V1–V16, B1–B14, and R1–R8, for the violet, blue, and red lasers, respectively. The wavelengths detected by each detector (channel) increase across the array. See the table on page 52 for details.

For excitation, a proprietary flat-top laser design enables a constant power distribution across the width of the flow cell.

Software Overview

SpectroFlo software allows you to acquire and analyze samples and adjust instrument settings. Once you log into the software, a Get started menu appears with six modules from which to choose.



Six options provide workspaces that allow you to perform various functions.

Module	Description
QC & Setup	Daily QC ensures that the instrument is in optimal condition for use. Run SpectroFlo QC beads daily to assess system performance and allow the software to adjust settings for day-to-day variation. Levey-Jennings reports keep track of trends in system performance. Setup allows you to create Reference Controls. See “QC & Setup” on page 21 for more information.
Acquisition	The Acquisition workspace allows you to create experiments to acquire and analyze data. Experiments can be created through a guided wizard or created from previously saved templates. See “Acquisition” on page 31 for more information.
Analysis	Here, FCS files can either be unmixed or compensated using virtual filters. See “Advanced Unmixing” on page 47 for more information.
Library	The Library allows you to store experiment templates, worksheet templates, user settings, fluorescent tags, SpectroFlo QC bead information, and label information. See “Library” on page 55 for more information.
Preferences	Software preferences can be changed to customize the software. Default plot sizes, fonts, gate colors, print layouts, statistics box table option, and more can all be changed in the Preferences. See “Preferences” on page 59 for more information.
Users	The Users workspace contains user management options and administrative controls. See “Users” on page 67 for more information.

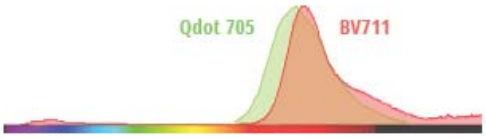
Spectral Unmixing

Spectral unmixing is an important concept to understand how data is generated and analyzed using the Aurora flow cytometer with SpectroFlo software. Spectral unmixing is used to identify the fluorescence signal for each fluorophore used in a given experiment.

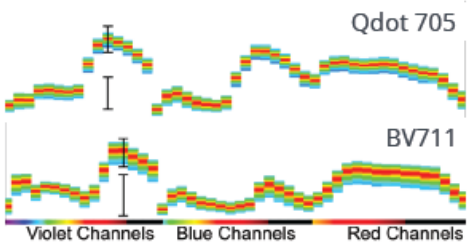
Understanding Full Spectrum Flow Cytometry

Because fluorophores emit light over a range of wavelengths, optical filters are typically used to limit the range of frequencies measured by a given detector. However, when two or more fluorophores are used, the overlap in wavelength ranges often makes it impossible for optical filters to isolate light from a given fluorophore. As a result, light emitted from one fluorophore appears in a non-primary detector (a detector intended for another fluorophore). This is referred to as spillover. In conventional flow cytometry spillover can be corrected by using a mathematical calculation called compensation. Single-stained controls must be acquired to calculate the amount of spillover into each of the non-primary detectors.

The Aurora's ability to measure a fluorochrome's full emission spectra allows the system to use a different method for isolating the desired signal from the unwanted signal. The key to differentiate the various fluorochromes is for those to have distinct patterns or signatures across the full spectrum. Because the system is looking at the full range of emission of a given fluorochrome, and not only the peak emission, two dyes with similar emission but different spectral signatures can be distinguished from each other. The mathematical method to differentiate the signals from multiple fluorochromes is call spectral unmixing. Just as for compensation, single-stained controls, identified in SpectroFlo software as Reference Controls, are still necessary, as they provide the full fluorescence spectra information needed to perform spectral unmixing.



Spectrum plots from conventional spectrum viewer shows heavy overlap between Qdot 705 and BV711.



Spectrum plots from Aurora show distinct signatures for Qdot 705 and BV711.

Reference Spectra

Reference Controls, obtained by running single-stained and unstained samples, provide the individual fluorescence spectra necessary to unmix the data. Either beads or cells can be stained for use as Reference Controls. These controls can be acquired in the Reference Group of the experiment during acquisition, or they can be acquired as Reference Controls in the QC & Setup workspace. If Reference Controls are acquired in the QC & Setup workspace, they are stored and can be used as Reference Controls for subsequent experiments.

3

Startup & Shutdown

Filling the Sheath and Emptying the Waste

The color-coded sheath and waste quick-connects and the waste level sensor connector are located at the lower-left corner of the front panel.

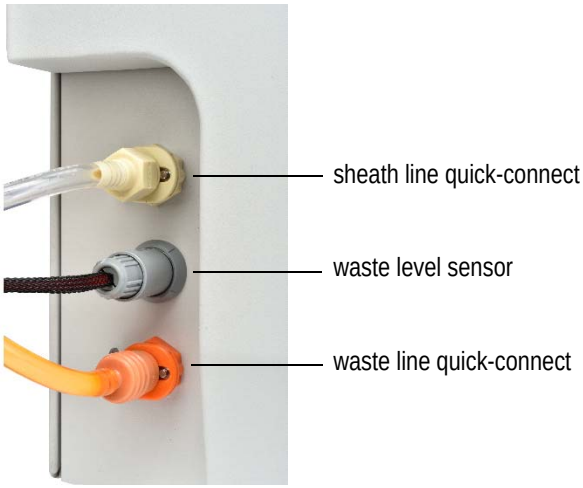


Figure 9. Sheath and waste line quick-connect and waste level sensor

Filling the Sheath

Fill the sheath container with manufacturer-provided sheath solution, MilliQ™ water, phosphate-buffered saline (PBS), or DI water.

Sheath can be drawn from either the supplied 4-L sheath tank or directly from a 20-L cubitainer.

Sheath solution can be added to the sheath container while the instrument is running. The plenum provides 5 minutes of run time at any flow rate while the tank is being filled or replaced.

Before turning on the cytometer, visually inspect all containers. Wear the recommended protective laboratory attire such as protective gloves, eyewear, and lab coat.

Fill the sheath container as needed. Use only the appropriate sheath solution. Never use tap water in the sheath container.

Filling Sheath into a Cytek 4-L Sheath Tank or a 20-L Cubitainer:

1. Remove the sheath fluidic line cap from the cubitainer or sheath tank lid from the Cytek sheath tank.
2. Add the appropriate sheath solution.
3. Replace the fluidic line cap or sheath tank lid. Do not over-tighten.
4. If the cytometer is powered on and the software is connected, verify that the software sheath indicator is green.

Emptying the Waste

Waste can be expelled into either the supplied 4-L waste tank or directly into an empty 20-L cubitainer.

Empty the waste container when filling the sheath container or as needed to prevent leakage. The software indicator for waste will be yellow or red when the container needs to be emptied. Take care to avoid damaging the fluid level sensor in the waste tank.

Biological samples are potentially dangerous and/or life threatening. Adhere to proper handling procedures for samples and reagents. Wear appropriate laboratory attire such as protective gloves, eyewear, and lab coat during this procedure.

Always treat the contents of the waste container with bleach (10% of the total volume). Contents of the waste container may contain biohazardous material.

Removing Waste from a Cytek 4-L Waste Tank or a 20-L Cubitainer:

1. Disconnect the waste line quick-connect from the front of the cytometer.
2. Disconnect the waste line orange quick-connect from the cubitainer cap or 4-L waste bottle. Disconnect the waste level sensor.

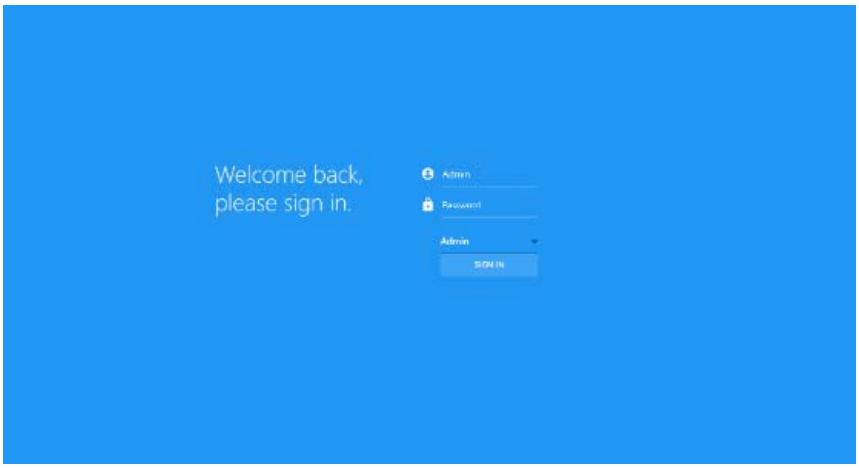
   The waste level sensor connector for the cubitainer is on the cubitainer cap. The waste level sensor connector for the 4-L tank is on the front of the cytometer.
3. Remove the waste cap from the cubitainer or the lid from the 4-L waste tank, taking care not to damage the liquid level sensor.
4. Dispose of the waste per local regulations.
5. Add 2 L of undiluted bleach to the waste cubitainer, or 400 mL of bleach to the waste tank.
6. Replace the waste cap/lid to the container. Hand-tighten the cap/lid until it is fully closed.
7. Reattach the waste line and level sensor line to the cap/lid and front of the cytometer.
8. If the cytometer is powered on and the software is connected, verify that the software waste indicator is green.

Starting Up the System

1 Turn on the workstation, then turn on cytometer.

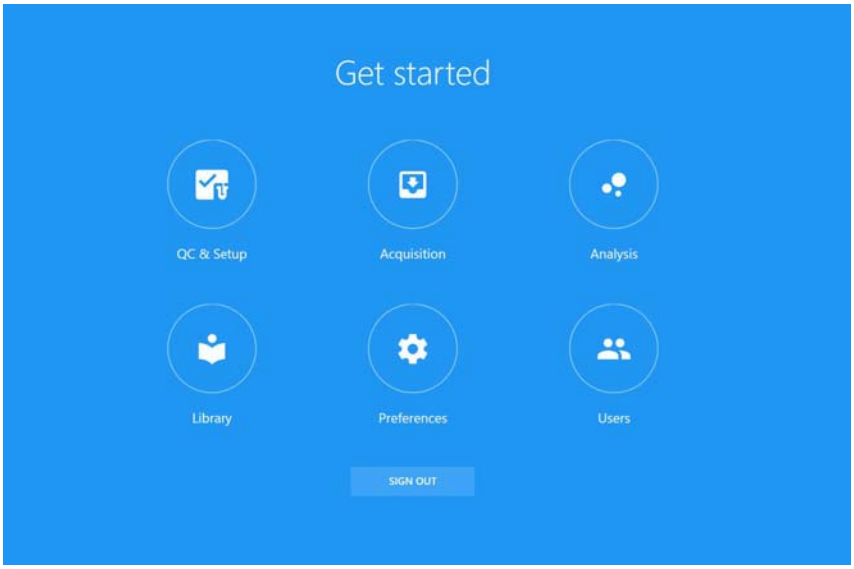
■ **NOTE:** Ensure that a tube of DI water is loaded on the SIP before launching SpectroFlo software. The tube is required for the SIT depth calibration.

2 Launch SpectroFlo software and log in.



The cytometer initialization procedure begins. Sheath fluid is flushed through the fluidics lines to eliminate any saline buildup. The system calibrates the SIT depth and the sample flow rate.

3 Select QC & Setup from the Get Started screen.



4 Check the status indicator in the lower-right corner of the screen. Ensure the indicator for Connected is a green checkmark. If the indicator shows the instrument is not connected, check to ensure that the USB connection between the cytometer and workstation is plugged into the appropriate ports. See “Back of Cytometer” on page 11.

5 Check the sheath and waste level checkmark indicators. Ensure the status checkmark indicators for sheath and waste are green before proceeding.

Fluid Indicator	Meaning
Yellow sheath indicator	Sheath tank is low and requires refilling (see “Filling the Sheath” on page 17).
Red sheath indicator	Sheath tank is empty and requires refilling (see “Filling the Sheath” on page 17).
Yellow waste indicator	Waste tank is nearing capacity and requires emptying (see “Emptying the Waste” on page 18).
Red waste indicator	Waste tank is full and requires emptying (see “Emptying the Waste” on page 18).

6 The cytometer is now ready for Daily QC. See “Performing Daily QC” on page 21.

Shutting Down the System

The shutdown procedure flushes the flow cell and sample lines with bleach and DI water. The software provides instructions during the shutdown procedure. The SIT will remain protracted from the SIP at the end of the shutdown procedure to ensure the SIT does not dry and form clogs.

1 In the Cytometer menu from either the QC & Setup or Acquisition workspace, select Fluidics Shutdown.

2 Place a tube containing 3 mL of 10% bleach on the SIP. Once loaded, the instrument begins drawing in the tube contents. This takes approximately 2 minutes.

3 When prompted, remove the tube and place a tube containing 3 mL of DI water on the SIP. Once loaded, the instrument begins drawing in the tube contents. This takes approximately 2 minutes.

4 Leave the tube of DI water on the SIP. Make sure the SIT is submerged in the DI water at the end of the Fluidics Shutdown procedure.

5 Exit SpectroFlo software by clicking the X in the upper-right corner of the application window.

6 Turn off the cytometer and workstation.

4

QC & Setup

Daily QC

Run Daily QC using SpectroFlo QC beads prior to acquiring samples to ensure that the cytometer is performing optimally. Daily QC assesses the instrument's optical alignment and the system performance drift by measuring rCVs and gain needed to place the beads at the target locations for each detector. During QC, laser delays and area scaling factors are optimized and gain settings are adjusted to ensure day-to-day repeatability. Upon completion of Daily QC, a QC report is generated. QC reports can be reviewed under the Reports tab.

Performance can be tracked and charted over time in the Levey-Jennings tab. The software can be configured to display a warning if the QC result on the QC report exceeds user-defined criteria. See "Alarm Ranges" on page 30.

Performing Daily QC

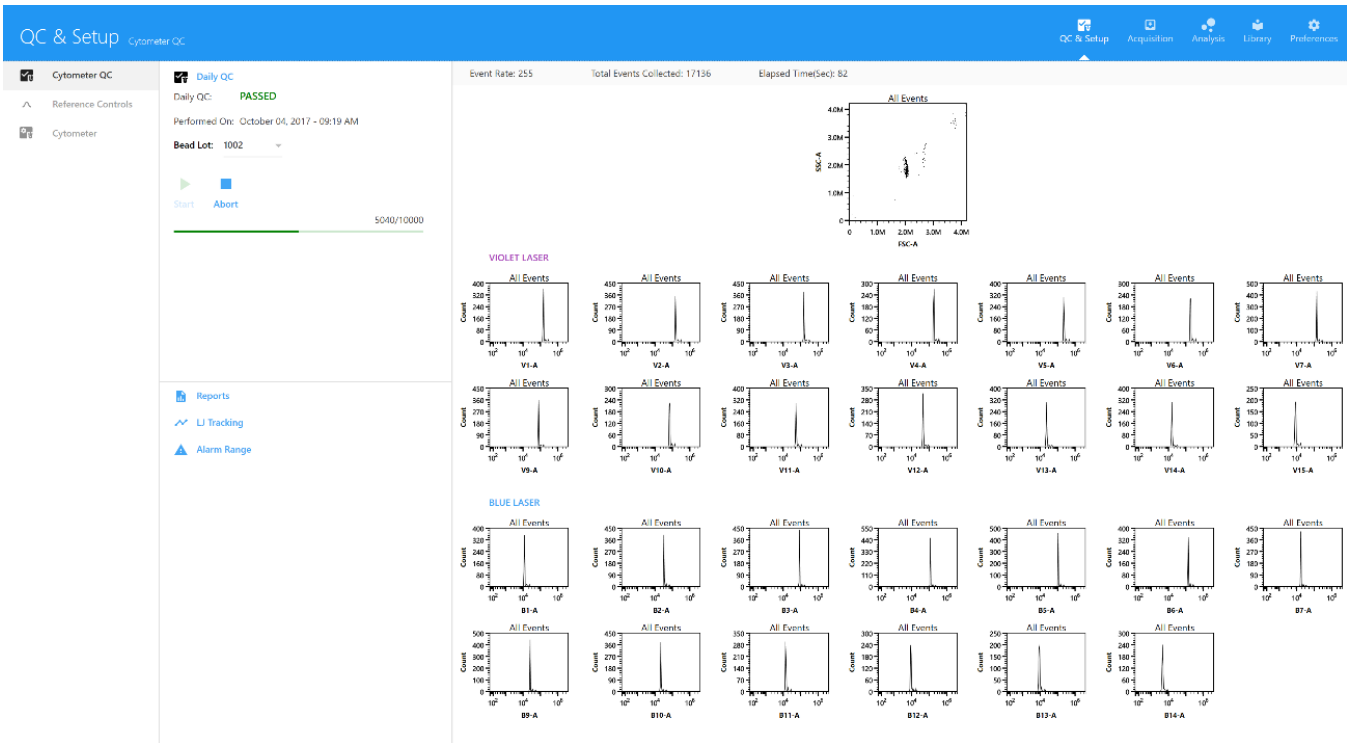
Daily QC ensures that the instrument is performing optimally. Instrument performance is characterized and tracked, laser delays and area scaling factors are determined, and user gain settings are adjusted to account for day-to-day instrument variability.

1. Allow at least 30 minutes to elapse after turning on the instrument to ensure the lasers are warmed up.

2. Load a 12 x 75-mm tube of SpectroFlo QC beads (1 drop in 0.5 mL sheath, PBS, or DI water) onto the SIP.

   The SpectroFlo QC beads are hard dyed polystyrene beads that have a single fluorescence intensity. They can be excited by each laser and emit fluorescence in all detector channels.

3 In the QC & Setup workspace, select Daily QC.

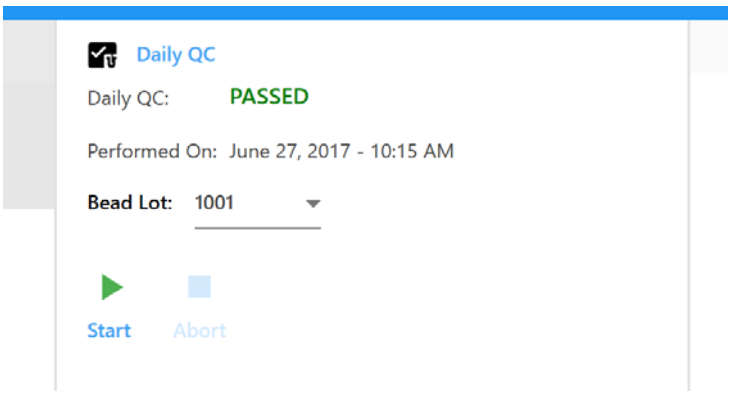


4 Select the appropriate bead lot from the Bead Lot menu.

Each time you open a new lot number of SpectroFlo QC beads you must import the bead lot ID into the Library so it is accessible when you run QC.

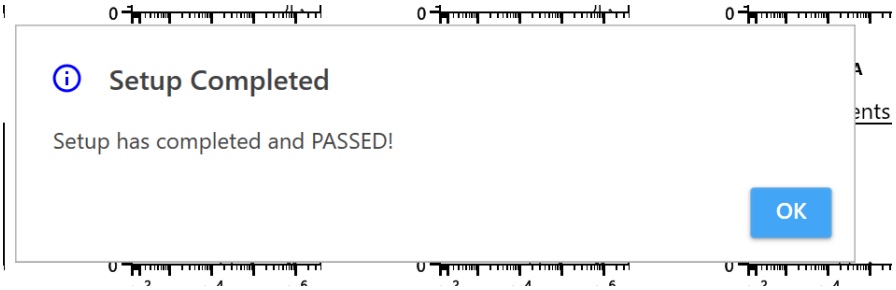
Different bead lots have different fluorescent intensities. Always select the correct bead lot when performing Daily QC.

5 Select Start to begin the Daily QC run.



The instrument begins acquiring the QC beads. The procedure takes approximately 3 to 5 minutes to complete.

6 When Daily QC passes, the following message is displayed.



You are now ready to acquire samples.

QC Report

At the completion of the Daily QC run, a QC report is generated. The report includes the following sections:

- The header section contains the name of the instrument, date the Daily QC was run, user who ran the Daily QC, instrument configuration, instrument serial number, SpectroFlo QC bead lot and expiration date, and Pass/Fail status of the run.
- The results section contains the gain, gain change, median fluorescent intensity of the daily QC bead, %rCV, and a pass/fail indicator for each detector channel. The center wavelength of the detector is shown in parentheses next to the detector name.
- The Laser Settings section contains the laser delays for all non-primary lasers, and area scaling factors for all lasers and the FSC detector

Pass/Fail Criteria - The pass/fail criteria are the following:

- %rCV must not exceed 6% for the FSC channel
- %rCV must not exceed 8% for the SSC channel
- %rCV must not exceed 6% for the V3 channel
- %rCV must not exceed 6% for the B3 channel
- %rCV must not exceed 6% for the R3 channel
- Delta gain for all channels must not exceed 100 from the last Daily QC run performed by Cytek Service personnel.

The number of reports listed in the Reports screen can be set in the Preferences. See “QC Setup” on page 66 for more information.

Daily QC Report

Setup Status:	PASSED	Date:	October 28, 2017 - 17:03 PM
Cytometer Name:	My Aurora	User:	Admin
Configuration:	3-Lasers-V16-B14-R8	Serial Number:	R0001

QC Beads

Lot ID: 1002 Expiration Date: December 31, 2019

Laser	Detector (nm)	Gain	Gain Change	Median (x1000)	% rCV	Status
Blue	FSC	174	-26	1,843.2	2.57	✓
Violet	SSC	342	9	2,087.8	4.45	✓
Violet	V1 (428)	381	55	202.2	3.96	✓
Violet	V2 (443)	212	19	205.7	3.97	✓
Violet	V3 (458)	201	17	202.5	4.11	✓
Violet	V4 (473)	153	19	244.1	3.98	✓
Violet	V5 (508)	197	13	302.0	4.04	✓
Violet	V6 (528)	248	12	243.1	4.04	✓
Violet	V7 (549)	233	13	182.7	4.01	✓
Violet	V8 (571)	256	19	123.1	3.79	✓
Violet	V9 (594)	251	14	102.5	3.80	✓
Violet	V10 (618)	381	18	91.0	3.78	✓
Violet	V11 (664)	638	46	72.6	3.73	✓
Violet	V12 (692)	974	59	60.6	3.71	✓
Violet	V13 (720)	530	36	31.5	3.75	✓
Violet	V14 (750)	531	40	21.0	3.88	✓
Violet	V15 (780)	793	75	10.8	5.96	✓
Violet	V16 (812)	461	35	4.2	7.93	✓
Blue	B1 (508)	231	2	14.1	2.62	✓
Red	R7 (783)	895	-9	79.4	5.85	✓
Red	R8 (812)	326	0	39.3	6.11	✓

Laser Settings

Laser	Laser Delay	Area Scaling Factor
Violet	-24.95	1.19
Blue	0.00	1.20
Red	27.50	0.85

FSC Area Scaling Factor: 1.24

Specifications

FSC	% rCV:	< 6	(Recommended)
SSC	% rCV:	< 8	(Recommended)
V3	% rCV:	< 6	(Recommended)
B3	% rCV:	< 6	(Recommended)
R3	% rCV:	< 6	(Recommended)
All Channels	% Gain Change:	< 100	(Recommended)

Instrument Setup - Reference Controls

Reference Controls must be acquired and recorded to ensure accurate spectral unmixing of the data. References are obtained by acquiring particles stained with individual fluorescent tags. Either beads or cells can be used as single-stained controls for acquiring references. You can select whether to create new Reference Controls or update Reference Controls already stored in the Library.

- Reference Control - references stored and retrieved from the Library
- Reference Group - references acquired and recorded in the experiment

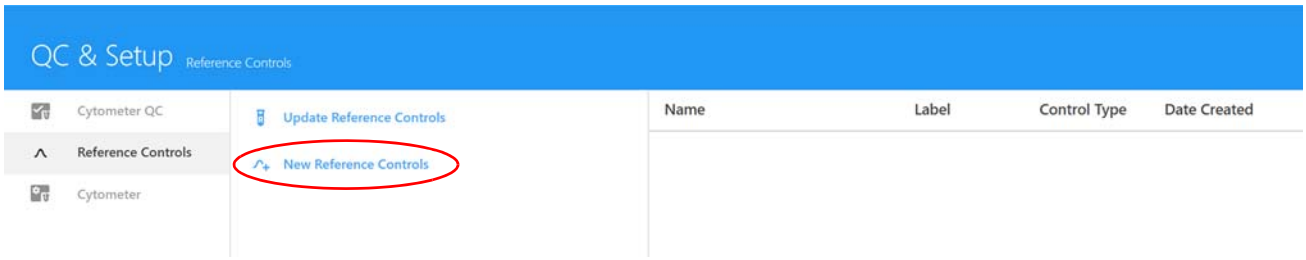
A step-by-step wizard guide you through recording Reference Controls.

Creating Reference Controls

To create Reference Controls you will need to define the fluorescent tags, define the controls, then label the fluorescent tags.

1 Select New Reference Controls from the Reference Controls tab in the QC & Setup workspace.

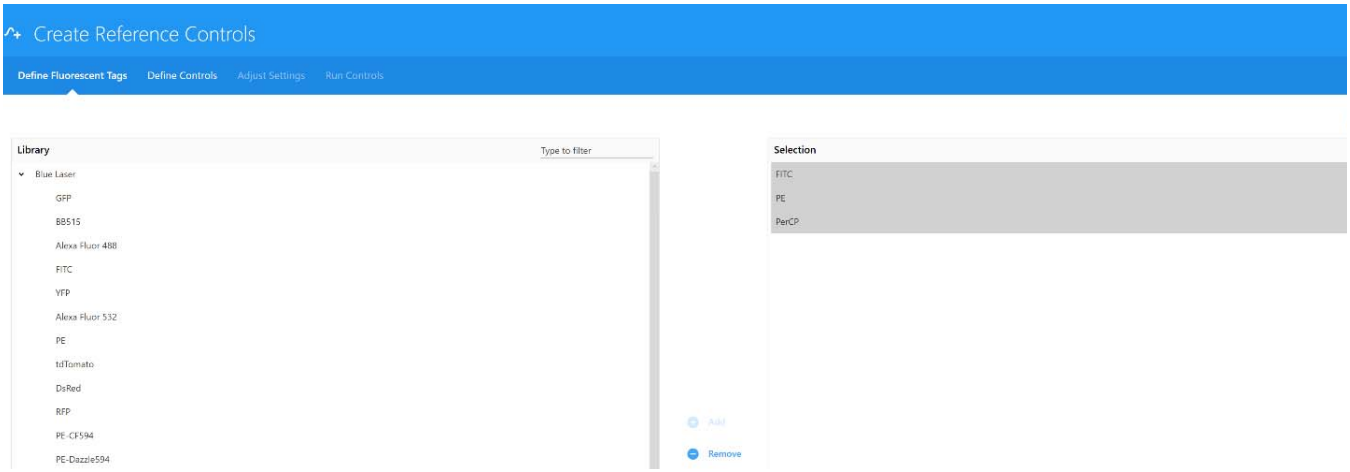
A wizard opens allowing you to create new Reference Controls.



2 Select fluorescent tags. The left pane displays the fluorescent tag groups found in the Library.

- Click the arrow to the left of the fluorescent tag group name to view the fluorescent tags associated with the group. (The default fluorescent tag groups are Blue Laser, Red Laser, and Violet Laser and contain a list of commonly used fluorescent tags excited by their respective lasers).
- From the expanded list of fluorescent tags, select the fluorescent tags used in the experiment. Once selected the fluorescent tags appear in the selection pane on the right side of the Define Fluorescent Tags window. You can select fluorescent tags by dragging and dropping, double-clicking, or using the Add button. Multiple tags can be chosen at one time. Confirm the tags selected, then click Next.

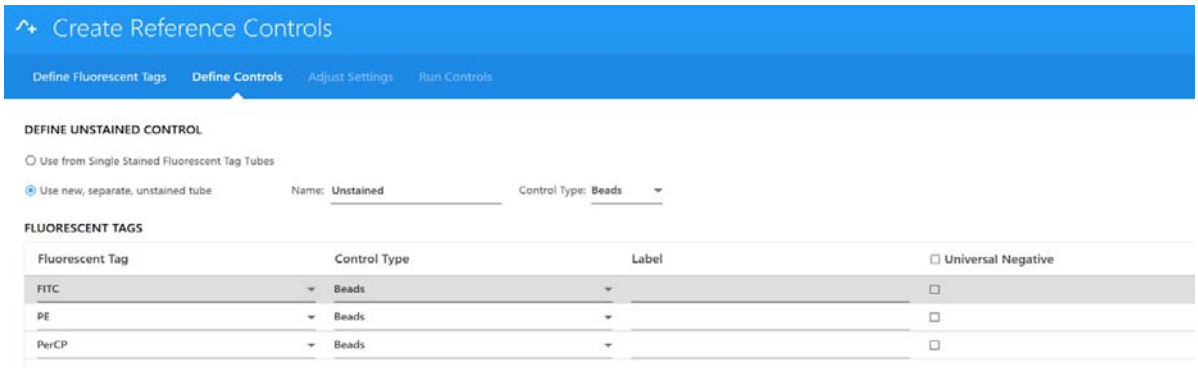
■ NOTE: The list of fluorescent tags can be edited in the Library. You can use the Library to add fluorescent tags that are not present in the default list. See “Fluorescent Tags” on page 55 for more information.



3 Define the control type for the fluorescent tags, as well as the unstained controls. Once the controls have been defined, select **Next**.

Either beads or cells can be stained and defined as control types. This allows you to keep track of control types. If any of the fluorescent tag controls lack a negative population and are of the same type as the unstained control, check the **Universal Negative** checkbox at the right.

4 (Optional) Enter labels associated with the fluorescent tag for identification and tracking.

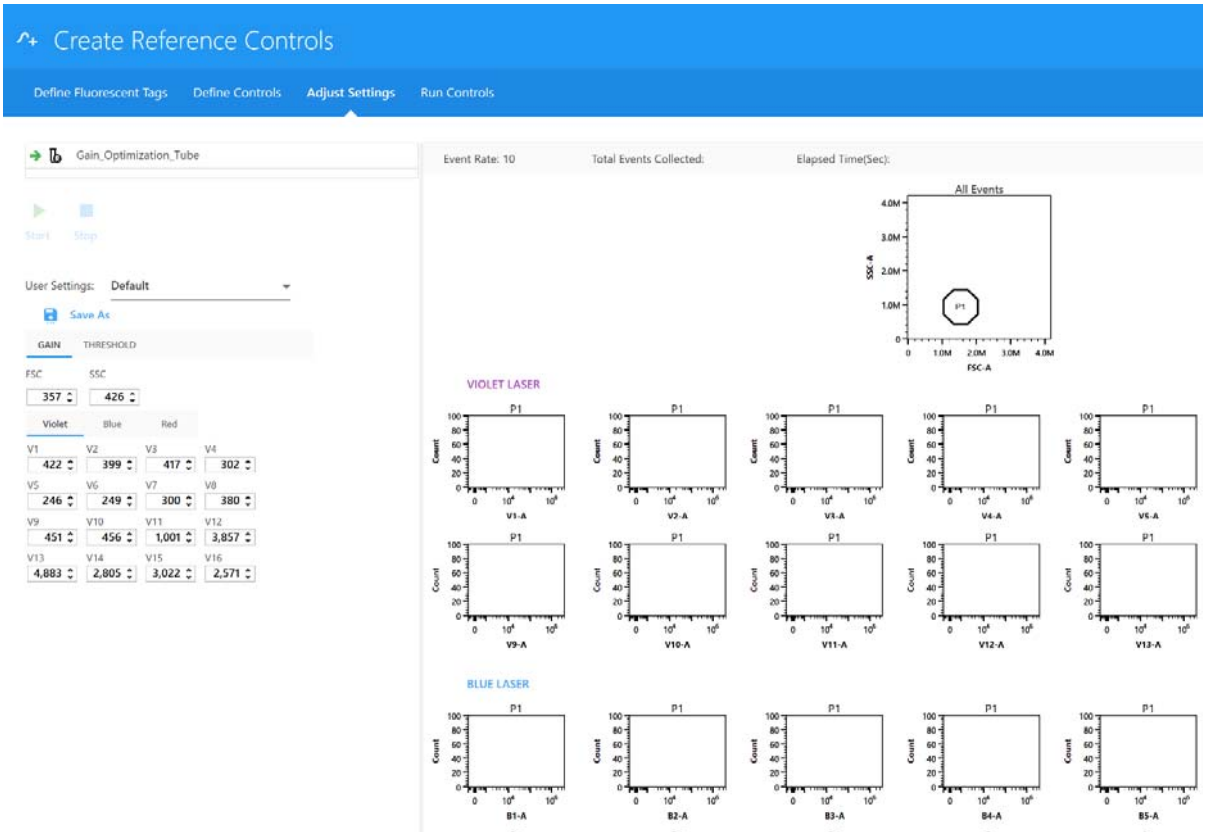


5 If necessary, adjust gain settings. Place the appropriate sample on the SIP and click **Start** to view the data.

Gain settings for all channels can be selected from the User Settings drop-down menu, or they can be individually adjusted for each channel using the detector gain spinboxes (V1-V16, B1-B14, and R1–R8).

The Adjust Settings screen allows you to view the data to ensure that the positively stained fluorescent particles are not off scale. FSC gain can be adjusted from 0-1000. SSC and detector channel gain can be adjusted from 0-10,000. If the positive population is off-scale for any detector channels, lower the gain setting for that channel. If the positive population is not sufficiently separated from the negative population within a specific channel, adjust the gain setting for that channel.

■ **NOTE:** Dim markers may not separate from the negative population regardless of how much the gain is increased.



6 Select Next when you are satisfied with the gain settings. Proceed to running controls.

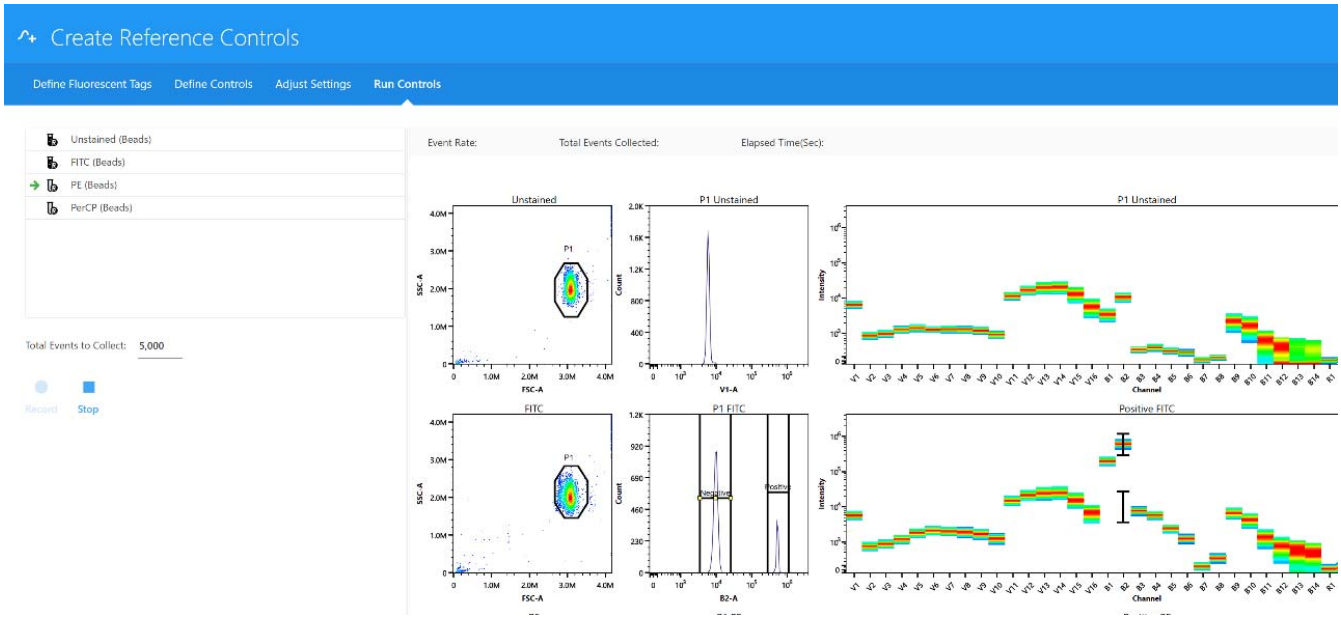
Running Reference Controls

Once gain settings have been confirmed, unstained and Reference Controls are ready for acquisition.

1 Place a tube of the appropriate single-stained particles on the SIP. Click Record to begin acquiring.

Make sure to follow the order listed in the left-hand panel.

During acquisition the spectra plot for each fluorescent control is displayed. The plots show all the channels across all lasers in the x-axis vs mean fluorescence intensity (MFI) of the fluorescent tag.



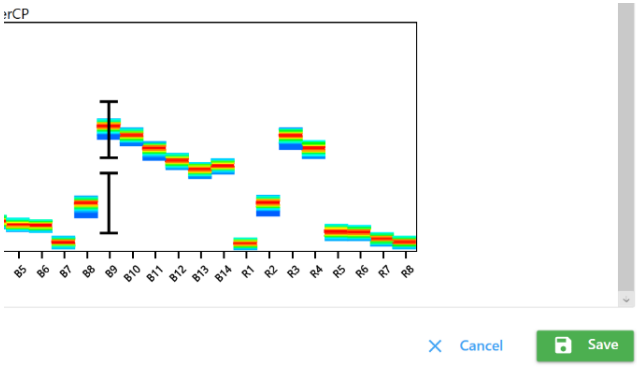
2 During acquisition obtain spectral information by moving the polygon gate on the FSC-A vs SSC-A plot to include the population of interest.

Hold down the Ctrl key while adjusting the gate to move the polygon gates for all the fluorescent tags at once. The gated population appears in the histogram, which is set to the peak emission channel of the fluorescent tag to be acquired. The emission spectrum of the population is displayed in the spectrum plot.

Adjust the positive gate on the histogram. The software automatically displays the emission spectrum of the positive particle in the spectrum plot. SpectroFlo software sets the default gate on the peak emission channel. The gate can be selected manually. It is best to set the gate on the brightest emission as this can make distinguishing the positive and negative population easier.

Readjust the positive and/or negative gate on the histogram, if necessary.

3 Select Save to save the Reference Controls to the Library.



Updating Reference Controls

You may wish to update the Reference Controls if any of the following occur:

- Major service performed on the instrument
- Fluorochrome exhibiting signs of instability
- Instrument exhibiting signs of instability

The Reference Controls tab displays the Reference Controls saved in the Library. Click the arrow next to the control name to display the details.

To Update Reference Controls:

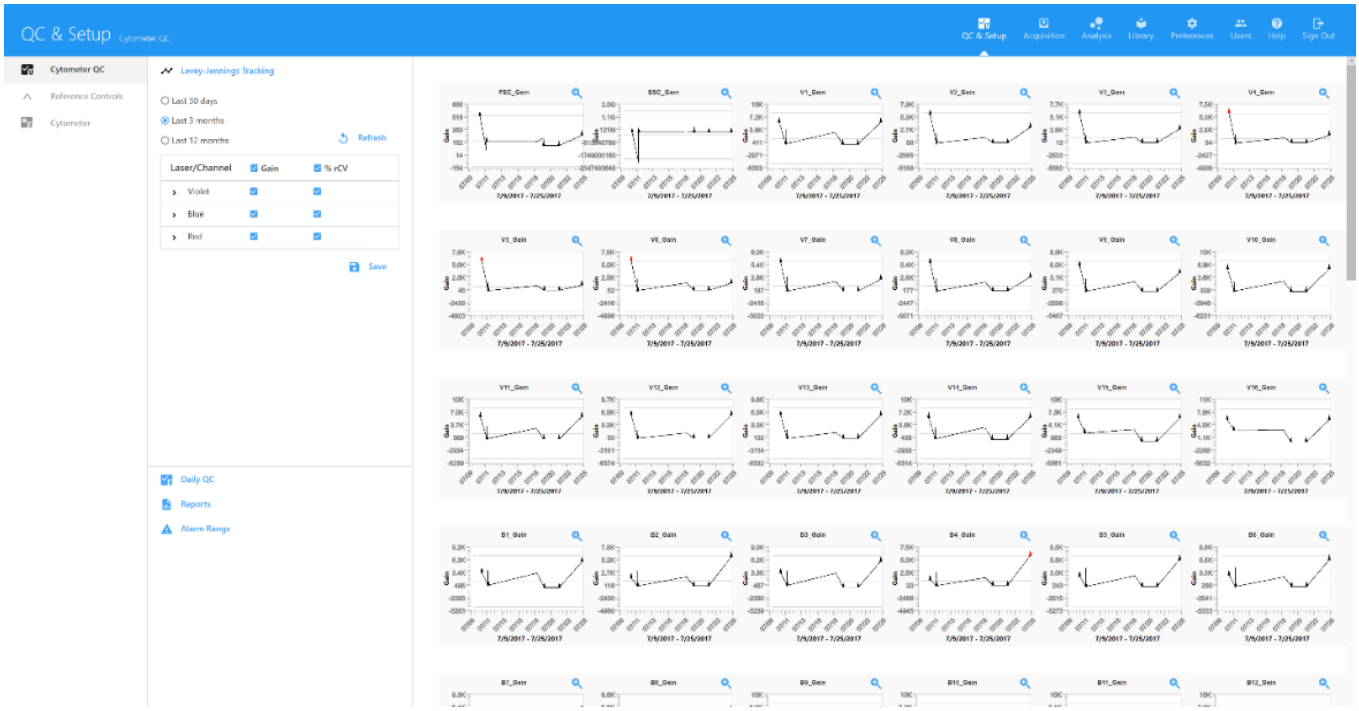
1 Select Update Reference Controls from the Reference Controls tab in the QC & Setup workspace.

A wizard opens allowing you to update Reference Controls.

2 Follow steps 2 through 6 in “Creating Reference Controls” on page 25.

Levey-Jennings Tracking

Levey-Jennings reports track %rCV and gain for all detector channels over time, allowing you to view the system’s performance and ensure that the system is reproducing consistent results. The graphs in the report show you random errors or shifts and trends in the data for each parameter. Data from the last 30 days, 3 months, or 12 months can be included in the reports.

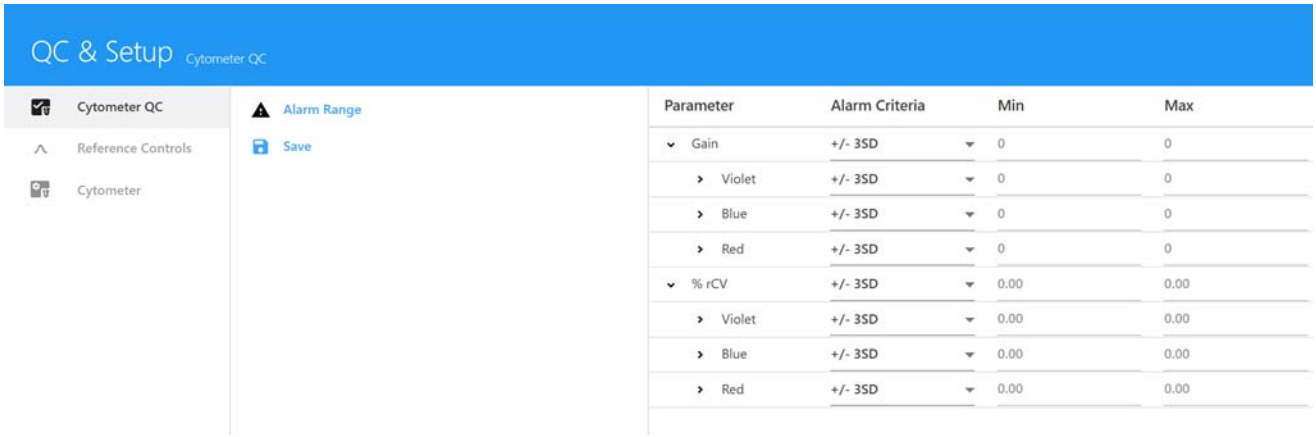


Gain Settings

The amount of signal amplification applied to each detector channel can be modulated by increasing or decreasing the amount of gain applied. The gains for every detector channel can be saved and are collectively known as the user settings. User gain settings are stored as a ratio against the Daily QC. Every time Daily QC is performed, User Settings will be adjusted accordingly.

Alarm Ranges

You can set an alarm to warn you when the gain and %rCV exceeds the passing criteria that you define. This changes the outliers (shown in red) in the LJ graphs. Select Alarm Range from the Cytometer QC tab, then adjust the SD range (plus or minus) for individual detectors for each laser.



5

Acquisition

Raw vs Unmixed data

SpectroFlo software saves flow cytometry data in the FCS 3.1 format. Data is saved in both raw and unmixed formats. Raw data contains all the fluorescence information from each detector. Each detector channel is designated by its excitation laser and position in the array. For example, B3 is the third channel of the blue laser detector array.

Unmixed data has been spectrally deconvolved based on a set of fluorescent tags and their corresponding Reference Controls. Fluorescence information in unmixed data is classified according to the reference spectra.

The Acquisition workspace provides the necessary tools that allow you to lay out an experiment worksheet. An experiment is a set of tubes, instrument settings, acquisition criteria (stopping rule), fluorescent tags, labels, and worksheets designed for the acquisition of samples.

New and saved experiments can be created or accessed in the Experiments tab of the Acquisition workspace.

Unmixing and Compensation

Raw FCS files can be spectrally unmixed in the following ways:

- Reference Group from the Experiment – Reference controls collected as FCS files along with the experiment can be used to unmix using the Unmixing wizard in the Acquisition workspace.
- Using Reference Controls – Reference controls stored in the Library can be used to unmix using the Unmixing wizard in the Acquisition workspace.
- Unmixing from the Analysis workspace – FCS files collected from different experiments can be unmixed in the Analysis workspace. FCS files can be imported and unmixed in this workspace.

Raw FCS files can also be compensated with the conventional method using the Virtual Filters tab in the Analysis workspace. Detector channels can be binned together to simulate the analysis of the data as if it were acquired using a filter. See “Advanced Unmixing” on page 47 for more information.

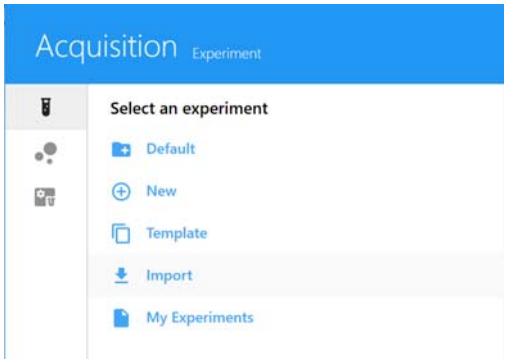
Setting Up an Experiment

Setting up the experiment in SpectroFlo software involves:

1. (Optional) Providing a name and description for the experiment. A default name is provided.
2. Specifying the fluorescent tags used in the experiment.
3. Defining the Reference Group as acquired in the experiment, originating from the Library, or a combination of both.
4. Selecting which acquisition worksheet to use—either new or from a template.
5. Defining the acquisition criteria (stopping rule based on events or time).
6. Adjusting gains for the appropriate detectors.

The Acquisition workspace provides the necessary elements for data collection. Flow cytometer data can be acquired from experiments. Experiments can be created either through the new experiment wizard or using experiment templates.

New or saved experiments can be created or accessed in the Experiment tab of the Acquisition workspace.



New experiments can be created using several different methods:

Method	Description
Default	Opens a new experiment with a list of tubes in groups and a set of labels and fluorescent tags in a default experiment worksheet template. The default experiment is user configurable. The Default experiment is the quickest way to access the experiment workspace to begin sample acquisition. Change made to the default worksheet can be saved, however it is not recommended.
New	Opens the New Experiment Wizard to guide you through creating an experiment.
Template	Allows you to select from a list of previously created templates.
Import	Imports template files that have been exported.

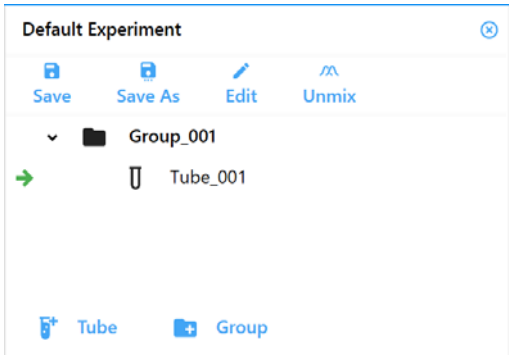
Method	Description
My Experiments	Allows you to select from a list of saved experiments. Experiments are organized into two categories—original (raw data) and unmixed. **NOTE:** Original experiments can be duplicated without data, which is equivalent to opening an experiment template.

Experiment Display

The experiment display in the Acquisition workspace includes the following panes:

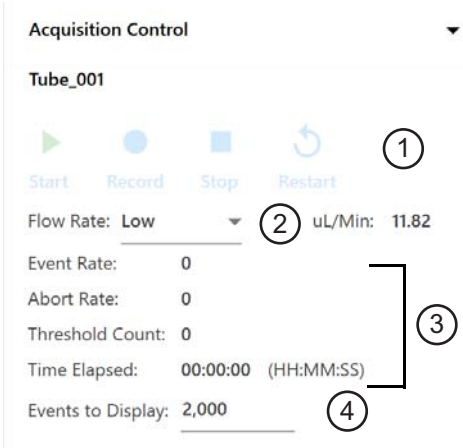
Sample List and Hierarchy

The samples are listed in the upper left of the workspace. Samples can be organized into groups.



Acquisition Control

The Acquisition Control pane allows you to acquire a tube, record data, stop acquisition, and restart acquisition. The acquisition controls are enabled only when a tube is present on the SIP.

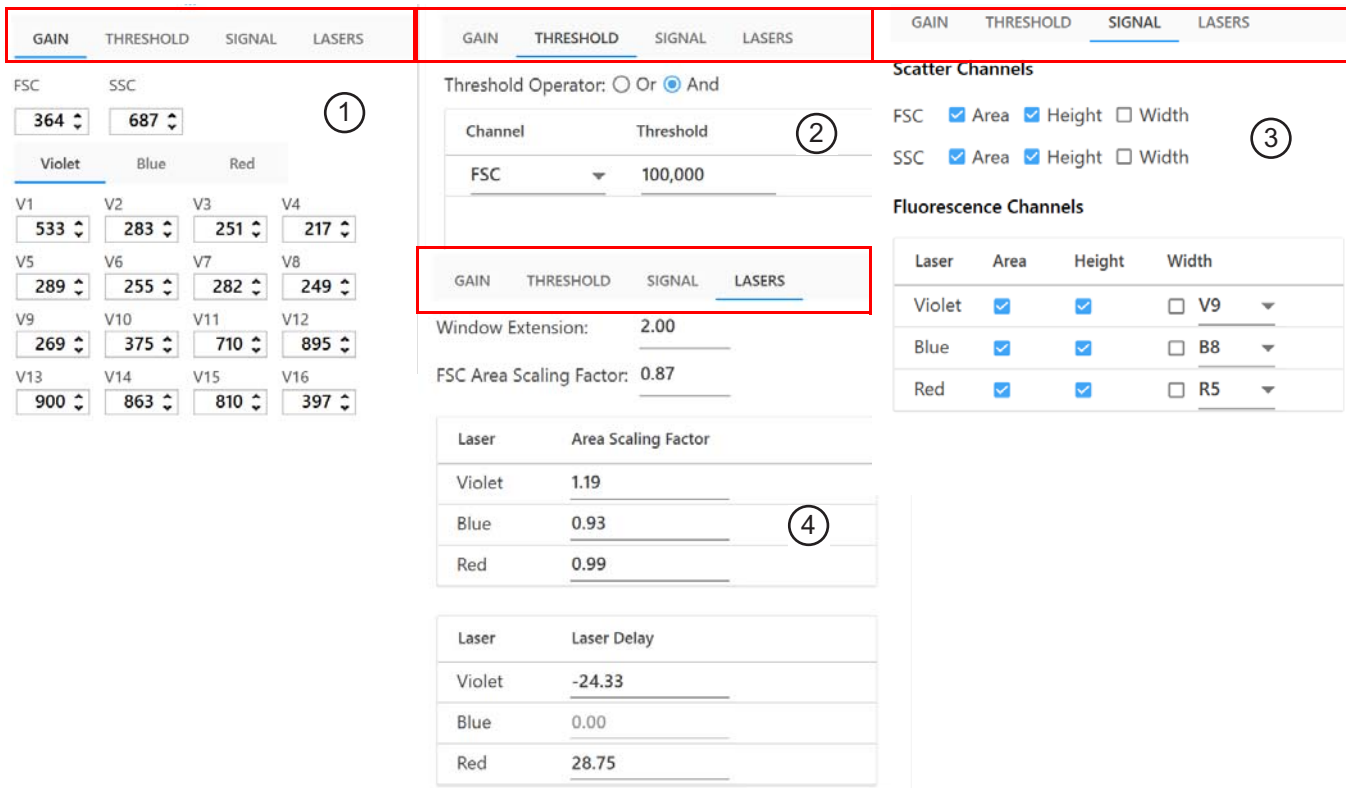


The following table describes the controls in the Acquisition Control pane.

No.	Control	Description
1	Start/Record/Stop/ Restart	Start and Record are enabled when a tube is present on the SIP. Select Start to start acquisition Select Record to record data. Record can also start acquisition. Select Stop to stop acquisition. Select Restart to restart the acquisition counters. All events and results displayed are refreshed. Stop and Restart are enabled once Start is selected.
2	Flow Rate	Select Low (15 µL/min), Medium (30 µL/min), or High (60 µL/min)
3	Events Rate, Abort Rate, Threshold Count, Time Elapsed	Displays the real-time counts during acquisition.
4	Events to Display	Enter the number of events you want displayed during acquisition.

Instrument Control

The Instrument Control pane consists of the Gain, Threshold, Signal, and Lasers tabs for use in adjusting the instrument.

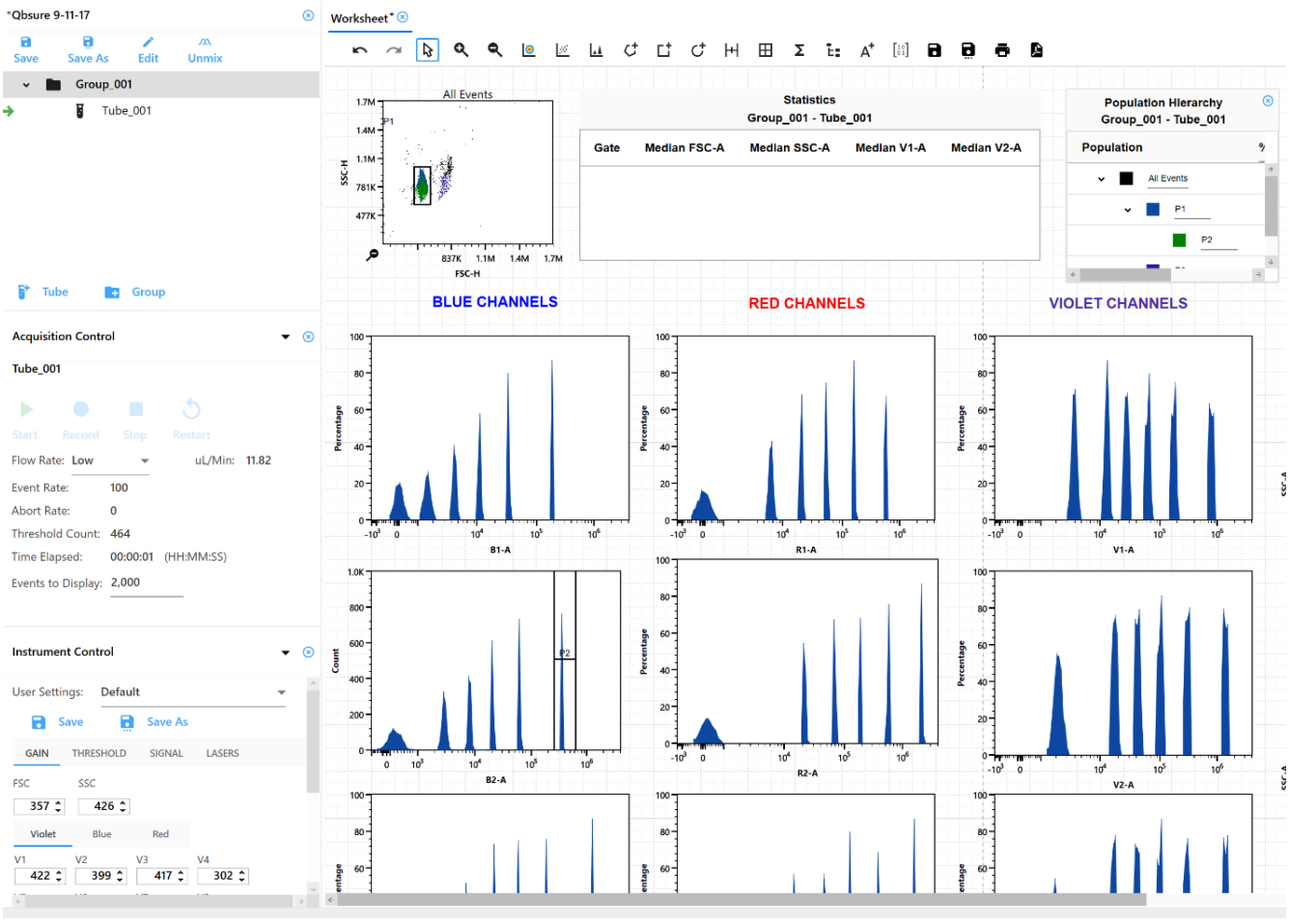


The following table describes the tabs in the Instrument Control pane.

No.	Tab	Description
1	Gain	Gains can be adjusted for all detector channels for all lasers using the gain spinboxes. FSC gain can be adjusted from 0–1,000. SSC and fluorescence detector gains can be adjusted from 0–10,000. To change the value that the gain increments, see the acquisition preferences on page 59.
2	Threshold	Use the Threshold tab to set the threshold parameter and minimum threshold channel value. Multiple parameters can be set as a threshold using either the AND or OR operator. Use OR for at least one parameter to be available.
3	Signal	Use the Signal tab to select area, height, or width for each signal. Area and height can be selected for all channels. Width can be selected for only one channel per laser.
4	Lasers	Use the Lasers tab to set the area scaling factor and laser delay. These values are automatically set and updated in all user settings upon completion of the Daily QC.

Worksheet Area

The worksheet area allows you to view the data in plots and create plots, statistics, and gates.



Experiment Toolbar

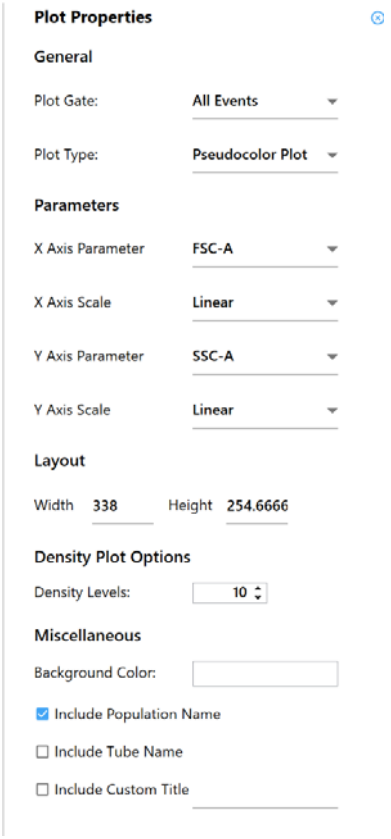
A toolbar at the top of the worksheet area allows you to undo/redo, zoom; create plots, gates, statistics, annotations; and save, print, and save a PDF of the worksheet. Hover the cursor over an icon to see a description and keyboard shortcut.

Plots

Three plot types can be created in the worksheet:

- dot plots
- pseudocolor plots (density plots)
- histogram plots

To change the properties of a plot, right-click the plot and select Plot Properties. You can select the plot type, parameters, scale, background color, and labels.

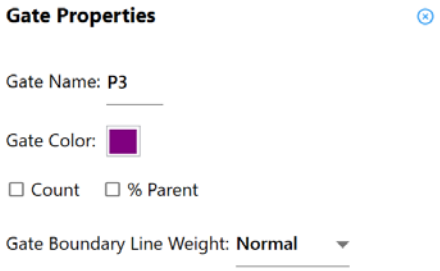


Gates

Gates types include:

- rectangle
- oval
- polygon
- quadrant
- interval

The properties of gates can be changed by right-clicking the gate. You can change the name of the gate, the color, and gate boundary line weight. You can also select whether to display gates and statistics.



Statistics

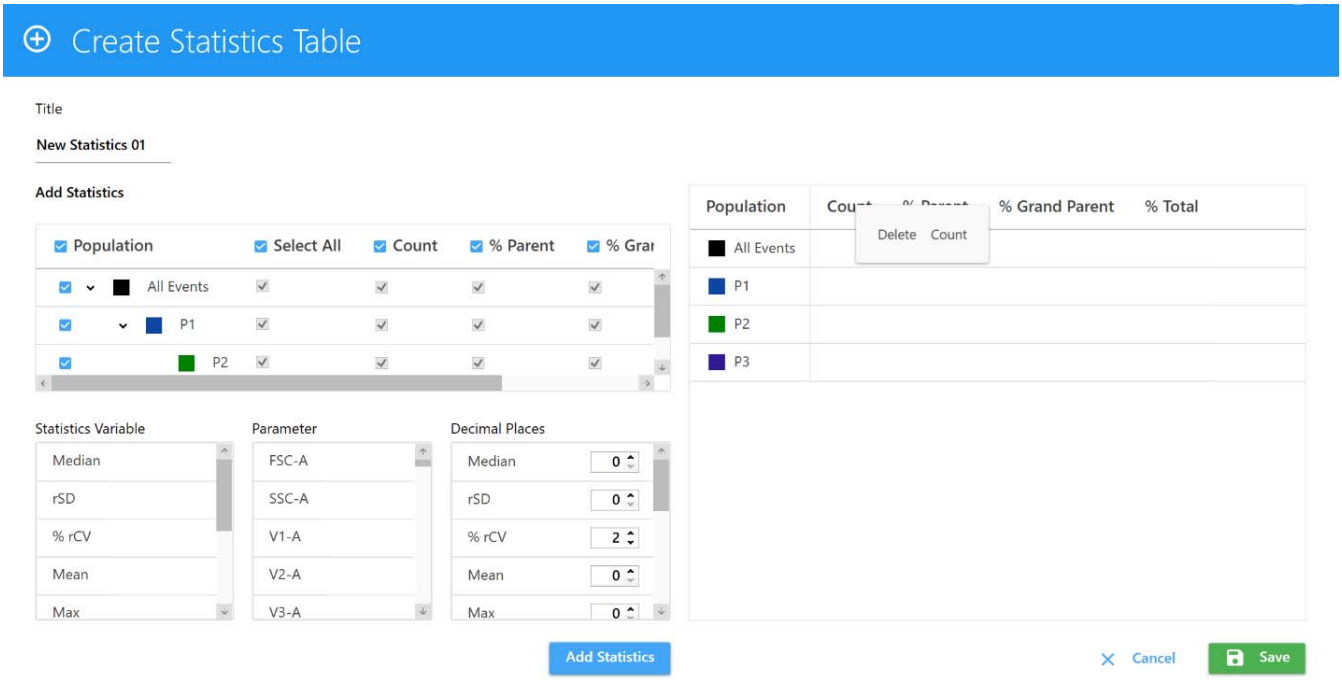
To create a statistics box, click the Statistics icon in the experiment menu toolbar.

Select the population checkbox next to the populations that have stats to display. To add a statistic, select the statistic from the Statistics Variable menu.

Select the parameter you would like to add for the statistics. Multiple parameters can be selected at once.

The precision of the statistics can be adjusted in the Decimal Places table.

To remove a statistic, right-click the column header and select **Delete**.

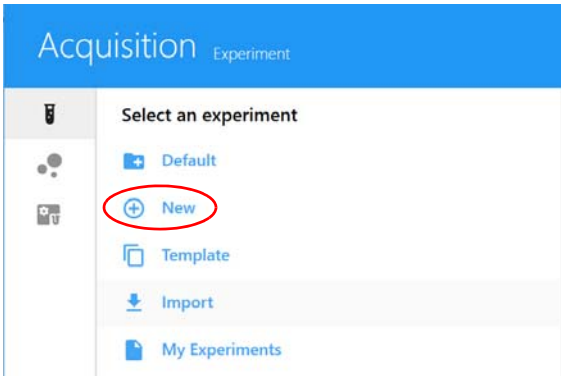


Creating a New Experiment

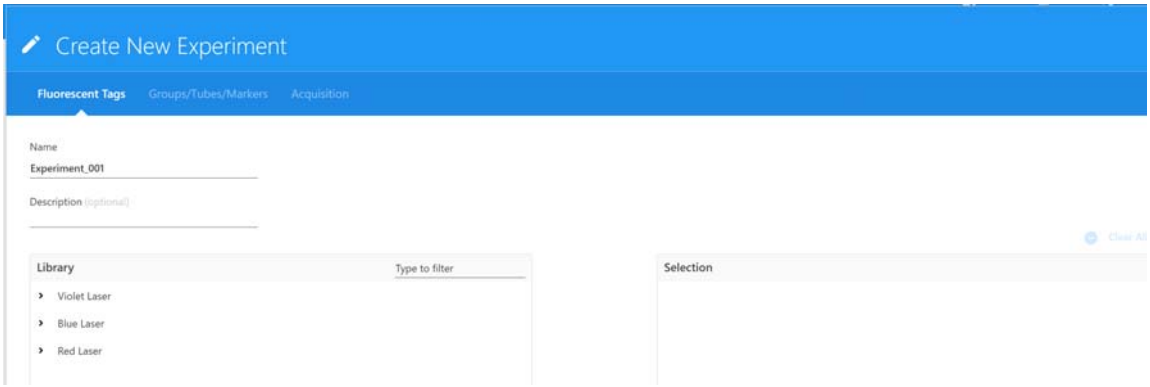
Selecting New in the Experiment tab opens the New Experiment wizard. The wizard walks you through creating a new experiment worksheet that is specific to your needs.

Creating a New Experiment With a Reference Group

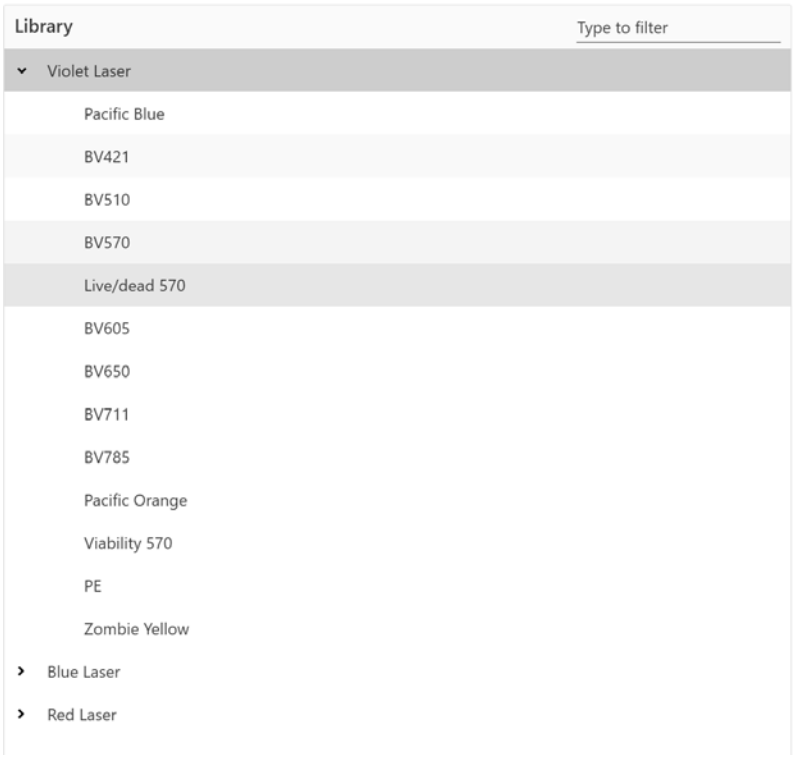
1 Select New in the Acquisition Experiment menu.



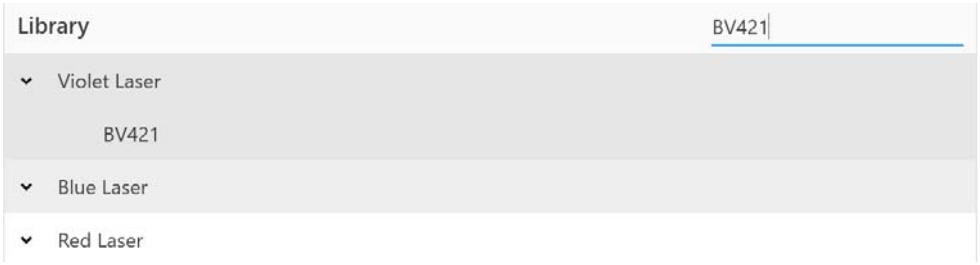
2 The Create New Experiment wizard opens. Specify a name for the experiment and/or type in a description.



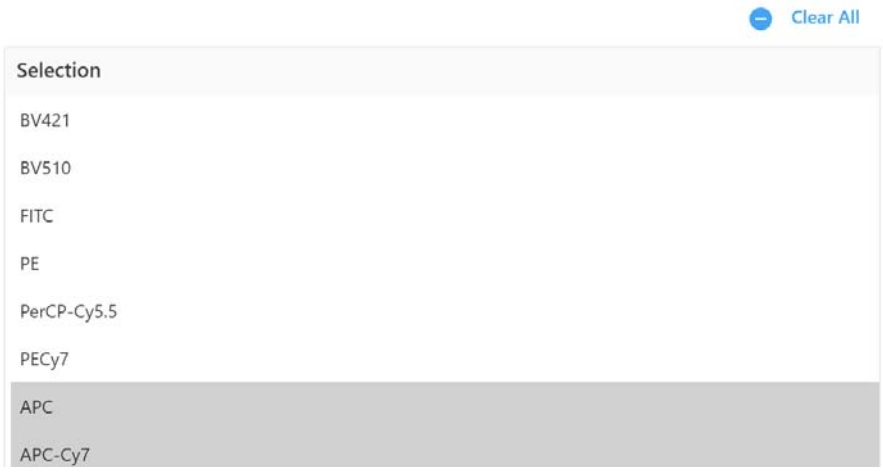
3 Select the fluorescent tags used in the experiment from the Library pane on the left. You must select all fluorescent tags present in the experiment, as this will determine which Reference Controls are to be used during spectral unmixing.



■ **NOTE:** Use the search box in the upper-right corner of the Library pane to search for the fluorescent tags of choice. A default list of fluorescent tags for each laser is available in the Library. See “Fluorescent Tags” on page 55.



Individual fluorescent tags can be removed. To remove all fluorescent tags, select Clear All.



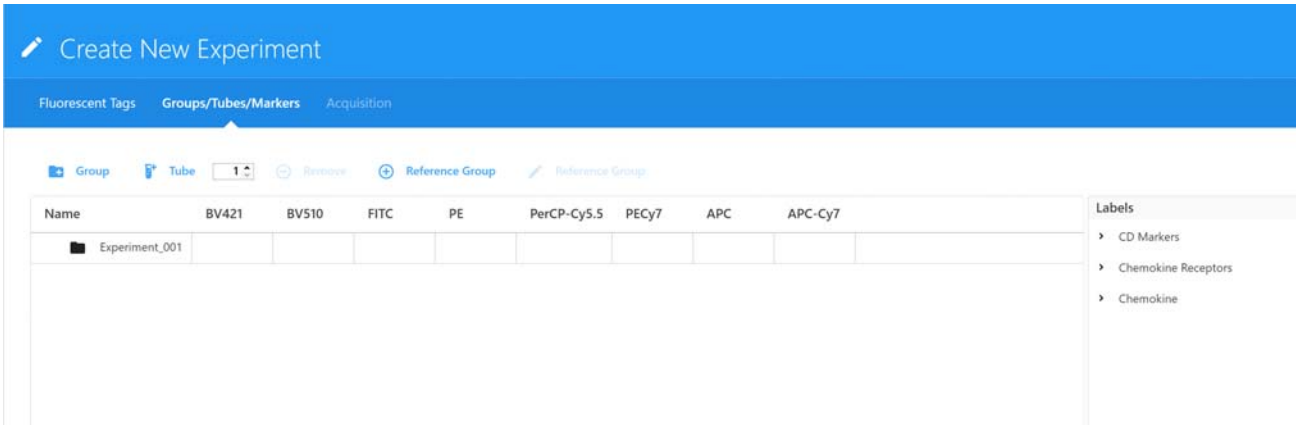
4 Once all fluorescent tags have been chosen from the Library, confirm the list in the selection pane, then click Next.

5 Select Reference Group if you are intending to unmix with controls acquired in this experiment.

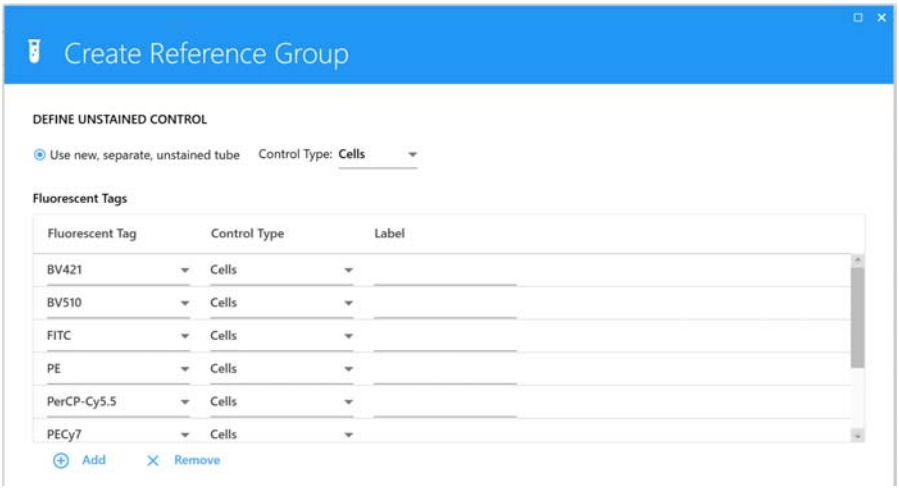
This creates a list of tubes for each fluorescent tag specified as part of the experiment.

■ **NOTE:** If you plan on unmixing the samples with Reference Controls only from the Library, steps 5-8 are not necessary.

■ **NOTE:** To mix and match references acquired in the experiment with Reference Controls stored in the Library, define the controls to acquire in the Reference Group, acquire the controls, then after selecting Unmix, select the remaining controls from the stored Reference Controls.

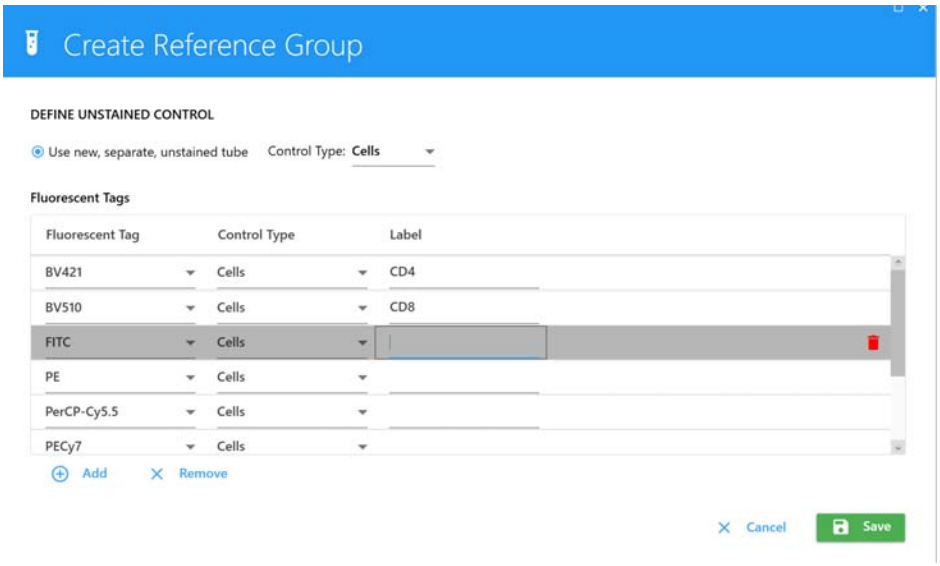


6 **IMPORTANT:** Define an unstained control by selecting its control type. The unstained control needs to be of the same type as the sample, as this will ensure accurate unmixing and autofluorescence quantitation.

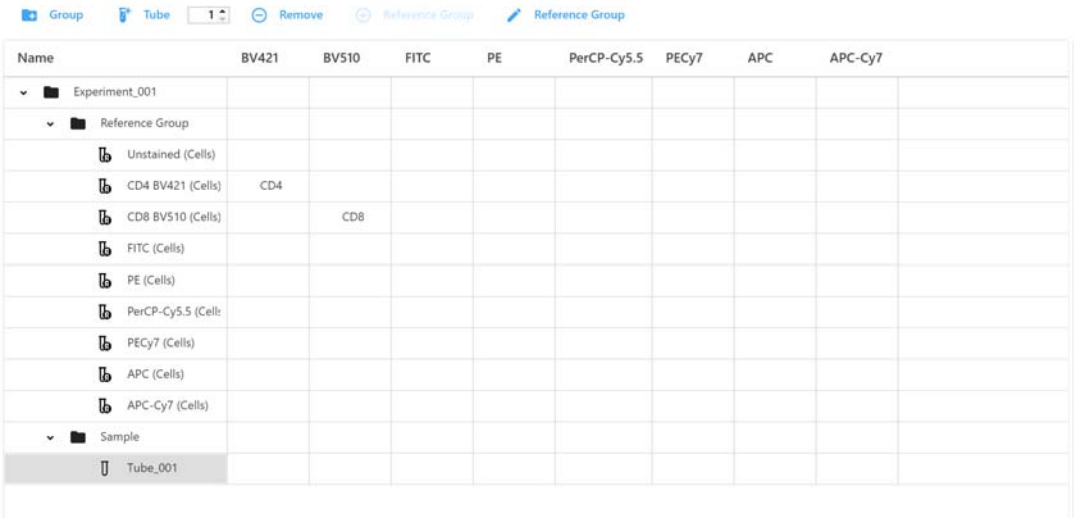


7 Select the control type for the single-stained Reference Controls. (Optional) Select the label that is conjugated to the fluorescent tag. Select Save.

Use the red trash can to delete one of the tubes from the Reference Group. This may be necessary if you wish to mix and match references acquired with stored Reference Controls. Any stored controls you plan to use should be deleted from the Reference Group.



8 Once the Reference Group has been created, entries for each of the references will be displayed. Each of the Reference Group tubes will have an icon (tube with the letter R) associated with it. Create sample groups and samples by selecting the add Group or Tube option in the upper left.



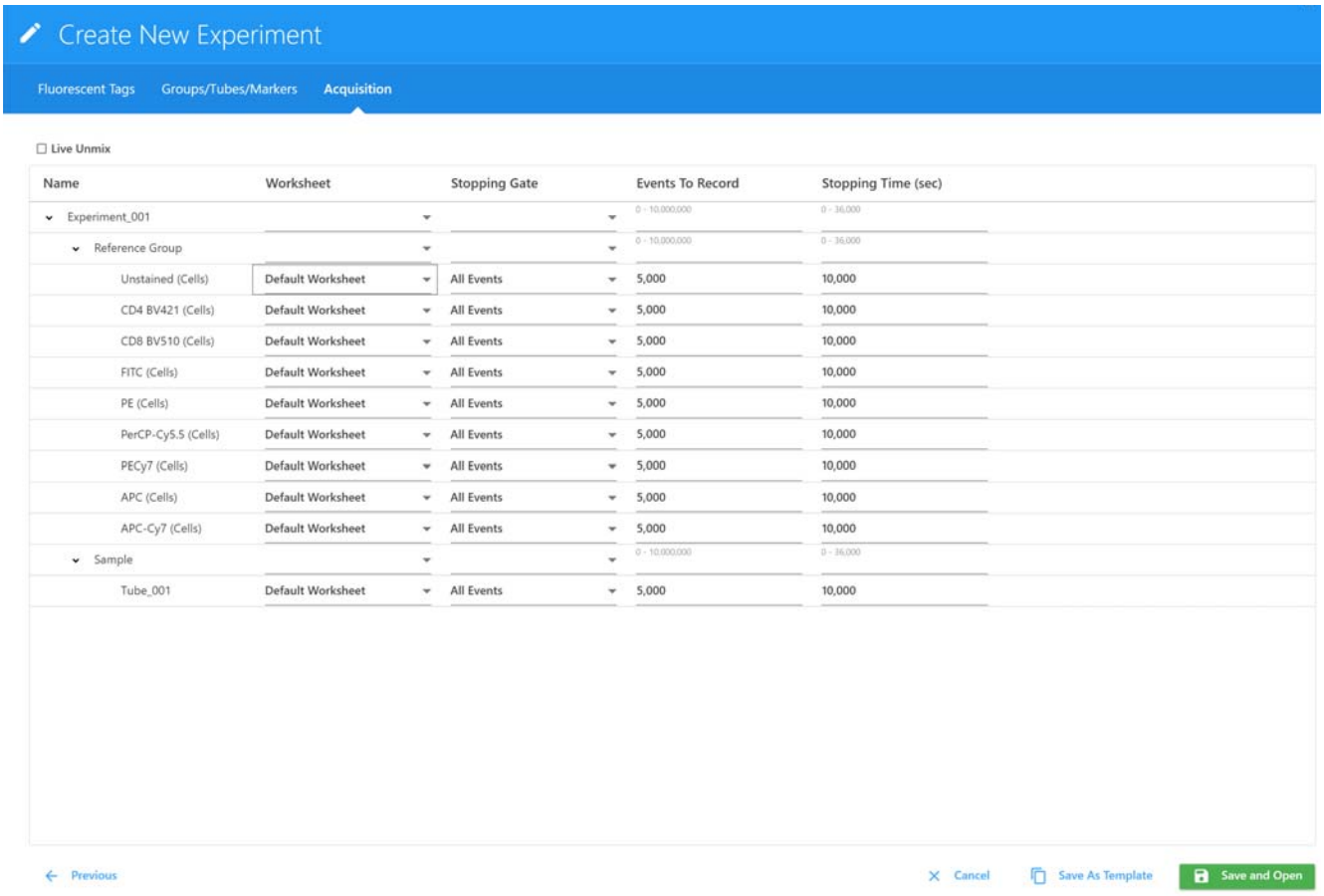
9 (Optional) Add labels to the remaining sample tubes before continuing. They can be chosen from the label list, entered directly into the table, or copied and pasted. Labels can be applied to multiple cells selected at once.

10 Select Next when all tubes have been created and labeled.

11 Select the worksheet for the sample tubes. Select the stopping gate, the number of events to collect, and the stopping time.

Acquisition stops when the first stopping criterion is met.

You can select a worksheet that applies to all the tubes in the experiment or group by choosing the pull-down menu that corresponds to the experiment or group.



12 Once worksheet and stopping criteria have been determined, click **Save and Open** to open the new experiment.

Unmixing Workflows

There are two data acquisition workflows available in SpectroFlo software:

- Live Unmixing
- Post-Acquisition Unmixing

When data is acquired with live unmixing, references are acquired as raw data either in the experiment as part of the Reference Group or previously acquired in the QC and Setup workspace as Reference Controls. References for *all* fluorochromes used in a given experiment must be present in the system in order for live unmixing of multicolor samples to occur. The live unmixing functionality allows you to visualize fully compensated data during acquisition.

Multicolor samples can be acquired as raw data and unmixed post acquisition as well. This can be done either in the Acquisition workspace or in the Analysis workspace.

Live Unmixing

Samples can be unmixed during acquisition. Live unmixing can be performed with the Reference Group acquired during the experiment, the Reference Controls (run during QC & Setup and stored in the system), or a combination of both.

For each sample tube that is live unmixed, two FCS files are generated, one that is composed of raw data and one that is composed of unmixed data.

Live unmixed data can be analyzed in unmixed worksheets in the Acquisition workspace. Unmixed worksheets are different from normal worksheets, as they only display fluorescence information categorized into the defined fluorescent tags for each of the experiments.

To Perform Live Unmixing:

1 Create a new experiment with fluorescent tags defined.

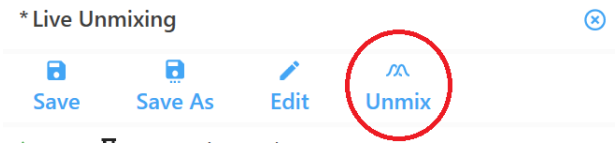
2 Create a Reference Group in the experiment with the fluorescent tags, if there are any that have not been stored as Reference Controls.

3 Acquire all Reference Control tubes.

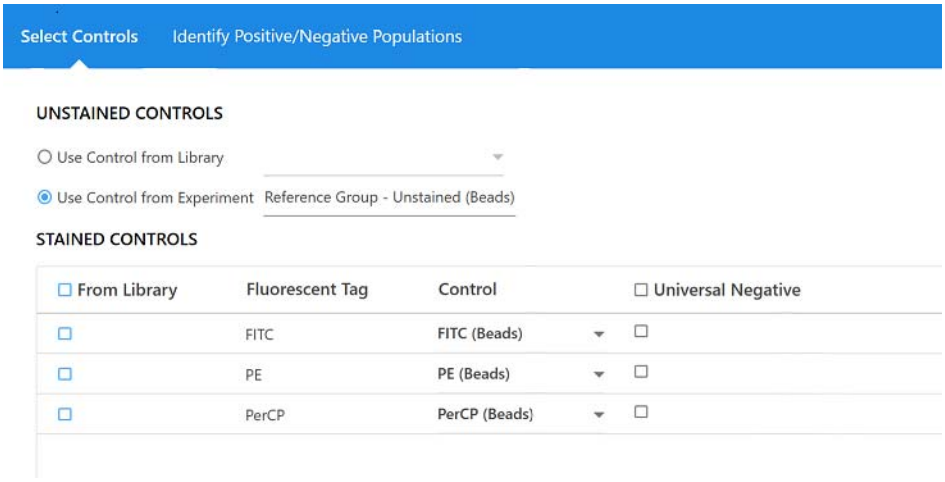
- If acquiring beads, we recommend collecting 5,000 singlet events.
- If acquiring cells, we recommend collecting 10,000 events.

■ **NOTE:** Avoid acquiring too many events in the Reference Group tubes. The more events you acquire, the longer it takes to compute the compensation matrix.

4 Select Unmix in the upper-left toolbar.



5 The Unmixing wizard appears with rows corresponding to the defined fluorescent tags. Select Use Control from Library if unmixing with the unstained Reference Controls. Select the From Library checkbox if unmixing with fluorescent tag Reference Controls. This checkbox is only active if Reference Controls for those fluorescent tags have already been saved to the Library.

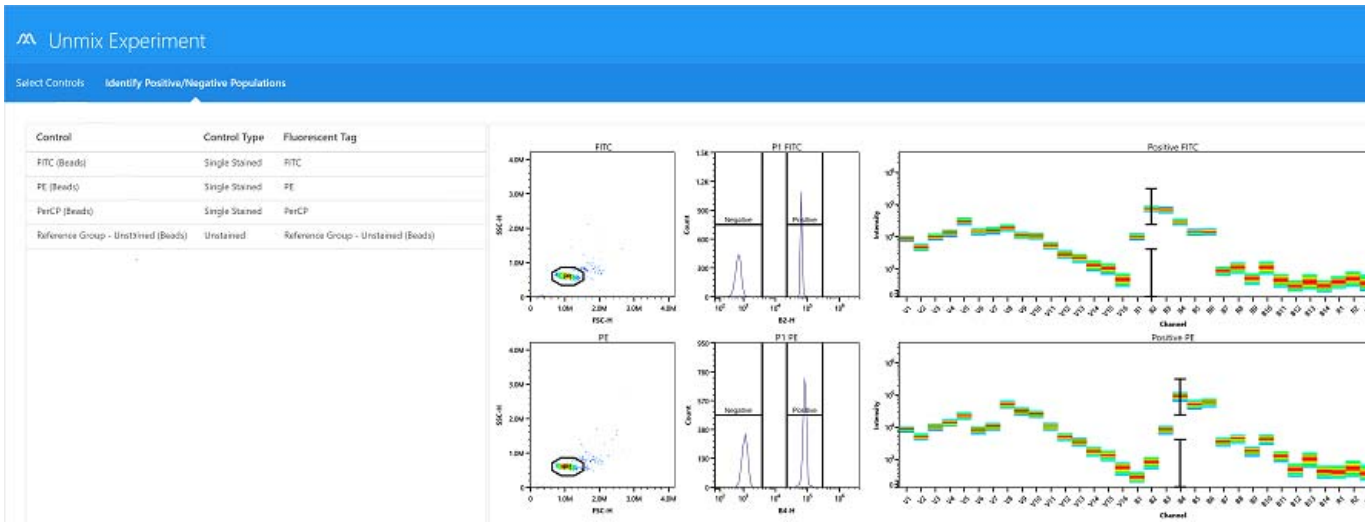


6 Use the Identify Positive/Negative Populations window to include the positive and negative populations for each fluorescent tag in the appropriate gate.

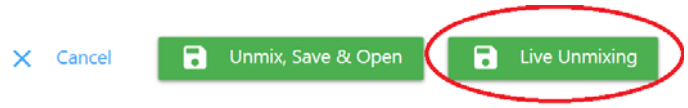
a. Move the polygon gate in the FSC vs SSC plot to include the singlet population. Hold down CTRL to move all the polygon gates at once.

b. Move the interval gate on the spectrum plot on the right to select the channel that exhibits the brightest fluorescence intensity. This channel is the peak emission channel for the fluorescent tag.

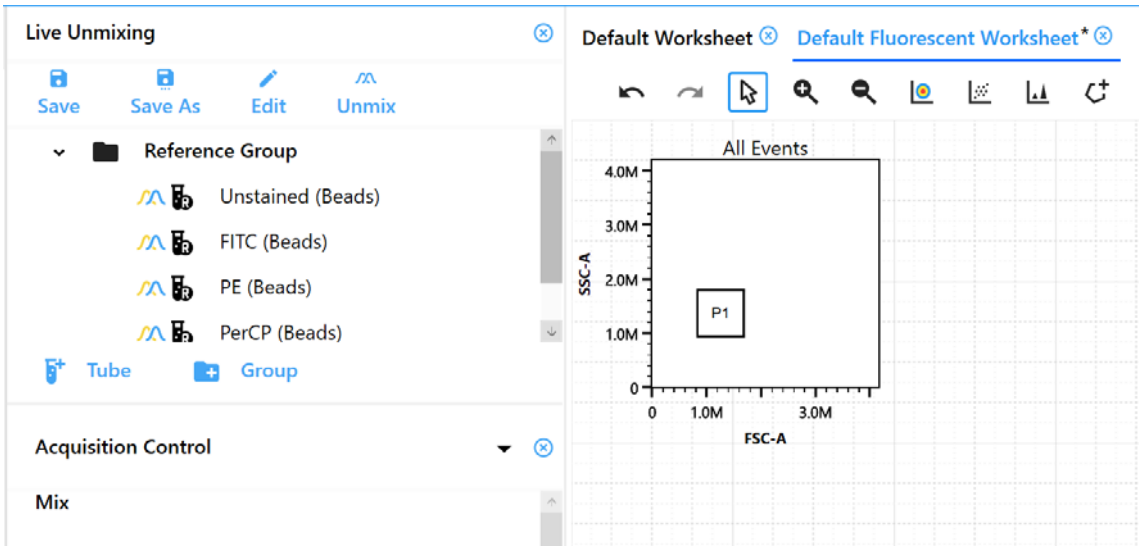
c. Move the interval gate in the histogram for the peak channel labeled *Positive* to include the positively stained population. Move the interval gate in the histogram for the peak channel labeled *Negative* to include the negative population.



d. Select Live Unmixing.



The wizard closes and the Acquisition workspace reappears. The Reference Group now has the unmixed icon to the left of the tube and a new unmixed worksheet appears.



Post-Acquisition Unmixing

Samples can be acquired as raw data and then unmixed after acquisition is complete in the experiment. This can be done through two methods:

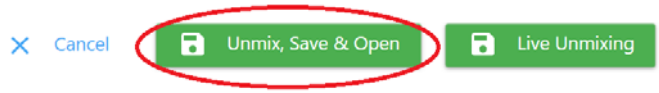
- post-acquisition unmixing in the Acquisition workspace
- post-acquisition unmixing in the Analysis workspace

Post-Acquisition Unmixing in the Acquisition workspace

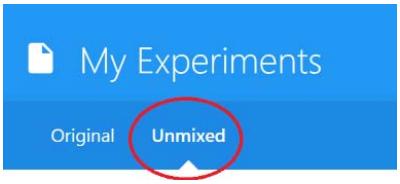
To perform post-acquisition unmixing in the Acquisition workspace, perform the same workflow as live unmixing EXCEPT the following:

1 Acquire all Reference Control tubes and sample tubes prior to selecting the unmix button in the upper-left pane.

2 Select Unmix, Save & Open.



3 Experiments that have been unmixed post-acquisition can be found in the Unmixed tab of the My Experiments menu.



Post-Acquisition Unmixing in the Analysis workspace

To perform post-acquisition unmixing in the Analysis workspace, see “Unmixing in the Analysis Workspace” on page 47.

■ **NOTE:** If one of the controls is questionable, you can reacquire it, overwriting the original file, then unmix again.

6

Advanced Unmixing

Unmixing in the Analysis Workspace

Post-acquisition unmixing with raw FCS files can be performed in the Acquisition workspace and can also be performed in the Analysis workspace. You can pick and choose which FCS files to unmix in the Analysis workspace (for example controls coming from different experiments or single-stained controls that were not run as part of the Reference Group). In contrast, the unmixing wizard in the Acquisition workspace limits the FCS files to be used as controls to those coming from the Reference Group of the Reference Control Library.

In addition, raw FCS files can also be conventionally compensated in this workspace through the Virtual Filters tab. This function can simulate the presence of filters and can compensate data using conventional methods that result in output like that obtained from a conventional cytometer.

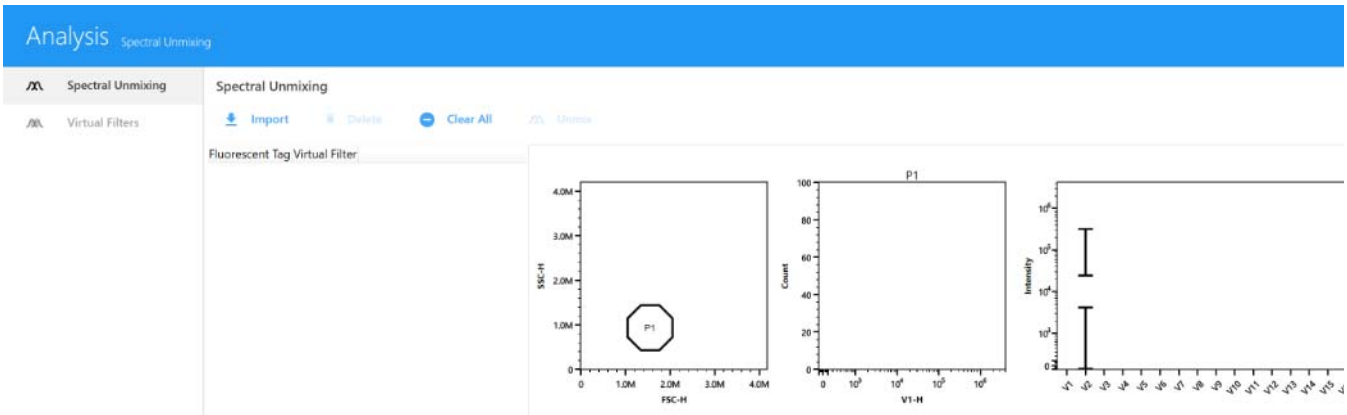
FCS files can be designated into three categories:

- Single Stained
- Unstained
- Sample

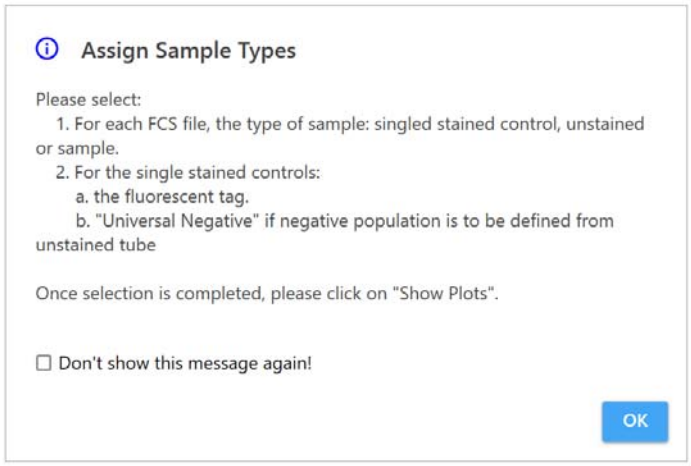
■ **NOTE:** There must be at least one single-stained FCS file and one unstained FCS file in the file list. Otherwise, unmixing cannot be performed.

To Unmix Raw Data Files:

1 Click **Import** to import raw FCS files for analysis.

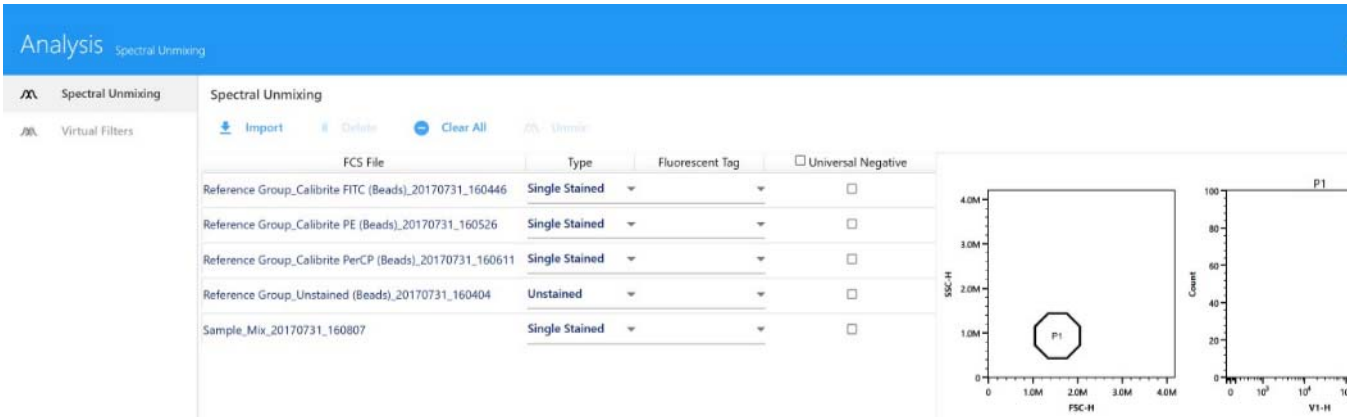


2 Upon importing, a dialog box on how to assign sample types appears. Read the instructions and click OK.

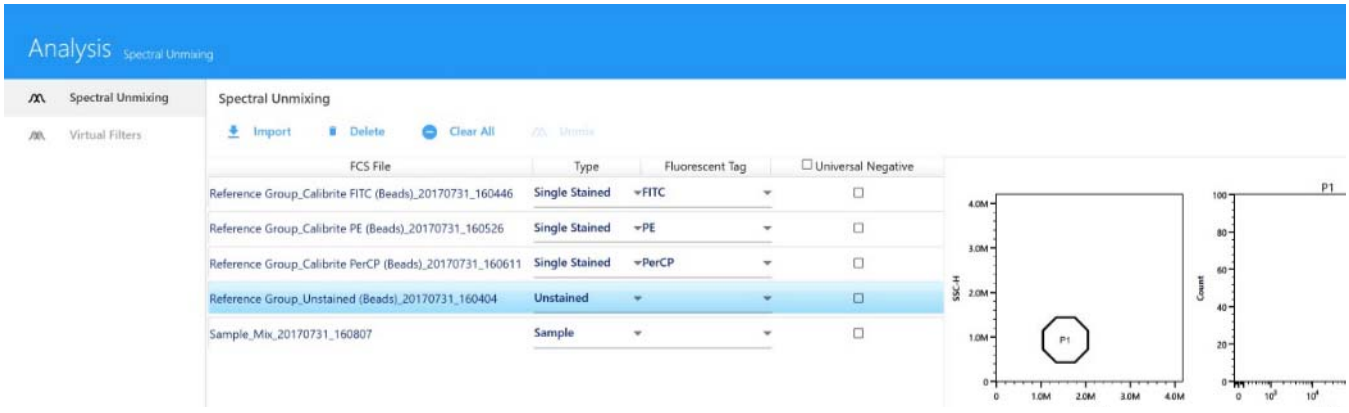


3 Once FCS files have been imported, the sample type for each FCS file needs to be designated as Single Stained, Unstained, or Sample. The software will automatically designate the type based upon the file name. This can be modified manually if the automatic designation is incorrect.

If the imported FCS files are incorrect, click Clear All to clear the entire imported list or select one or more FCS files to remove from the list and click Delete.



4 FCS files designated as single-stained will require a fluorescent tag designation to specify what reference spectrum will be provided for unmixing.



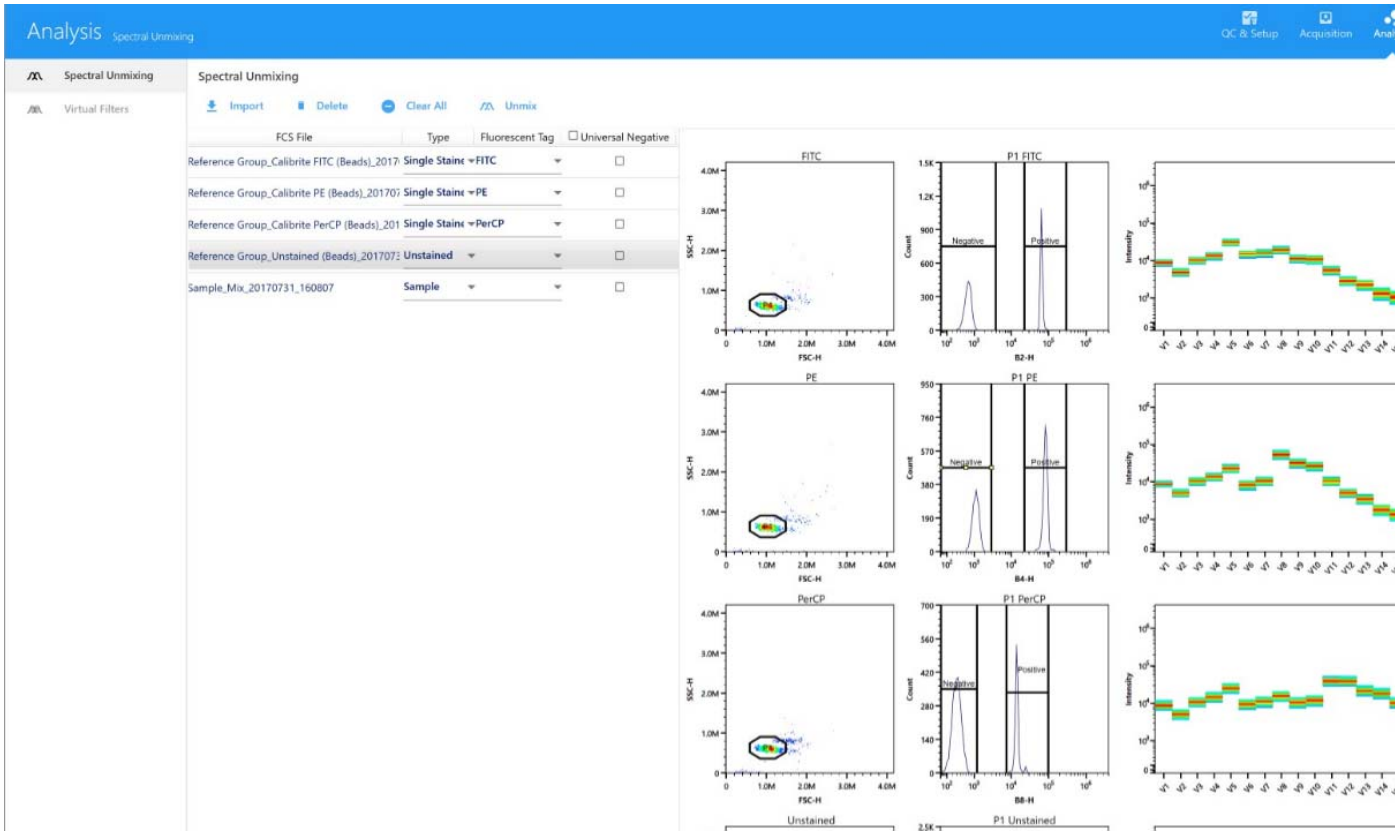
5 Select Universal Negative for single-stained FCS files that do not contain a negative population. In the bottom left of the screen, check whether Auto Fluorescence will be used as a fluorescent tag.

6 Select Refresh Plots to display the data in the FSC vs SSC plot, peak emission channel histogram, and spectrum plots. The positive and negative populations need to be identified through the appropriate placement of the existing gates.

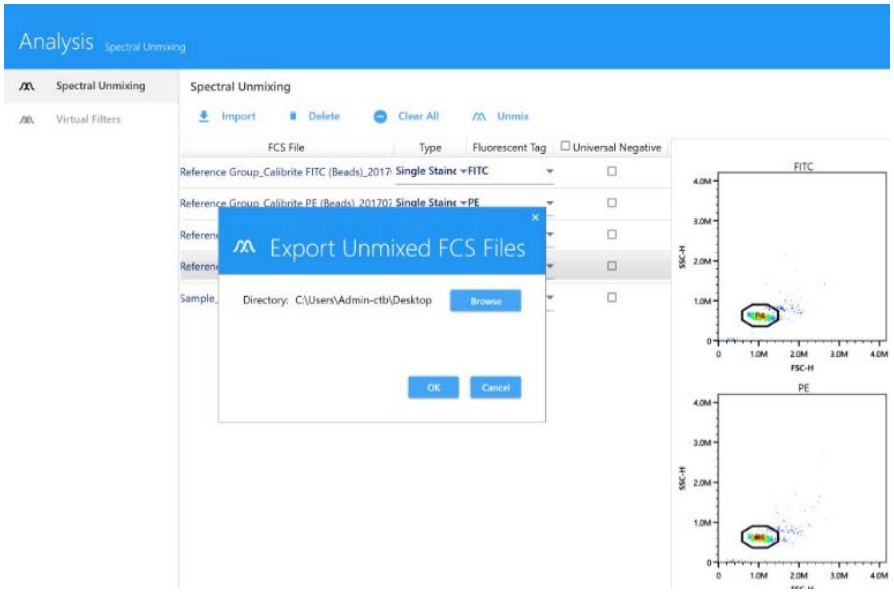
a. Move the polygon gate in the FSC vs SSC plot to include the singlet population. To apply this gate placement to all FSC vs SSC plots, hold the Ctrl key while moving the gate.

b. Move the interval gate on the spectrum plot to select the channel that exhibits the brightest fluorescence intensity. This channel is the peak emission channel for the fluorescent tag.

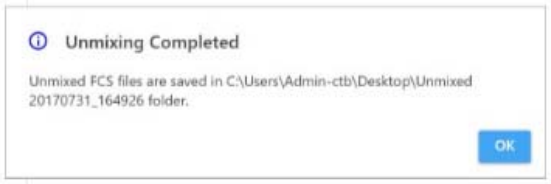
c. Move the interval gate in the histogram for the peak channel labeled *Positive* to include the positively stained population. Move the interval gate in the histogram for the peak channel labeled *Negative* to include the negative population.



7 Click **Unmixing** and select the directory to which the unmixed FCS files are exported. The default folder can be set in the Preferences workspace. See “Storage” on page 65.



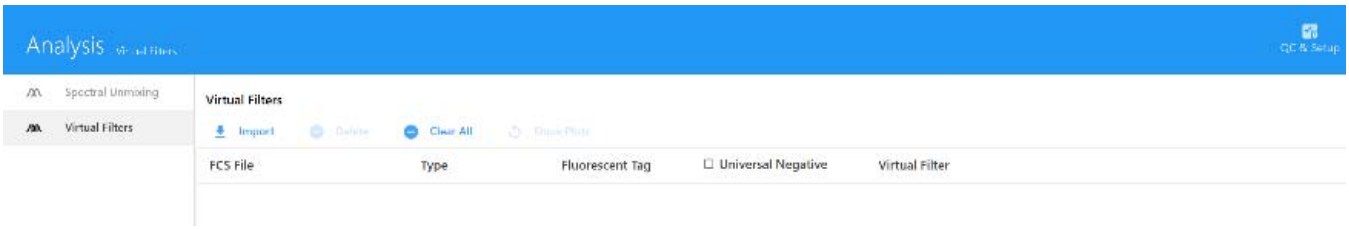
8 Once unmixing is complete, the unmixed FCS files are saved in the specified directory. These FCS files can then be imported to an experiment for analysis or analyzed using third-party software.



Virtual Filters

Raw FCS data can be compensated using conventional methods in the Virtual Filter tab.

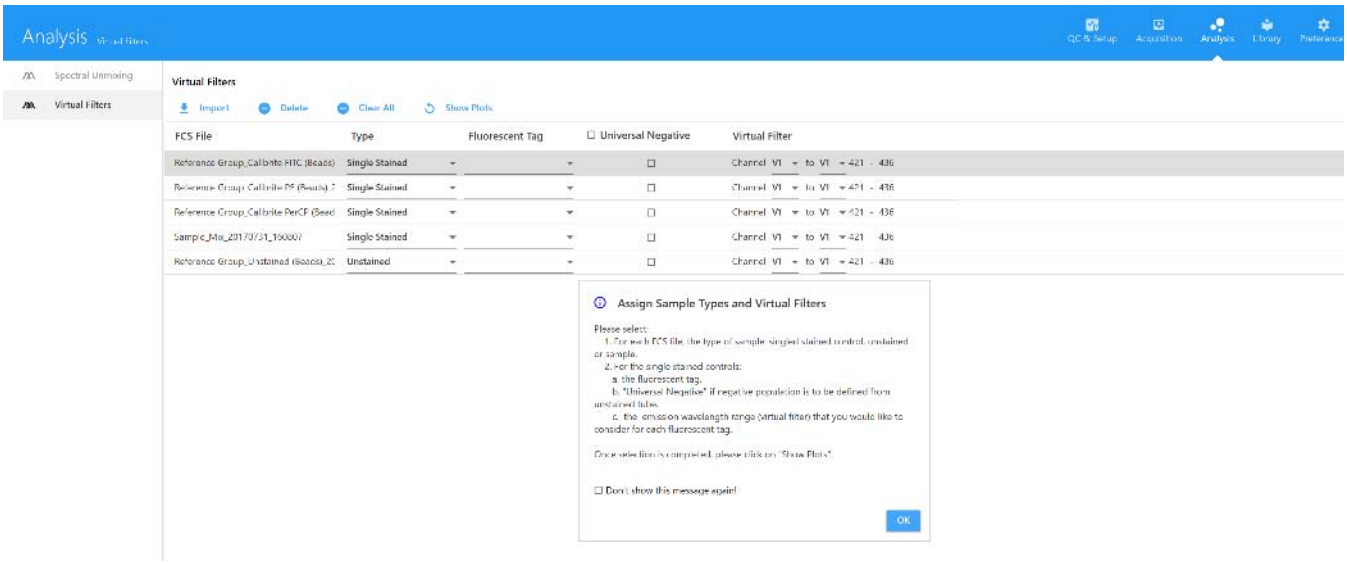
1 Click the Virtual Filter tab in the Analysis workspace.



2 Select **Import** to import raw FCS files for virtual filter analysis.

These FCS files can be single-stained Reference Control FCS files, unstained control FCS files, and/or sample FCS files. It is important to note that an unstained control FCS file must be included.

3 Upon importing, a dialog box on how to assign sample types appears. Read the instructions and click **OK**.



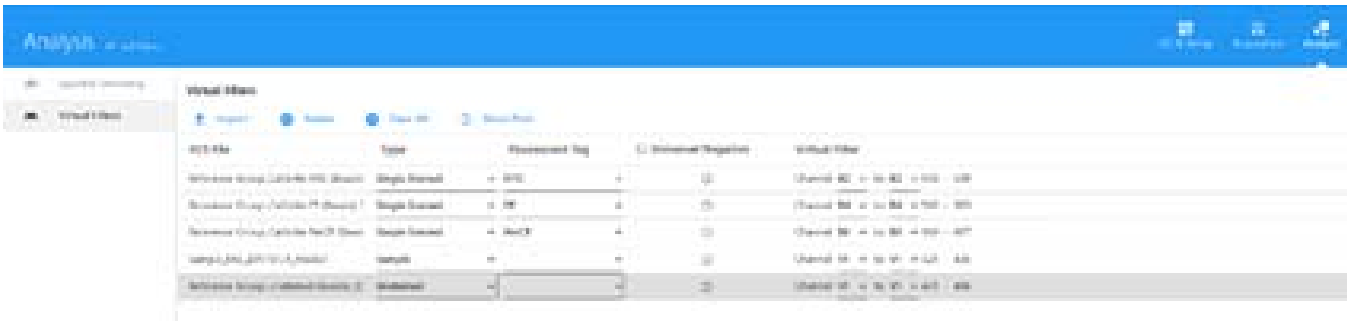
4 Once FCS files have been imported, the sample type for each FCS file needs to be designated as Single Stained, Unstained, or Sample. The software will automatically designate the type based upon the file name. This can be modified manually if the automatic designation is incorrect.

FCS files designated as single stained will require a fluorescent tag designation. If there is no negative population in the single-stained FCS file, select the **Universal Negative** option.

The virtual filter will automatically be assigned by the software based upon the fluorescent tag designation. To increase the bandwidth of the virtual filter, adjust the pull-down menus to capture the desired spectrum range. The following table shows the system's filter bandwidths.

Laser	Channel	Center Wavelength (nm)	Bandwidth (nm)	Wavelength Start (nm)	Wavelength End (nm)
Violet	V1	428	15	421	436
	V2	443	15	436	451
	V3	458	15	451	466
	V4	473	15	466	481
	V5	508	20	498	518
	V6	528	21	518	539
	V7	549	22	538	560
	V8	571	23	560	583
	V9	594	23	583	606
	V10	618	24	606	630
	V11	664	27	651	678
	V12	692	28	678	706
	V13	720	29	706	735
	V14	750	30	735	765
	V15	780	30	765	795
	V16	812	34	795	829
Blue	B1	508	20	498	518
	B2	528	21	518	539
	B3	549	22	538	560
	B4	571	23	560	583
	B5	594	23	583	606
	B6	618	24	606	630
	B7	660	17	652	669
	B8	678	18	669	687
	B9	697	19	688	707
	B10	717	20	707	727
	B11	738	21	728	749
	B12	760	23	749	772
	B13	783	23	772	795
	B14	812	34	795	829
Red	R1	660	17	652	669
	R2	678	18	669	687
	R3	697	19	688	707
	R4	717	20	707	727
	R5	738	21	728	749
	R6	760	23	749	772
	R7	783	23	772	795
	R8	812	34	795	829

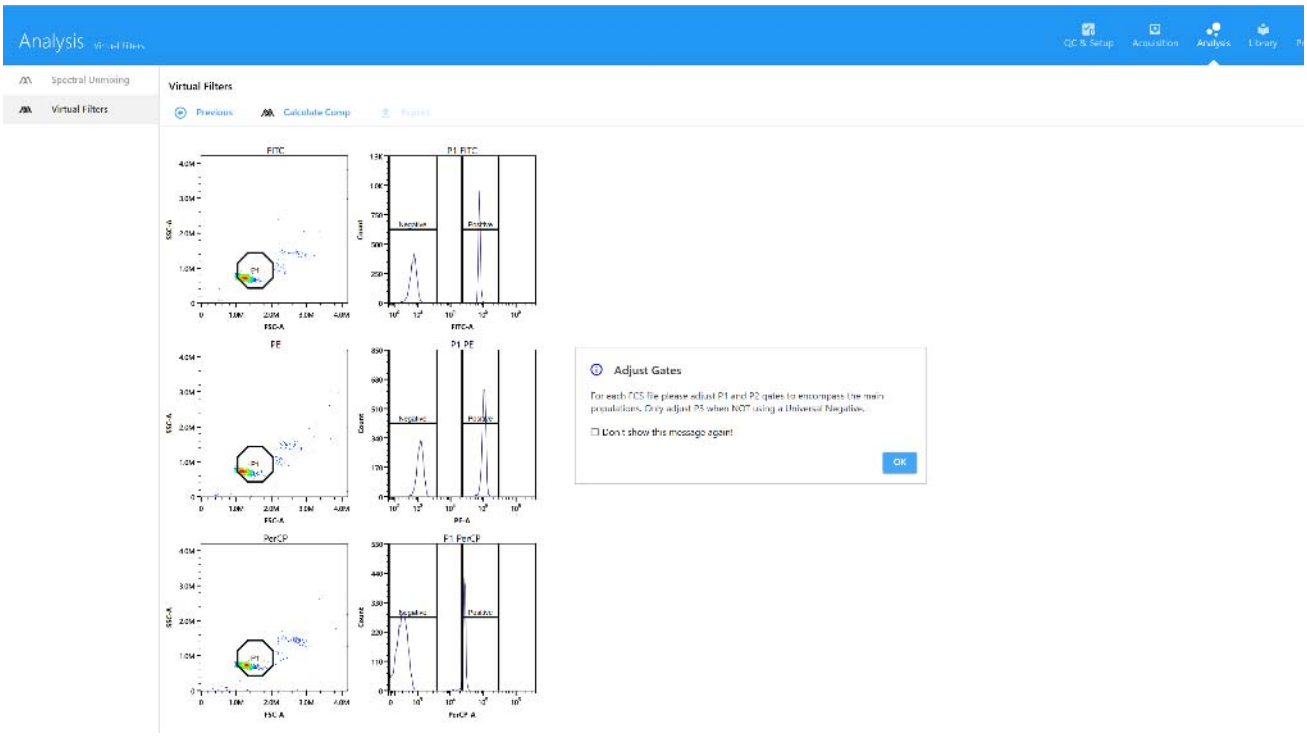
5 Select Show Plots to display the plots.



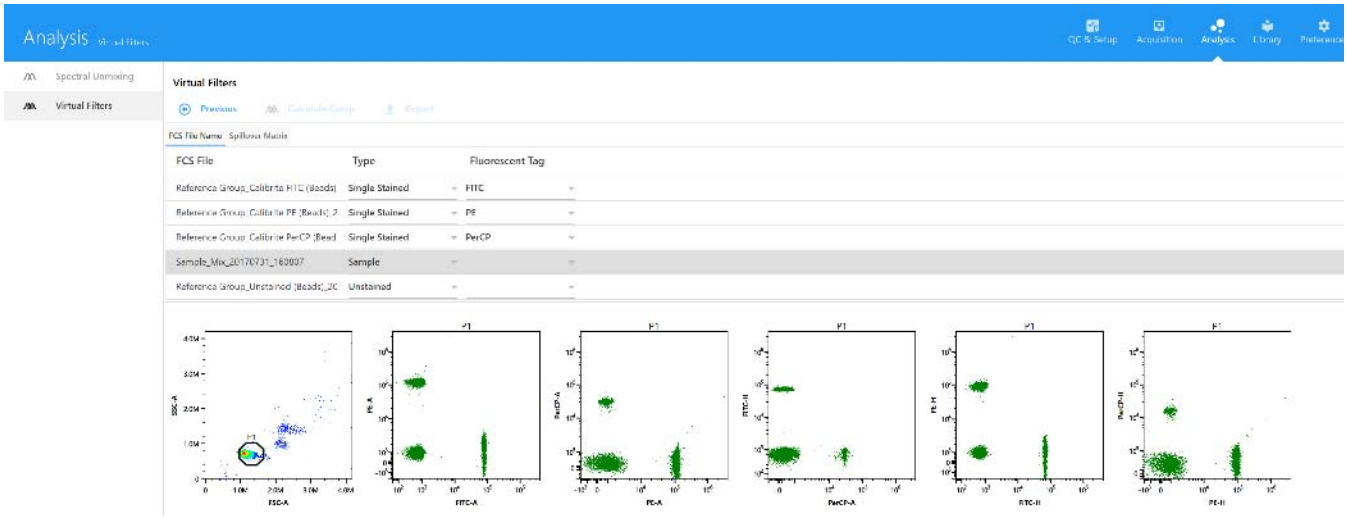
6 The data is displayed in the FSC vs SSC plot and fluorescent tag histogram plot. The positive and negative populations need to be identified through the appropriate placement of the gates.

a. Move the polygon gate in the FSC vs SSC plot to include the singlet population.

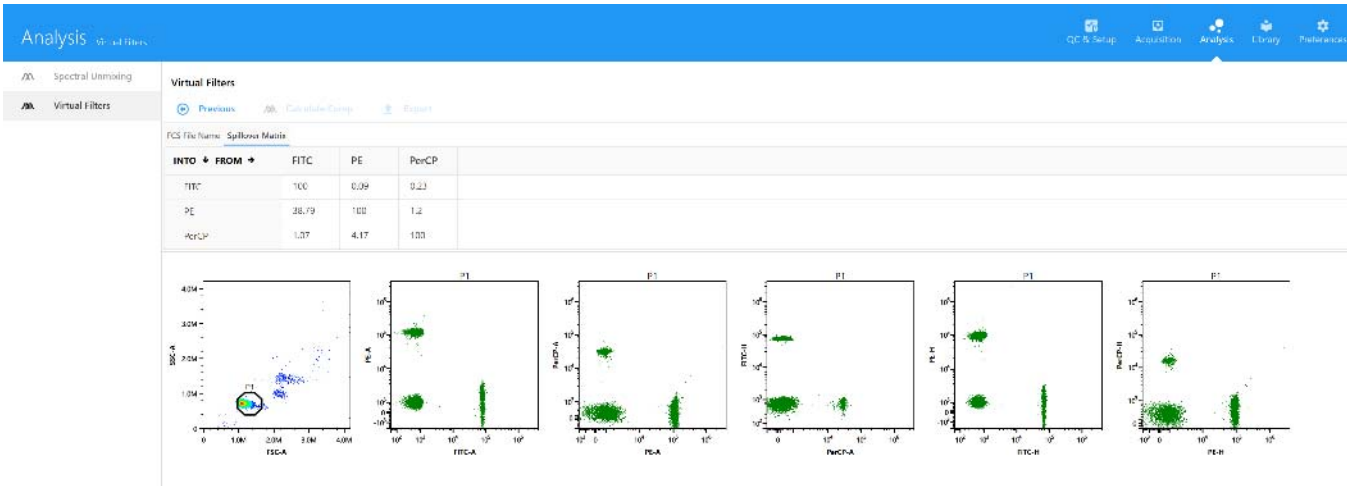
b. Move the interval gate in the histogram labeled *Positive* to include the positively stained population. Move the interval gate in the histogram labeled *Negative* to include the negative population. Do not adjust the negative gate when using the Universal Negative.



7 Select **Calculate Comp** once gates have been set correctly. The conventionally compensated data is displayed in the Analysis workspace.



The spillover matrix is also calculated and can be viewed under the Spillover matrix tab.



8 Select **Export** to export the conventionally compensated data.

7

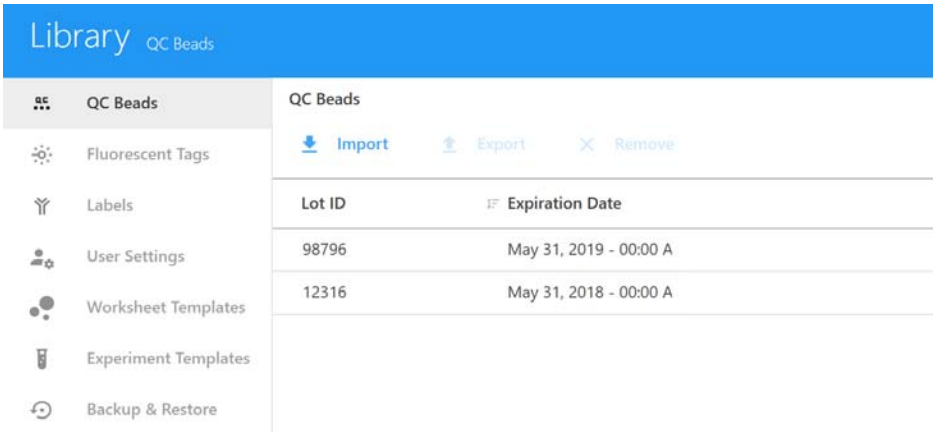
Library, Preferences, and Users

Library

The Library contains information for various elements used for the experiments. Information saved in the Library includes SpectroFlo QC bead lots, fluorescent tags, labels, user settings, worksheet templates, and experiment templates. Information stored in the Library can be saved, exported, and imported for reuse.

QC Beads

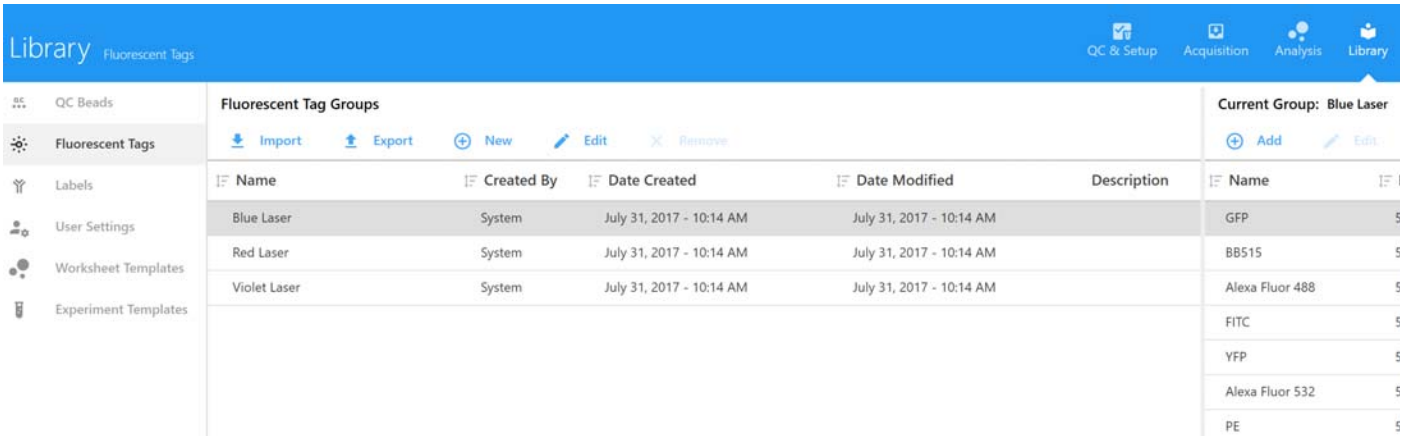
SpectroFlo QC bead lot IDs and expiration dates can be imported, exported, or removed from the Library. The QC bead lot for the beads used for Daily QC must be saved in the Library.



Fluorescent Tags

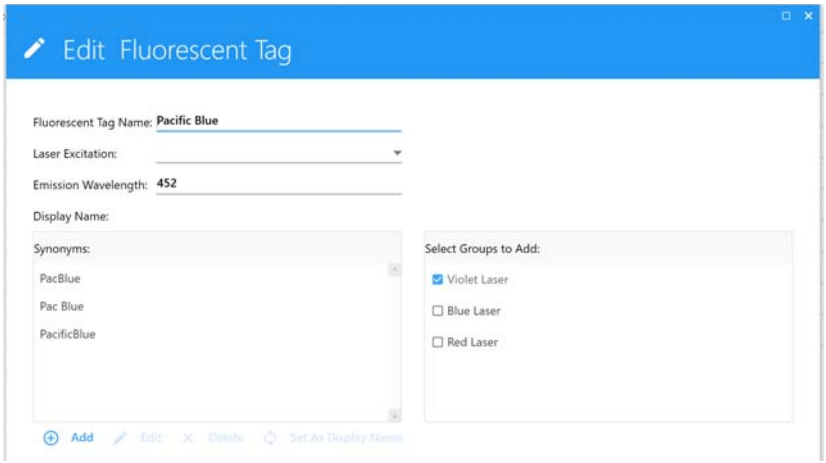
Fluorescent Tags are the designation given to each distinct fluorescent molecule that can be detected by the system. This includes for example, fluorophores, fluorescent proteins, and fluorescent viability dyes. Each unique fluorophore run on the instrument must be given a fluorescent tag name.

By default, three groups of fluorescent tags are pre-installed with the software—Blue Laser, Red Laser, and Violet Laser. These groups contain the most commonly used fluorophores excited by the system's three lasers. Additional tags can be added to these groups. The default tags that are included with the software can be edited, but cannot be deleted.



You can create groups of fluorescent tags by selecting New. Individual fluorescent tags can also be imported or exported.

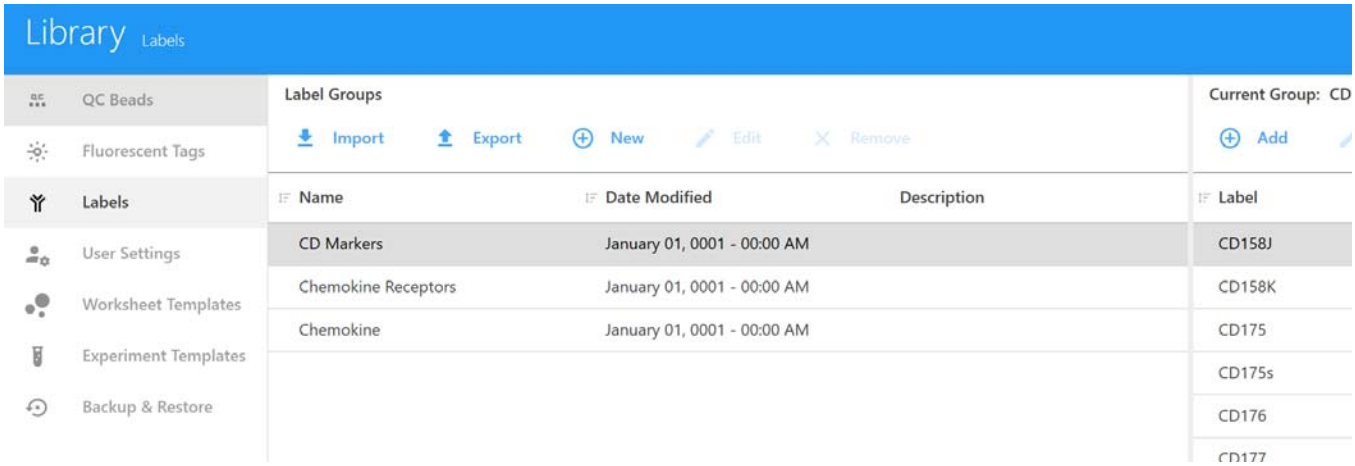
To edit the properties of the fluorescent tag, select the fluorescent tag of interest and select Edit. Properties that can be edited include fluorescent tag name, laser excitation wavelength, emission wavelength, and display name.



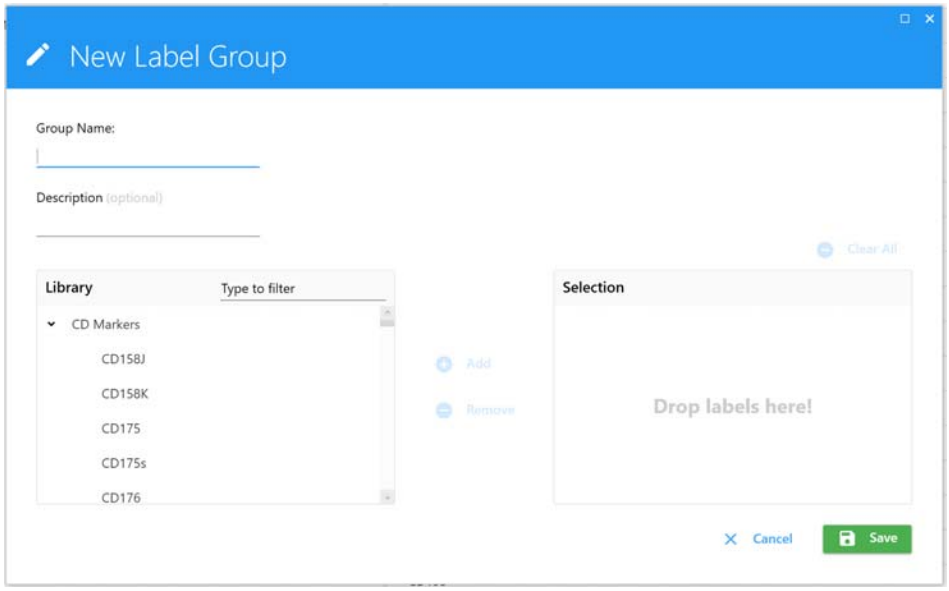
If the fluorophore is known by another name or identified by a different spelling, those additional names or spellings can be added as synonyms. The group in which the tag can be found can also be edited in this window.

Labels

Fluorescent tags can be conjugated or attached to proteins that can specifically bind to other proteins on the cell surface or within the cytoplasm. They can also be inherently fluorescent, such as fluorescent proteins that can be fused to a variety of cellular proteins using molecular cloning techniques. The proteins that are either bound or attached to fluorescent tags can be designated as labels. The software comes with an initial set of pre-installed labels that are categorized as CD Markers, Chemokines, Chemokine Receptors, and Cytokines. Additional labels can be added by using Add in the right pane.



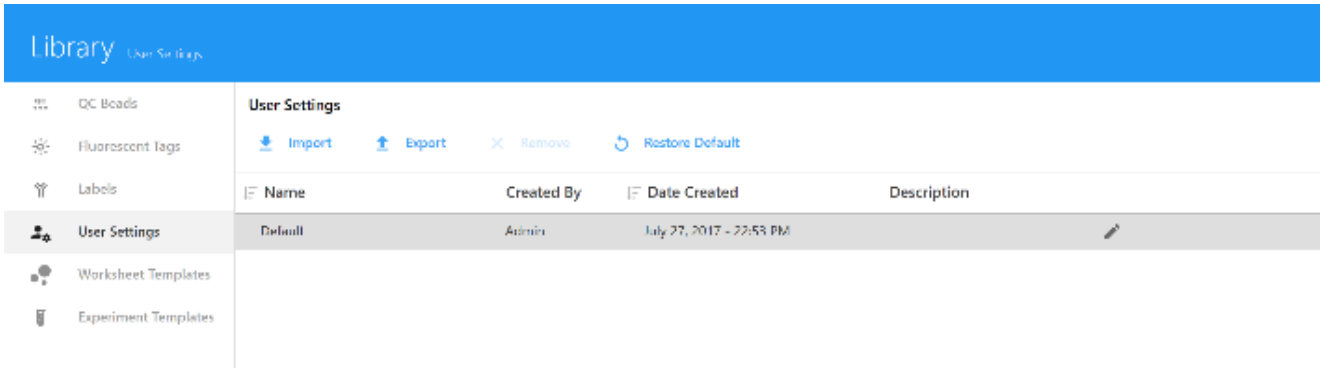
New label groups can be created by clicking New. Label groups can also be imported and exported for use on other systems. The default labels can be edited but *cannot* be deleted.



User Settings

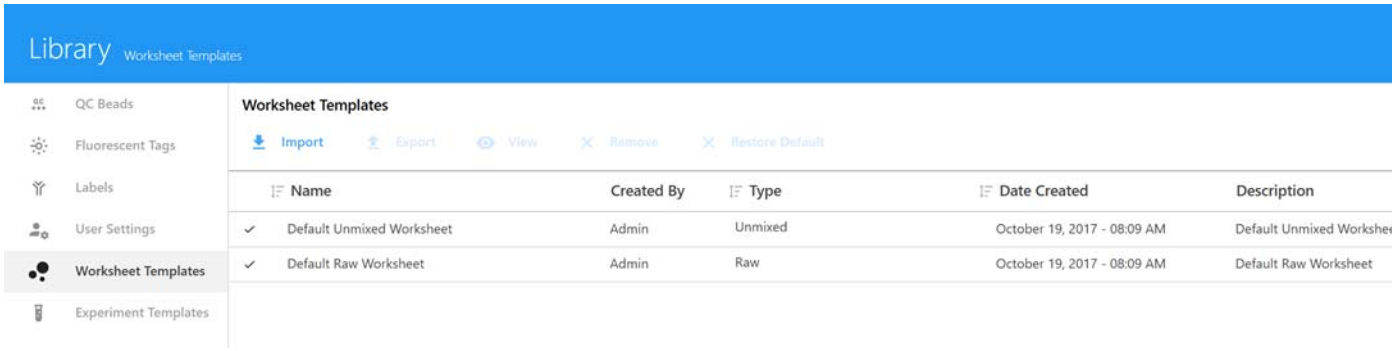
User Settings are the set of gain settings, threshold, and signal type for all detector channels. The name and description can be modified in this tab. The date when it was modified, as well as the user name of the creator are also saved.

User settings are adjusted daily based on the results of the Daily QC run.

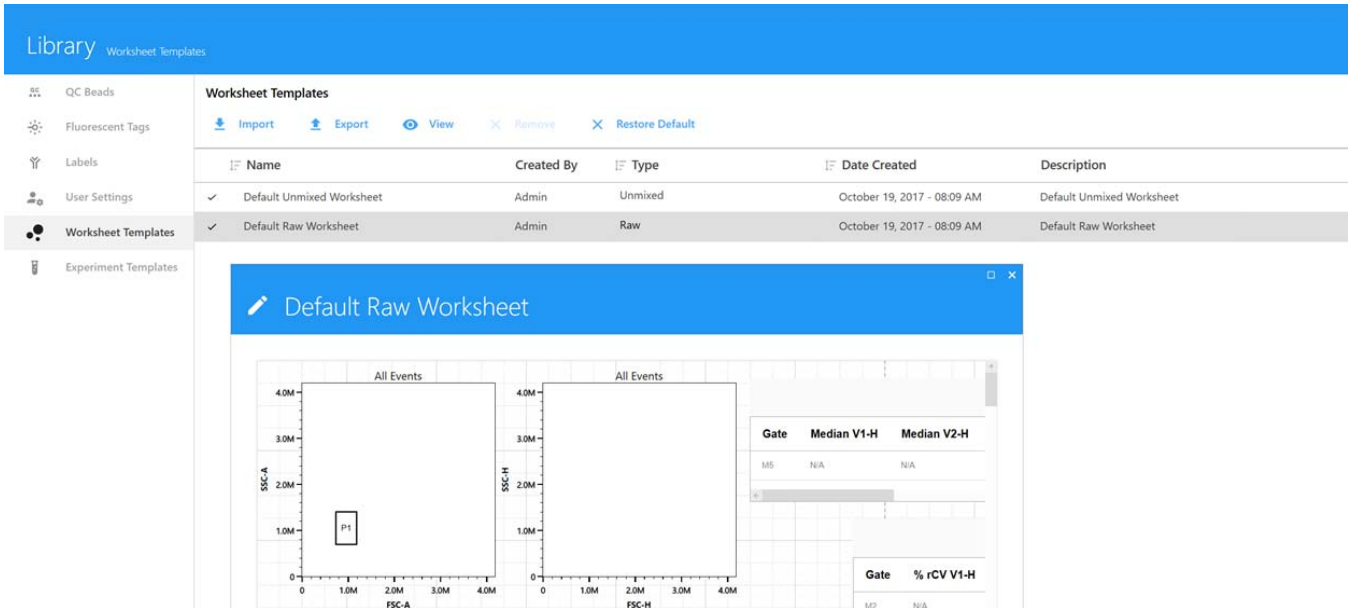


Worksheet Templates

All worksheets created in the Acquisition workspace are saved in the Library and can be accessed through the Worksheet Templates tab. Worksheets can be exported as .WTML files and imported for re-use. To remove a worksheet that is no longer needed, select it, then select Remove.

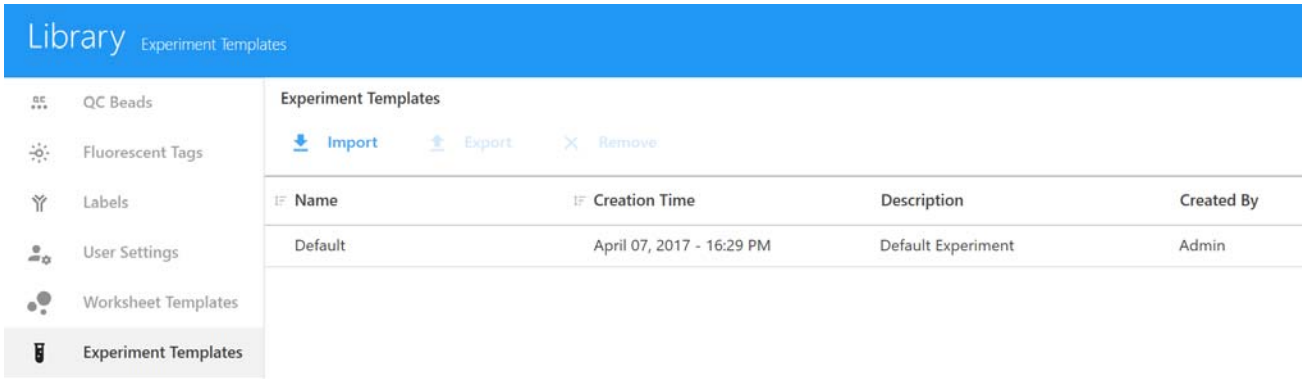


To view a worksheet, select View.



Experiment Templates

Experiment templates can be saved and stored in the Library. The name, creation date and time, description, and creator information is displayed. Experiment templates can also be imported and exported from this tab.

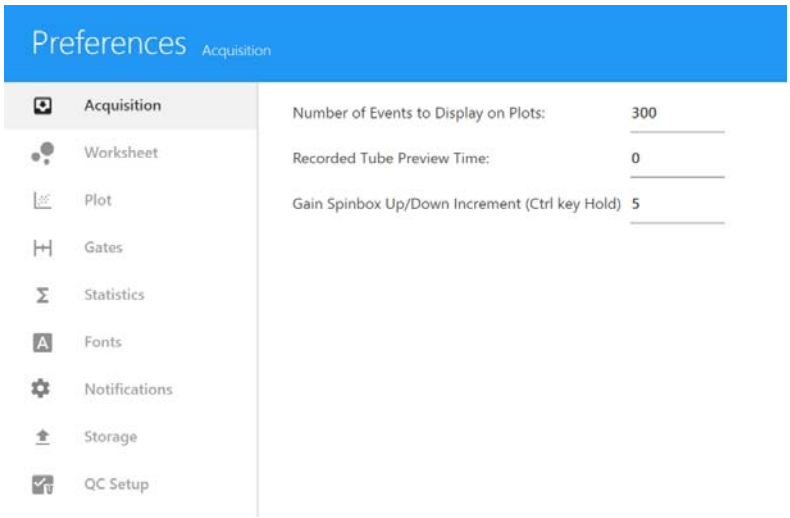


Preferences

The Preferences workspace allows you to change various functionality and display elements of the software user interface. The following section describes the options that can be changed in the Preferences workspace. Each section within the Preferences workspaces can be restored to its default settings by selecting Restore Default Preferences.

Acquisition

In the Acquisition tab, you can change the number of events displayed on plots during acquisition.

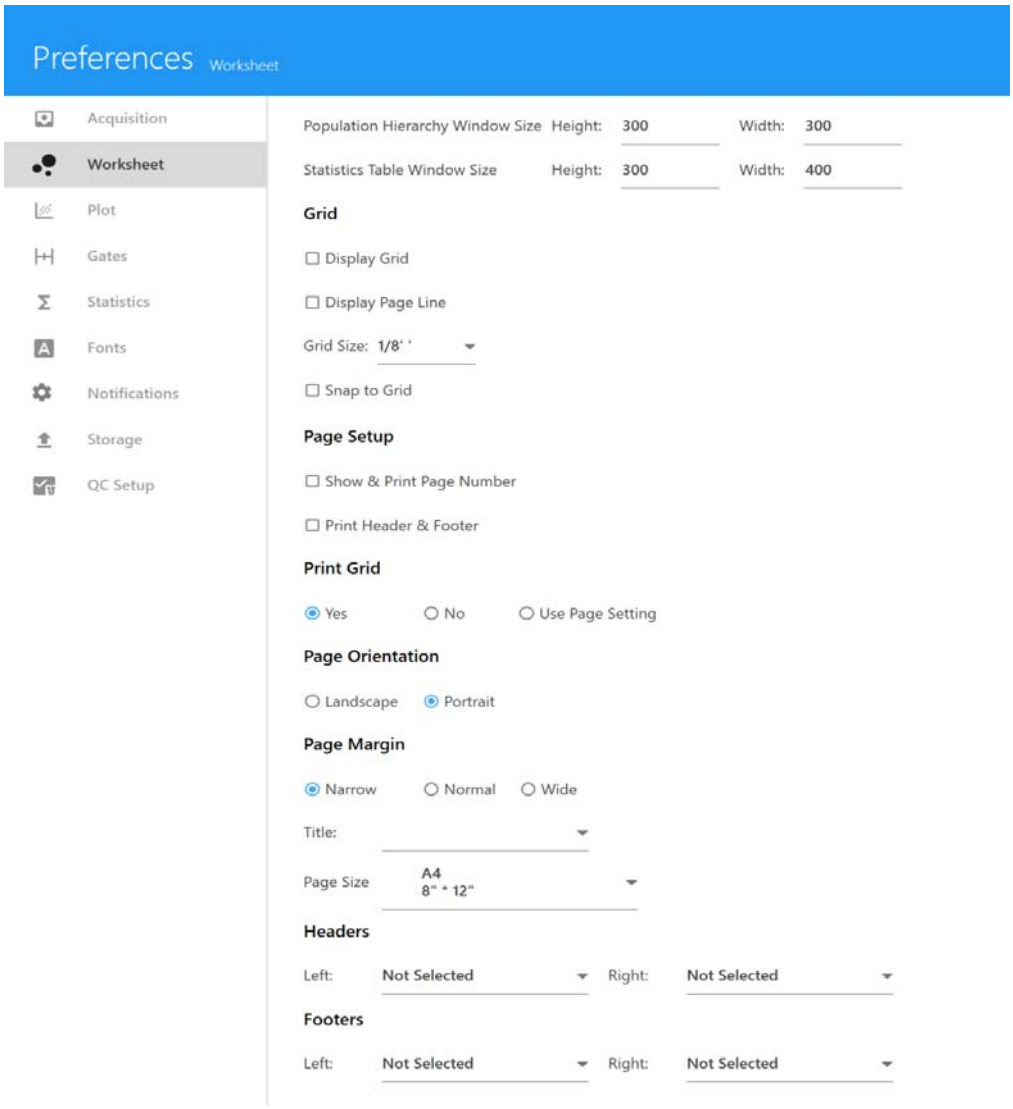


The following table describes the options in the Acquisition preferences.

Item	Description
Number of Events to Display on Plots	The number of events displayed in the pseudocolor plots, dot plots, and histograms. The default it 2,000 events.
Recorded Tube Preview Time	The number of seconds that elapse before the tube pointer moves to the next tube after the current tube is finished recording.
Gain Spinbox Up/Down Increment (Ctrl key Hold)	Increments the gain for each detector channel by the amount indicated when you hold the Ctrl key and select the up and down arrows of the Gain Spinbox.

Worksheet

The Worksheet tab allows you to change the way elements are displayed in the worksheet. Header and footer properties are also adjusted in the Worksheet tab.



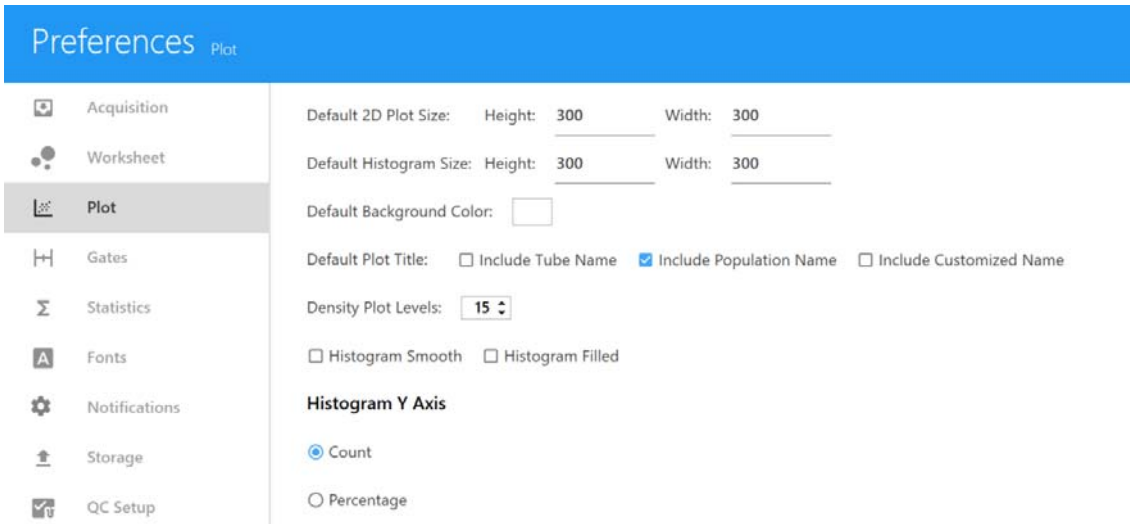
The following table describes the options in the Worksheet preferences.

Item	Description
Population Hierarchy Window Size	Sets the default height and width for the population hierarchy experiment element.
Statistics Table Window Size	Sets the default height and width for the statistics table.
Grid	Display Grid – Toggles on/off the display of grid lines in the worksheet. Display Page Line – Toggles on/off the page break line in the worksheet. Grid Size – Modifies the size of the grid squares. Options include 1”, 1/2”, 1/4”, and 1/8”. Snap to Grid – Toggles on/off the ability for the worksheet elements to snap to and line up with the grid lines on the worksheet.

Item	Description
Page Setup	Show & Print Page Number – Toggles whether the page number is shown and printed. Print Header & Footer – Toggles whether the header and footer are printed.
Print Grid	Toggles whether the grid is printed. Can also be set to use Page Setting.
Page Orientation	Toggles between landscape and portrait.
Page Margin	Sets the margins of the page to Narrow, Normal, or Wide. Title allows you to select the text displayed as the worksheet's title. The title is shown when the worksheet is printed or exported as a PDF.
Page Size	Sets the page width according to standard paper sizes.
Headers	Sets what text is displayed in the left and right headers.
Footers	Sets what text is displayed in the left and right footers.

Plot

The display properties of pseudocolor, dot, and histograms plots can be adjusted in the Plot tab.



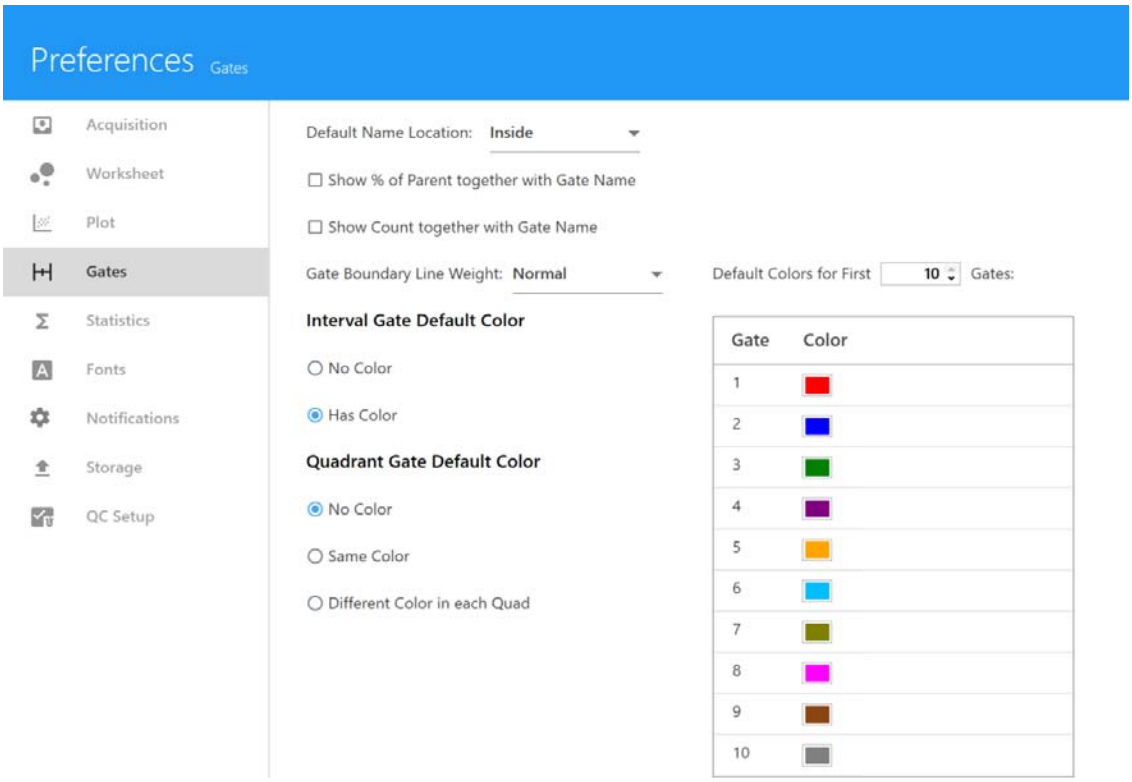
The following table describes the options in the Plot preferences.

Item	Description
Default 2D Plot Size	Set the default height and width of the pseudocolor plots and dot plots in pixels.
Default Histogram Size	Set the default height and width of histograms in pixels.
Default Background Color	Set the default background color for all plots.
Default Plot Title	Customize the title of all plots to include the tube name, population name, and/or a custom name.

Item	Description
Density Plot Levels	Increase or decrease the number of density levels displayed in the pseudocolor plot.
Histogram Smooth	Set whether histogram distributions are smoothed.
Histogram Filled	Set whether histogram distributions are filled.
Histogram Y Axis	Set the scale of the histogram y-axis to a count or a percentage.

Gates

Gate properties can be adjusted in the Gates tab.



The following table describes the options in the Gates preferences.

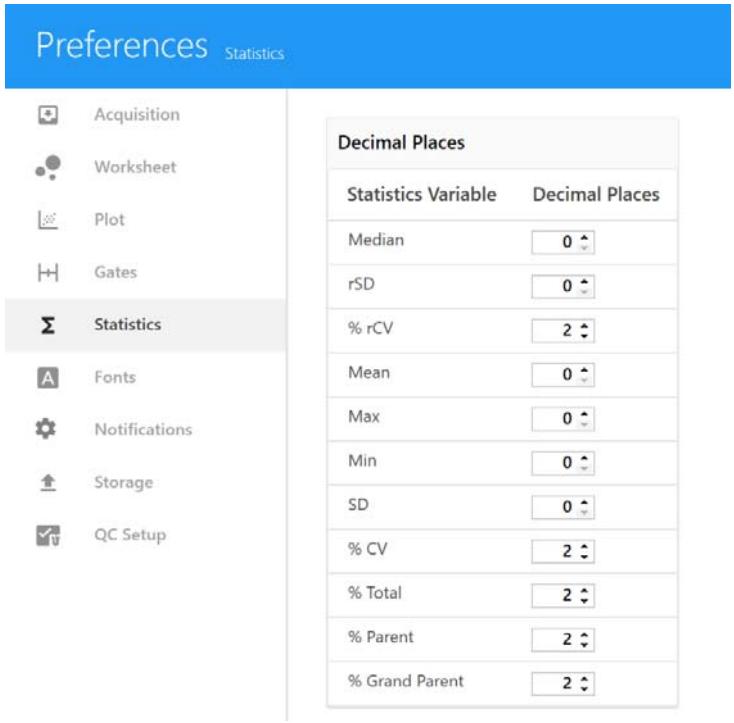
Item	Description
Default Name Location	Select where the gate name is displayed with respect to the gate itself.
Show % of Parent together with Gate Name	Toggles on/off the display of the % of Parent with the gate name.
Show Count together with Gate Name	Toggles on/off the display of the population count with the gate name.
Gate Boundary Line Weight	Sets the thickness of the line drawn by the gate.

Item	Description
Interval Gate Default Color	Toggles on/off whether the population captured by the interval gate has a default color.
Quadrant Gate Default Color	Select whether the population captured by the quadrant gate has a default color.
Default Colors for First X Gates	Set the number of gates that will follow the color scheme detailed in the gate color table. The order in which the colors appear can be changed.

Statistics

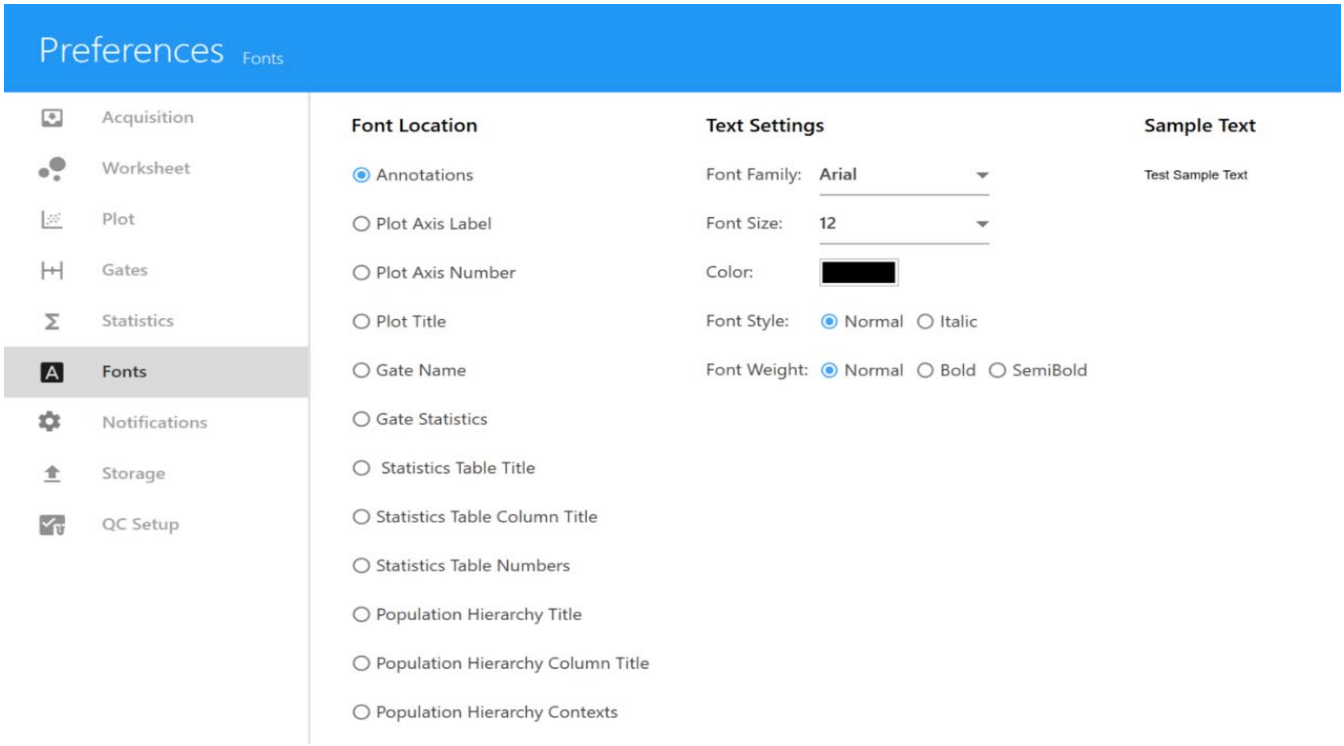
The default degree of precision (number of decimal places) of the statistics displayed in the worksheet can be modified in the Statistics tab.

The precision for the following statistics can be adjusted: Mean, rSD, % rCV, Mean, Max, Min, SD, % CV, % Total, % Parent, and % Grand Parent.



Fonts

The Fonts tab allows you to change the font properties of each display element.

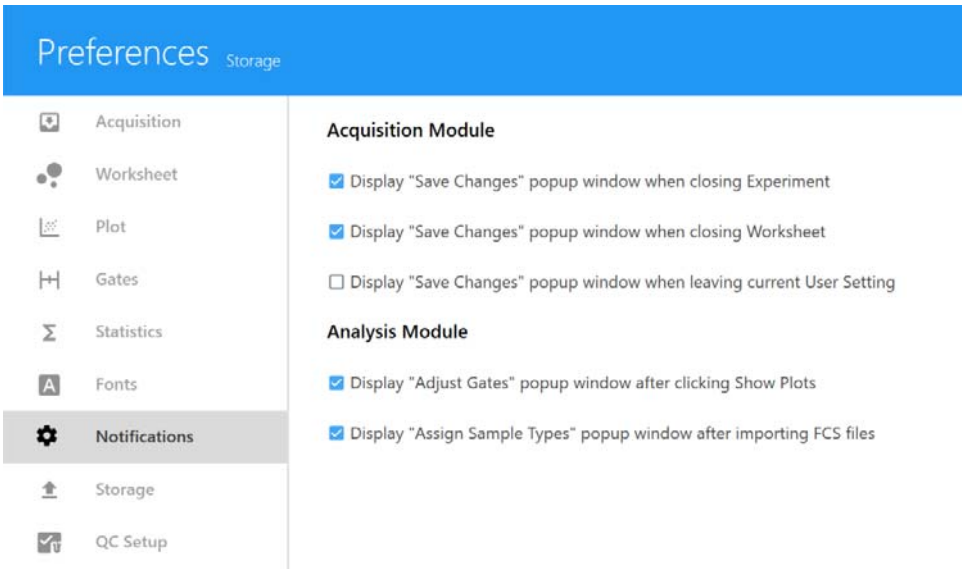


The following table describes the options in the Fonts preferences.

Item	Description
Font Locations	Select which display element's font to modify.
Text Settings	Font Family – Select the font family. Font Size – Select the font size. Color – Select the font color. Font Style – Toggles between normal and italic. Font Weight – Select normal, bold, or semibold.
Sample Text	Example preview text with the properties set in the Text Settings.

Notifications

The Notifications tab allows you to change certain notification settings in the Acquisition and Analysis workspaces.

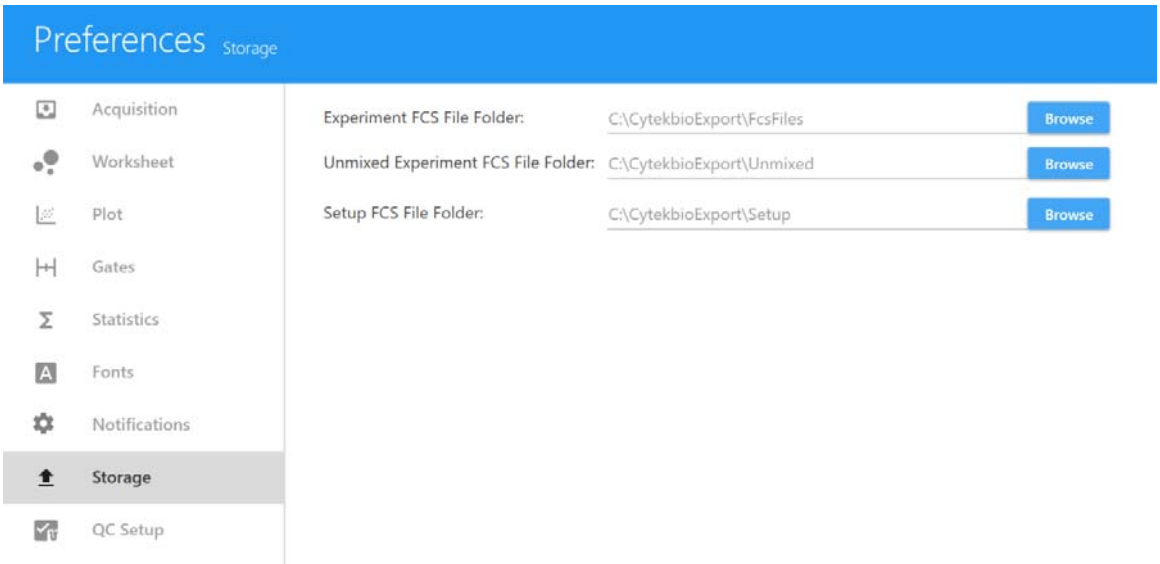


The following table describes the options in the Notifications preferences.

Item	Description
Acquisition Module	Toggles whether to display the Save Changes pop-up window when closing an Experiment, Worksheet, or User Settings.
Analysis Module	Toggles whether to display the instructional dialog boxes in the Analysis workspace.

Storage

The Storage tab allows you to set the default storage locations for the experiment FCS files, unmixed experiment FCS files, and setup FCS files.

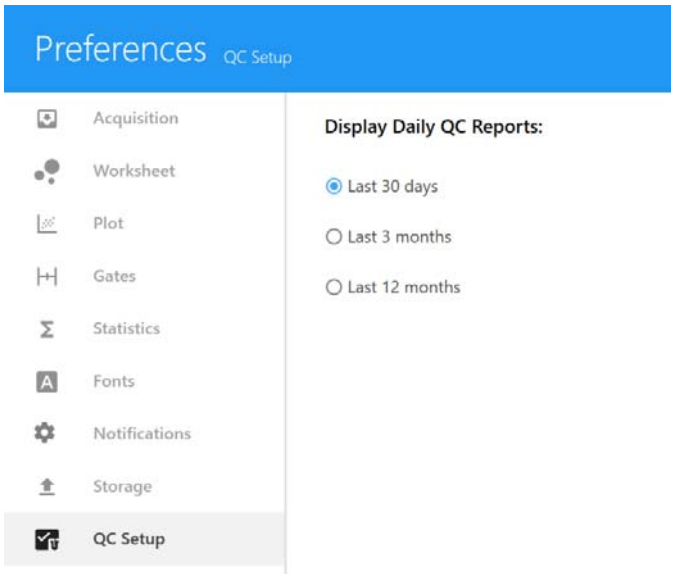


The following table describes the options in the Storage preferences.

Item	Description
Experiment FCS Files Folder	Select the folder where Experiment FCS files are saved.
Unmixed Experiment FCS Files Folder	Select the folder where unmixed Experiment FCS files are saved.
Setup FCS Files Folder	Select the folder where FCS files generated by QC & Setup procedures are saved.

QC Setup

The QC Setup tab allows you to select the days/months of QC reports to display in the Cytometer QC Reports menu.



The following table describes the option in the QC Setup preferences.

Item	Description
Display Daily QC Reports	Select the number of Daily QC reports to be displayed in the Cytometer QC Reports menu.

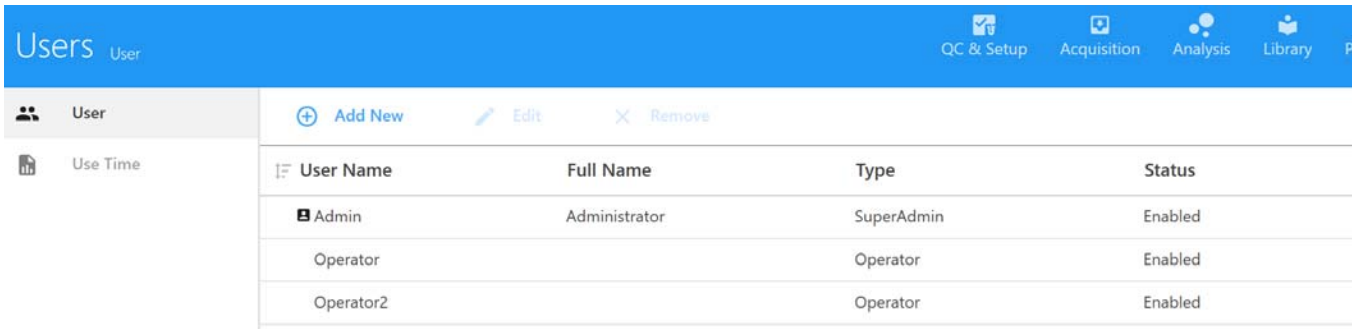
Users

User accounts can be can be managed in the Users workspace. User account information and use time are stored in the workspace.

There are two types of user accounts—administrator and operator. Only administrators can manage user accounts.

Managing Users

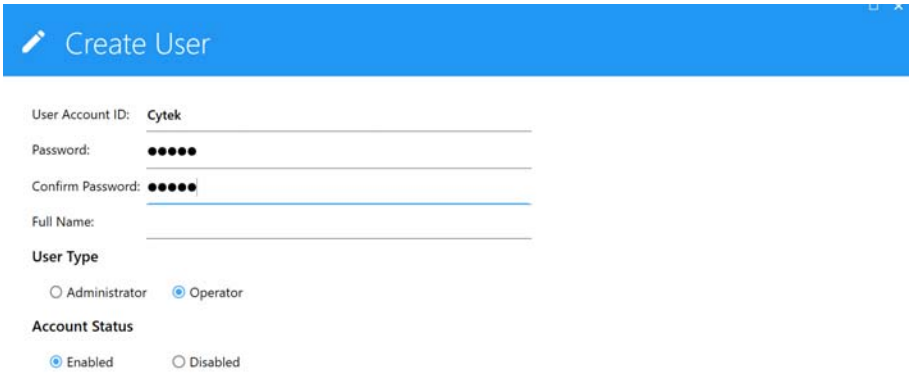
Administrators can add, remove, edit and disable user accounts from the User tab of the Users workspace. The User tab lists all users and displays the type and status of each.



Adding a New User Account

1 Click Add New in the User tab of the Users workspace.

The option is available only for administrators.



2 Enter a user account ID and password, then enter the password again to confirm.

3 (Optional) Enter the user's name.

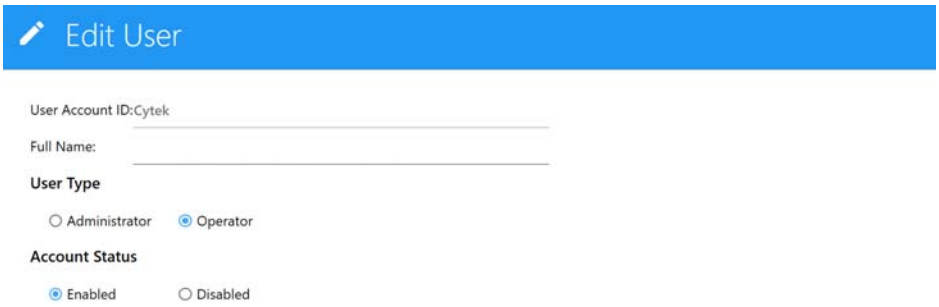
4 Select the user type—administrator or operator

5 Select the user status—enabled or disabled.

6 Click Save.

Editing a User Account

1 Select the user from the User tab of the Users workspace, then click Edit.



2 You can edit or add a user name. You can also change the user type and/or account status.

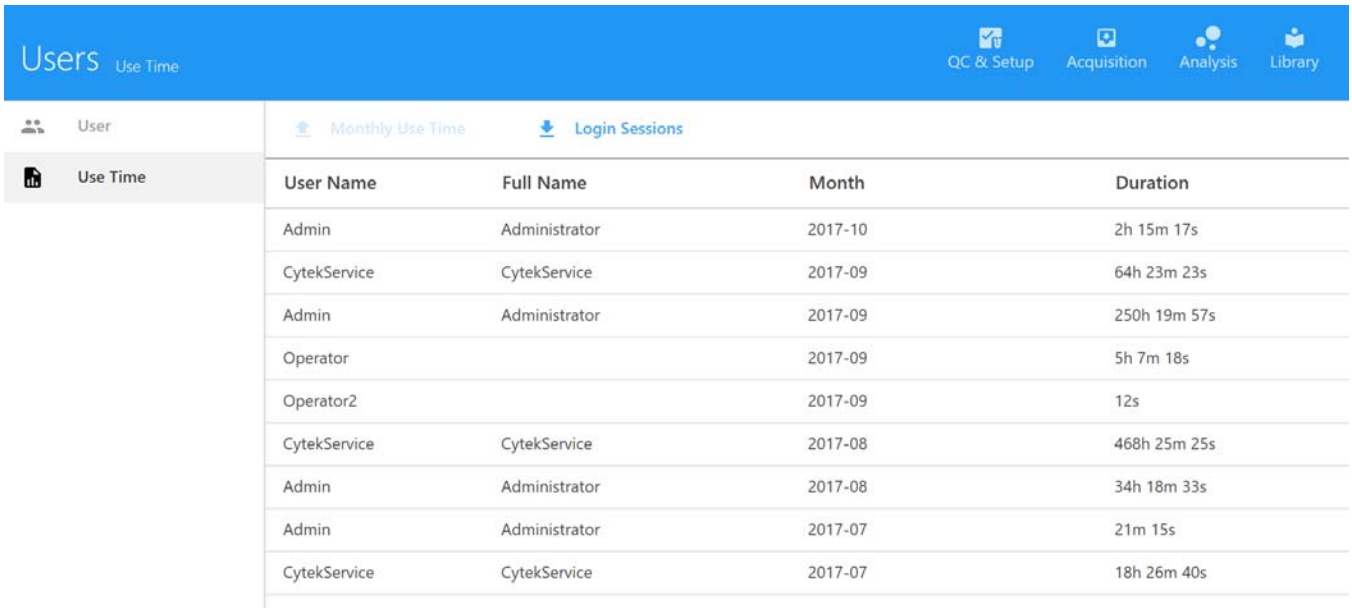
3 Click Save.

Removing a User Account

To remove a user account, select the user from the User tab of the Users workspace, then click Remove.

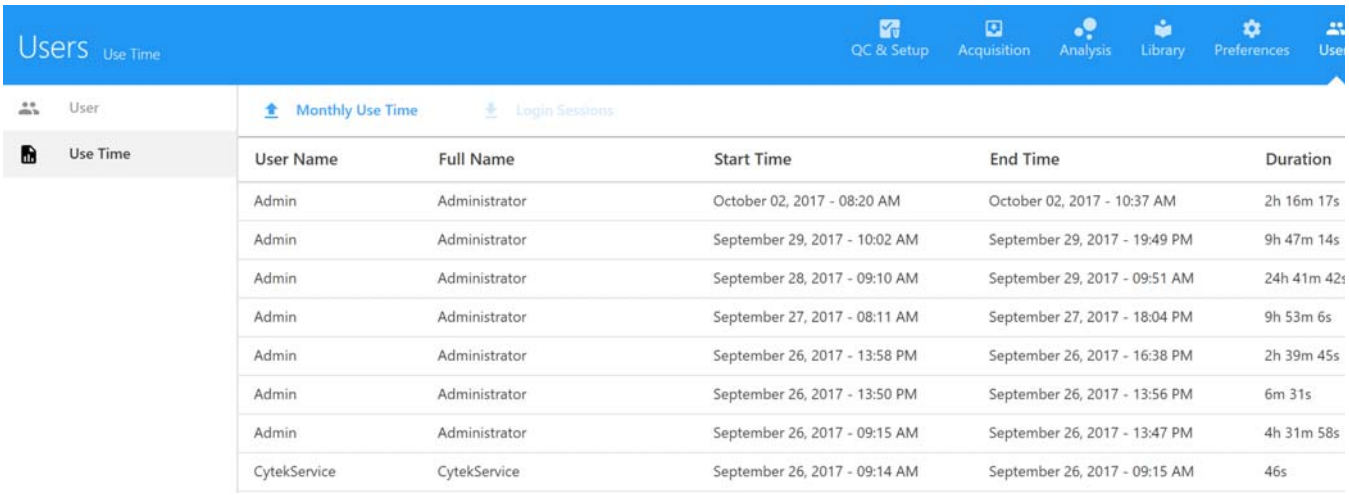
Use Time

The amount of time that each user is on the system is tracked. Click Monthly Use Time to see the total monthly use time (duration) for each user.



User Name	Full Name	Month	Duration
Admin	Administrator	2017-10	2h 15m 17s
CytekService	CytekService	2017-09	64h 23m 23s
Admin	Administrator	2017-09	250h 19m 57s
Operator		2017-09	5h 7m 18s
Operator2		2017-09	12s
CytekService	CytekService	2017-08	468h 25m 25s
Admin	Administrator	2017-08	34h 18m 33s
Admin	Administrator	2017-07	21m 15s
CytekService	CytekService	2017-07	18h 26m 40s

To see the individual login sessions for each day, click Login Sessions. The log on and log off times for each session, as well as the session duration is displayed.



User Name	Full Name	Start Time	End Time	Duration
Admin	Administrator	October 02, 2017 - 08:20 AM	October 02, 2017 - 10:37 AM	2h 16m 17s
Admin	Administrator	September 29, 2017 - 10:02 AM	September 29, 2017 - 19:49 PM	9h 47m 14s
Admin	Administrator	September 28, 2017 - 09:10 AM	September 29, 2017 - 09:51 AM	24h 41m 42s
Admin	Administrator	September 27, 2017 - 08:11 AM	September 27, 2017 - 18:04 PM	9h 53m 6s
Admin	Administrator	September 26, 2017 - 13:58 PM	September 26, 2017 - 16:38 PM	2h 39m 45s
Admin	Administrator	September 26, 2017 - 13:50 PM	September 26, 2017 - 13:56 PM	6m 31s
Admin	Administrator	September 26, 2017 - 09:15 AM	September 26, 2017 - 13:47 PM	4h 31m 58s
CytekService	CytekService	September 26, 2017 - 09:14 AM	September 26, 2017 - 09:15 AM	46s

8

Maintenance

Maintenance Schedule

Any instrument surface in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning the instrument or replacing parts. Wear suitable protective clothing, eyewear, and gloves.

Routine maintenance of the Aurora cytometer includes periodic replacement of parts. For part numbers, see “Supplies and Replacement Parts” on page 91.

Scheduled Maintenance

The following table describes the scheduled maintenance procedures for your cytometer.

Maintenance Procedure	Description	Frequency
Replacing the sheath filter	Ensures debris-free sheath fluid	Every 6 months, or as needed
Decontaminating the fluid system (Long Clean)	Cleans the fluidic lines with bleach	Once a month and prior to service calls

Unscheduled Maintenance

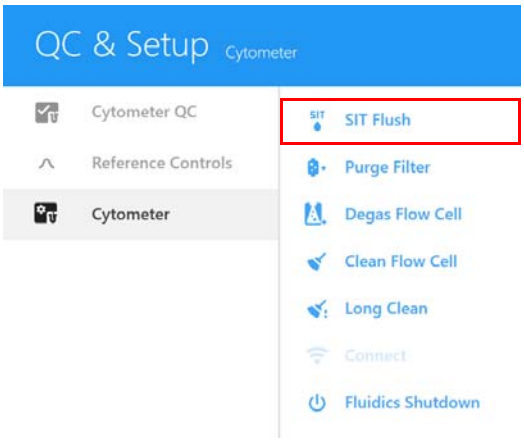
The following table describes the list and frequency of unscheduled maintenance procedures for your cytometer.

Maintenance Procedure	Description	Frequency
Cleaning the SIT (SIT Flush)	Backflushes the SIT	As needed, or after running sticky dyes
Purging the sheath filter (Purge Filter)	Removes bubbles from the sheath filter	If bubbles are present in the sheath filter, or if the plenum or sheath tank run dry
Removing air bubbles from the flow cell (Degas Flow Cell)	Removes bubbles from the flow cell	A needed
Cleaning the flow cell (Clean Flow Cell)	Runs 10% bleach solution followed by DI water through the flow cell	As needed, or after running sticky dyes
Cleaning external surfaces	Keeps surfaces free from salt buildup	As needed

Cleaning the SIT

A sample line backflush is performed whenever a tube is removed from the SIP after sample acquisition. If the sample line exhibits signs of carryover or becomes clogged after completing an experiment with a sticky dye such as propidium iodine, acridine orange, or thiazole orange, the sample line should be manually backflushed.

1 In the Cytometer tab, from either the QC & Setup or Acquisition workspace, select SIT Flush.



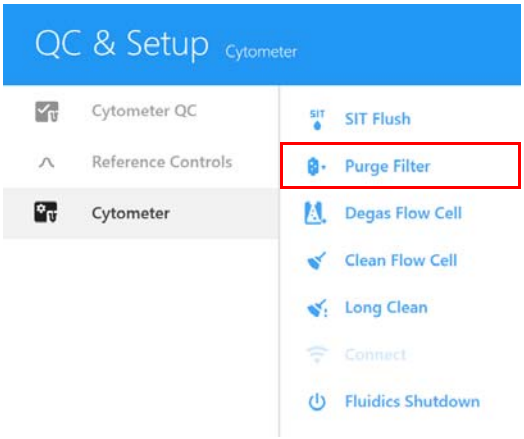
2 If carryover or a clog persists, place a tube of 10% bleach on the SIP and acquire at High flow rate for 5 minutes. Afterwards, acquire a tube of DI water at High flow rate for 5 minutes.

■ **NOTE:** If running large quantities of beads or large cells, we recommend running a tube of 10% bleach followed by a tube of DI water, each for 5 minutes, between experiments.

Purging the Sheath Filter

Perform this procedure if air bubbles are visible in the sheath filter, or if the plenum or sheath tank have run dry and air is present in the fluidics system.

1 In the Cytometer tab, from either the QC & Setup or Acquisition workspace, select Purge Filter.



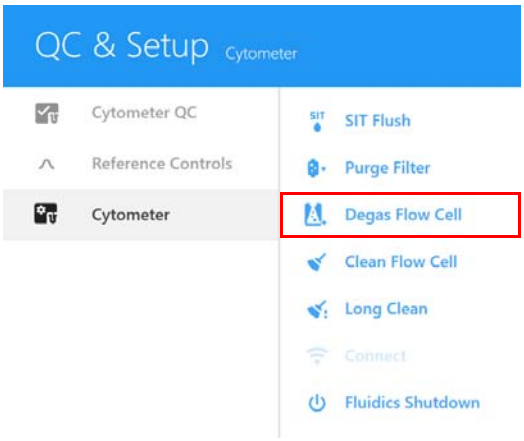
The vent valve connected to the sheath filter will open releasing any air bubbles trapped inside the sheath filter.

2 Repeat the Purge Filter fluidic mode until there are no visible bubbles inside the sheath filter.

Removing Air Bubbles from the Flow Cell

Perform this procedure if the FSC and SSC signals appear abnormal. Air bubbles may be trapped in the flow cell, disrupting the sample flow.

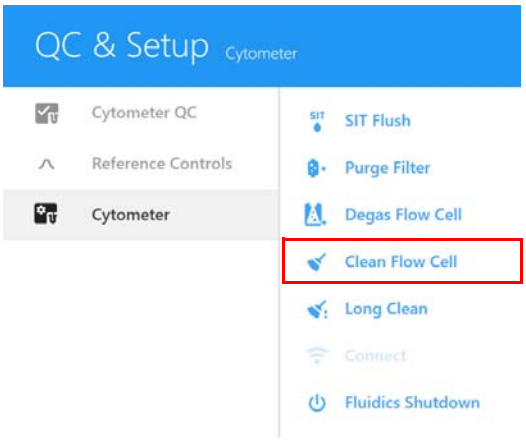
1 In the Cytometer tab, from either the QC & Setup or Acquisition workspace, select Degas Flow Cell.



Cleaning the Flow Cell

Clean the flow cell after completing an experiment with a sticky dye such as propidium iodine, acridine orange, or thiazole orange. Cleaning the flow cell is also recommended after acquiring large quantities of highly concentrated bead solutions.

1 In the Cytometer tab, from either the QC & Setup or Acquisition workspace, select Clean Flow Cell.



2 Follow the instructions that appear. Install a tube of 10% bleach solution on the SIP.

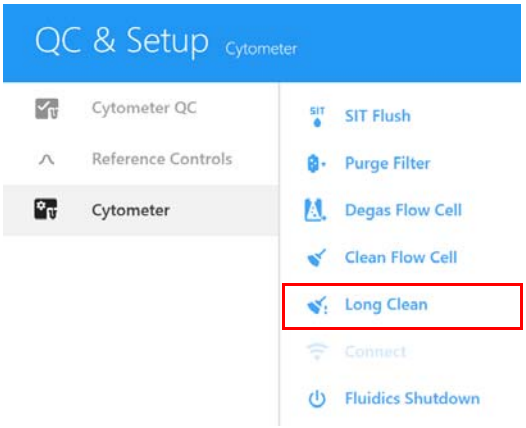
3 When the cleaning is complete, replace the tube of bleach with a tube of DI water and select Clean Flow Cell again.

Decontaminating the Fluidics System

Decontaminate the fluidics system monthly by running the Long Clean fluidic mode. Run the Long Clean just prior to service calls and if you run high volumes of unwashed samples or samples stained with propidium iodide, acridine orange, or thiazole orange.

Do not run bleach or detergent through the sheath filter. This can damage the filter, resulting in paper fragments that could clog the flow cell.

1 In the Cytometer tab, from either the QC & Setup or Acquisition workspace, select Long Clean.



2 Follow the instructions that appear. Prepare the appropriate cleaning tubes and fluidic tanks.

3 Empty the waste tank. Replace the sheath filter with the sheath filter bypass (long clean tubing) assembly.

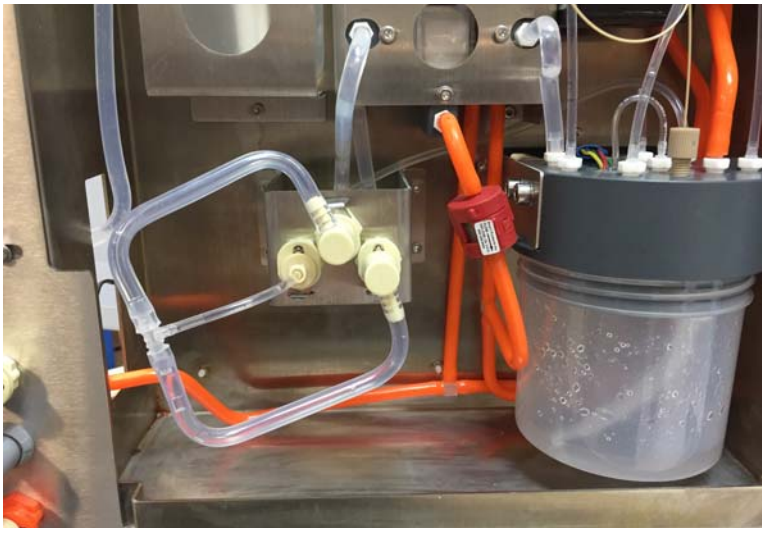


Figure 10. Long clean tubing assembly connected in place of sheath filter

4 Detach the sheath tank and replace it with a tank containing a 10% bleach solution.

5 Install a tube containing 3 mL of a 10% bleach solution on the SIP.

6 Proceed with the Long Clean in the software.

7 Once the bleach cleaning cycle is complete, reattach the sheath tank.

8 Remove the tube of 10% bleach from the SIP and replace with a tube of 3 mL of DI water.

9 Proceed with the Long Clean in the software.

10 When prompted, remove the long clean tubing assembly and re-install the sheath filter.

Cleaning the External Surfaces

Periodically check for saline residue.

1 Dampen a cloth with a mild cleaning solution and wipe the surfaces of the instrument.

2 Dampen a cloth with DI water and wipe the surfaces again to remove residual cleaning solution.

3 Dry the surfaces with a clean, dry cloth.

Inspecting the Fluidics Lines

Check the cytometer periodically for fluid leaks. If any evidence of a leak is detected, contact Cytek Technical Support immediately. Do not attempt to repair the instrument.

1 Visually inspect for fluid leaks by looking for small pools of liquid near any of the quick-connects.

2 Visually inspect for dried residue or slight discoloration in the spaces surrounding the cytometer.

Replacing the Sheath Filter

The sheath filter traps debris and air bubbles before they reach the flow cell. Replace the filter assembly every 6 months or when you see increased debris in an FSC vs SSC plot.

Wear appropriate safety attire such as protective gloves, lab coat, and eyewear while performing this procedure.

1 Turn off the cytometer.

2 Open the front cytometer panel.

3 Press the two fluidics line quick-connects and the vent line quick-connect to the right of the sheath filter.

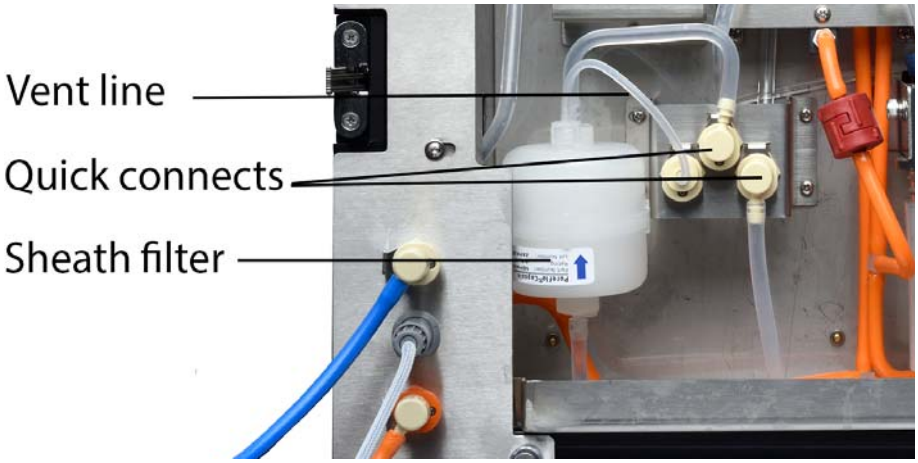


Figure 11. Sheath filter and sheath filter quick-connects

4 Disconnect the vent line.

5 Discard the filter according to standard laboratory protocol and local regulations.

6 Install a new sheath filter with the arrow pointing up.

7 Run the Purge Filter fluidic mode to remove air bubbles (see “Purging the Sheath Filter” on page 72). Repeat this step until all air bubbles are purged from the filter.

8 Close the front panel.

Replacing the SIT

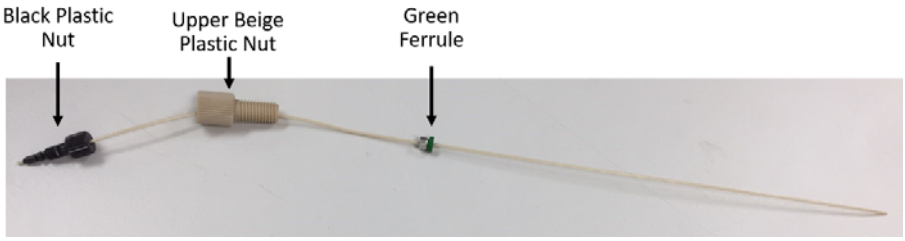
Replace the SIT if the tubing is clogged even after repeatedly cleaning and flushing the SIT.

1 Ensure the SIT is protracted in a tube of DI water and the cytometer is turned off.

If the cytometer was shut down properly using the Fluidics Shutdown procedure, the SIT will already be protracted and left is water.

■ **NOTE:** If the SIT is not protracted, turn on the cytometer and run Fluidics Shutdown. Then turn off the cytometer.

2 Obtain a SIT tubing assembly.



3 Open the SIT door. Identify the four components shown below.

The SIT door is located above the SIP.

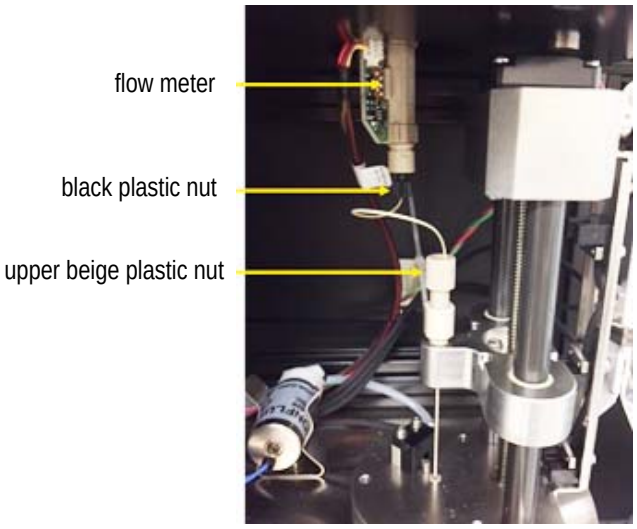
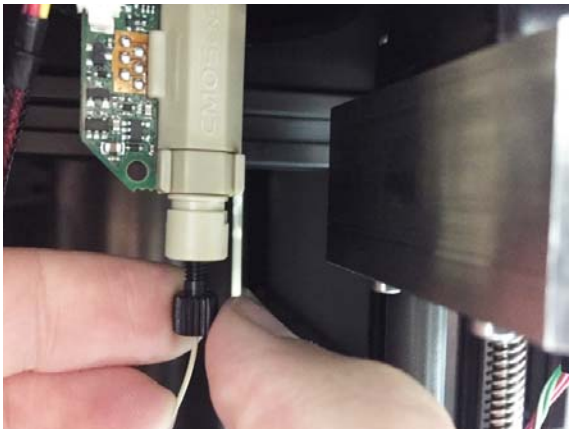
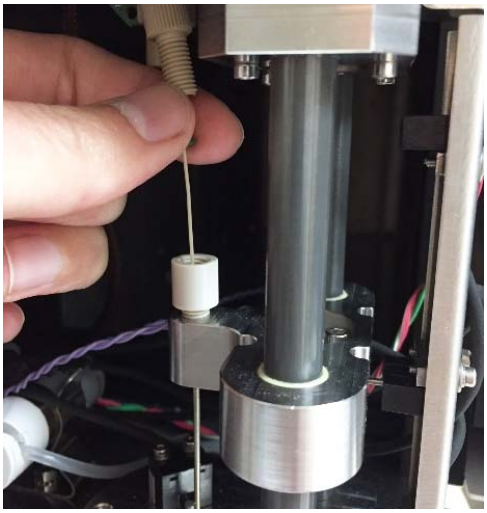


Figure 12. Inside SIT door

4 Twist off and carefully remove the black plastic nut from the bottom of the flow meter.

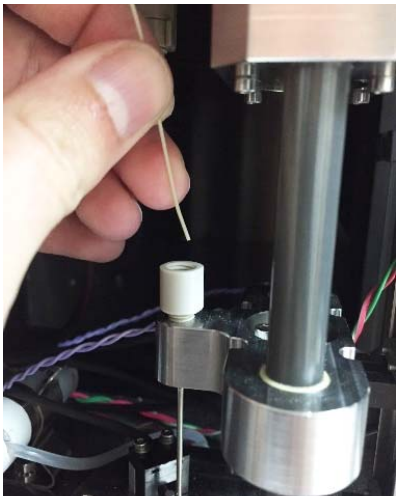


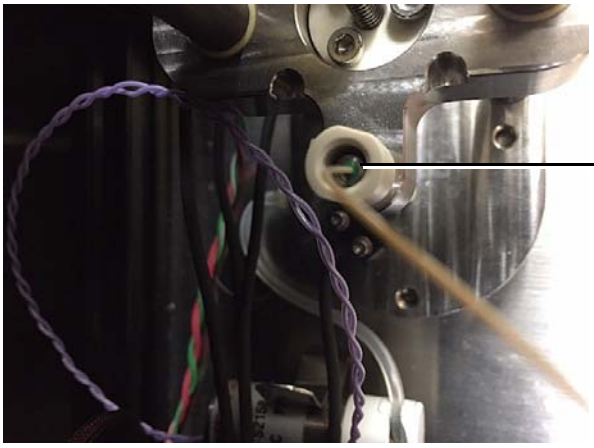
5 Follow the tubing from the black nut down to the beige plastic nut. Twist off the beige nut and gently pull the beige nut and tubing out from the SIP.



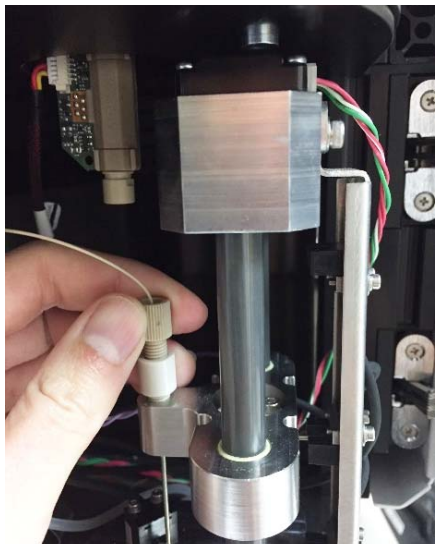
6 Discard the SIT tubing assembly.

7 Insert the new sample tubing through the hole. Feed the tubing through the hole until the green ferrule contacts the surface around the hole.

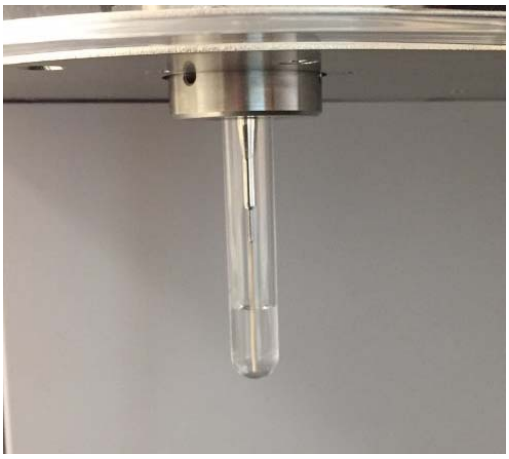




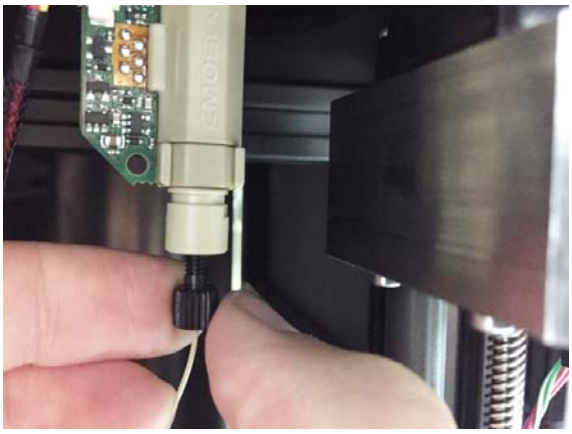
8 With the green ferrule flush with the surface around the hole, slide the beige nut down into the hole and turn the nut until it is securely attached.



9 Verify that the tubing contacts the bottom of the tube on the SIP. If the end of the tubing is not positioned properly, ensure you performed steps 7 and 8.



10 Secure the black nut to the bottom of the flow meter. Turn the nut until it is firmly attached.



11 Close the SIT door.

9

Troubleshooting

This section provides tips to help you identify and resolve issues that might occur on your flow cytometer. If additional assistance is required, contact Cytek Biosciences. Please have the following information available: serial number, error messages, and details of recent performance.

For instrument support within the US, call 1-877-92-CYTEK. Visit our website, www.cytekbio.com, for up-to-date contact information.

Observation	Possible Causes	Recommended Solutions
Air in sheath filter	Cytometer was not in use for a prolonged period	Run a Purge Filter. Check that all sheath connectors are securely attached. Check for leaks or cracks in the sheath plenum. Replace, if needed.
	Empty sheath tank	Fill the sheath tank.
No events displayed	Insufficient gain for threshold parameter	Increase the gain for the threshold parameter.
	Threshold too high	Lower the threshold.
	Laser delay not correct	Ensure the laser delay values match those from the latest Daily QC run. See “Instrument Control” on page 34 for the laser delay location. If the values do not match, rerun Daily QC.
	Threshold set to incorrect parameter	Set the threshold to the appropriate parameter for the application (usually FSC).
	No sample in tube	Add sample or install a new sample tube.
	Improperly mixed sample	Mix sample to suspend cells/particles.
	Clogged SIT	Run a SIT Flush. Then run Clean Flow Cell with 10% bleach, followed by a Clean Flow Cell with DI water. If the clog persists, replace the sample line.
	Gated plot with no data in gate	Delete or move the gate.

Observation	Possible Causes	Recommended Solutions
Low sample event rate	Threshold too high	Lower the threshold.
	Insufficient gain for threshold	Increase the gain for the threshold parameter.
	Sample not mixed	Mix sample to suspend cells/particles.
	Sample too dilute	Concentrate the sample. If the flow rate is not critical to the application, set the flow rate to Medium or High.
	Clogged SIT	Run a SIT Flush. Then run Clean Flow Cell with 10% bleach, followed by a Clean Flow Cell with DI water. If the clog persists, replace the sample line.
Erratic event rate	Partially blocked SIT	Run a SIT Flush. Then run Clean Flow Cell with 10% bleach, followed by a Clean Flow Cell with DI water.
	Contaminated sample	Prep the specimen again, ensure the tube is clean.
	Clumpy sample	Vortex or disaggregate the sample.
Data in scatter parameters appear distorted	Incorrect instrument settings	Optimize the settings.
	Air bubble in flow cell	Run a Degas Flow Cell.
	Air in sheath filter	Run a Purge Filter.
	Dirty flow cell	Run a Clean Flow Cell.
	Poor sample health	Check the viability of the cells.
	Hypertonic buffers	Check the pH of the buffers and fixative.
High CVs	Air bubble in fluidics	Run a Purge Filter and a Degas Flow Cell.
	Sample flow rate set to High	Set the sample flow rate to Low or Medium.
	Dirty flow cell	Run a Clean Flow Cell.
	Questionable sample prep	Verify sample prep technique.
	Air in sheath filter	Run a Purge Filter.
	Sample not diluted in same fluid as sheath	Dilute the sample in the same fluid as the sheath solution.

10

Glossary

auto-fluorescence	The inherent fluorescence arising primarily from cell structures such as mitochondria and lysosomes. Auto-fluorescence can hinder detection of dim fluorescent signals.
compensation	The process by which spillover fluorescence from secondary parameters is accounted for so that fluorescence values for a parameter represent only the fluorescence of the primary fluorophore.
data file	A collection of measured values from a single sample combined with text describing the data that has been stored as a flow cytometry standard (.fcs) file to disk.
deconvolve	An algorithm-based process used to reverse the effects of convolution (or overlapping) on recorded data.
detector	A device that responds to a specific stimulus. Photodiodes and photomultiplier tubes are two types of detectors in cytometers. They convert light signals into electronic signals.
dot plot	A graphical representation of two-parameter data. Each axis of a plot displays values of one parameter.
electronic noise	Random fluctuation in electronic signals, a characteristic of all electronic circuits.
event rate	The rate at which cells or particles are acquired.
FCS	Flow cytometry standard, a standard format for flow cytometry data files.
filter	An optical device that blocks the passage of part of the incident light, allowing the rest to pass virtually unchanged.

flow cell	The flow cell enables hydrodynamic focusing of the sample so that the individual cells or particles of interest can be interrogated by the laser(s) sequentially.
flow cytometry	A technology that simultaneously measures and analyzes multiple characteristics of single cells or particles as they pass through a laser beam.
flow rate	The amount of fluid passing through a point per unit of time.
fluorescence	The emission of light of longer wavelengths that occurs when a substance absorbs light of shorter wavelengths.
fluorophore	A fluorescent dye. A molecule capable of absorbing light energy, then emitting light at a longer wavelength (fluorescence) as it releases this energy.
gain	Amplification of a signal. Increasing gain results in a larger output signal for a given input signal.
gate	A numerical or graphical boundary (region) that defines a subset of data. Gates can be single- or multi-dimensional.
laser	Light Amplification by Stimulated Emission of Radiation. A light source that is highly directional, monochromatic, coherent, and bright. The emitted light is in one or more narrow spectral bands, and with most lasers, is concentrated in an intense, narrow beam.
laser delay	Amount of time between signals from different laser intercepts.
photodiode	A device for measuring light intensity. A photodiode generates an output current proportional to the incident light intensity.
PMT	Photomultiplier tube. A sensitive device for measuring light intensity. PMTs produce an output current proportional to the incident light intensity.
rSD	Robust standard deviation. The robust SD is based on the deviation of individual data points to the median of the population.
reference	Spectral profile of a fluorescent tag in all detectors for all lasers.

resolution	A measure of a cytometer's ability to distinguish between two populations with differing fluorescence or light scatter intensities.
SIP	Sample injection port. The area of the cytometer where the sample is placed.
SIT	Sample injection tube. The probe that pulls sample from the sample tube to the flow cell.
spectral overlap	The phenomenon of different fluorophores emitting light within the same detection range. In multi-color experiments, compensation must be performed to correct for spectral overlap.
spillover	Emitted light from a fluorophore entering the detector of another fluorophore.
voltage	Measure of electric potential. The voltage applied to a PMT affects its amplification gain.

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Specifications

Cytometer

Optics

Item	Specification
Optical platform	Fixed optical assembly configured with three spatially separated laser beams. Laser delays are automatically adjusted during instrument QC.
Lasers	405 nm: 100 mW 488 nm: 50 mW 640 nm: 80 mW
Beam geometry	Flat-top laser beam profile with narrow vertical beam height optimized for small particle detection.
Emission collection	Fused silica cuvette coupled to high NA lens for optimum collection efficiency to optical fibers.
Forward scatter detector and filter	High-performance semiconductor detector with 488-nm bandpass filter
Violet side scatter detector	High-performance semiconductor detector with 405-nm bandpass filter
Fluorescence detectors	Proprietary high-sensitivity Coarse Wavelength Division Multiplexing (CWDM), 16-channel semiconductor detector array per laser, enabling more efficient spectrum capture for dyes emitting in the 400-nm to 900-nm range. No filter changes required for any fluorophore excited by the 405-nm, 488-nm, and 640-nm lasers.
Standard optical configuration	Violet detector module: 16 channels uneven spaced bandwidth from 420 nm–830 nm. Blue detector module: 14 channels uneven spaced bandwidth from 500 nm to 890 nm. Up to 16 channels available. Red detector module: 9 channels uneven spaced bandwidth from 650 nm to 890 nm standard. Up to 16 channels available.

Fluidics

Item	Specification
General operation	Vacuum driven fluidics with the following fluidics modes: Long Clean, SIT Flush, Purge Filter, Degas Flow Cell, Clean Flow Cell, Fluidics Shutdown.
Compatible tubes	12 x 75-mm polystyrene and polypropylene tubes
Fluidic reservoirs	4-L fluid tanks with level-sensing provided. Compatible with 20-L sheath and waste cubitainers.
Sample flow rates	Three preset flow rates: • Low: 15 µL/min • Medium: 30 µL/min • High: 60 µL/min
Data acquisition rate	Up to 35,000 events/s

Fluorescence Sensitivity

Item	Specification
Fluorescence sensitivity threshold	FITC: 100 molecules of equivalent soluble fluorophore (MEFL-FITC) PE: 30 molecules of equivalent soluble fluorophore (MEFL-PE) APC: 15 molecules of equivalent soluble fluorophore (MEFL-APC) Pacific Blue: 200 molecules of equivalent soluble fluorophore (MEFL-Pacific Blue) **NOTE:** Measurements performed using SPHERO Rainbow Calibration Particle (RCP-30-5A) based on its single-peak detection channel.
Fluorescence linearity	FITC $R^2 \geq 0.995$ / PE $R^2 \geq 0.995$
Forward and side scatter sensitivity	Enables separation of fixed platelets from noise.
Forward and side scatter resolution	Performance is optimized for resolving lymphocytes, monocytes, and granulocytes, as well as microparticles.
Side scatter resolution	Capable of resolving 0.2-µm beads from noise.

Workstation

Item	Specification
Operating system	Microsoft® Windows® 10, 64-bit Professional
Processor	Intel® Core™ i7-6700T, 3.0 GHz
RAM	16 GB, 16,000 MHz DDR4 SO-DIMM
Hard drive	500 GB SATA 3.0 GB/s
Video processor	Intel® HD Graphics 530
Monitor	28-in UHD

Installation Requirements

Item	Specification
Dimensions	54 x 52 x 52 cm (21.3 x 20.5 x 20.5 in)
Weight	61 kg (134.5 lb)
Workstation	3.5 x 18.3 x 17.9 cm (1.4 x 7.2 x 7.0 in)
Recommended workspace	152.4 x 61 x 132 cm (60 x 24 x 52 in)
Power	100-240 V, 50/60 Hz, 2A max
Heat dissipation	500 Watts with all solid-state lasers
Temperature	15°–28°C
Humidity	20% to 85% relative non-condensing
Air filtering	No excessive dust or smoke
Lighting	No special requirements

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Supplies and Replacement Parts

Item	Part Number	Description
4L Tank	60-30060-00	4-L tank for sheath or waste
Lid for sheath tank	02-62001-00	Lid fits 4-L sheath tank and includes liquid level sensor.
Lid for waste tank	02-62002-00	Lid fits 4-L waste tank and includes liquid level sensor.
Cubitainer waste cap	N7-32014-0A	Cap fits 20-L waste cubitainer.
Cubitainer sheath cap	N7-32015-0A	Cap fits 20-L sheath cubitainer.
Reservoir holder	18-22008-00	Holds the sheath and waste tanks.
SpectroFlo QC Beads	97-30310-00	Contains 2 mL of beads at 10^7/mL concentration. Beads provide a single peak fluorescence intensity for use with Daily QC.
Sheath filter (0.2 µm)	41-40018-00	0.2-µm sheath filter, 1/4 barb fitting on top and bottom. Bleed-off port adjacent to sheath solution output.
Sheath filter assembly	02-20664-00	Sheath filter assembly with quick-connect fittings and manifold.
Sample line	N7-22007-0A	SIT tubing assembly.
Sheath filter bypass (Long Clean tubing)	N7-22010-0A	Replaces the sheath filter during a Long Clean.

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