Cytek® Aurora CS 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
Class I laser product.

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

History

Revision	Date	Change
N9-20077 Rev. A	1/2022	Initial release. Added features to the software including the Sorting Theory and Troubleshooting chapters.

Contents

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Introduction

About this Guide

This guide provides information on the Aurora CS cell system, daily workflow, SpectroFlo® CS software features, steps on setting up and sorting, and cytometer specifications.

Safety

Safety Symbols

The Aurora CS cell system 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
	Laser radiation
	Mechanical pinch hazard

General Safety

- Read all safety instructions completely before using the equipment. Keep the instructions in a safe place.
- Follow all instructions when operating the instrument.

- If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.
- Do not place any object on top of the instrument.
- Do not block any ventilation openings.
- Do not place the unit near any heat sources such as radiators, heat registers, stoves, or other devices (including amplifiers) that produce heat.
- Use only attachments/accessories specified by the manufacturer.
- Use only with the cart, stand, tripod, bracket, or table specified by the manufacturer or sold with the equipment. When a cart is used, use caution when moving the cart/equipment combination to avoid injury from tipping over.
- Unplug the instrument when it will not be used for long periods of time.
- Refer all servicing to qualified service personnel. Service is required when the unit has been damaged in any way, such as: if the power-supply cord or plug is damaged, if the unit is dropped, if liquid is spilled onto the unit or objects fall into the unit, if the unit is exposed to rain or moisture, or if the unit does not operate properly.
- Do not expose the instrument to temperatures outside the range of 15°C to 28°C.
- Concentrations of sodium hypochlorite (bleach) higher than 10%, as well as other cleaning agents, can damage the instrument. Use a 10% solution of household bleach to clean, where indicated. A 10% bleach solution is prepared by adding 1 part household bleach to 9 parts deionized water.

  ■ **NOTE:** Household bleach contains 5–7% sodium hypochlorite.
- Before turning on the cytometer, visually inspect all containers. Wear appropriate personal protective equipment (PPE), including, but not limited to, gloves, eyewear, and lab coat.
- Fill the sheath container as needed. Never use tap water or EtOH (as it can damage the de-gasser) as sheath solution. Never use surfactant-based sheath solutions.
- 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 current 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.
- Do not use this equipment near water.

WARNING: To reduce the risk of fire or electric shock, do not expose this apparatus to rain or moisture.

- Use only the power supply cord specified by the manufacturer. The power cord of the unit is equipped with a 10A three-prong power plug. Do not remove the ground pin of the power plug under any circumstances. Make sure the plug is securely plugged into the power outlet to prevent fire. If the power supply cord needs to be replaced, the cross section area of the conductor should be at least 16 AWG. This is to prevent electric fire or shock.

- Use only the fuse specified by the manufacturer. The fuse is 250 VAC, 5 A, size 5 x 20 mm.
- Protect the power cord from being tread upon or pinched, particularly at the plug and the point where if emerges from the equipment. Be sure that the power outlet is located near the equipment so that it is easily accessible.

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. Follow local, state, and national regulations when disposing of waste.
- Biological samples are potentially dangerous and/or life threatening. Adhere to proper handling procedures for samples and reagents. Wear appropriate personal protective equipment (PPE), including, but not limited to, 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.
- Cell sorters create aerosolized droplets, which under certain circumstances, can create a risk for the operator if infectious materials are being sorted. For this reason, careful characterization of the class of risk the sample presents, and the precautions necessary to sort these samples, should be undertaken before any sort is performed. Current recommendations for sorting biohazardous samples can be found at the ISAC Biosafety guidelines: https://isac-net.org/.

Laser Safety

The Aurora CS is a Class 1 Laser Product and complies with the US FDA Center for Devices and Radiological Health 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007. No laser radiation is accessible to the user during normal instrument operation.

The lasers are fully contained within the instrument and do not require any special work area safety except when service procedures are being performed. These procedures are performed only by Cytek service personnel.

CAUTION: Modification or removal of the optics covers or laser shielding could result in exposure to hazardous laser radiation. To prevent damage to skin and eyes, do not remove the optics covers or laser shielding, or attempt to service the instrument where laser warning labels are attached.

Use of controls or adjustments or performance of procedures other than those specified in this user's guide may result in hazardous radiation exposure.

To prevent exposure to laser radiation:

- Do not defeat any safety interlocks on the instrument.
- Do not use controls, make adjustments, or perform procedures other than those specified in this user's guide.
- Do not attempt to perform service procedures on the lasers.

Technical Support

For instrument support within the US, call 1-877-92-CYTEK (1-877-922-9835); option 1.

Outside the US, call 1-510-657-0102. In Europe, call +31207653440.

Email Cytek at technicalsupport@cytekbio.com.

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 or screen shots of recent system performance
- SpectroFlo CS software version and system firmware version, located in the Help module

2

Overview

Cytek® Aurora CS System

The Aurora CS system consists of the Aurora CS cell sorter, sheath and waste tanks, and a computer workstation running SpectroFlo® CS software for acquisition and analysis. SpectroFlo® QC beads and SpectroSort™ beads are also included. The system is intended for analyzing and/or sorting cells in the fields of immunology, biochemistry, biology, oncology, and hematology research. Cell sorters have also been used to isolate bacteria, marine organisms, plant cells, and yeast. Cell sorters can be used for any application where a desired population of particles with a certain size range (0.2–25 µm) need to be separated from a mixture of particles.

The Aurora CS cell sorter is an air-cooled, compact benchtop cytometer. It is equipped with up to five lasers, 64 detection channels for fluorescence, and three channels for scatter. The blue laser is used for forward scatter (FSC) and side scatter (SSC). The violet laser is also used for SSC, allowing for better detection of smaller particles. Sheath and waste fluids are contained in 10-L tanks, included with the system. Software indicators notify you when the sheath is getting low and the waste is getting full.

The sorter is capable of sorting into collection tubes of various sizes (1.5 mL and 5 mL) and into 96-well microplates.

The workstation is a dedicated USB-compatible PC with two monitors, keyboard, and mouse. It runs Microsoft® Windows® 10 Pro with a 64-bit operating system.

Cytometer Overview

Doors on the front of the instrument open, allowing access to different components. The hinged panel on the left folds down to reveal the fluidics compartment. The top-right door lifts up allowing access to the sort chamber, which consists of the flow cell, nozzle, deflection plates, and stream aspirator. Below the sort chamber are two doors. The door on the left allows access to the DDU (droplet deposition unit) chamber, which accommodates the sort collection devices and the plate puck. The windowed door on the right opens to reveal the sample chamber where sample tubes are loaded into the sample loading station (SLS). The sample pressure cylinder, located above the loading station, lowers to enclose the sample tube, creating a pressurized compartment.

The cytometer power button is located in the top-right corner on the left side of the instrument. When the instrument is powered on, the power button is illuminated.

Connectors located on the front of the instrument connect the air line, fluidic lines, and fluidic sensor lines to the instrument.

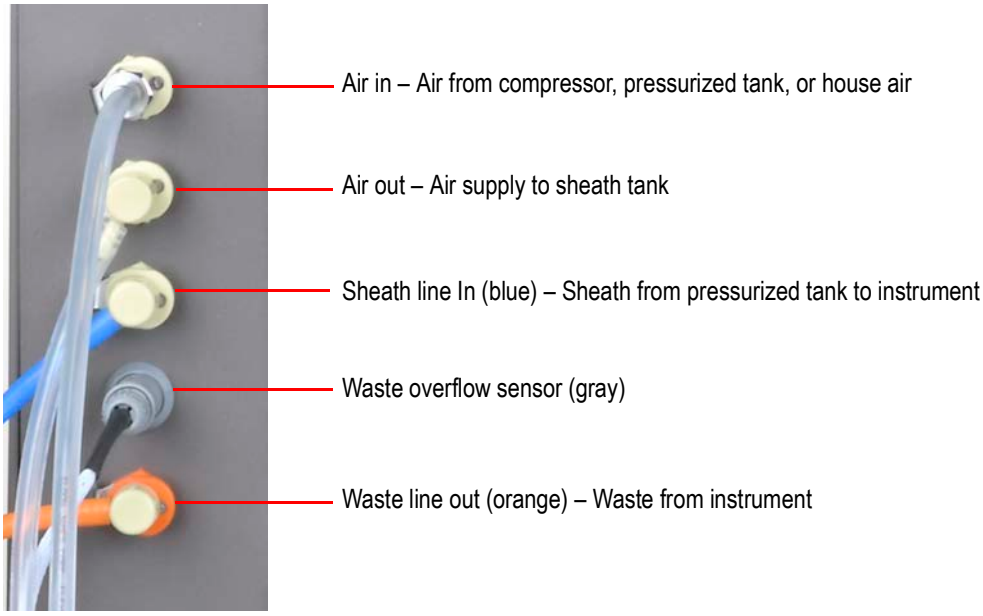
Front of Cytometer

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.

Connectors

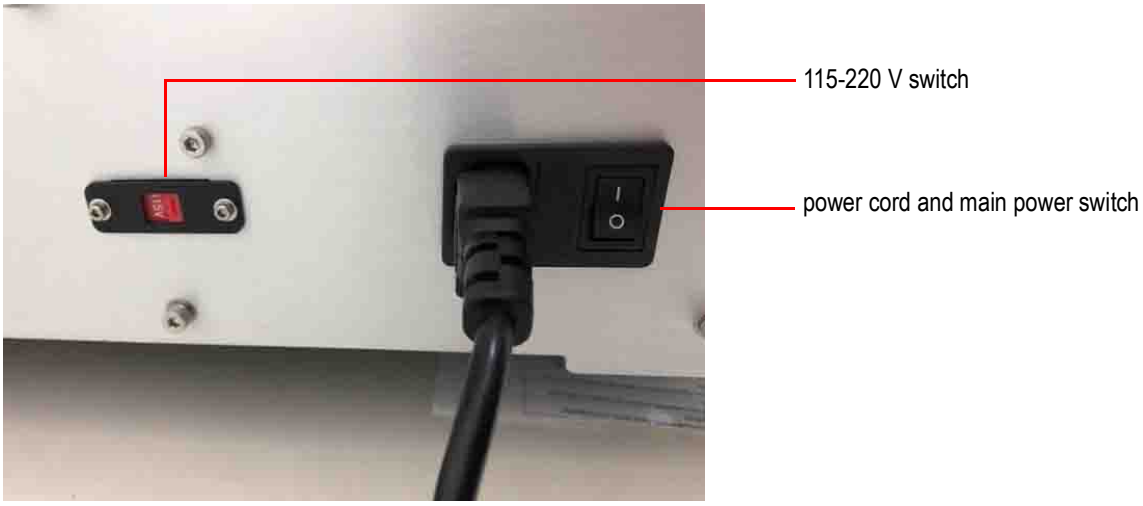
Quick-connects in the bottom-left corner of the instrument allow you to connect the air, sheath and waste fluid tubing, and waste level sensor. The blue fluid line is for sheath solution. The orange fluid line is for waste.



Back of Cytometer

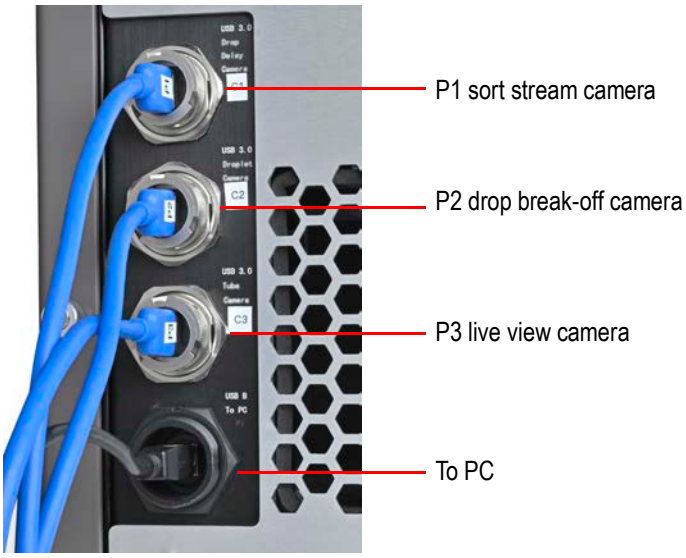
Allow 30.5 cm (12 in) between the back of the cytometer and the wall for proper ventilation.

The main power switch should remain on at all times. The power button to turn the instrument on/off is located on the left side of the instrument in the upper-right corner.



Connectors

Four USB 3.0 connectors are located at the bottom-left corner at the back of the instrument. These ports connect the three system cameras to the workstation and the workstation to the instrument. All cables can be plugged into any USB 3.0 port on the back of the workstation only.

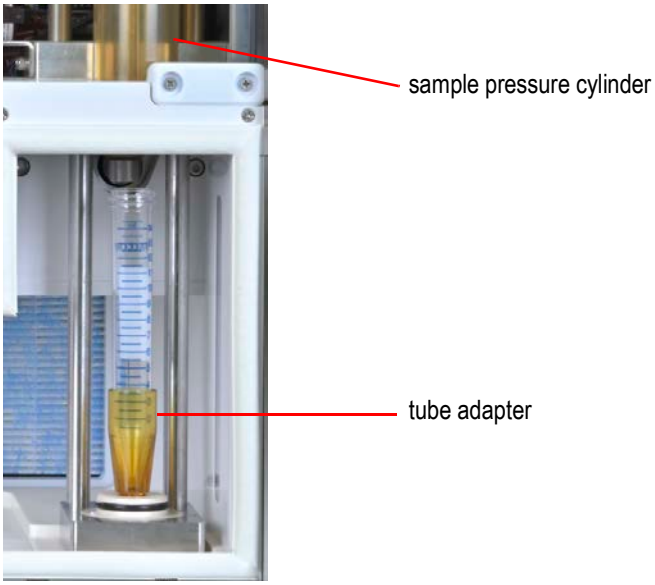


Fluidics System

The Aurora CS instrument uses a pressurized fluidics system. Air from a compressor, pressurized tank, or house air pressurizes both the sheath tank and the sample chamber, displacing sheath and sample fluids at predetermined flow rates.

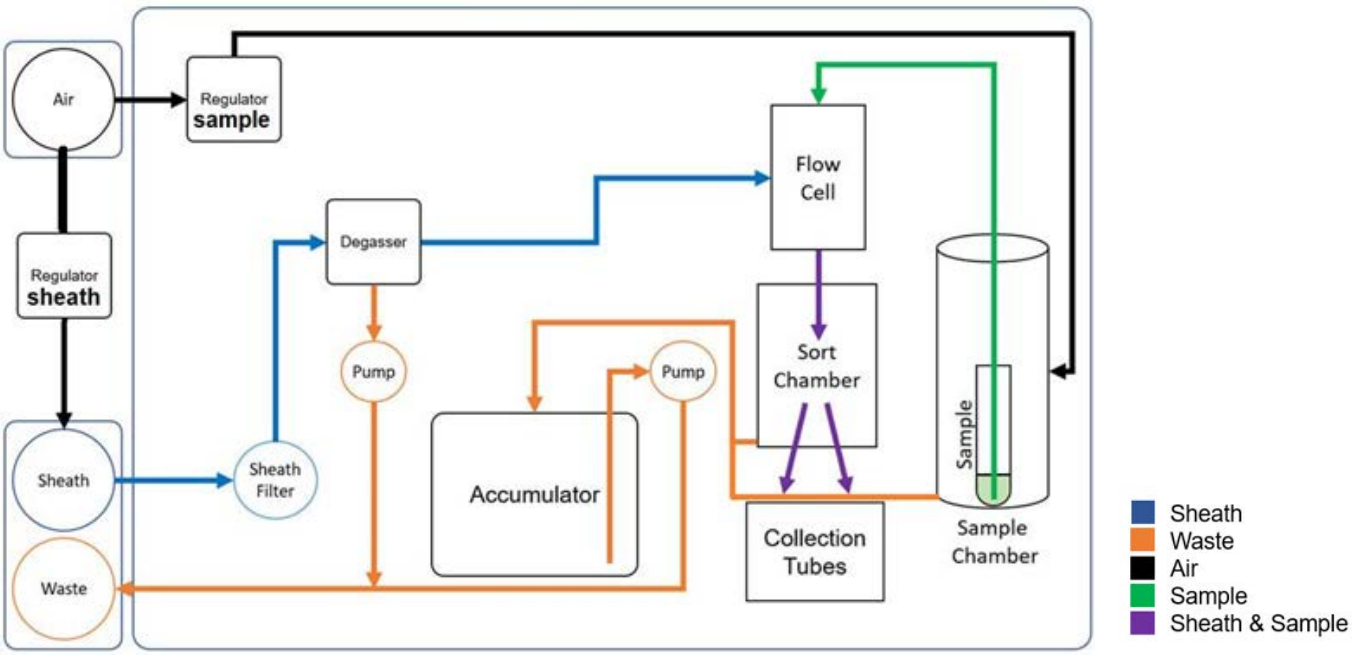
Sample Loading Station

Sample tubes are loaded in the sample loading station. The loading station can accommodate a 5-mL (12 x 75-mm) tube or a 15-mL conical tube. A tube adapter for each tube size is included with the system. The adapter fits into a motor that spins at a user-defined speed to keep the cells or particles in suspension. Sample enters the cytometer through the sample introduction tube (SIT). The sample pressure cylinder lowers to encompass the tube and pressurizes it at a variable pressure, which you can adjust using the flow rate adjuster in the software.



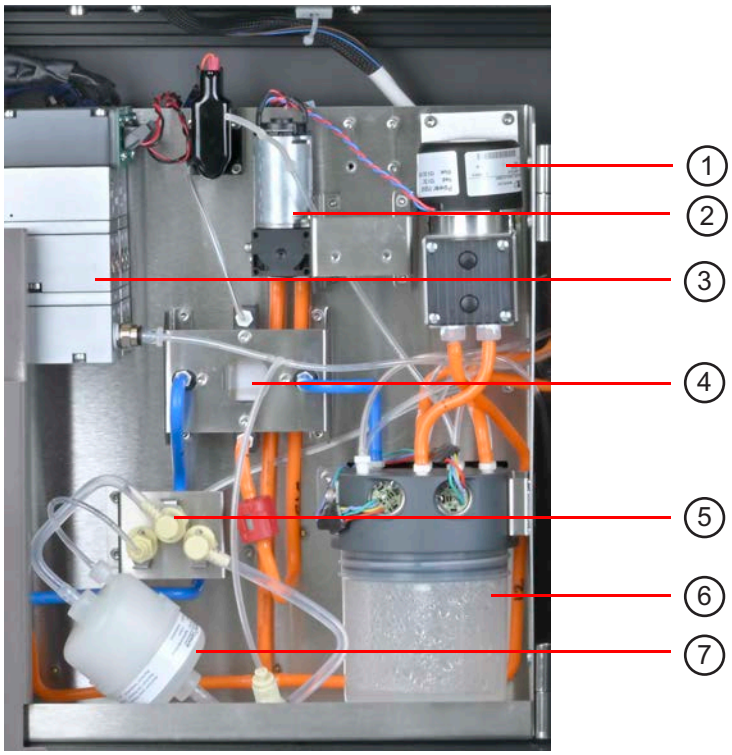
Fluid Flow

The Aurora CS incorporates a pressurized fluidics system. Air from a compressor, pressurized tank, or house air pressurizes both the sheath tank and the sample chamber, displacing sheath and sample fluids at predetermined flow rates. Sheath is pushed from the sheath tank, into 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 and dissolved gas. After passing the laser interrogation point, the combination of sheath solution and sample passes through a nozzle, which creates a stream-in-air jet. The jet is broken up into droplets. The droplets pass through the deflection chamber for sorting or are sent to waste via the stream aspirator.



Fluidics Compartment

Open the fluidics compartment to access the fluidics components. The following figure shows the main components.



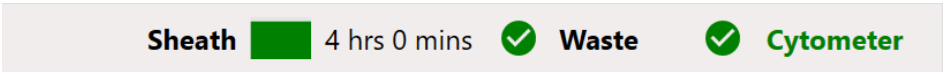
The following table describes the fluidics components.

No.	Component	Description
1	Aspirator pump	Vacuum source for the waste aspirator and aspirator buckets
2	Degasser pump	Applies suction on the degasser
3	Sample and sheath pressure regulators	Regulates sample and sheath pressure
4	Degasser	Pulls air bubbles from the sheath fluid
5	Sheath filter quick connects (x3)	Sheath filter fluid input, fluid output, and vent line quick-connects
6	Accumulator	Stores the vacuum that is used to aspirate waste
7	Sheath filter	Filters debris and particles from the sheath fluid

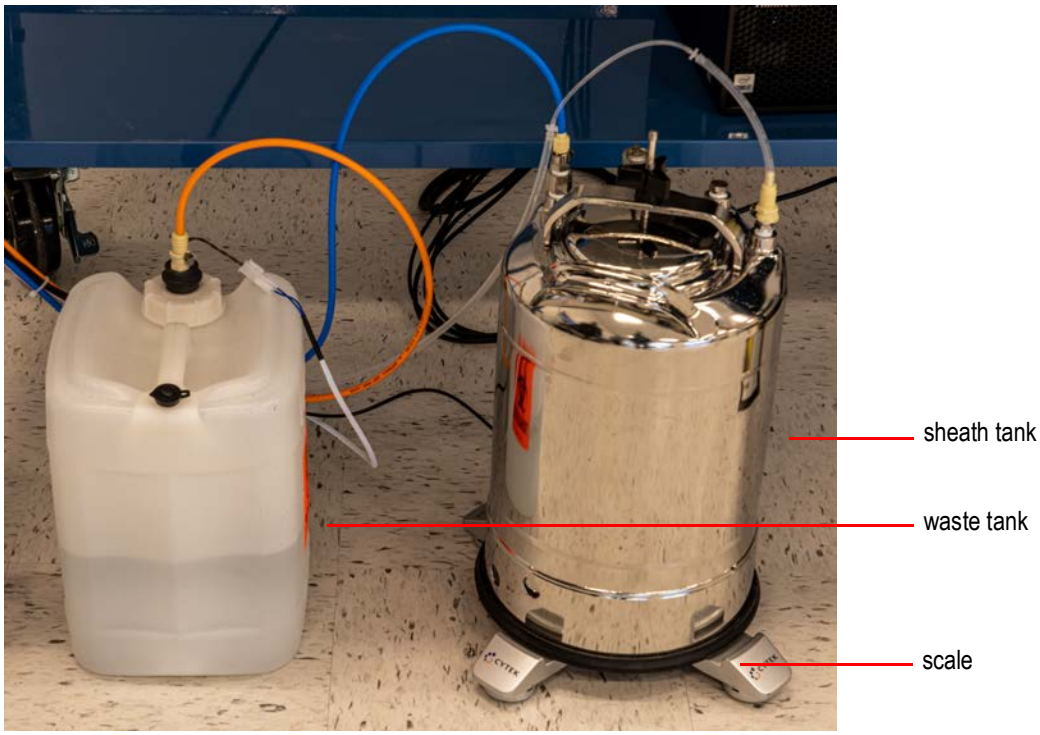
Fluid Containers

The Aurora CS draws sheath solution from a pressurized 10-L sheath tank. It expels waste into an empty 10-L tank. The fluid tanks can sit on the floor beneath the cytometer.

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 connected to a scale where the sheath tank sits. The scale measures the weight of the tank. The weight is then converted to a time, which is displayed in the status indicators in the lower-right corner of the software.



Both sensors are monitored by the software. For more information on the sheath and waste tanks, see “Running Fluidics Startup” on page 35.



Sorting Components

Nozzles

Below the flow cell, the sample flows through a nozzle. You can choose from various nozzle sizes compatible with the system, 70, 100, and 130 µm, which generate different sized droplets for sorting different sizes of particles at corresponding pressures. Smaller nozzles create smaller and more droplets per second to allow faster sorting speed, while larger nozzles create larger and fewer droplets per second resulting in slower sorting speeds. Nozzle size must be carefully chosen according to cell size (to avoid clogging) and cell fragility.

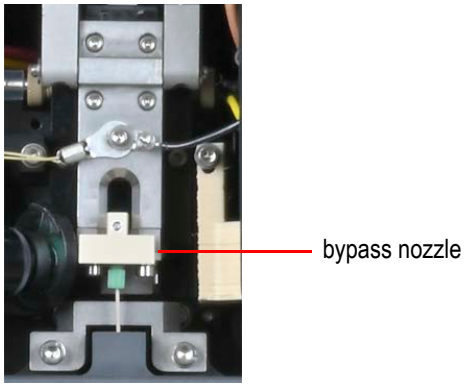
Each nozzle size has default settings associated with it. The default settings are optimized by Cytek for each instrument. The following table lists the default settings for achieving optimal stream

stability for each of the four supported nozzle sizes. You can also save and store custom nozzle settings in the Library for future use.

Cytek Default Nozzle Settings

	Nozzle Settings			
Parameter	**70 µm**	**100 µm**	**130 µm**	**Bypass (90 µm)**
Pressure (PSI) range	60-66	16-21	6-10	19-27
Drop-drive frequency (DDF) (drops/s)	70k–90k	22k–35k	11.5k–16k	N/A
Amplitude	1–40k	1–55k	1–40k	N/A
Drop center	150-250	200-300	250-350	N/A
Drop interval	6-15	15-35	25-45	N/A
Plate voltage (V)	5k-6k	3k-4k	2k-3k	N/A
Drop compensation (Drop 1,2,3,4)	15-25	3-7	1-4	N/A

A bypass nozzle is provided with the system. The bypass nozzle directs the sheath and sample fluid directly to the waste. It is used for fluidics startup and shutdown as well as other cleaning procedures.



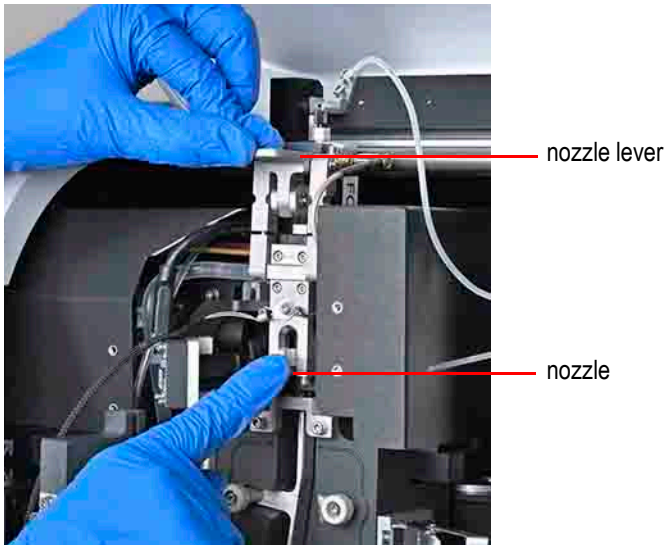
Installing a Nozzle

1 To install a nozzle, lower the nozzle release lever and remove the bypass or existing nozzle by pulling it straight out.

2 Check the o-ring in the nozzle that you are installing to ensure it is seated.

3 Insert the nozzle securely into the nozzle chamber by sliding it in, following the guide rails at the bottom of the chamber. Push the nozzle all the way in until it stops, then push lightly down on

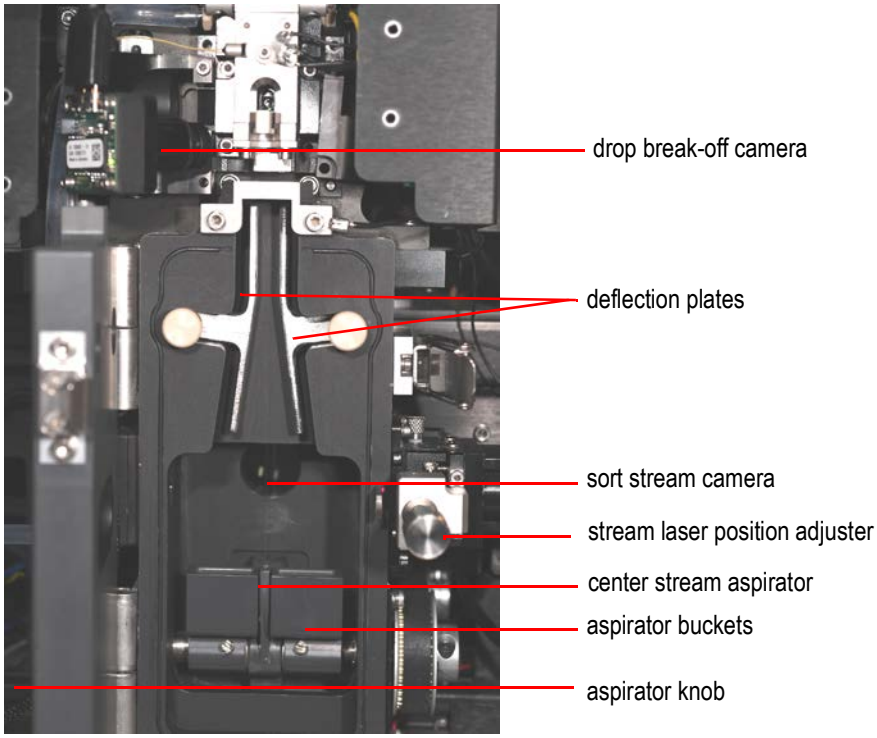
the nozzle as you lift the nozzle lever to secure the nozzle in place. Note: Once the nozzle is secured in place in the nozzle chamber, you should not move or apply pressure to it as it can cause damage to the o-ring or flow cell assembly.



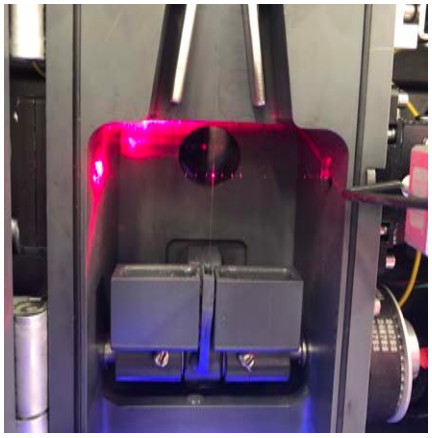
Sort Chamber

Open the sort chamber door to gain access to the deflection plates and aspirator assembly (center stream aspirator and aspirator buckets). The sort chamber door limits exposure to the deflection plates and aerosols during sorting. A knob located on the left side of the sort chamber allows you to move the aspirator assembly right/left to center the stream within the aspirator.

Two cameras allow you to view the stream. The drop break-off camera allows you to view an image of the drop profile. The sort stream camera allows you to view the center and side streams, and it is also used for determining drop delay.



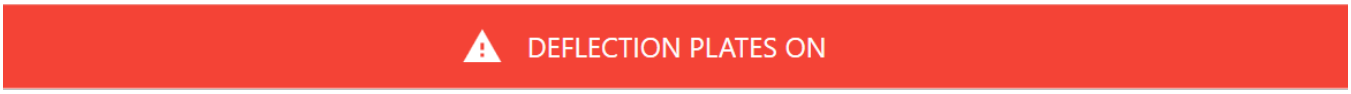
The side stream laser illuminates the center and side streams when the sort chamber door is closed. Use the stream laser position adjuster knob at the right of the chamber to achieve the best illumination of all streams. The illuminated streams are viewed by the sort stream camera and displayed in the Sort Stream Adjuster area of the sorter controls.

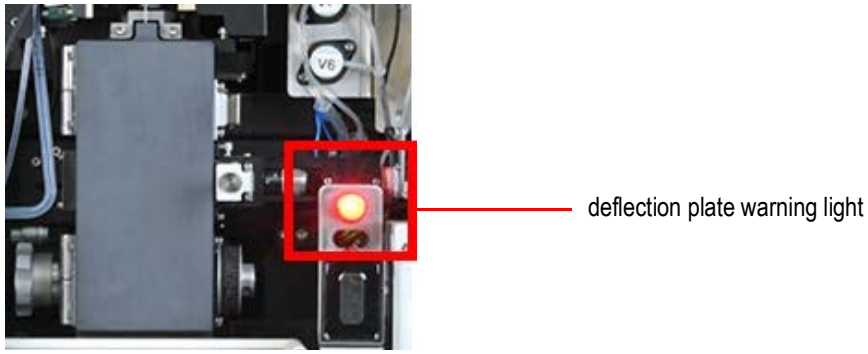


The deflection plates provide a charged field which attracts the charged droplets towards the collection vessels. The deflection plates are turned on/off in the Sort Stream Adjuster window of the software and are also automatically turned on when you start a sort or when Test Sort is activated. See "Sort Stream Adjuster" on page 99 for more information.

WARNING: The deflection plates can be on even when the sort door is open. The electrical field generated by the plates is a 12,000 V differential. A red LED illuminates when the plates are on.

When the plates are on, a red warning light located to the right of the sort chamber is illuminated and the software displays the following warning.



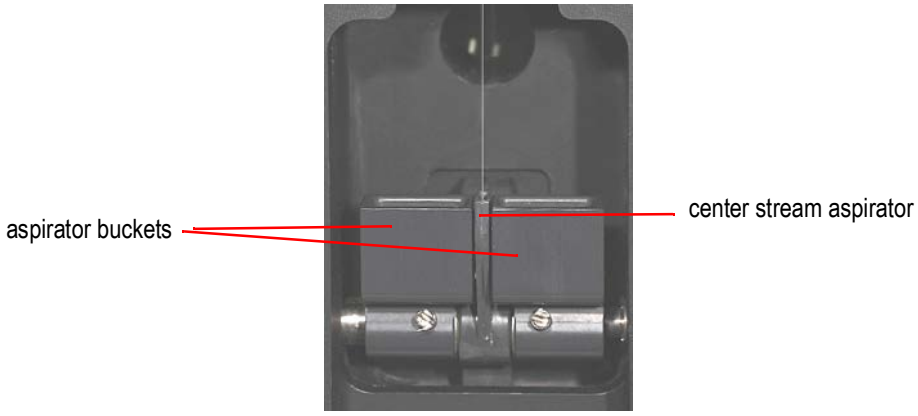


Aspirator Assembly

The aspirator assembly consists of the center stream aspirator and the aspirator buckets.

MECHANICAL HAZARD: Keep fingers away from the aspirator assembly when it is in motion.

The aspirator buckets collect side streams generated during the setup process and protect the collection tubes from contamination during a sort if a clog is detected by the Sort Monitoring system. The buckets automatically open (move back) when sorting starts. They automatically close (move forward) when a clog is detected and when sort monitoring is enabled, and when sorting stops. The buckets can also be opened and closed from the Sort Stream Adjuster window in the software.

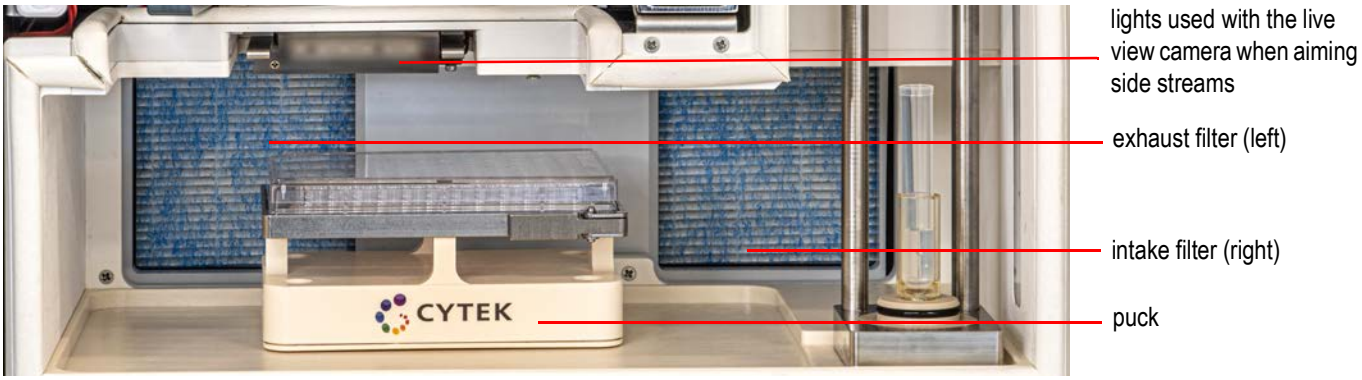


DDU

The DDU chamber accommodates the collection vessels (1.5-mL tubes, 5-mL tubes, or a 96-well microplate). It features an aerosol evacuation system and temperature control to heat or cool the chamber.

Two HEPA filters are located at the back of the DDU chamber. Fans located behind the left and right HEPA filter compartments control the flow of air in the chamber. When temperature control is turned on, clean air is pulled into the DDU chamber through the HEPA filter compartment on the right and passes a thermoelectric cooling (TEC) unit that cools or heats the air. The cooled or heated air circulates through both the sample loading station and the DDU chamber. This cools or heats both the pre-sorted sample and the sorted samples. The air then exhausts through the HEPA filter compartment on the left.

When sorting into a 96-well plate, a magnetic puck is used to hold the plate. The puck is driven by a magnetic puck driver underneath the DDU chamber.



Aerosol Evacuation System

The Aurora CS comes equipped with a primary aerosol evacuation system (AES) that is activated whenever an emergency evacuation condition occurs. See “Emergency Evacuation Solutions” on page 23.

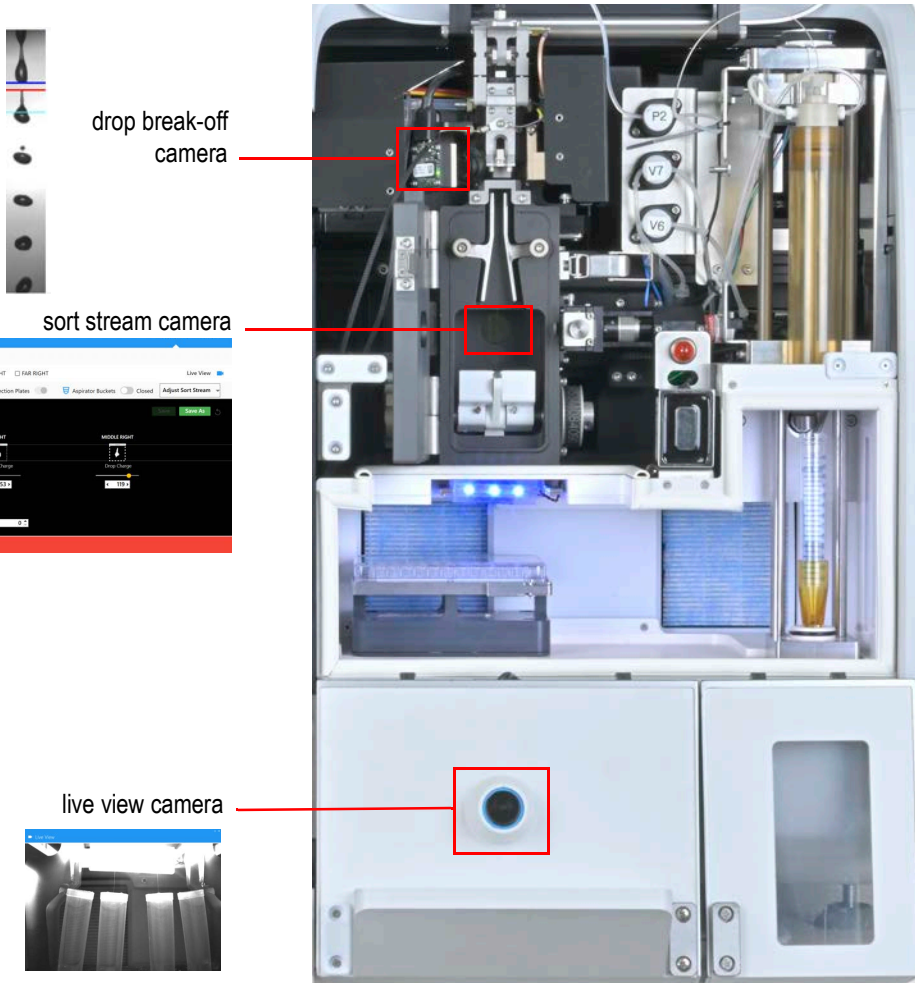
When the system is activated air is exhausted through the HEPA filter located in the DDU and sample chambers to clear the chambers.

When the aerosol evacuation system is activated, the following actions occur:

- The DDU and sample chamber access doors lock.
- High-evacuation mode turns on to exhaust aerosols from the chambers via a HEPA filter.
- The chamber doors unlock after high-evacuation mode has completed, allowing access to the chambers.

Cameras

The instrument is equipped with three cameras that assist with visualizing the stream during sorting and sort setup. The drop break-off camera displays an image of the drop profile. The sort stream camera is used for optimizing the center and sides streams, and during the drop delay procedure optimizing the center and side streams. The live view camera is used to view and aim the sides streams into the collection vessels.



Sort Collection Devices

The Aurora CS can sort into 1.5-mL tubes, 5-mL tubes, and 96-well microplates.

The various collection tube sizes are contained in specially sized receptacles. Each receptacle slides into a collection tube holder retainer. For aseptic sorting, the collection tubes can be loaded and unloaded without having to touch the top of the tubes.

Emergency Evacuation Solutions

The system provides three emergency evacuation solutions:

- Automatically through clog detection (when Sort Monitoring is enabled)
- When the Quick Stop button on the instrument is pressed
- When the software Quick Stop button in the Sorter Control window is pressed

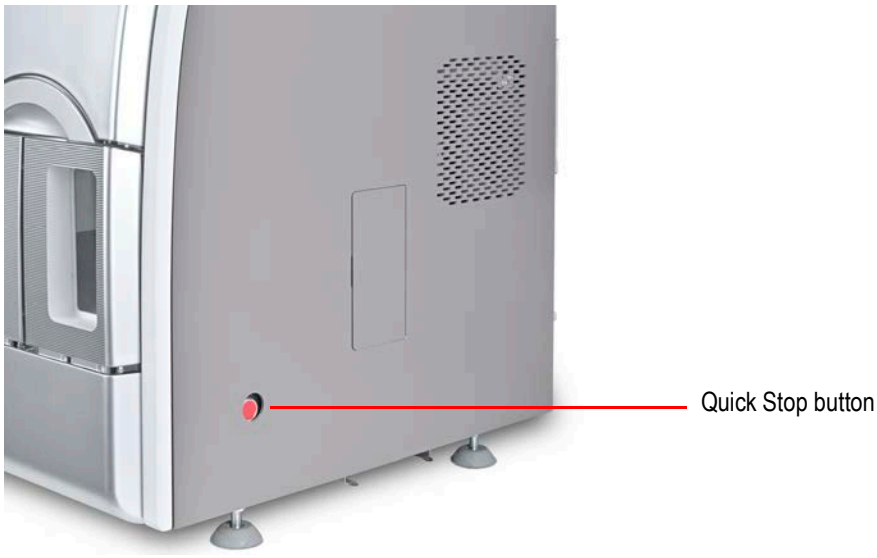
When one of these instances occurs, the following happens:

- Aspirator buckets close
- Defection plates turn off
- Drop charge turns off
- Stream is turned off
- Sample pressure cylinder depressurizes and raises
- If the sort was into a plate, plate movement is stopped
- The aerosol evacuation system is activated and the chamber doors lock
- Software message appears, explaining how to proceed

Hardware Quick Stop Button

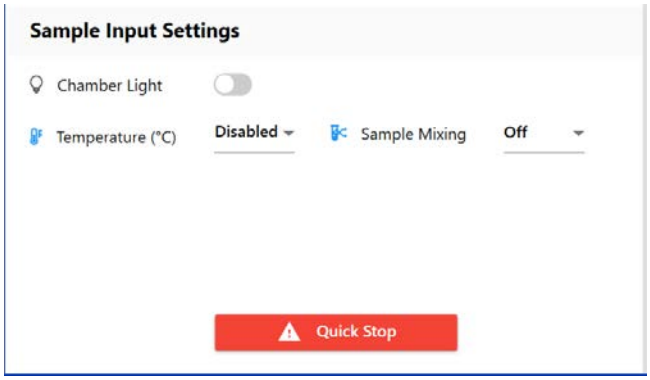
If you encounter a situation that requires an immediate halt of all sorting operations, press the red Quick Stop button located on the right side of the instrument. Once pressed, wait until the aerosol evacuation system has cleared any aerosols and the chamber doors unlock. At this point you can continue operation.

To continue operation, press the button again to reset. There is a warning in the software to remind you if you have not reset the button.



Software Quick Stop

The software provides two ways for activating the aerosol evacuation system—when a clog is detected via Sort Monitoring and manually when you press the Quick Stop button in the Sort Control window.



The software Quick Stop is similar to the hardware Quick Stop button. When Sort Monitoring cannot maintain a consistent droplet interval and detects a clog or when you press the Quick Stop software button, the aerosol evacuation system is activated and the software automatically stops all sorting operations.

Optics

Unlike conventional flow cytometers that direct specific bandwidths of fluorescence light into discrete detectors or photomultiplier tubes (PMTs), the Aurora CS uses a solid-state, multi-channel, narrow-beam detector APD array for each laser. Each array can be individually configured with up to 16 detectors that capture a part of the emission spectrum from each particle passing through the laser beam. Up to 5 laser-associated detector arrays 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 from a reference control to the total collected signal in the sample.

For excitation, a proprietary flat-top laser design enables a constant power distribution across the width of the sample core stream.

Laser information is as follows:

Laser	Excitation	Channels for detection	Detector names
Ultraviolet	355 nm	16	UV1–UV16
Violet	405 nm	16	V1–V16
Blue	488 nm	14	B1–B14
Yellow Green	561 nm	10	YG1–YG10
Red	640 nm	8	R1–R8

■ **NOTE:** Different laser configurations are available.

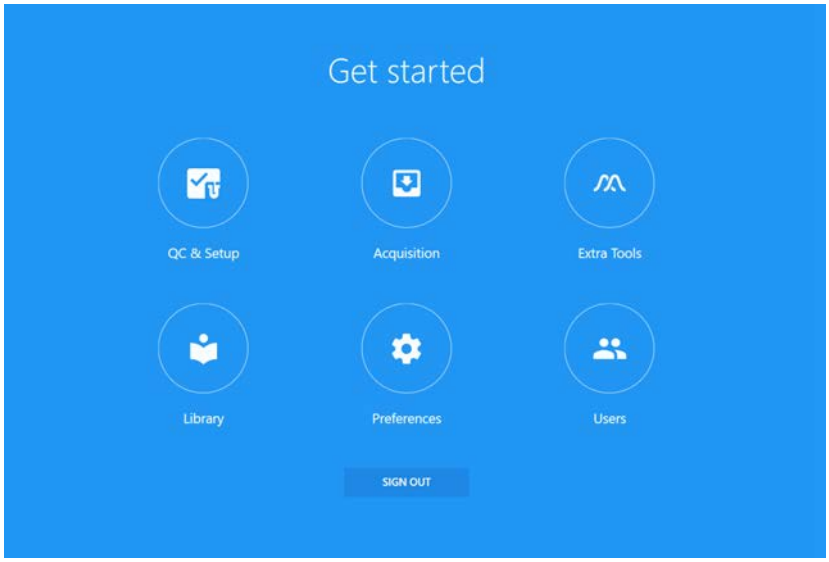
The wavelengths detected by each detector (channel) increase across the array. See "Detector Bandwidths Specifications" on page 80.

Software Overview

SpectroFlo CS software allows you to acquire and analyze samples, control sorting operations, and adjust instrument settings. Once you log into the software, a Get started menu appears.

Get Started Menu

The Get started menu provides six modules that allow you to perform various functions. These same six modules are also accessible across the upper-right corner of each module screen.



The following table describes options in the Get started menu.

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 to adjust settings to account for day-to-day variation. Levey-Jennings reports keep track of trends in system performance. Setup allows you to create reference controls. See "Instrument Setup and QC" on page 45 for information.
Acquisition	The Acquisition module allows you to create experiments to acquire and analyze data and sort. Experiments can be created through a guided wizard or created from previously saved templates. See "Acquisition" on page 51 for information.
Extra Tools	Here, FCS files can either be unmixed or compensated using virtual filters. See "Unmixing and Compensation" on page 67 for information.
Library	The Library allows you to store experiment templates, worksheet templates, user settings, fluorescent tags, SpectroFlo QC bead information, label information, keywords, sorting related settings (sort modes, nozzle configuration etc.), and spillover values. See "Library" on page 123 for 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 132 for information.

Module	Description
Users	The Users module contains user management options and administrative controls. See "Users" on page 142 for information.

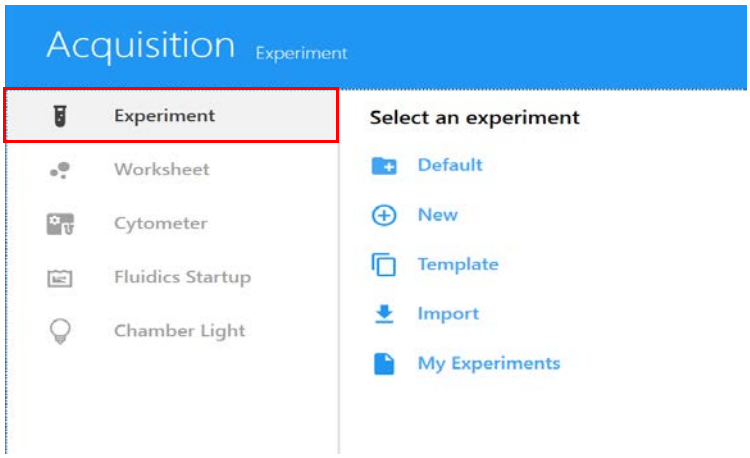
About Experiments

The Acquisition module provides the tools necessary to acquire data, such as the acquisition controls used to start, stop, and record data, and the instrument controls used to set the threshold and adjust the detector gains. See "Experiment Display" on page 52 for more information on these controls. Experiments contain the fluorescent tags and labels used in the experiment, the stopping criteria, and the groups of tubes run, which can include the reference control group. You can create groups for your samples, if you wish, to conveniently organize samples by type or staining panels, for example.

Opening an Experiment

Click Experiment from the Acquisition tab to open the Experiment menu. The Experiment menu allows you to open a default or template experiment, create a new experiment, or import an experiment. A wizard walks you through the steps to create a new experiment.

■ **NOTE:** By default the Acquisition menu in the left pane is collapsed, showing only the icons for Experiment, Worksheet, Cytometer, Fluidics Startup. To expand the menu to show the labels, click the arrows (>>) at the bottom of the pane.

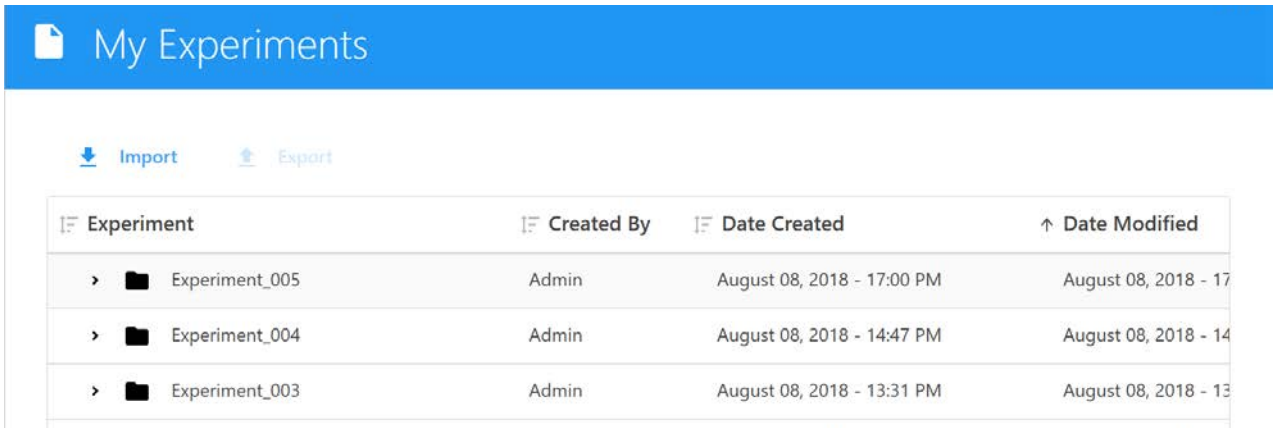


Experiments can be created using several different methods. The following table describes the options in the Acquisition Experiment menu:

Method	Description
Default	Opens a new experiment with one group containing one tube and a set of labels and fluorescent tags in a default experiment worksheet template. The default experiment is user configurable. It is the quickest way to begin sample acquisition.
New	Opens the New Experiment Wizard to guide you through creating an experiment.
Template	Allows you to select from a list of saved experiment templates (see page 28).
Import	Imports an experiment ZIP file that was exported.

Method	Description
My Experiments	Allows you to select from a list of saved experiments. **NOTE:** Original experiments can be duplicated without data, which is equivalent to opening an experiment template. Right-click an experiment in My Experiments and select Duplicate (with or without data).

Completed experiments can be accessed through the My Experiments option in the Acquisition Experiment menu. Use the column headers to sort the list of experiments. For every tube recorded, two FCS files are saved, one raw and one unmixed. Use My Experiments to open experiments you already ran, as you may want to review the data or acquire more samples. You can also export experiments from My Experiments (below). A ZIP file is exported, containing all the raw data files, if applicable (and unmixed files for unmixed experiments), as well as the worksheet templates and experiment template.



FCS Files

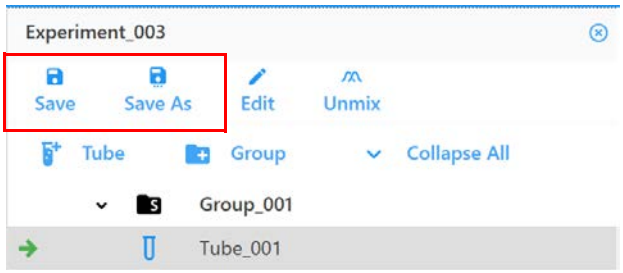
FCS files generated from an experiment are stored in the CytekBioExport folder by default, or the folder you set as the default. See "Storage Preferences" on page 140 for information. Experiments can contain the following types of FCS data files for each tube run:

- raw data files only (for samples that were acquired in an experiment)
- raw data files + unmixed data files (for samples that were acquired and unmixed live during acquisition)
- unmixed data files only (for samples that were unmixed post acquisition)

Experiment Templates

Use the Save As option above the experiment's tube/group (hierarchy) list to save the current experiment as a template, which can then be used for running similar experiments. Experiment templates include fluorescent tags used in the experiment, reference controls, groups/tubes, labels, worksheets, and stopping criteria. Templates are saved in the Library. To open and use a

template, select **Template** from the Acquisition Experiment menu. See “Experiment Templates” on page 126 for more information on experiment templates.



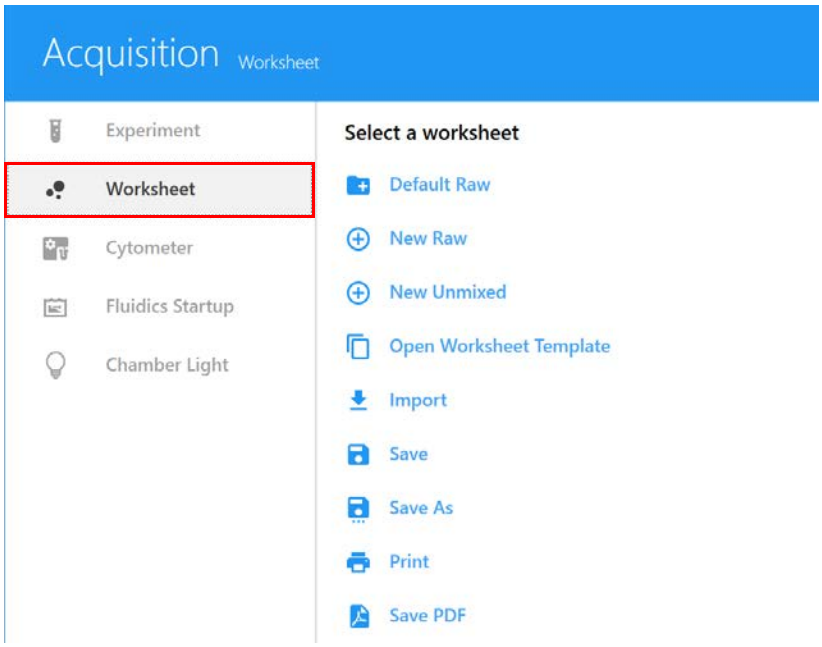
About Worksheets

Worksheets are used to visualize the data in the experiment. Each experiment requires at least one worksheet. A worksheet allows you to view the data in plots during acquisition, as well as perform analysis functions. Worksheets contain the tools necessary to create plots, gates, annotations, statistics, and the population hierarchy. Worksheets are saved with the experiment and can be saved separately and reused across experiments.

Two types of worksheets are available—worksheets for raw data and worksheets for unmixed data. You must select the appropriate worksheet to view the corresponding type of data. When viewing raw data, the parameters on the plots in a raw worksheet reflect the channel names, for example, B1-A, R1-A, V1-A. When viewing unmixed data, the parameters on the plots in an unmixed worksheet reflect the fluorescent tags, for example, PerCP-A.

Opening a Worksheet

To select a worksheet, click **Worksheet** in the Acquisition menu. The Select a worksheet menu appears (below). You can open a new raw or new unmixed worksheet. These worksheets open with a single FSC vs SSC plot. You can also open a default worksheet. Use the worksheet tool bar to add plots and other analysis elements. All worksheets are saved as template files (WTML) and can be opened using the **Open Worksheet Template** option. You can also import worksheets that were exported, as well as save, print, and save a worksheet PDF.

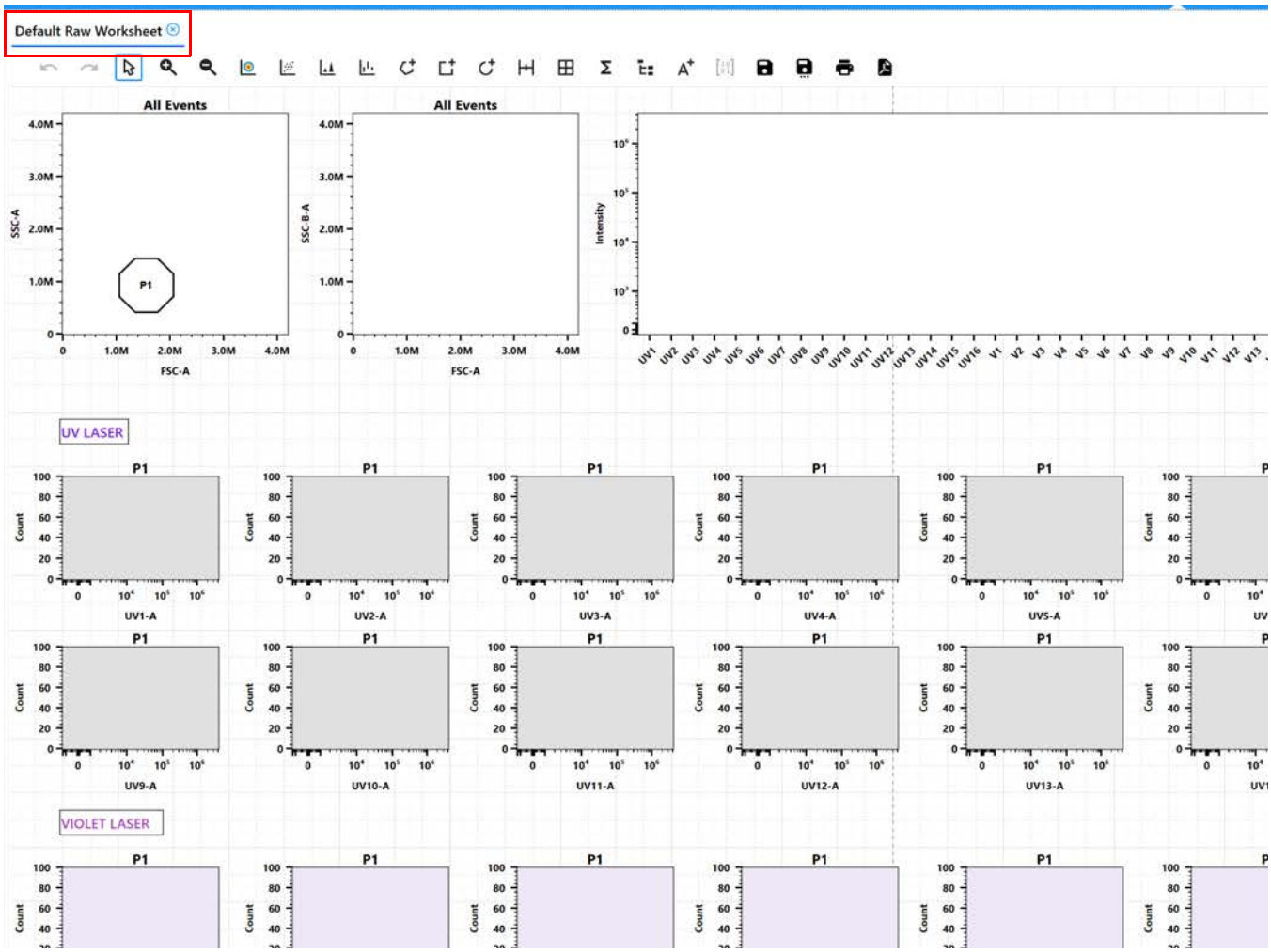


The following table describes the options in the Acquisition Worksheet menu:

Method	Description
Default Raw	Opens a default raw worksheet that can be used for experiments where reference controls will be acquired. Do not overwrite this worksheet. Always use Save As to save this worksheet with a new name.
New Raw	Opens a new raw worksheet.
New Unmixed	Opens a new unmixed worksheet.

Method	Description
Open Worksheet Template	Allows you to select from a list of saved worksheet templates. A default raw and default unmixed worksheet are provided.
Import	Imports a worksheet template that was exported. Tip: If you have an Aurora flow cytometer, you can set things up on the analyzer and then import into the Cell Sorter.
Save, Save As, Print, Save PDF	Saves the worksheet, saves the worksheet with a new name, prints the worksheet, saves a PDF of the worksheet.

You can have multiple worksheets open at a time. The currently displayed worksheet appears with a blue line under the worksheet name. When sorting, the current worksheet associated with the tube selected is used to select the sorting gates. You can select different worksheets for different groups or tubes in an experiment, and each tube will need a worksheet associated with it.



Each user can define a default raw worksheet and a default unmixed worksheet. Open the default worksheet and set it up for your experiment, then select Save As to save this worksheet with a new name. The worksheet will be available to select when you create an experiment. You can use this worksheet, open a template worksheet, or create a new worksheet.

All worksheets are saved in the Library. See "Worksheet Templates" on page 126 for more information on worksheet templates.

3

Startup & Shutdown

Instrument Startup

Startup Workflow

Turn on the system and log in to the software to begin the 30-minute warm-up cycle. Run the fluidics startup procedure. The instrument does not need to be warmed up to run fluidics startup.

Task	See...
Start up the system	“Starting Up the System” on page 33
Run Fluidics Startup	“Running Fluidics Startup” on page 35

Starting Up the System

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

1 Before starting the system, ensure the sample door is closed, fill the sheath tank and empty the waste tank. See “Running Fluidics Startup” on page 35.

   Ideally the tanks were filled and emptied the last time the system was shut down.

2 Turn on the workstation.

3 Turn on the air supply from the source. Ensure the air pressure is 80–85 psi.

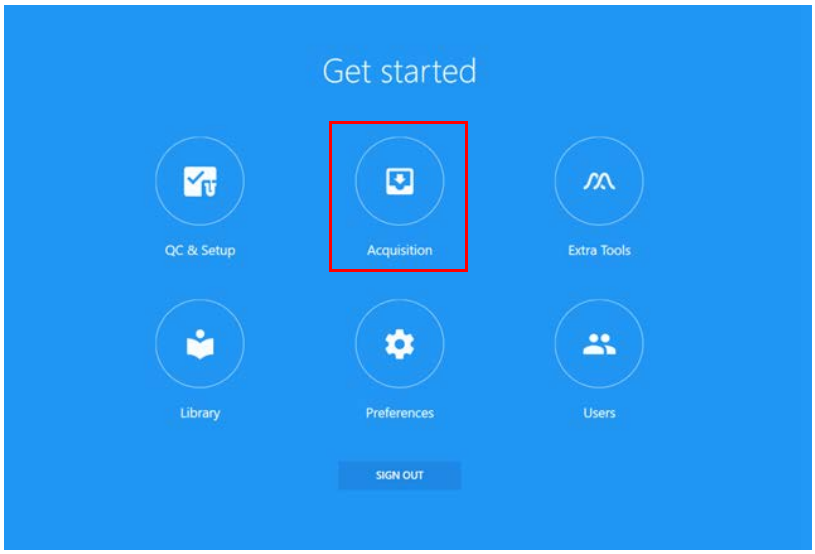
4 Turn on the cytometer and allow it to initialize.

5 Open SpectroFlo CS software.

6 Log in by entering your user name and password, then clicking SIGN IN.

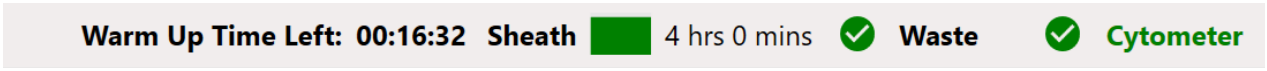
   You can start typing your user name to display a list of names beginning with the letter(s) you type.

7 Select Acquisition from the Get started menu to launch the fluidics startup wizard.



8 Check the status indicators in the lower-right corner of the screen. It may take a few minutes for the indicators to update.

- Ensure the indicator for Cytometer is a green checkmark. This confirms that the cytometer is connected.



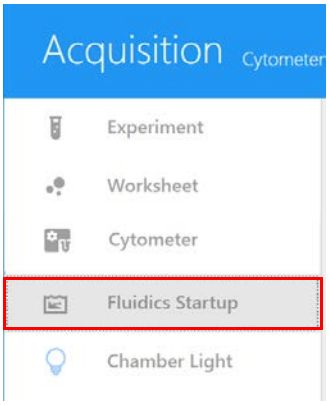
  If the indicator shows the cytometer is not connected, check to ensure that the USB connection between the cytometer and workstation is plugged into the appropriate ports. See "Back of Cytometer" on page 13. Note: If connections are seated, try exiting the software and rebooting the cytometer.
- Ensure the status indicators for sheath and waste are green before proceeding. If the indicators are yellow or red, fill or empty the tank, as necessary.

Fluid Indicator	Meaning
Yellow sheath	Sheath tank is low. User still has approximately 2 hours of run time.
Red sheath	Sheath tank is empty. Acquisition will continue for 5 minutes before the sheath is empty.
Yellow waste	Waste tank is nearing capacity.
Red waste	Waste tank is full.

Running Fluidics Startup

Run the fluidics startup at the start of each day. The fluidics startup takes approximately 5 minutes. No other fluidic modes listed in the Cytometer menu are available until you run the fluidics startup.

1 Click **Fluidics Startup** from the Acquisition tab.



2 Follow the fluidics startup steps.

a Ensure that the sheath tank is depressurized, then fill the tank with sheath solution or PBS. See "Filling the Sheath Tank" on page 41.

b Empty the waste tank. See "Emptying the Waste Tank" on page 43.

c Ensure that the sheath, air, and waste lines are connected to the sheath and waste tanks.

d Turn on the air at the source to pressurize the sheath tank.

e Ensure that the bypass nozzle is installed. See "Installing a Nozzle" on page 18.

6 Click **Start**.

7 Click **Done** when the fluidics startup is complete.

You are now ready to perform instrument setup if you plan to sort samples (see "Instrument Setup" on page 101). Daily QC is part of instrument setup.

Instrument Shutdown

Shutdown Workflow

Run the fluidics shutdown procedure at the end of each day. After you power down the system, fill the sheath and empty the waste so the tanks are ready the next time the system is used.

Task	See...
Run Fluidics Shutdown	"Running Fluidics Shutdown" on page 36
Power down the system	"Turning Off the System" on page 40
Fill the sheath and empty the waste	"Filling the Sheath and Emptying the Waste" on page 41

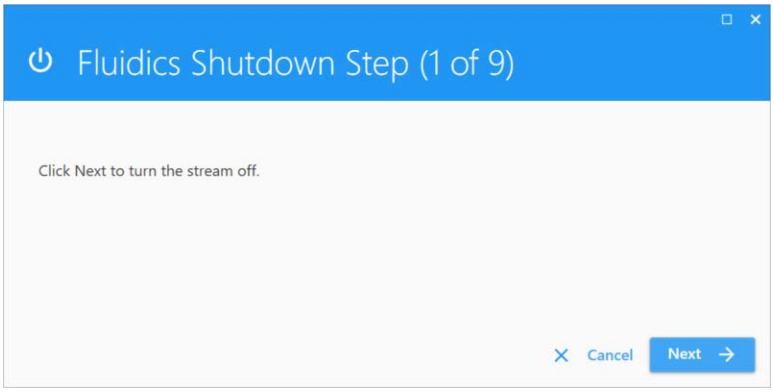
Running Fluidics Shutdown

The fluidics shutdown wizard is a nine-step procedure that uses 10% bleach, DI water, and 30% Contrad 70 to clean the flow cell and fluidic lines. Run the Fluidics Shutdown procedure at the end of each day that you use the instrument. The procedure takes approximately 10 minutes.

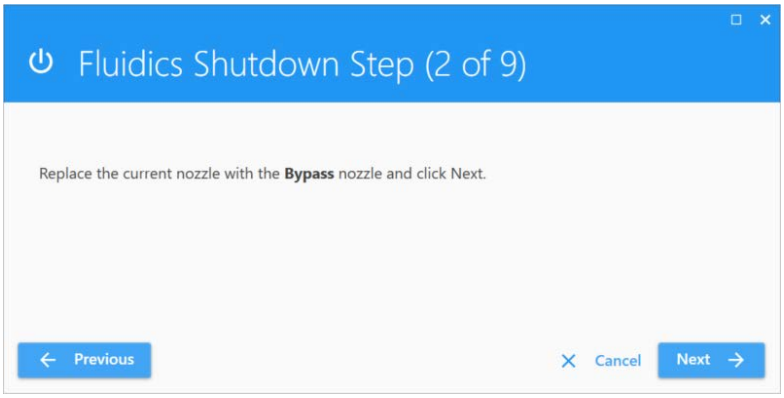
1 In the Acquisition window, click on Cytometer.

2 Click Fluidics Shutdown.

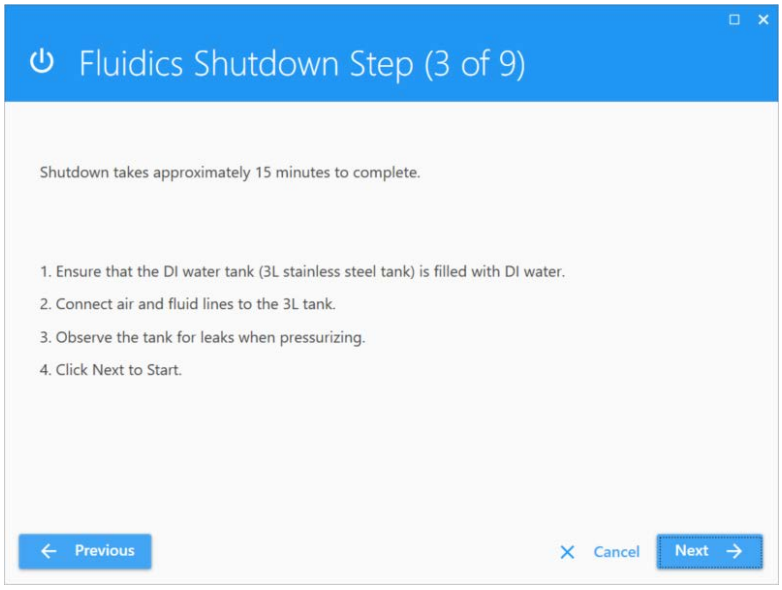
3 Follow the steps of the wizard. Turn off stream.



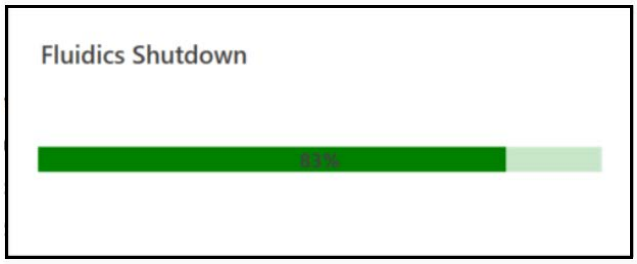
4 Replace nozzle with Bypass nozzle.



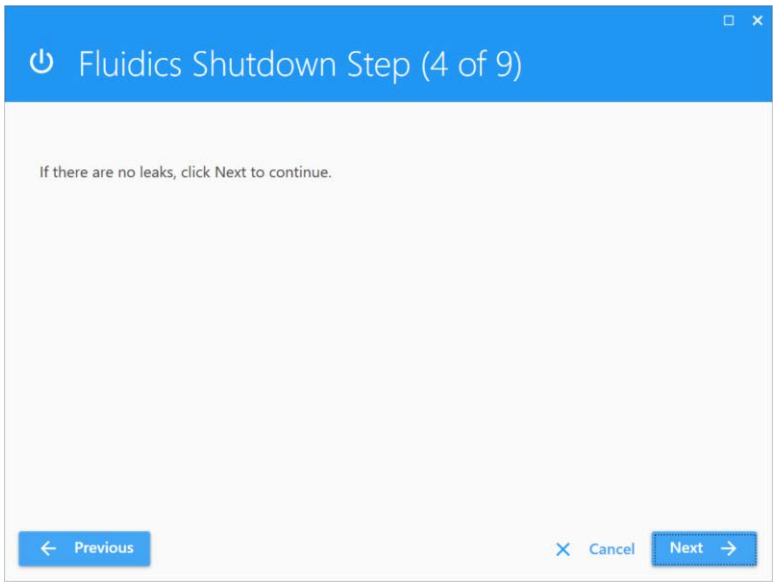
5 Disconnect fluid line and air line from the sheath tank and connect to the 3L tank containing DI water.



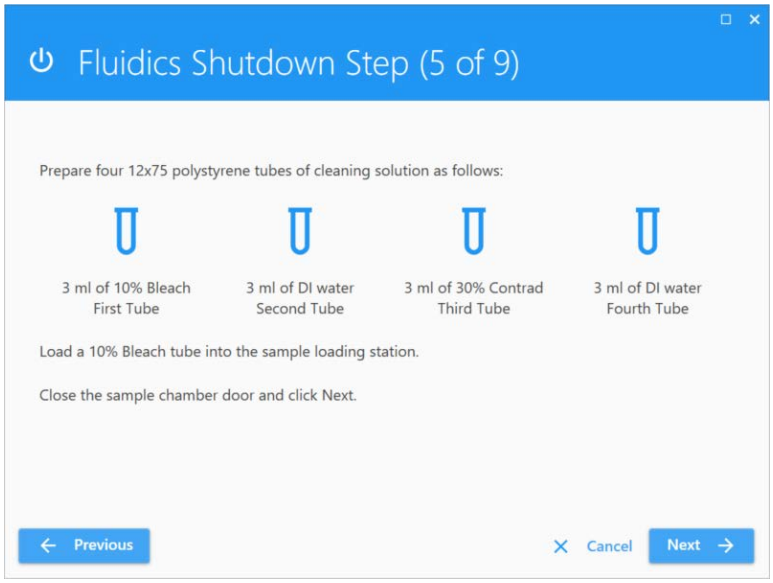
6 Click Next. The progress bar appears.



7 Check for leaks on the DI tank. Shutdown won't complete properly if the tank isn't sealed properly.



8 Click Next. Prepare 4 tubes of the cleaning solutions in Step 5 of the wizard.



9 Load the cleaning tubes when prompted by the wizard.


Fluidics Shutdown Step (7 of 9)
Load 30% Contrad tube into sample loading station.
Close the sample chamber door and click Next.
Previous
Cancel
Next


Fluidics Shutdown Step (8 of 9)
Load second DI water tube into sample loading station.
Close the sample chamber door and click Next.
Previous
Cancel
Next

10 Shut off the air supply to the system for the final step.

11 Log out of the software and shut down the instrument.

Turning Off the System

1 Close SpectroFlo CS software and turn off the workstation.

2 Ensure the air supply is turned off at the source.

3 Turn off the cytometer.

4 Vent the sheath tank, if desired, by pulling up on the bleed valve to relieve the pressure. Or, if not performed, the tank will slowly depressurize over time.

Filling the Sheath and Emptying the Waste

Fill the sheath and empty the waste after you shut down the system at the end of the day. If necessary, you can also perform these tasks when the system is on.

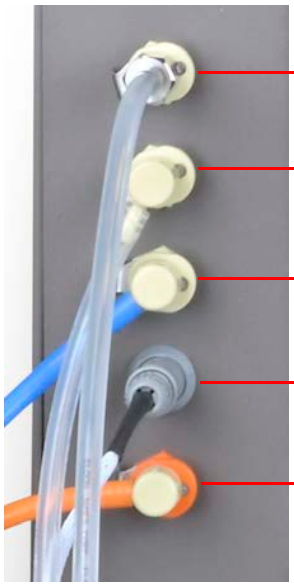
Empty the waste container when filling the sheath container, or as needed to prevent leakage. The software indicator for waste will be yellow or red when the container needs to be emptied. Take care to avoid damaging the fluid level sensor in the waste tank.

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

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

Sheath, Air, and Waste Lines

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



Air in – Air from compressor, pressurized tank, or house air

Air out – Air supply to sheath tank

Sheath line In (blue) – Sheath from pressurized tank to instrument

Waste overflow sensor (gray)

Waste line out (orange) – Waste from instrument

Filling the Sheath Tank

Fill the 10-L sheath tank with manufacturer-provided sheath solution or phosphate-buffered saline (PBS). Pressure to the sheath tank must remain between 80 and 85 PSI and can be supplied by an air tank. a compressor, or house air. Note the following settings:

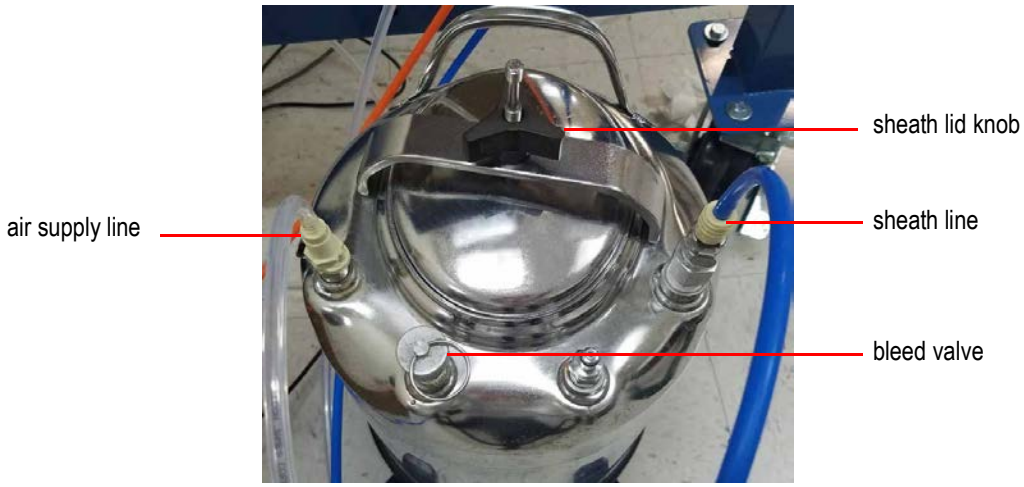
- with an air tank using a regulator set to 80 PSI

- with house air, if the pressure is 80–85 PSI

Fill the sheath container as needed. Use only the appropriate sheath solution. Never use tap water, surfactant-based sheath solution, or ethanol (EtOH) through the system as a cleaning solution.

To fill the sheath tank

1 Disconnect the sheath fluidics line and air supply line from the tank.



2 Ensure that the sheath tank is depressurized before filling it. If necessary, pull up on the bleed valve to release the pressure.

3 Remove the sheath tank lid by unscrewing the lid knob to loosen the clamp, then removing the lid.

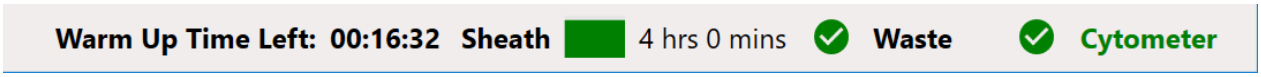
4 Add the appropriate solution, filling to the weld line to allow space for proper pressurization of the tank.

5 Replace the sheath tank lid and ensure a tight seal.

Re-tighten the lid after the system is turned on and pressure is applied to the tank.

6 If the cytometer is powered on and the software is connected, verify that the software sheath indicator is green.

The software displays the time that the system can operate before running out of sheath.



Emptying the Waste Tank

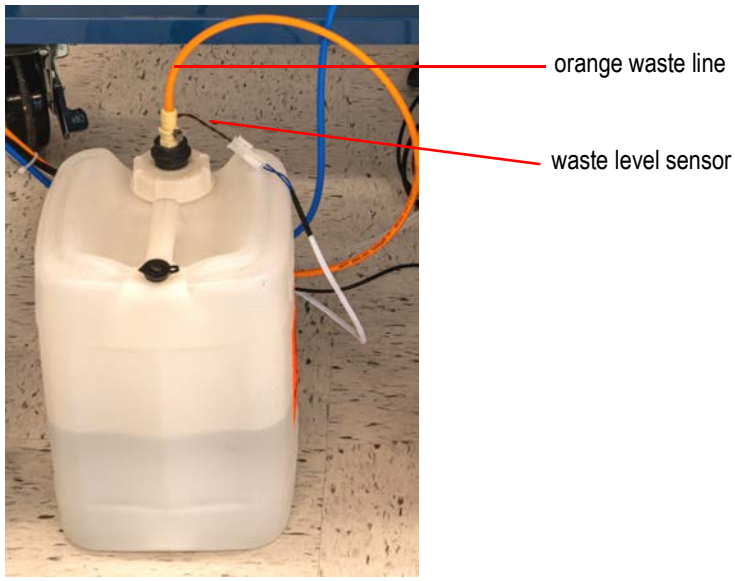
Waste is expelled into the 10-L waste tank supplied. Empty the waste tank and add 1 L of undiluted bleach for a total volume of 10%. If bleach is not allowed, use the appropriate disinfectant allowed for your locale.

Biological samples are potentially dangerous and/or life threatening. Adhere to proper handling procedures for samples and reagents. Wear appropriate personal protective equipment (PPE), including gloves, eyewear, and lab coat.

Contents of the waste container may contain biohazardous material. Always treat the contents of the waste container with household bleach (10% of the total volume). Undiluted household bleach contains 5–7% sodium hypochlorite (NaClO).

To empty the waste tank

1 Disconnect the orange waste line from the 10-L waste tank.



2 Disconnect the waste level sensor on the tank.

3 Remove the lid from the 10-L waste tank.

4 Dispose of the waste according to local, state, and national regulations.

5 Add 1 L of undiluted bleach or appropriate disinfectant to the waste tank.

6 Replace the waste tank lid by hand-tightening it until it is fully closed.

7 Reattach the waste line and level sensor line to the tank.

8 If the cytometer is powered on and the software is connected, verify that the software waste indicator is green.

4

Instrument Setup and QC

Daily QC

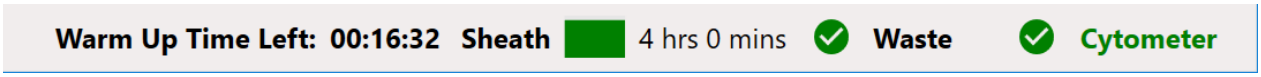
Run Daily QC using SpectroFlo QC beads prior to acquiring samples to ensure that the cytometer is performing optimally. Daily QC assesses the instrument's optical alignment and the system performance drift by measuring rCVs and gains needed to place the beads at the target locations for each detector. During QC, laser delays and area scaling factors are optimized and gain settings are adjusted to account for day-to-day instrument variability. 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 49.

Performing Daily QC

Perform the instrument setup procedure before running daily QC. Perform the "Instrument Setup" on page 101 prior to running Daily QC. Once a stable droplet break-off profile is achieved, perform daily QC.

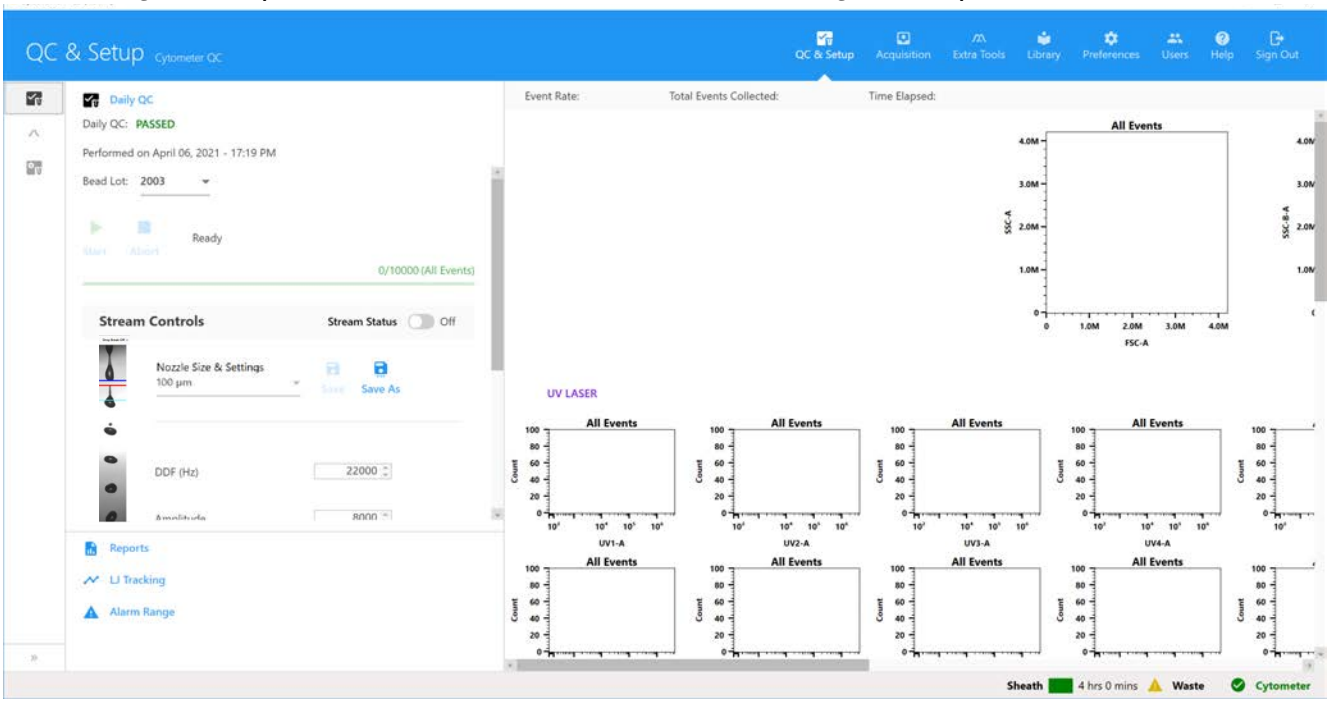
Allow 30 minutes to pass after turning on the system to ensure the optics compartment is warmed up. The warm-up time is displayed in the status indicators in the lower-right corner of the software.



1 Once a stable droplet break-off profile is achieved, perform daily QC.

■ NOTE: The nozzle setting defaults to the nozzle you selected during the instrument setup workflow. You must use the same nozzle when running daily QC that you used for instrument setup. If you acquiring samples only and will not be sorting, select the Bypass nozzle.

2 Select QC & Setup from the Get Started menu, or click the QC & Setup tab.



3 Prepare SpectroFlo QC beads (1 drop of beads in 0.3 mL of sheath solution or PBS in a 12 x 75-mm tube).

SpectroFlo QC beads are 3-µm hard-dyed, polystyrene beads that have a single fluorescence intensity. They can be excited by each laser and emit fluorescence in all detector channels.

4 Select the current 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. Bead lot files can be downloaded from the Resources section at http://www.cytekbio.com.

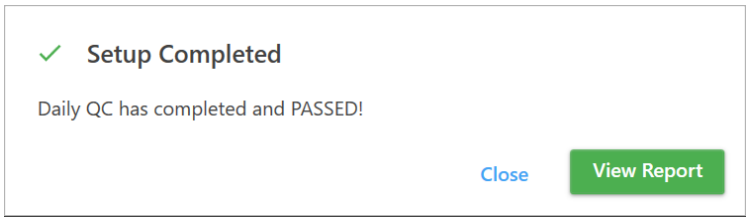
Different bead lots have different fluorescence intensities. Always select the correct bead lot when performing Daily QC.

5 Load the beads on the instrument and click Start.

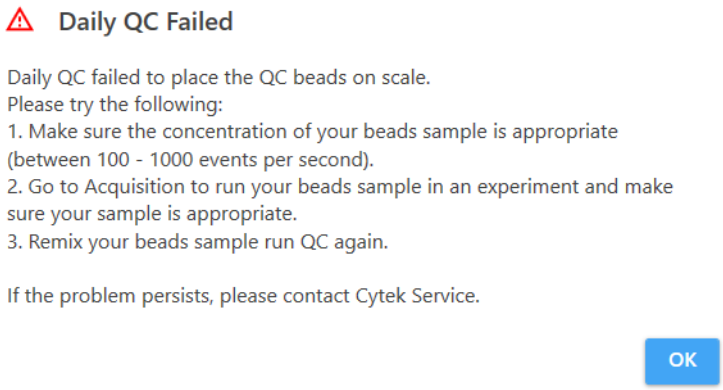
As the instrument begins acquiring the QC beads, they appear in the scatter plot. The laser delays are initially set to 0, then optimized thereafter. The performance measurements are established and compared to the pass/fail criteria (see “Pass/Fail Criteria” on page 47).

The procedure takes approximately 3 to 5 minutes to complete. Once acquisition is complete, two SIT Flushes are automatically performed to clear the beads from the sample line.

The following message is displayed when Daily QC passes. To view the QC report, click **View Report** (see "QC Report" on page 47).



If QC fails, remove the tube and follow the guidelines that appear. The recommended solution will vary depending on the reason for the failed test.



You are now ready to:

- create an experiment and acquire samples (see "Creating an Experiment" on page 59)
- perform instrument setup to prepare for sorting (see "Instrument Setup" on page 101)

QC Report

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

- The header section contains the Pass/Fail status of the run, name of the instrument, instrument configuration, date the Daily QC was run, user who ran the Daily QC, instrument serial number, nozzle size, sheath pressure, and SpectroFlo QC bead lot and expiration date.
- The results section contains the gain, gain change, median fluorescent intensity of the 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.

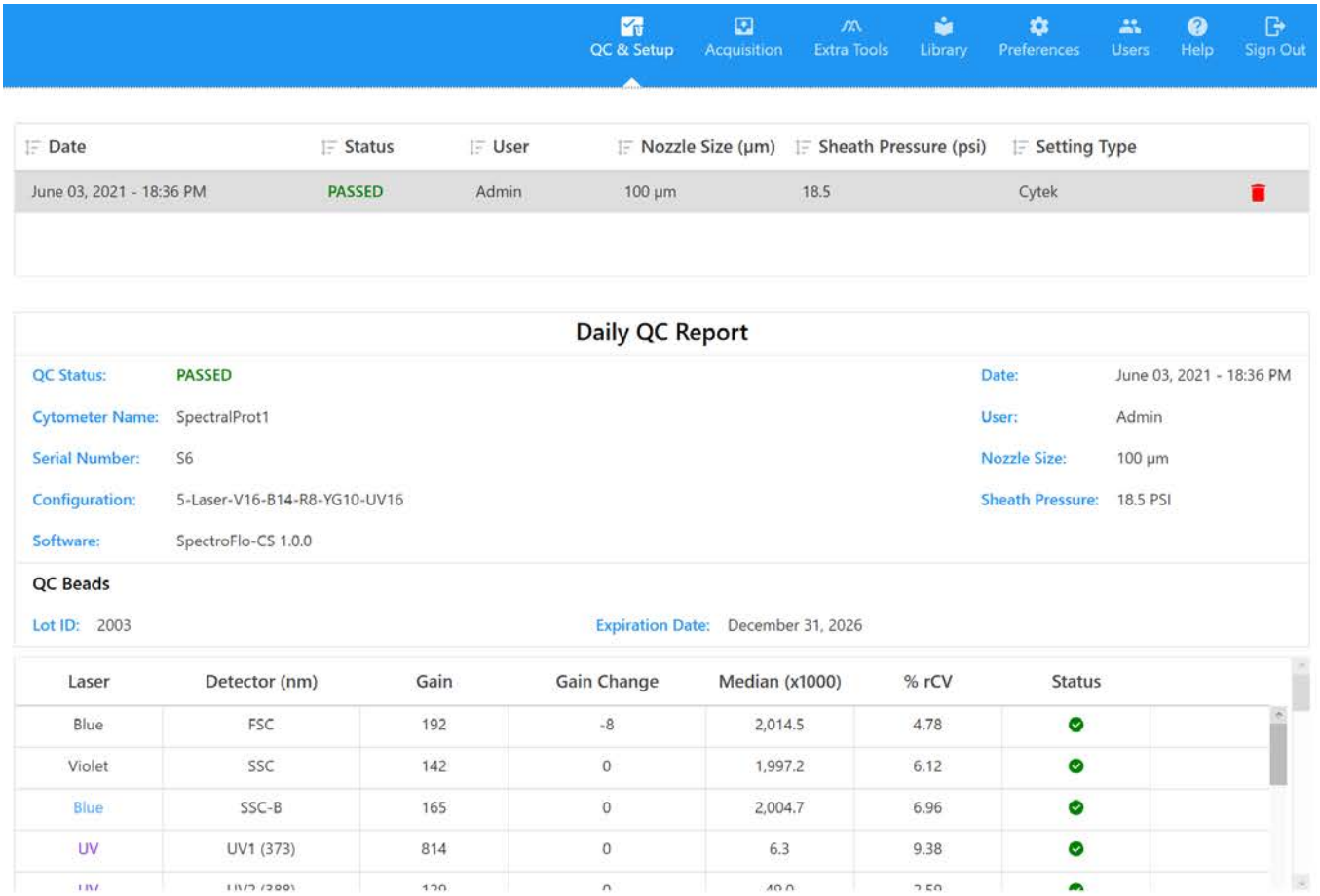
Pass/Fail Criteria

The pass/fail criteria are the following:

- %rCV must not exceed 6% for the FSC channel
- %rCV must not exceed 8% for the SSC-B channel
- %rCV must not exceed 6% for the third channel of each laser (V3, B3, R3, YG3, and UV3)
- % delta gain change for all channels must not exceed 100% from the last Daily QC run performed by Cytek Service personnel.

QC reports are automatically exported as CSV files to the Setup folder (C:\CytekbioExport\Setup). The number of reports listed in the Reports screen can be set in the Preferences. See "QC Setup Preferences" on page 140 for more information.

An example QC Report is shown.



Levey-Jennings Tracking

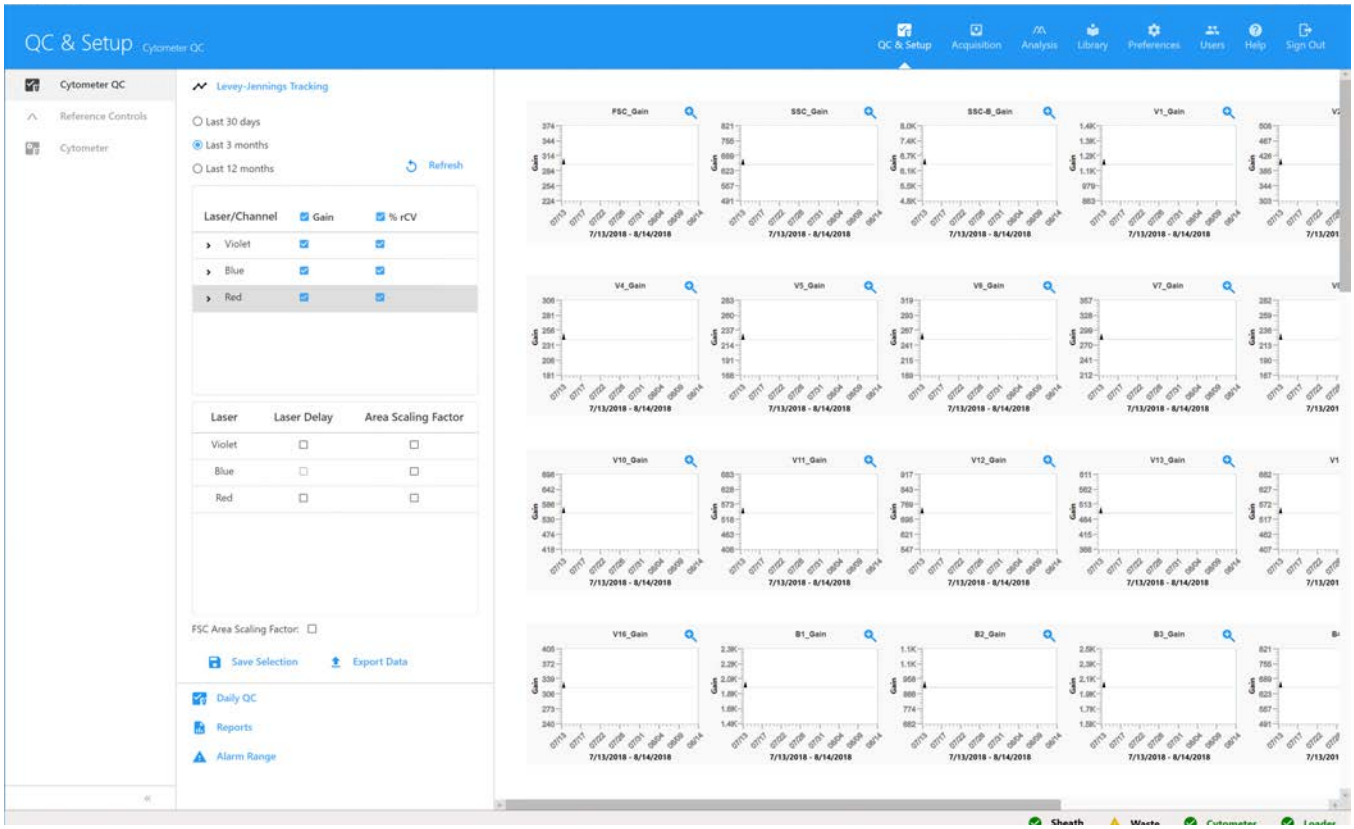
Levey-Jennings reports track the gain, %rCV, laser delay, and area scaling factor for all detector channels over time, allowing you to view the system's performance and ensure that the system is reproducing consistent results. Select the parameter(s) you wish to track.

The graphs in the report show you 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.

To plot Levy-Jennings plots (for example to check Area Scaling factor), select the Area Scaling Factor checkbox and click refresh to plot Levey-Jennings for area scaling factor.

Use Save Selection to save the LJ tracking settings.

To export Levey-Jennings data to a .csv file, click **Export Data**.

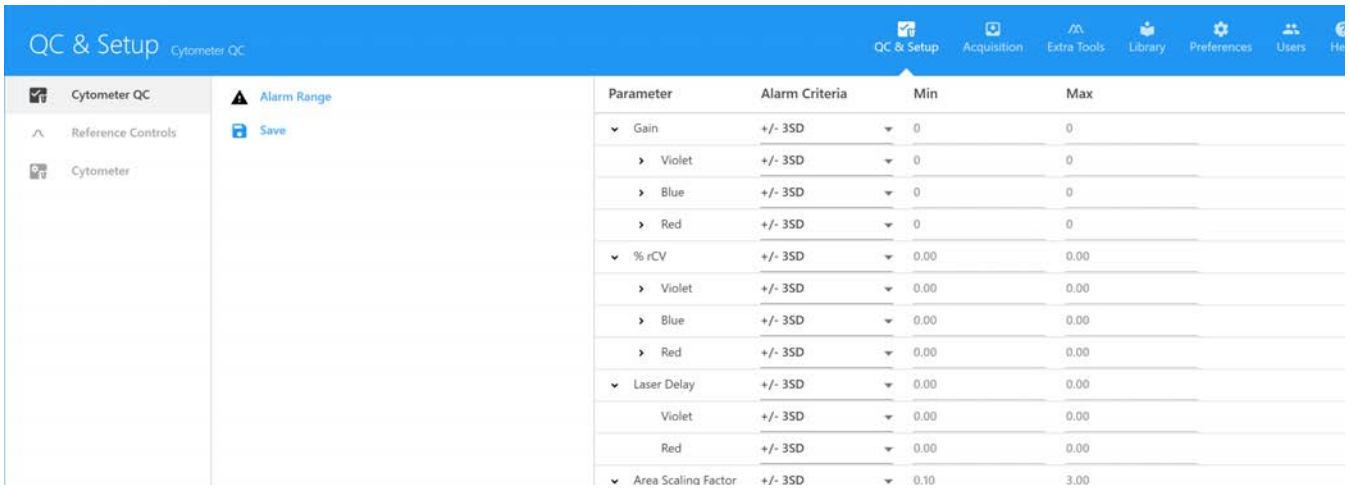


Gain Settings

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 versus Unmixed Data

SpectroFlo CS 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 (ie, V1, V2, V3, etc). 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 contains all the fluorescence information from each fluorescent tag in the experiment. To unmix data, single stained controls (or reference controls) for each of the fluorescent tags (as well as an unstained control) are required. During unmixing a mathematical algorithm is used for the decomposition of the fluorescent components in the sample using the reference controls. Parameters in unmixed data will display as the fluorescent tag name along with their associated labels.

The Acquisition module provides the tools that allow you to create an experiment. An experiment is a set of tubes, instrument settings, acquisition criteria (stopping rule), fluorescent tags, labels, and worksheets designed for the acquisition of samples. See "About Experiments" on page 27.

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

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 within the experiment can be used in the Unmixing wizard in the Acquisition module. In order to sort, you will need to use Live Unmixing during sample acquisition.
- Unmixing from the Extra Tools module – FCS files collected from different experiments can be unmixed in the Extra Tools module. FCS files can be imported and unmixed in this module. Note: The Extra Tools module cannot be used to sort samples since Live Unmixing is not available in this tool.

Raw FCS files can also be compensated with the conventional method using the Virtual Filters tab in the Extra Tools module. Detector channels can be binned together to simulate the analysis of the data as if it were acquired using a filter. See "Virtual Filters" on page 79 for more information.

Setting Up a New Experiment

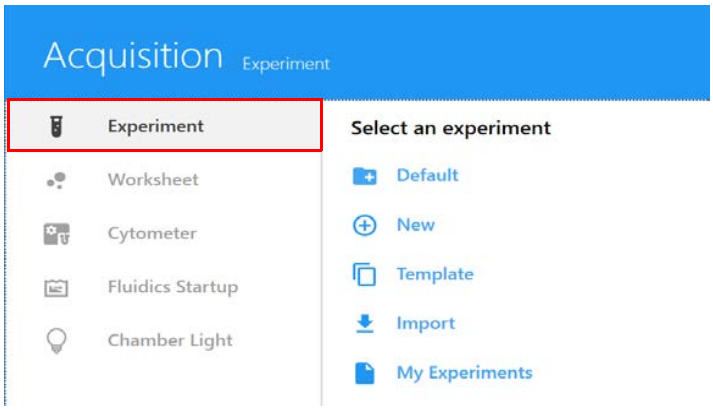
An experiment can be saved as a template or created for one time use. Setting up the experiment in SpectroFlo CS software involves:

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 with associated reference tags, labels, and lot numbers, as needed.
4. Adding labels and lot numbers to each of the fluorescent tags.
5. Adding custom keywords. Custom keywords can be defined in the Library.
6. Selecting an acquisition worksheet—either new or a template.
7. Defining acquisition criteria (stopping rule based on events, time, or volume).

Acquisition Experiment Overview

The Acquisition module provides the necessary elements for data collection within the experiment.

Click Experiment in the left pane to open a template, the default, or a new experiment using the wizard.



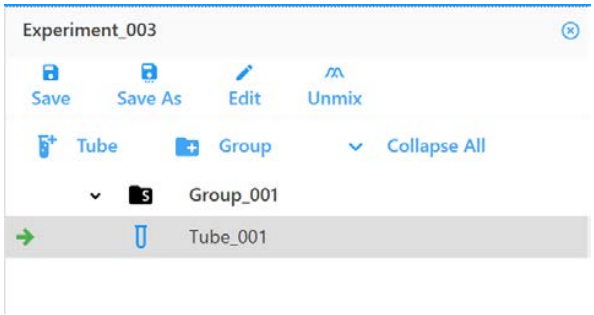
Experiment Display

The experiment display in the Acquisition module includes the following panes. To show, hide, or undock (float) these panes from the experiment panel, click the corresponding icons in the top-right corner of the pane.

Group-Tube List and Hierarchy

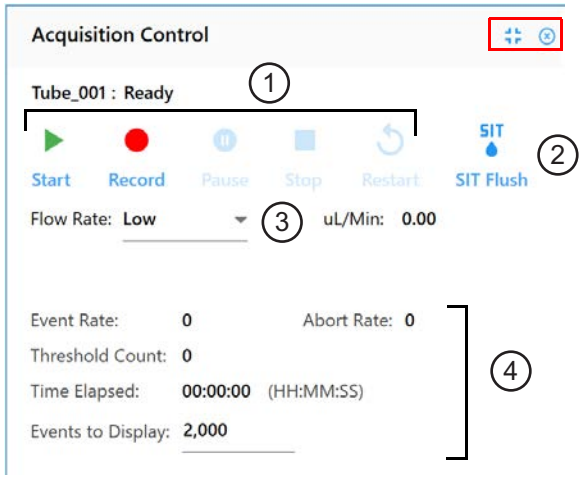
The samples are listed in the upper left of the screen. Samples can be organized into groups. Use the Tube and Group icons to add tubes and groups.

Click Save to save changes to the experiment, or click Save As to save an experiment template. Click Edit to edit the experiment. Worksheets can be applied to the experiment, groups, or individual tubes.



Acquisition Control

The Acquisition Control pane allows you to start, stop, and pause acquisition, record data, and restart acquisition counters. The acquisition controls are enabled when a tube is present in the sample loading station. To show, hide, or undock (float) this pane from the experiment panel, use the dock/undock and hide icons in the top-right corner.



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

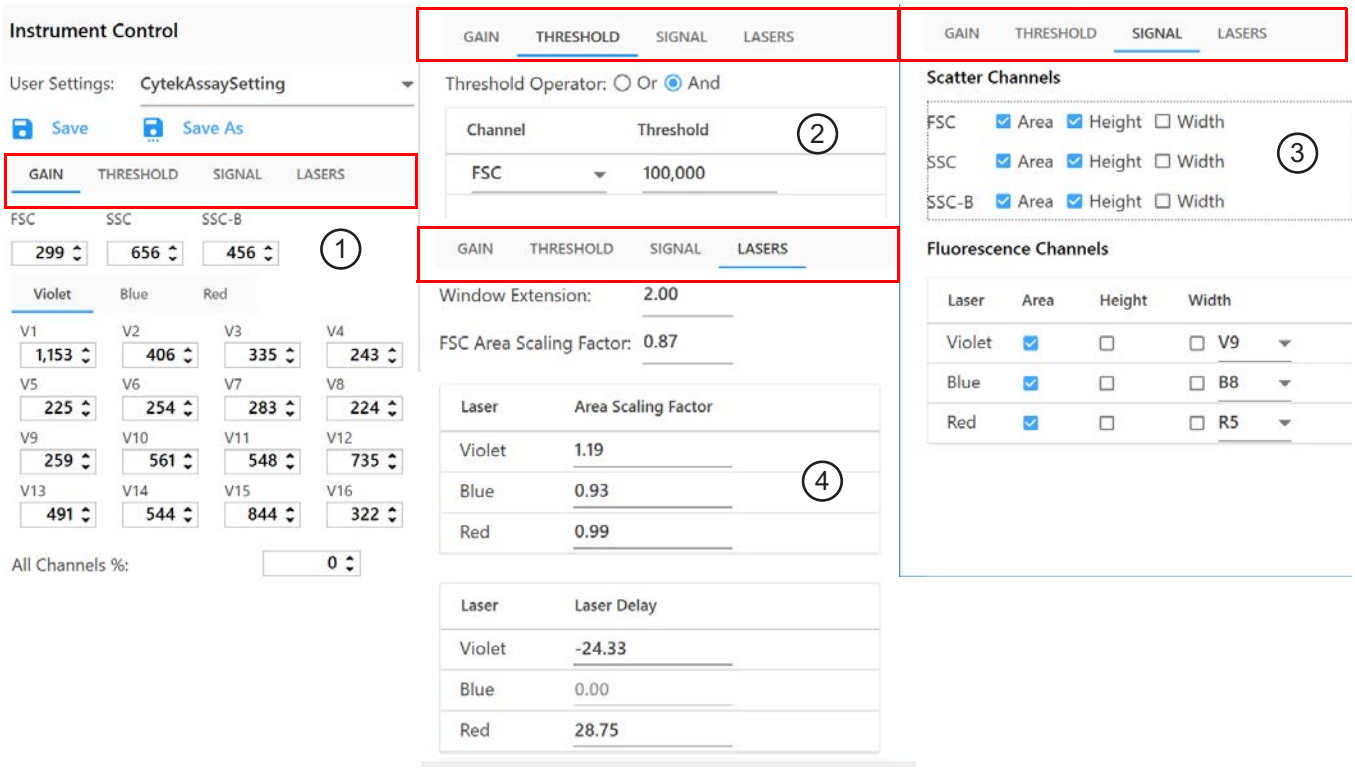
No.	Control	Description
1	Start/Record/Pause/ Stop/Restart	Start and Record are enabled when a tube is present. Select Start to start acquisition Select Record to record data. Record can also start acquisition. Select Pause to pause recording. While paused you can adjust the flow rate. Select Record again to continue. 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. Stop and Pause are enabled once Record is selected.
2	SIT Flush	Select to perform a SIT Flush
3	Flow Rate	SpectrofloCS has a range of 1-10. 1 = 10 µL/min, 10 = 80 µL/min

No.	Control	Description
4	Event Rate, Abort Rate, Threshold Count, Time Elapsed	Displays the real-time counts during acquisition.
5	Events to Display	Enter the number of events to display during acquisition.
	Sample Return	Returns 35 µL of sample back to sample tube before Sit Flush.

Instrument Control

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

User Settings allow you to select CytekAssaySetting, Default, or any saved user settings for the experiment. We recommend using CytekAssaySetting as a starting point. This setting provides the optimal resolution for each channel, accommodates bright signals, and minimizes spread.



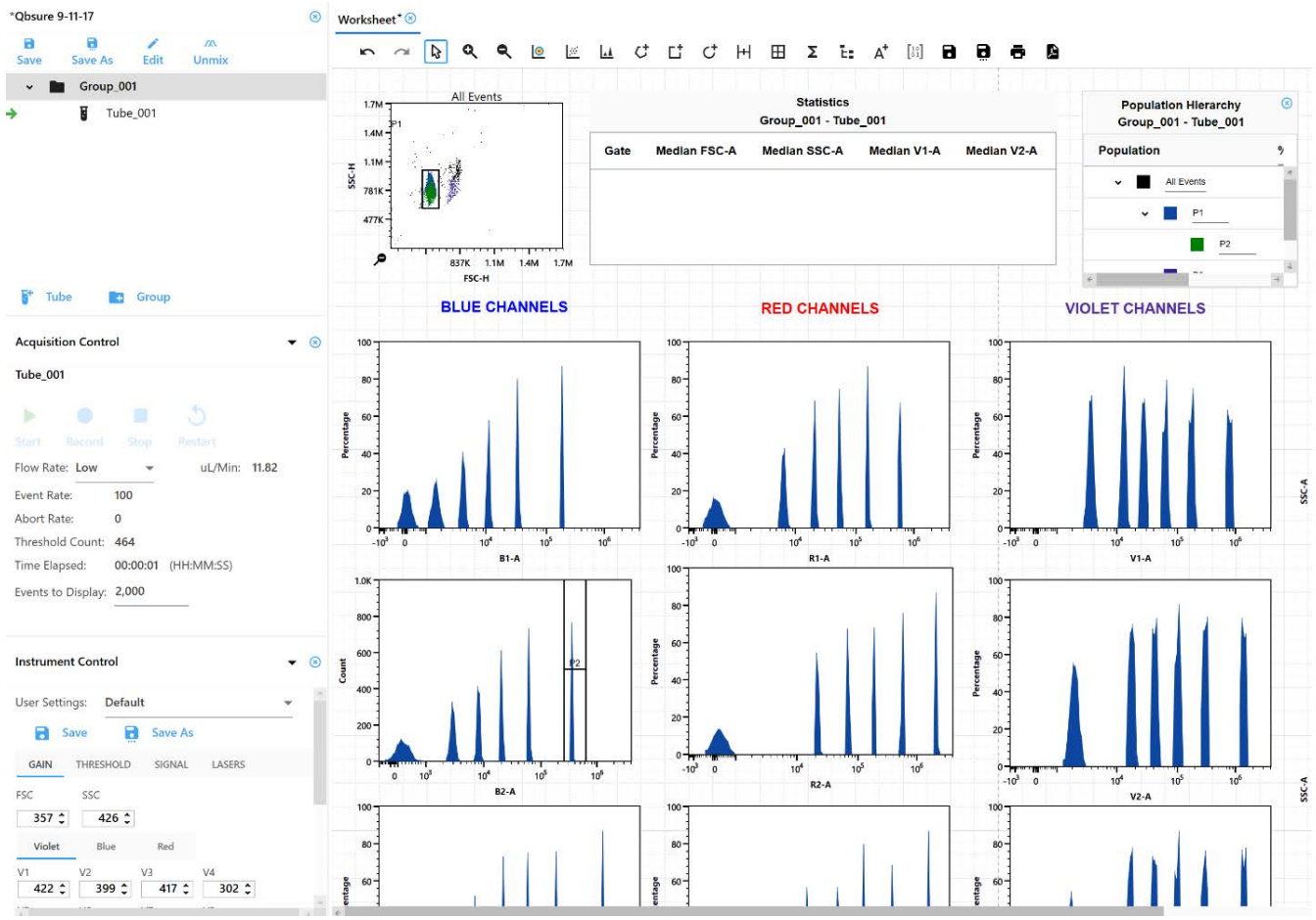
The following table describes the tabs in the Instrument Control pane.

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 1–1,000. SSC and fluorescence detector gains can be adjusted from 10–10,000. To change the value that the gain increments, see "Acquisition Preferences" on page 132. Use All Channels % to increase/decrease all gains for a selected laser by the percentage you select.

No.	Tab	Description
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 when at least one parameter is 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. ■ **NOTE:** If you run large cells and the lowest FSC gain setting is not low enough to see your cells, lower the FSC area scaling factor (for example, 0.5).

Worksheets

The worksheet allows you to view the data in plots and create plots, statistics, population hierarchy, and gates. Click the worksheet icon in the far left pane to select, import, save, and print worksheets.



Worksheet Toolbar

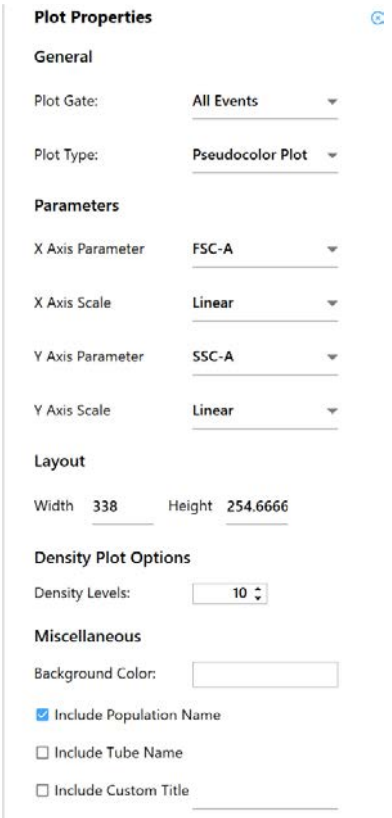
A toolbar at the top of the worksheet area allows you to undo/redo, zoom; create plots, gates, statistics, population hierarchy, annotations; and save, print, and save a PDF of the worksheet. Hover the cursor over an icon to see a description and keyboard shortcut.

Plots

Four plot types can be created in the worksheet:

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

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



Gates

Gates types include:

- rectangle
- oval
- polygon
- interval

- quadrants

Gate Properties

Gates properties 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 the count and/or the % parent events within the gate, as well as the gate parameters.

Statistics

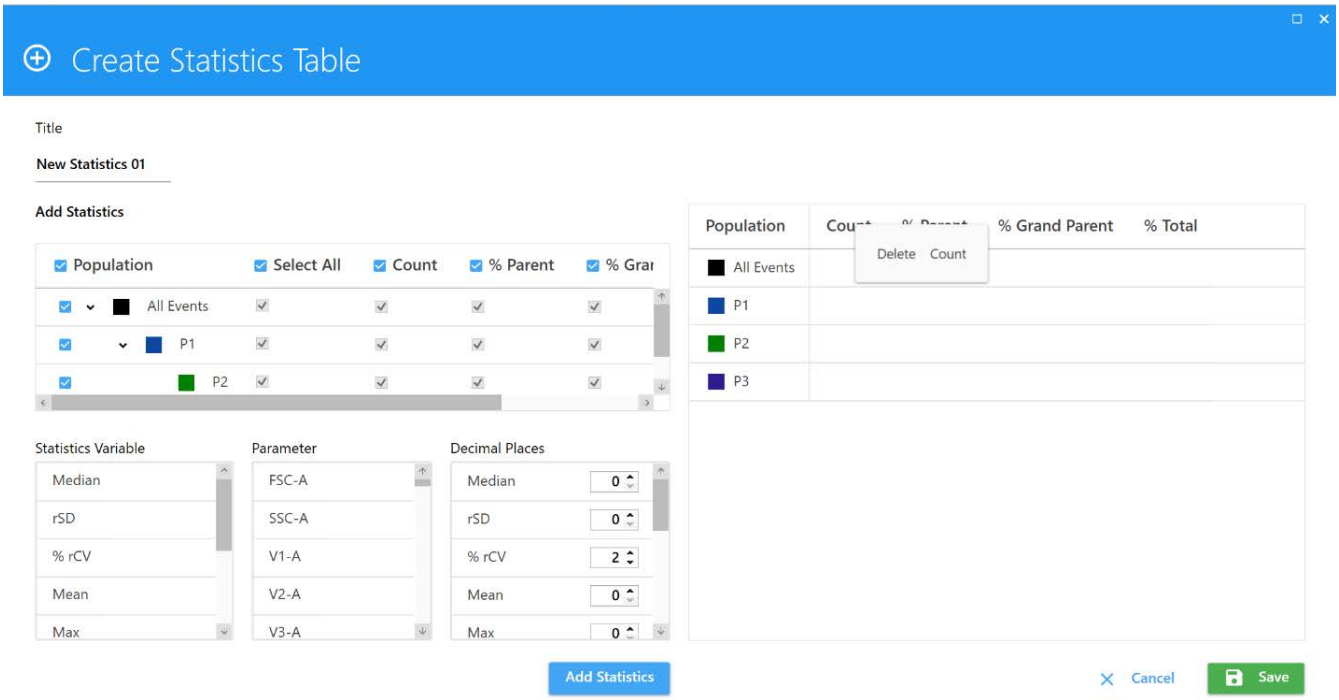
To create a statistics box, click the Statistics icon in the worksheet toolbar, then click in the worksheet area.

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

Select the parameter you would like to add for the statistics. Multiple parameters can be selected at once by pressing either the Shift or Ctrl keys then select the desired parameters.

To adjust the precision of the statistics, select the decimal place in the Decimal Places table. To remove a statistic, right-click the column header and select **Delete**.

Once the statistics are complete, you can export the stats as a CSV file to the location you choose. Statistics output can be appended or overwritten if you export the stats into the same CSV file.



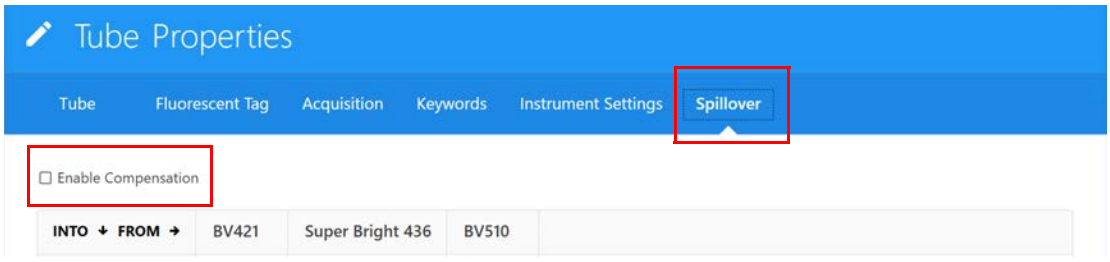
Adjusting Spillover

You can adjust the fluorescence spillover for a selected tube. To apply spillover to other tubes, right-click a tube, select **Copy Spillover**, then select the other tubes and paste. You can save these spillover settings to the Library and apply them to samples in any experiment.

1 Right-click on the sample tube in the group-tube hierarchy list and select **Edit Properties**.

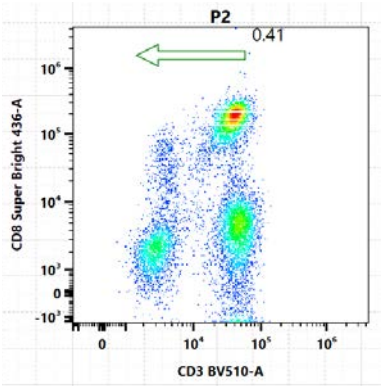
The Tube Properties window opens.

2 Select the **Spillover** tab and click the **Enable Compensation** checkbox. The Adjust Spillover icon [10 01] in the toolbar is now enabled.



3 Leaving the Tube Properties window open, click the Adjust Spillover icon [10 01], then click and drag in the plot in the direction you want to adjust.

Changes are reflected in the Spillover Matrix tab of the Tube Properties window.



4 Click Save to save the changes. To discard the changes, close the Tube Properties window and click No in the confirmation window.

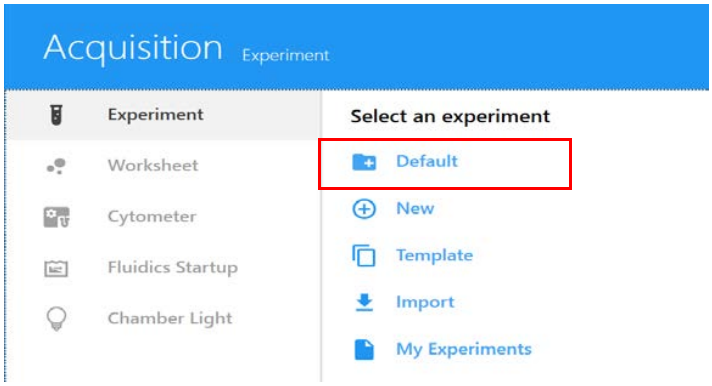
Creating an Experiment

You can create an experiment by using the default experiment, creating a new experiment, or opening a saved experiment or experiment template. You can also open an experiment created on the Aurora or Northern Lights system using SpectroFlo software, v2.2 or earlier.

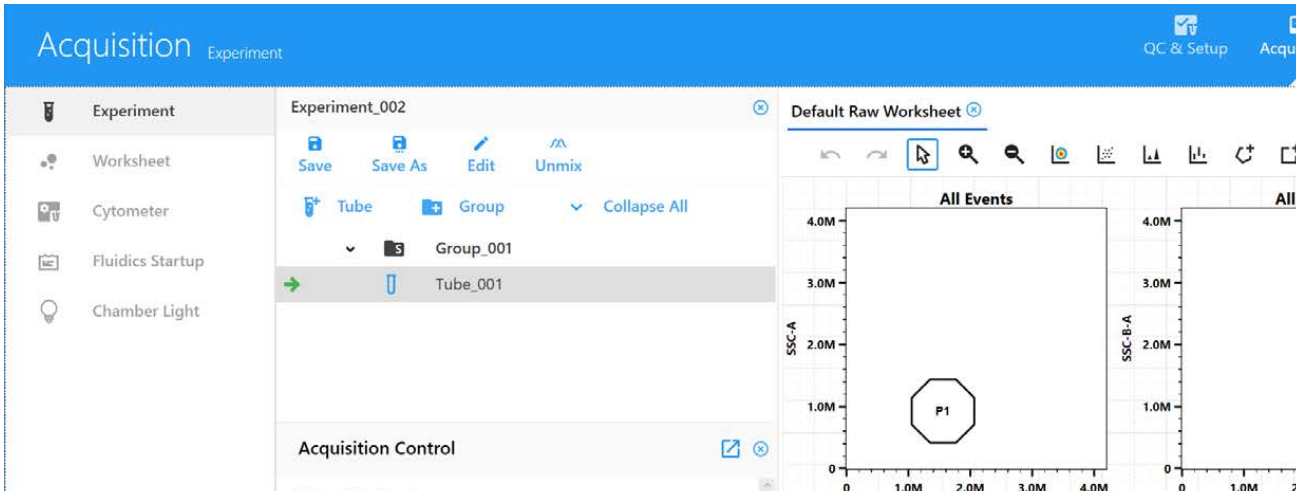
Creating a Default Experiment

Use the default experiment option to quickly begin an experiment. The default experiment contains a set of fluorescent tags, however, all parameters are user configurable.

1 Click Default in the Acquisition Experiment menu.



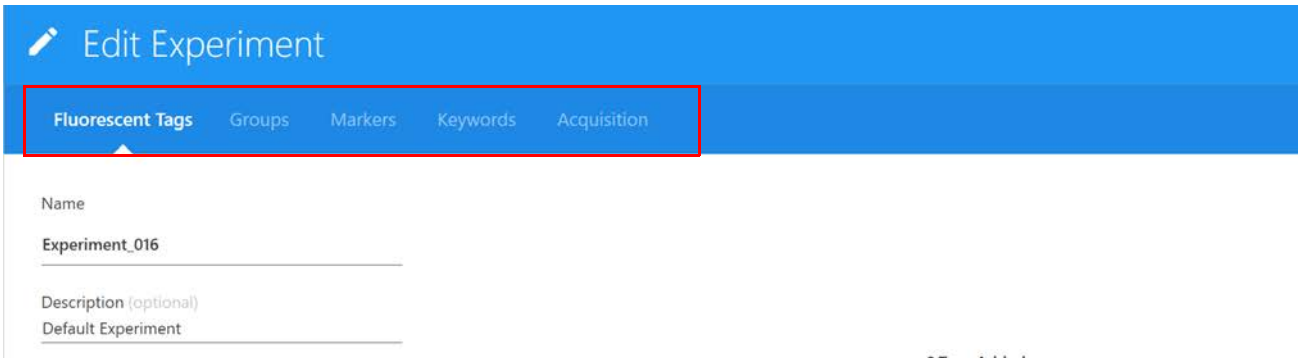
The default experiment opens with a default raw worksheet.



2 Click Edit.

The same wizard appears as when creating a new experiment. Follow the steps to select the fluorescent tags, add groups, and define makers, keywords, and acquisition settings/ worksheets.

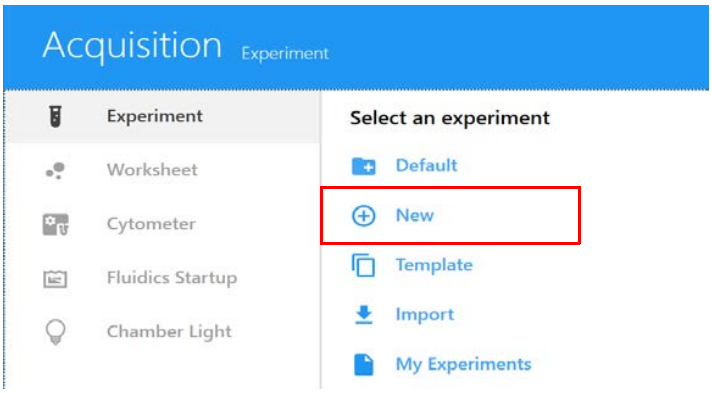
See "Creating a New Experiment" on page 60 for details on defining the information in the experiment wizard.



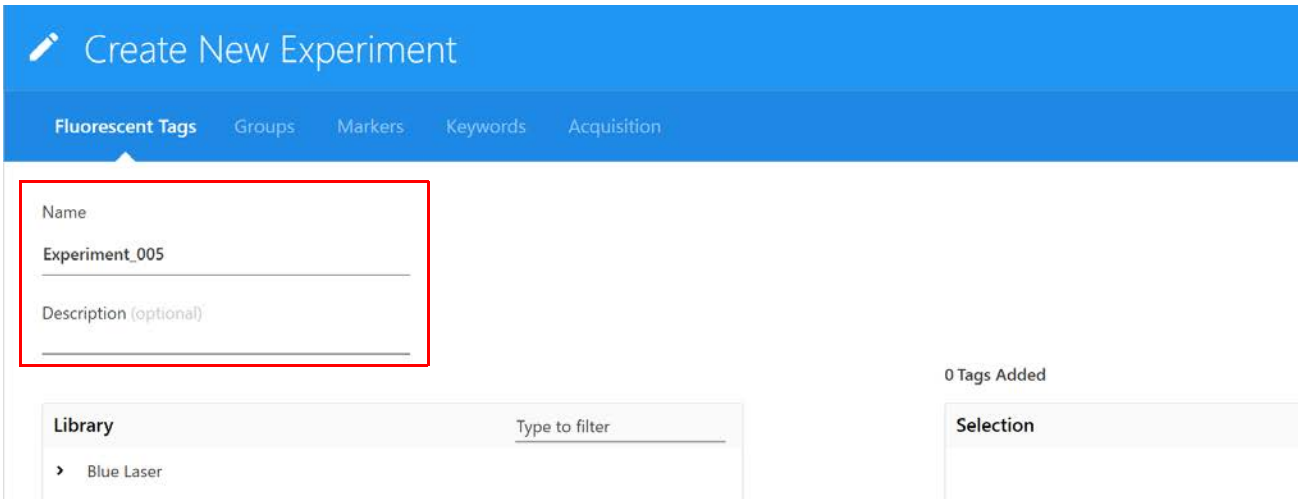
Creating a New Experiment

Selecting New in the Experiment menu opens the New Experiment wizard. The wizard walks you through the steps to create a new experiment.

1 Click New in the Acquisition Experiment menu.



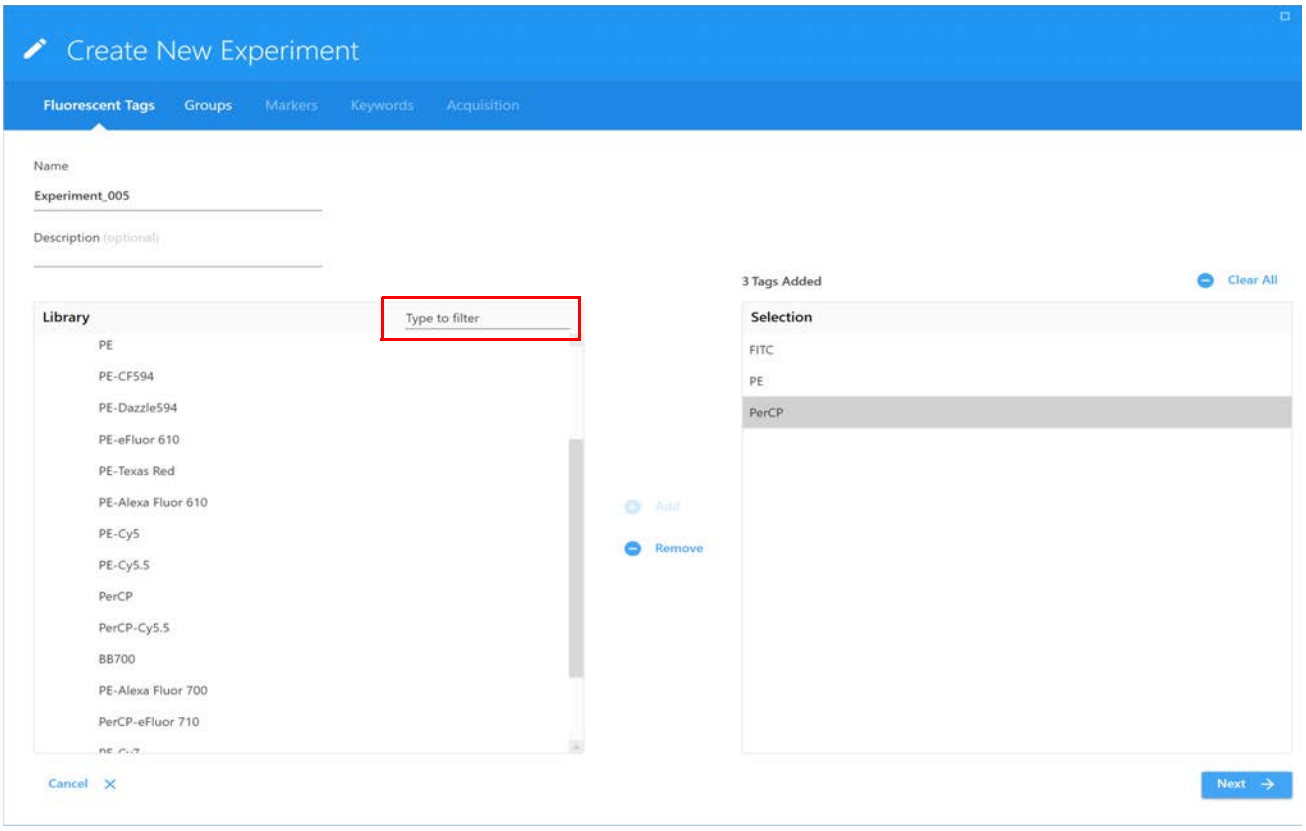
2 The Create New Experiment wizard opens. Specify a name for the experiment or use the default name. (Optional) Type in a description.



3 Click the arrow to the left of the group name (laser) in the Library pane on the left to display the list of its fluorescent tags. Select the fluorescent tags used in the experiment and click ⊕ Add to add them to the Selection list on the right. You can also double-click the tag to add it to the Selection list.

To quickly find a fluorescent tag, type the tag name in the Type to filter text box. A default list of fluorescent tags for each group is available in the Library. See "Fluorescent Tags" on page 123.

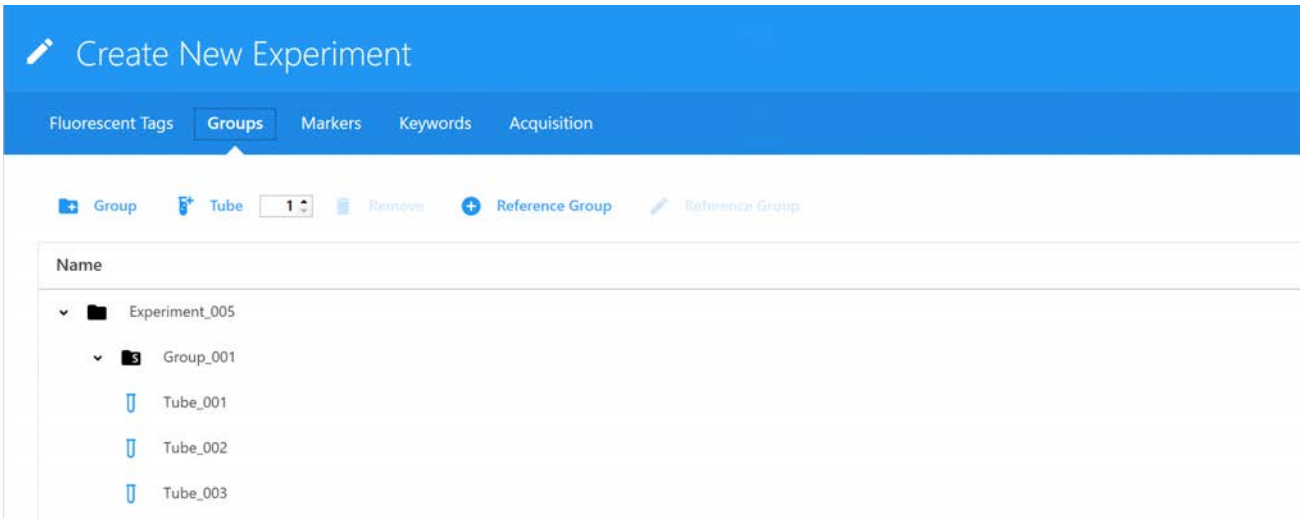
You must select all fluorescent tags present in the experiment, as this will determine which reference controls are to be used during spectral unmixing.



To remove individual fluorescent tags from the Selection list, click to select the tag, then click **Remove**. To remove all fluorescent tags, click **Clear All**.

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

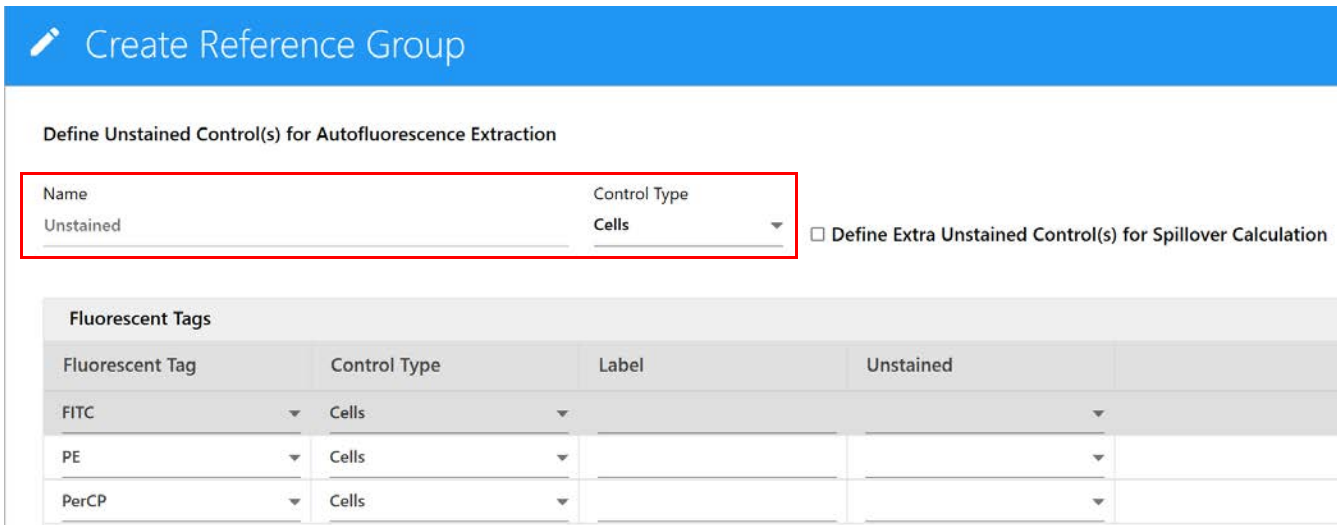
5 Create groups for your samples by clicking **Group**. Add tubes to the groups.



6 Select **Reference Group** if you are intending to unmix in this experiment.

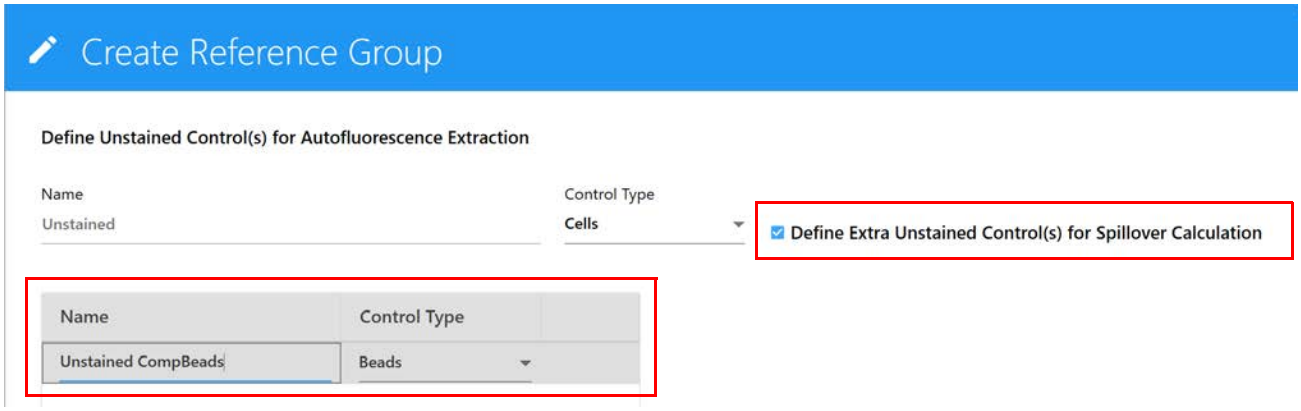
This creates a list of reference control tubes for each fluorescent tag specified as part of the experiment.

7 **IMPORTANT:** Define an unstained control for autofluorescence by selecting its control type (beads or cells). The unstained control needs to be of the same type and prepared in the same way as the samples, as this will ensure accurate unmixing and autofluorescence quantitation.



8 If applicable, select **Define Extra Unstained Control(s) for Spillover Calculation** to use a different unstained control to calculate spillover for your reference controls. Then enter a name and control type for this extra unstained control.

For example, if test samples are cells and the reference controls are beads, all with only positive peaks, you will need to run a separate tube of negative beads for the spillover calculation. An extra unstained control is not needed if your unstained autofluorescence control (and sample) is the same type as the reference controls.

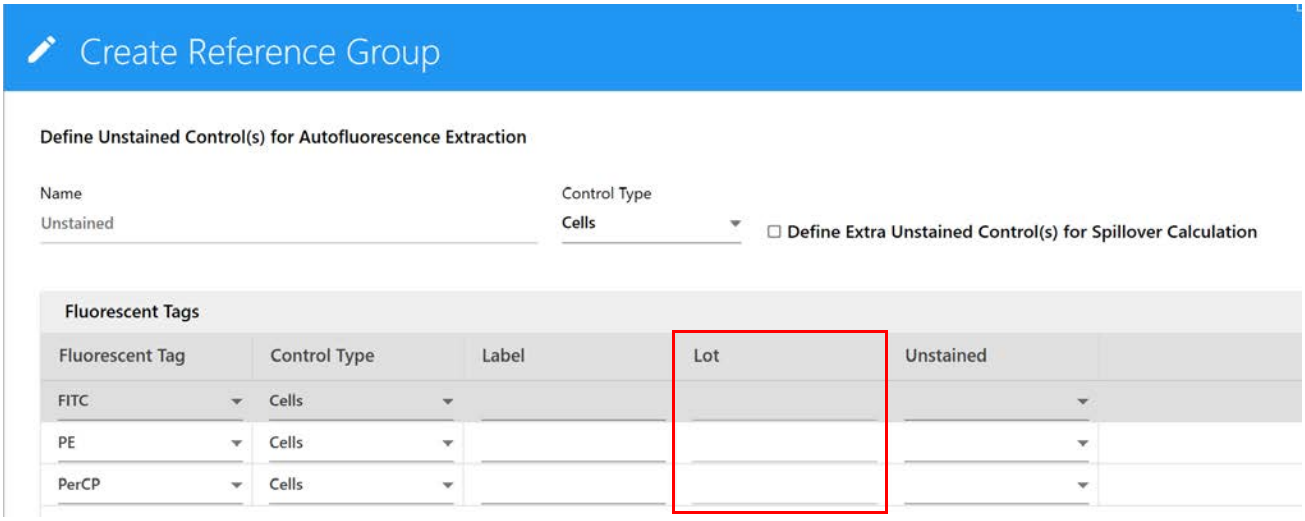


9 Select the control type (beads or cells) for the single-stained reference controls.

10 (Optional) Enter the label (for example, CD nomenclature) that is conjugated to the fluorescent tag.

11 If applicable, enter the lot number(s) of the reference controls.

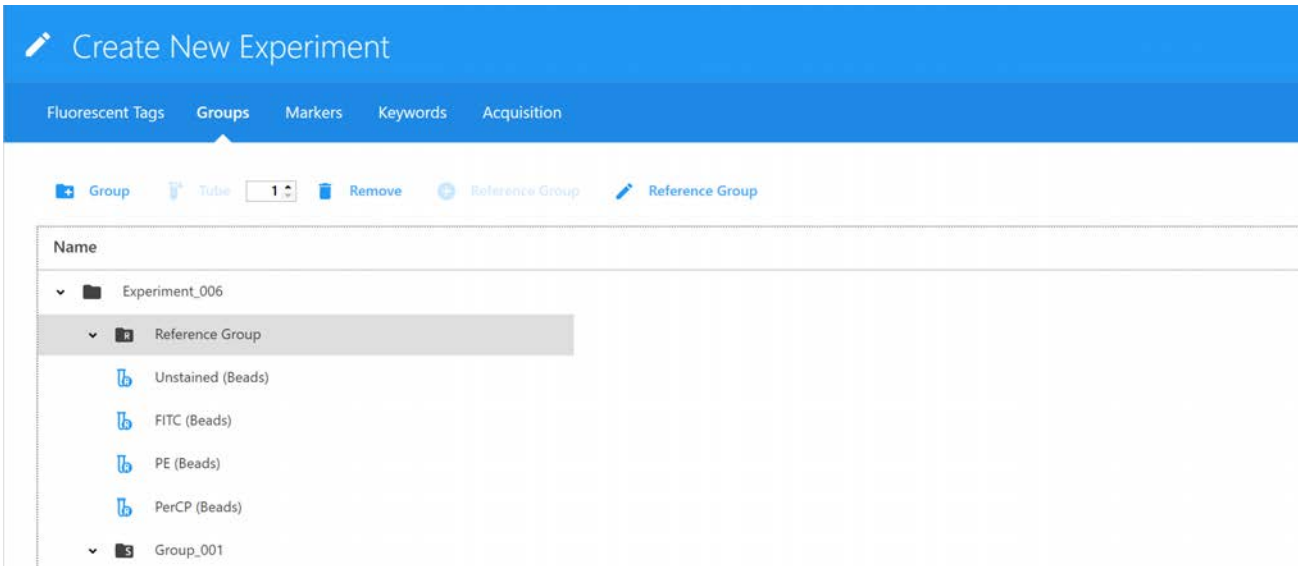
■ **NOTE:** If you selected **Label/Lot Specific Unmixing** in the Acquisition Preferences (see page 133), the software will search the experiment reference group for reference controls that have the same fluorescent tag, label, and lot information in order to use the corresponding control for unmixing.



■ **NOTE:** Use the red trash can icon to delete an individual tube from the reference group.

12 Click Save.

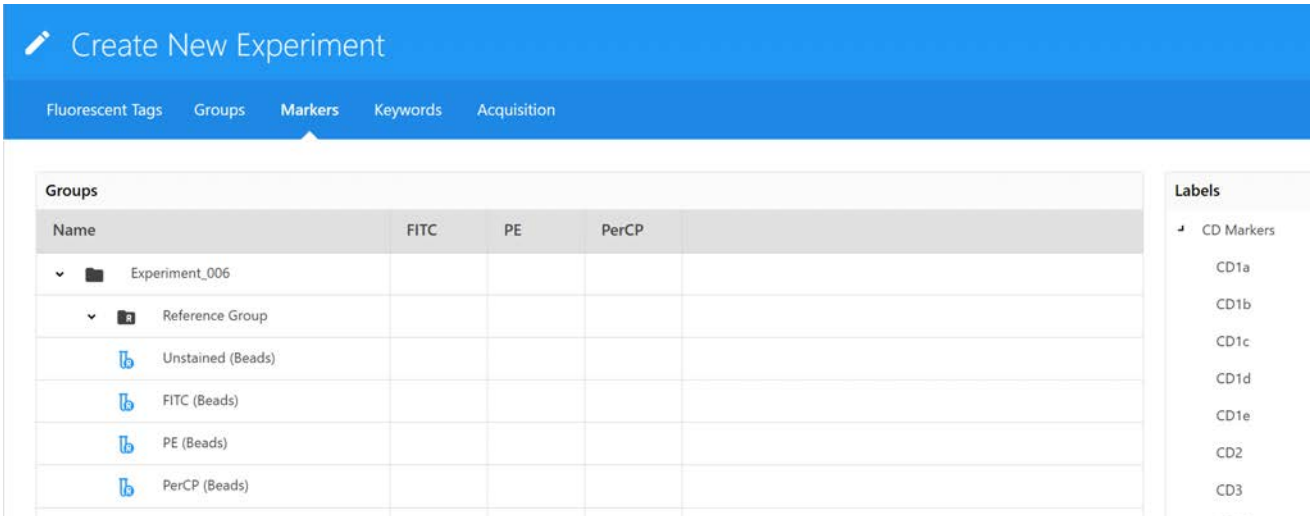
Once the reference group has been created, entries for each of the references will be displayed. Each of the reference group tubes will have an icon (with the letter R) associated with it under the reference group.



13 If necessary, continue adding tubes, click Next when all tubes are created.

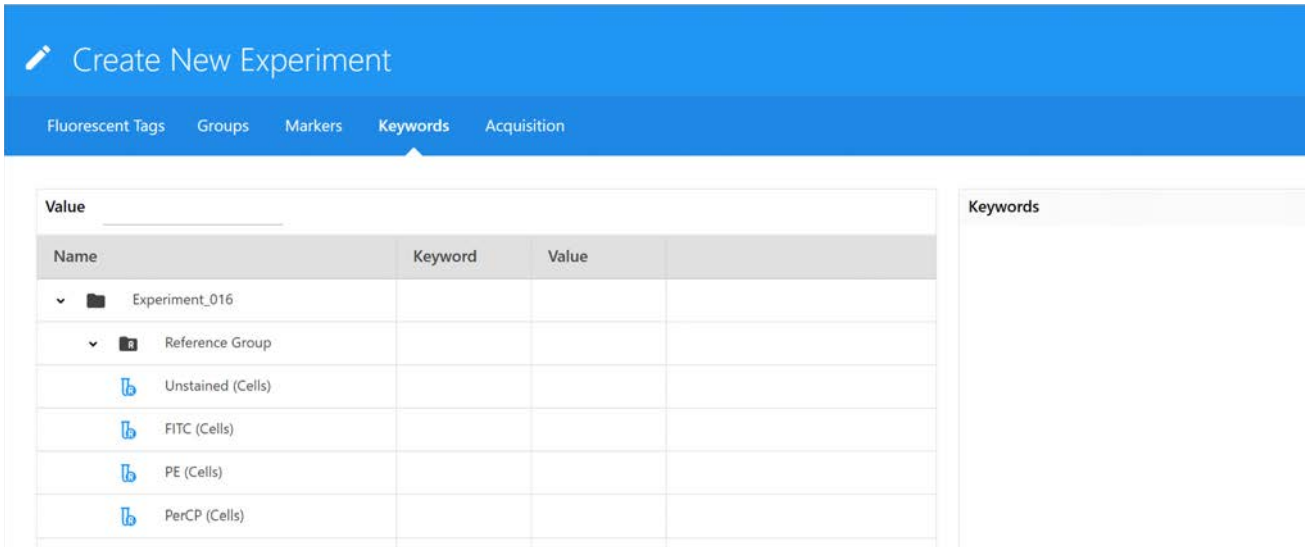
14 Add markers/labels to the remaining sample tubes before continuing. They can be chosen from the Labels list on the right using click and drag, or typed directly into the table, or copied and pasted. Labels can be added at the group or tube level and can be applied to multiple cells selected at once. Labels are required for reference controls if you selected Label/Lot Specific Unmixing in the Acquisition Preferences (see page 133). Click Next when all the tubes are labeled.

■ **NOTE:** If you selected Label/Lot Specific Unmixing, the software will search the experiment reference group for reference controls that have the same fluorescent tag, label, and lot information in order to use the corresponding control for unmixing.



15 (Optional) Enter custom keywords and click Next.

Custom keywords can be added at the experiment, group, or tube level. You must define the custom keywords in the Library before you can add them to an experiment (see "Keywords" on page 127 for information). Drag and drop the keywords from the Keywords list on the right to the experiment, group, or tube and enter keywords values as needed. You can also copy and paste custom keywords across different tubes in this wizard.



16 Select the acquisition settings and worksheet(s). The worksheet menu lists all the worksheets for the given user.

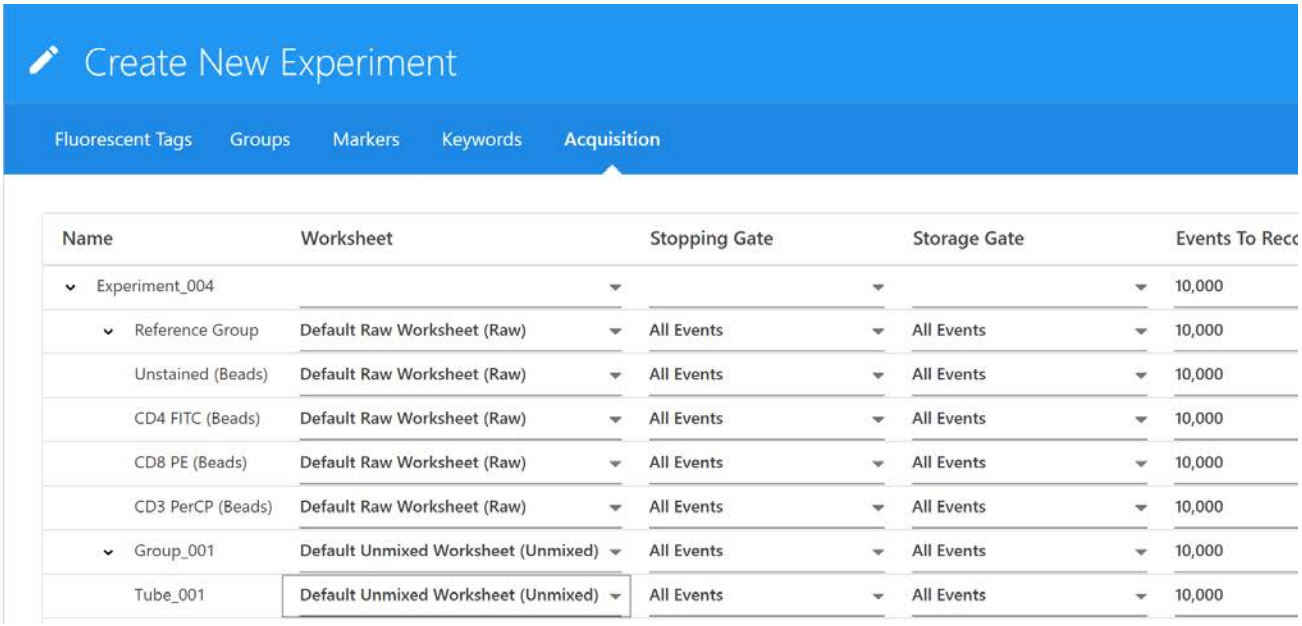
- Select the Default Raw Worksheet (Raw) for the Reference Group and for the sample groups if you plan to perform post-acquisition unmixing.
- Select the Default Unmixed Worksheet (Unmixed) or any user-created unmixed worksheet for your sample groups if you are performing live unmixing. Worksheets can be selected at the experiment, group, or tube level.

Select the stopping gate, storage gate, number of events to record, or stopping time (in seconds). Acquisition stops when the first of the stopping criteria is met (time, number of events). These criteria can be selected at the experiment, group, or individual level.

- If acquiring beads, we recommend collecting 5,000 singlet events.
- If acquiring cells, we recommend collecting 10,000 to 20,000 events of the desired population.

■ **NOTE:** The number of events to acquire depends on the target population. For example, you may need to acquire 10,000 to 20,000 events to get 2,000 of the desired population.

Approximately 1,000 to 2,000 events is needed in both the negative and positive populations of each control for accurate unmixing.



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

To make any changes to the experiment, click **Edit** above the group/tube hierarchy.

18 To acquire controls and samples and perform live unmixing, see "Live Unmixing" on page 70.

6

Unmixing and Compensation

Spectral Unmixing

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

Understanding Full Spectrum Flow Cytometry

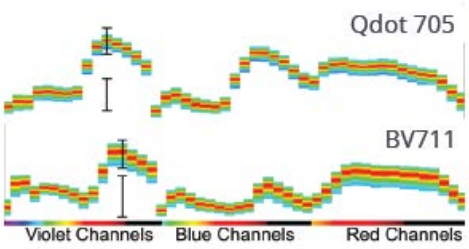
Because fluorophores emit light over a range of wavelengths, optical filters are typically used to limit the range of frequencies measured by a given detector. However, when two or more fluorophores are used, the overlap in wavelength ranges often makes it impossible for optical filters to isolate light 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 CS's ability to measure a fluorophore’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 fluorophores 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 fluorophore, 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 fluorophores/dyes is called spectral unmixing and results in an unmixing matrix that is applied to the data. While not mathematically identical to conventional compensation, the overall principal is the same. Just as for compensation, single-stained controls, identified in SpectroFlo CS software as reference controls, are still necessary, as they provide the full fluorescence spectra information needed to perform spectral unmixing.

One advantage of unmixing is the ability to extract the autofluorescence of a sample and treat it as a separate parameter. This is especially useful when running assays with particles that have high autofluorescence and for which that high background has an impact in the resolution of the fluorescent signals. Per experiment, you can define one unstained control or multiple unstained controls (one per group), depending on whether the multicolor samples have the same or different autofluorescence signatures.

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

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



Unmixing Workflows

Unmixing Overview

There are three unmixing workflows available in SpectroFlo CS software—two in the Acquisition module and one in the Extra Tools module:

- live unmixing during acquisition
- post-acquisition unmixing (in the Acquisition module)
- post-acquisition unmixing (in the Extra Tools module)

When data is acquired with live unmixing, references are acquired as raw data either in the experiment as part of the reference group as reference controls. References for *all* fluorescent tags 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 unmixed data during acquisition.

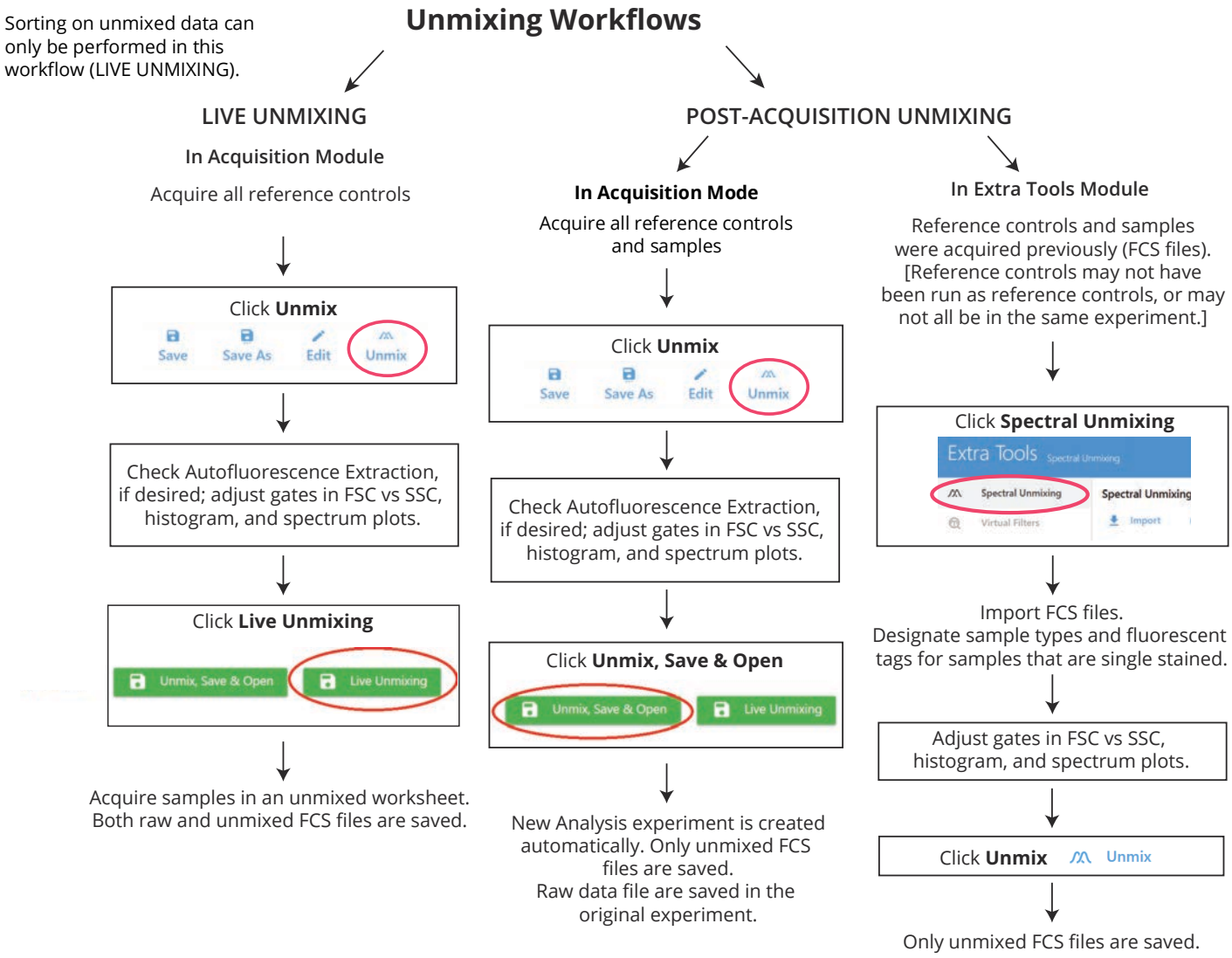
■ NOTE: In order to sort on unmixed data, Live Unmixing must be used.

Reference Controls for Unmixing

Depending on when you unmix the data, you will use the following controls for unmixing.

Unmixing	Reference Controls
Live unmixing	Reference controls run in the experiment
Post-acquisition unmixing in Acquisition module	Reference controls run in the experiment
Post-acquisition unmixing in Extra Tools module	Any FCS files from samples run in any experiment

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



Negative/Unstained Controls

In addition to positive reference controls needed for spectral unmixing, an unstained control is also necessary to assess **autofluorescence**. The unstained control needs to be of the same type and prepared in the same way as the samples, as this will ensure accurate unmixing and autofluorescence extraction, if desired. Ideally, your reference controls, unstained control, and samples will all be the same sample type and prepared in the same way.

In addition to assessing autofluorescence, **fluorescence spillover** must also be determined. To correct for spillover, the unstained autofluorescence control can be used if it matches the sample and reference control type. However, if your reference controls do not match your sample type and do not contain a negative population in each tube (have only positive peaks), you must use a separate spillover unstained control that matches your reference control type.

Sample	**Controls**
Cells	reference controls (can be beads or cells)
	autofluorescence unstained control (must be cells with same characteristics as samples)
	spillover unstained control (can be beads or cells, must match reference controls)

Live Unmixing

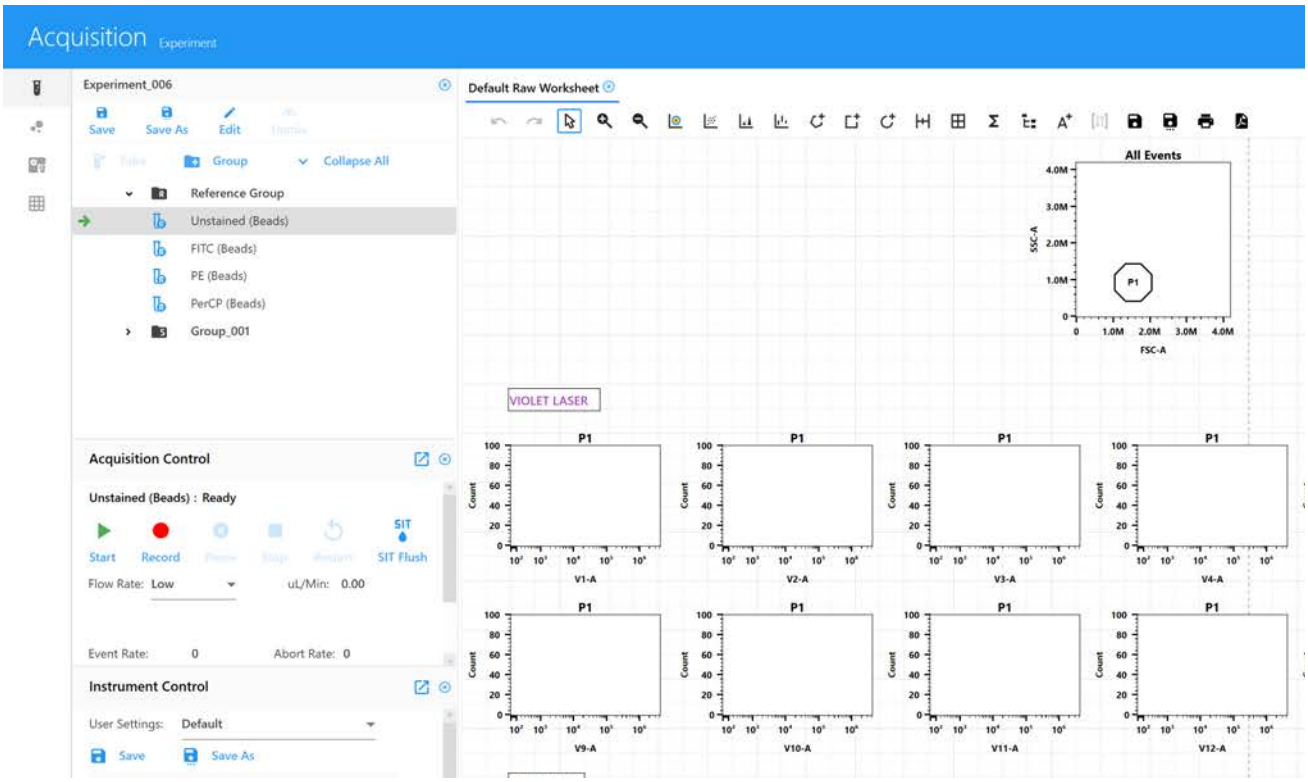
Samples can be unmixed during acquisition and sorting. Live unmixing can be performed with the reference group acquired live during the experiment, or by reusing a previously acquired reference control. Sorting on unmixed data can only be performed using the Live Unmixing function.

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 module. Unmixed worksheets are different from raw 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. Create a reference group in the experiment with the fluorescent tags. See "Creating a New Experiment" on page 60 for details.



2 To view the data for the reference control tubes, make sure CytekAssaySetting is selected, then click **Start**. If necessary, use the Instrument Controls to adjust the settings so that all events are on scale. View all the controls, as well as the multi-color tube, and make any instrument adjustments to ensure populations are on scale before you begin recording.

   To edit the acquisition criteria, click **Edit** at the experiment level and select the Acquisition tab. Or, to edit the properties of a single tube, right-click a tube and select Tube Properties.

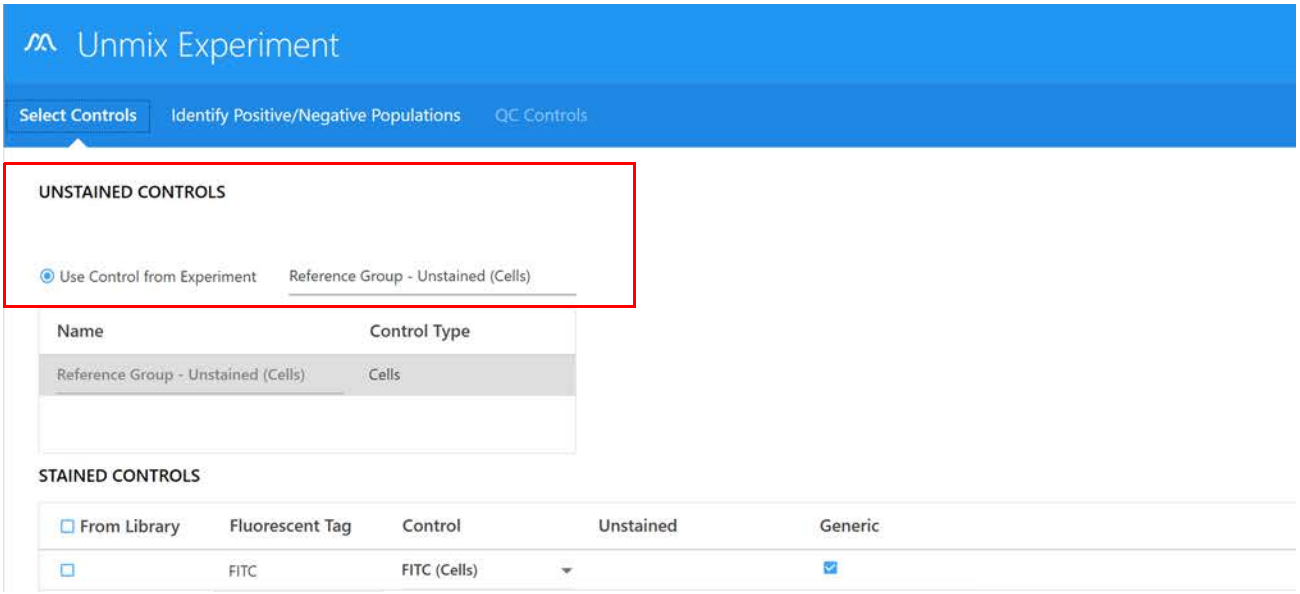
   ■ **NOTE:** Keep in mind the more events you acquire, the longer it takes to unmix the data.

3 Click **Record** when you are ready to begin acquisition. Acquisition stops when the first stopping criterion is met.

   ■ **NOTE:** If necessary, you can pause to change the flow rate.

4 When all reference controls are acquired, click **Unmix** in the upper-left toolbar.

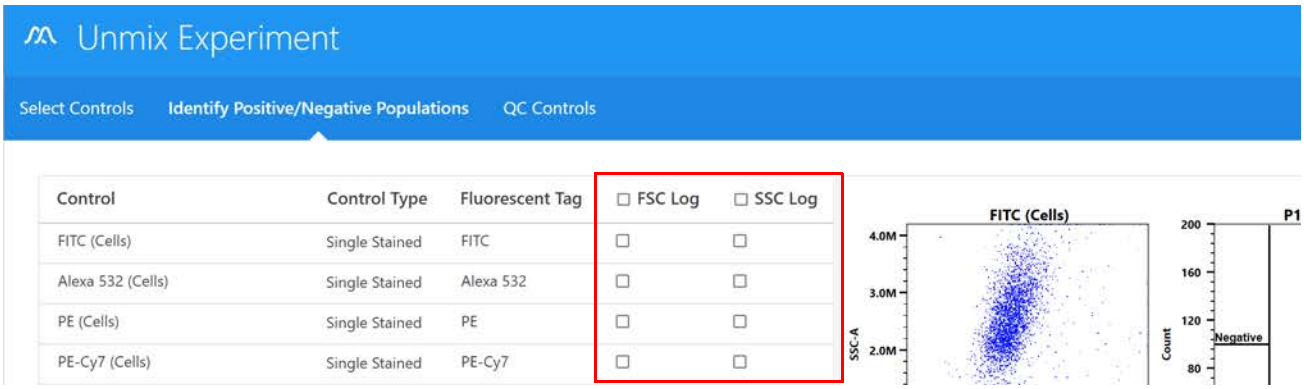
5 For the unstained controls, we recommend selecting **Use Control from Experiment** if unmixing with controls you acquired in the experiment.



6 Click **Next**.

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

■ **NOTE:** If you need to set the FSC and/or SSC axis to a log scale, select the Log checkbox.



You may find it more efficient to view the data in columns—adjust the gates in the FSC vs SSC plots first, then adjust the histogram gates.

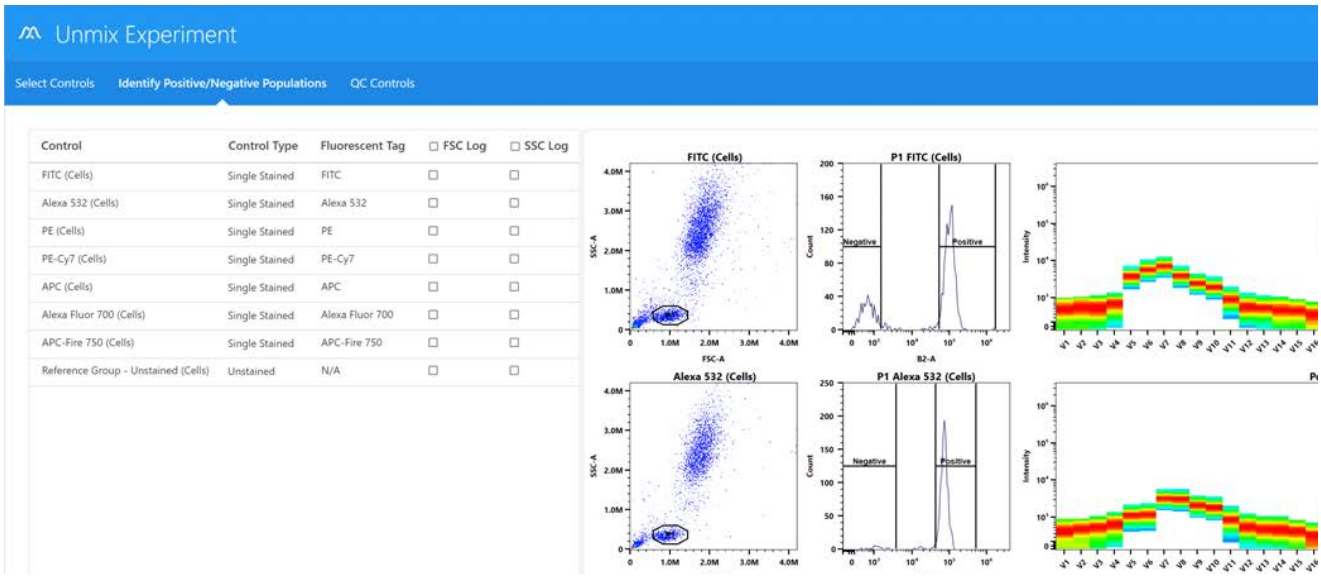
a. Move the polygon gate in the FSC vs SSC plot on the left to include the singlet population. Hold down Ctrl to move all the polygon gates at once.

b. Move the positive interval gate in the histogram to include the positively stained population. Move the negative interval gate to include the negative population.

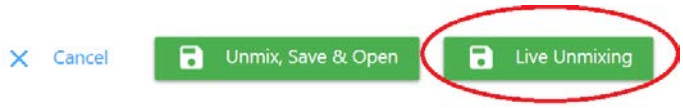
c. Move the interval gate on the spectrum plot on the right to select the channel that exhibits the brightest fluorescence intensity. This channel is the peak emission channel for the fluorescent tag.

d. Compare the fluorochrome signatures to the fluorochorome selection guide to ensure correct reference controls are used.

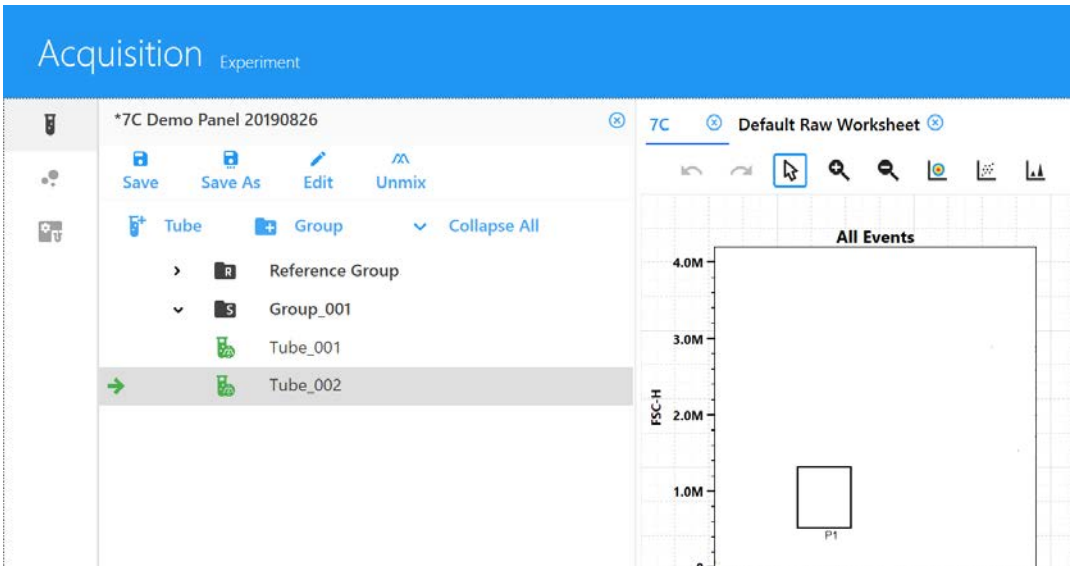
■ **NOTE:** If one of the controls is questionable or does not contain sufficient data, you can reacquire it or append to it, then unmix again.



8 Click Live Unmixing.

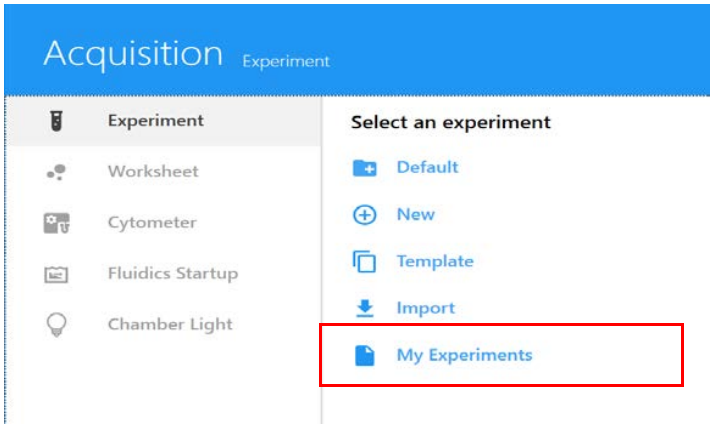


9 The wizard closes and the experiment reappears. The reference group now has the unmixed icon to the left of the tube(s). Select an unmixed worksheet to view the unmixed data.



10 Select the sample tube you wish to acquire. The green arrow indicates the tube is selected. Click **Start**, then **Record**.

Use My Experiments to open experiments you ran if you wish to review the data or acquire more samples.



FCS files are stored in the CytekBioExport folder by default, or the folder you set as the default. See "Storage Preferences" on page 140 for information. FCS files for live unmixed data are saved as both raw data and unmixed data.

Alternately, you can click My Experiments, select the experiments you want to export, right-click and select **Export**. This will export the entire experiment as a ZIP file with all of the FCS files and worksheet templates contained inside. This experiment can be imported into other instances of SpectroFlo CS software, or unzipped to access the FCS files for analysis using other analysis software.

Post-Acquisition Unmixing

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

- post-acquisition unmixing in the Acquisition module (see below)

- post-acquisition unmixing in the Extra Tools module (see page 76)

Post-Acquisition Unmixing in the Acquisition Module

The unmixing wizard in the Acquisition module limits reference controls to those coming from the reference group in the experiment.

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

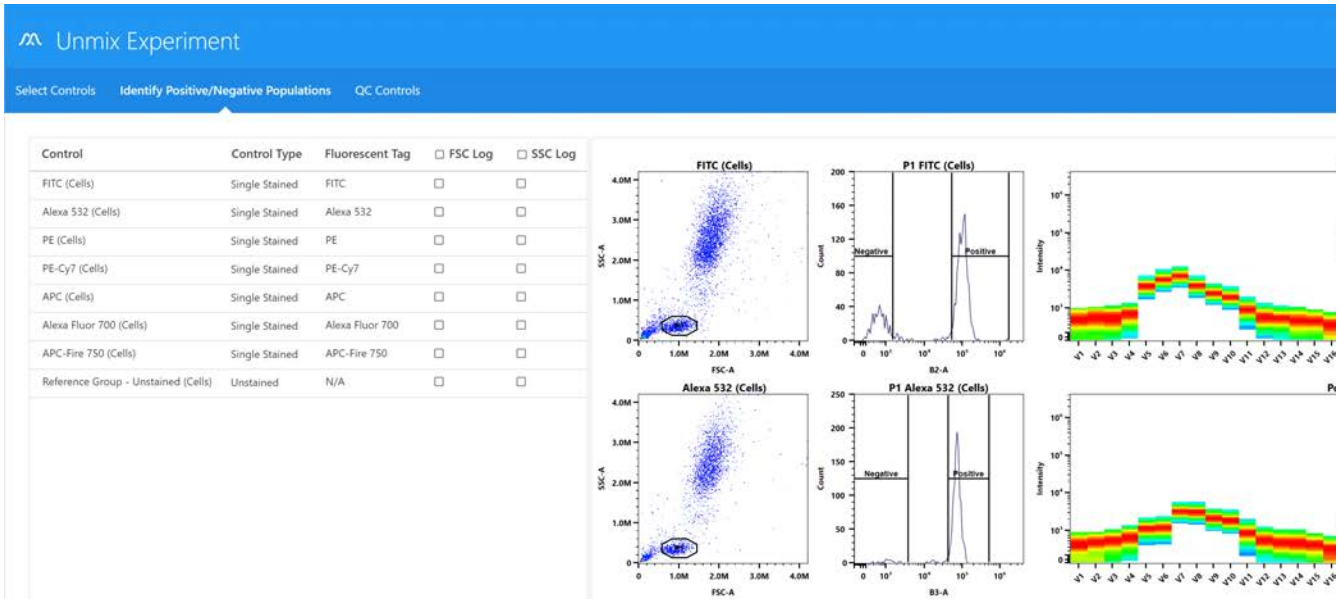
1 Acquire all reference control tubes and sample tubes prior to selecting the **Unmix** button in the upper-left pane.

2 You may find it more efficient to view the data in columns—adjust the gates in the FSC vs SSC plots first, then adjust the histogram gates. Finally, examine the spectra plots.

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

   b. Move the positive interval gate in the histogram to include the positively stained population. Move the negative interval gate to include the negative population.

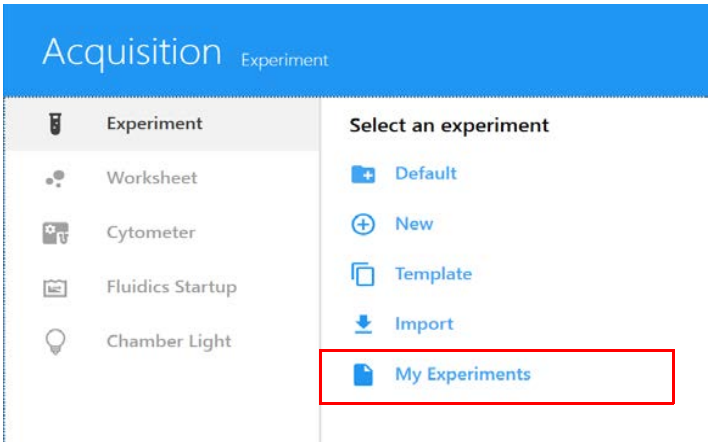
   c. Move the interval gate on the spectrum plot on the right to select the channel that exhibits the brightest fluorescence intensity. This channel is the peak emission channel for the fluorescent tag.



3 Click **Unmix, Save & Open**.

A new experiment opens with a new unmixed worksheet. Note: If sorting, use the workflow "Live Unmixing" on page 70.

Use My Experiments to open experiments you ran, if you wish to review the data or acquire more samples.



FCS files are stored in the CytekBioExport folder by default, or the folder you set as the default. See "Storage Preferences" on page 140 for information. FCS files for post-acquisition unmixed data are saved as unmixed data only.

Post Acquisition Unmixing in the Extra Tools Module

When performing post-acquisition unmixing in the Extra Tools module, you can pick and choose which FCS files to unmix (for example, controls coming from different experiments, or single-stained controls that were not run as part of the reference group).

FCS files can be designated into three categories:

- Single Stained
- Unstained
- Sample

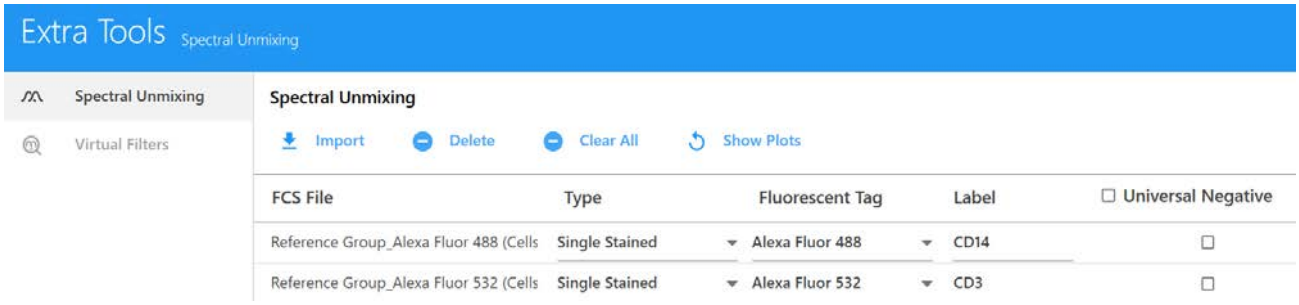
■ **NOTE:** There must be at least one single-stained FCS file and one unstained FCS file in the file list. Otherwise, unmixing cannot be performed.

In addition, raw FCS files can also be conventionally compensated in this module through the Virtual Filters tab. This function can simulate the presence of filters and can compensate data using conventional compensation methods (see "Virtual Filters" on page 79).

To unmix raw data files

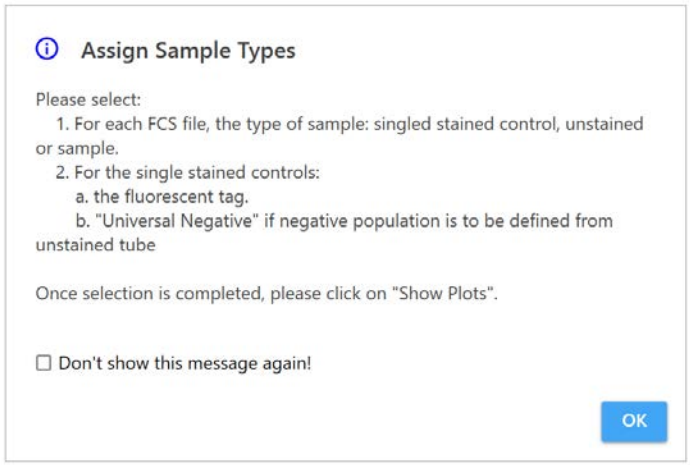
1 Select Spectral Unmixing from the Extra Tools module.

2 Click Import to import raw FCS files for unmixing.

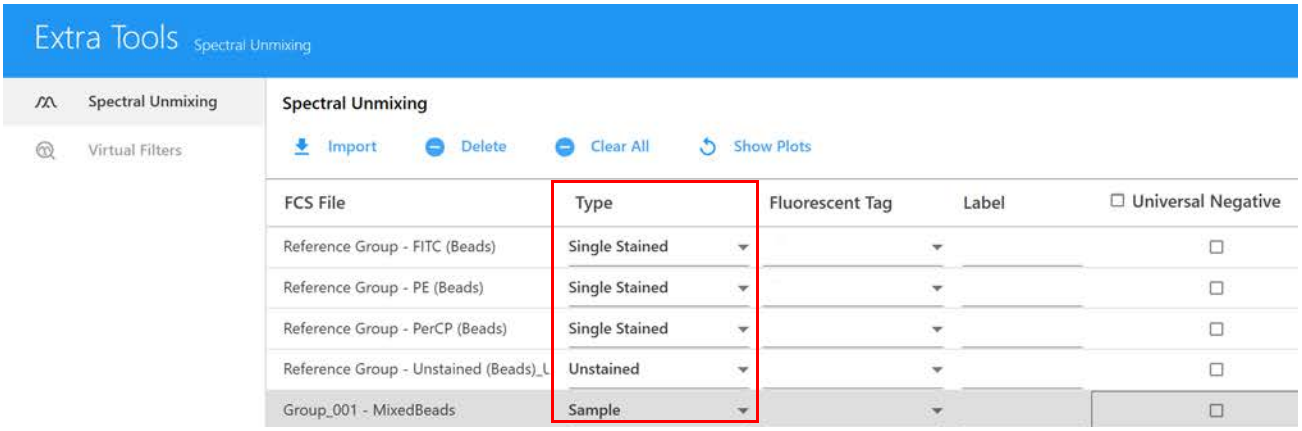


3 Select the files. Select multiple files using either the Shift or Ctrl key. Click **Open**.

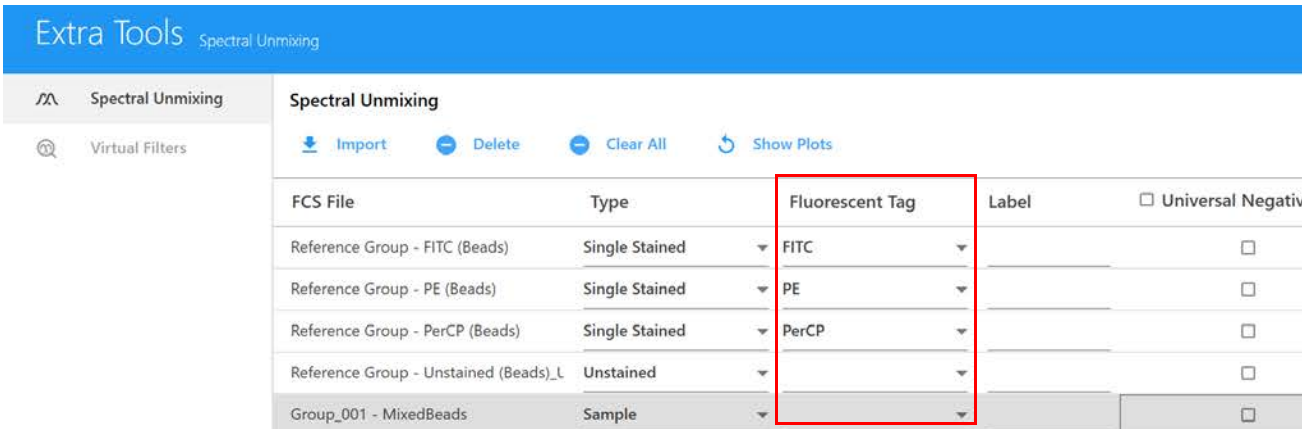
4 Upon importing, a dialog box on how to assign sample types appears. Read the instructions and click OK.



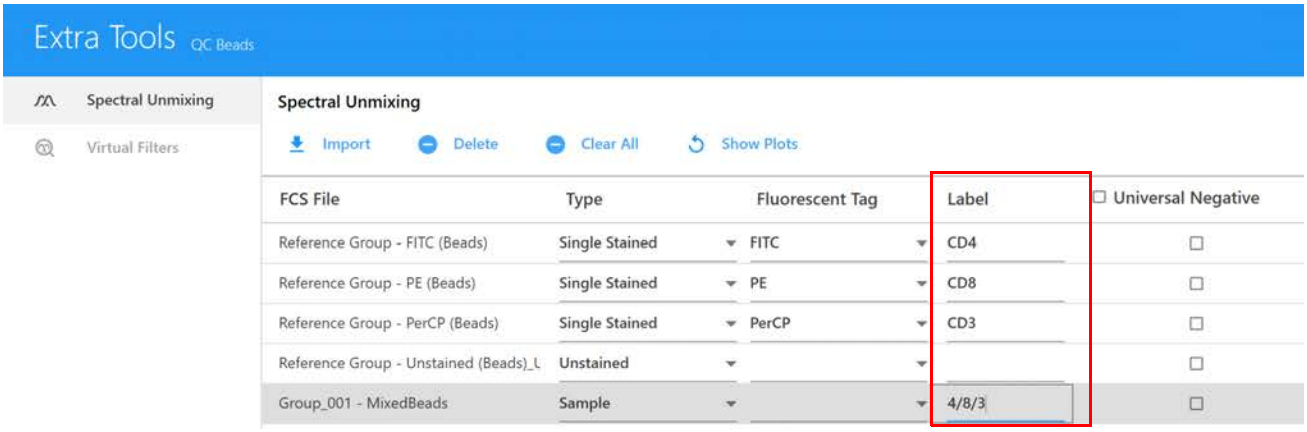
5 Once FCS files have been imported, select the sample type for each FCS file as Single Stained, Unstained, or Sample. The software will automatically designate the type based upon the file name. You can manually modify these if the automatic designation is incorrect.



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



7 Enter a label for each single-stained control and sample.



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

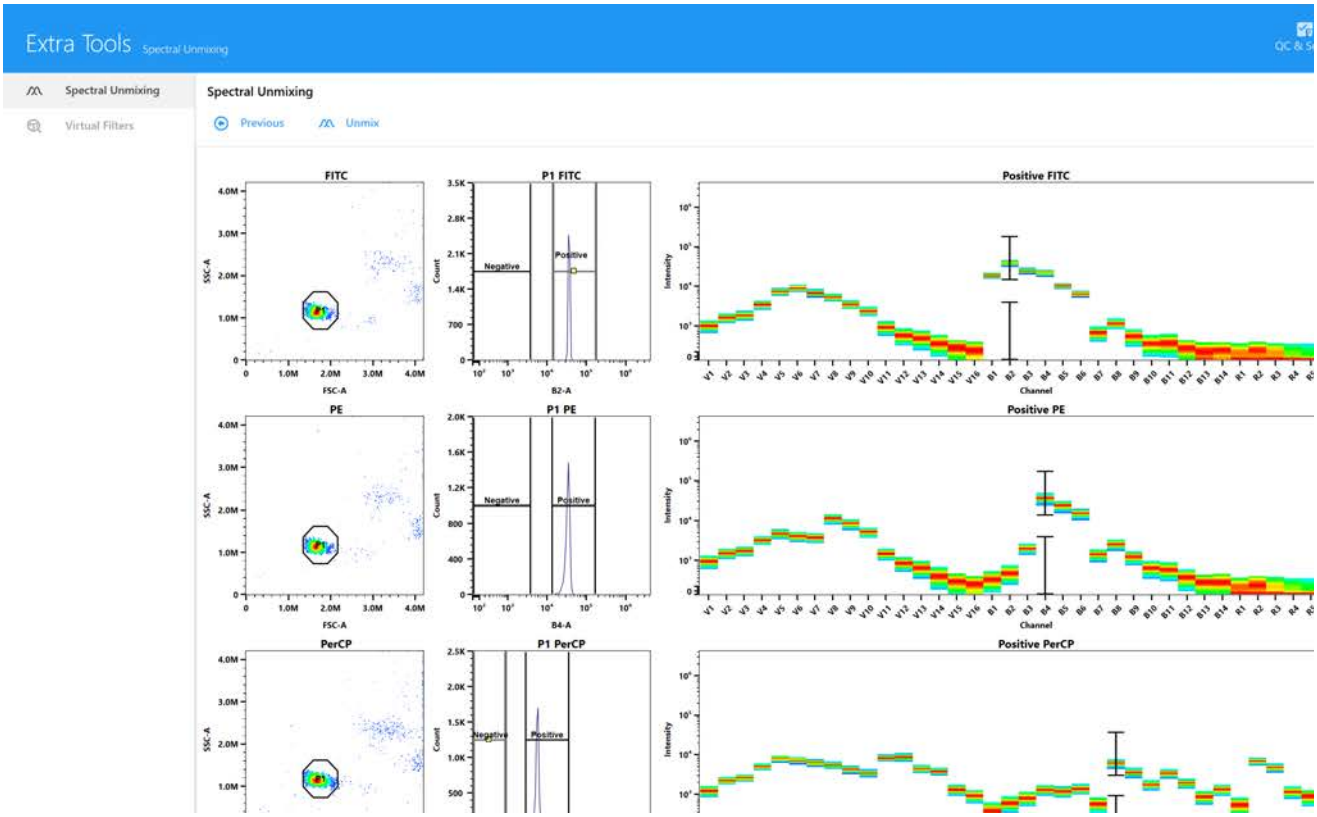
9 Click **Show Plots** to display the data in the FSC vs SSC plot, peak emission channel histogram, and spectrum plots.

10 The positive and negative populations need to be identified through the appropriate placement of the existing gates. Click **OK** to adjust the gates.

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

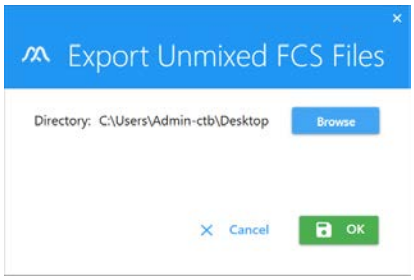
b. Move the interval gate in the histogram labeled *Positive* to include the positively stained population. Move the interval gate in the histogram labeled *Negative* to include the negative population. Do not adjust the negative gate when using the Universal Negative.

c. 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.



11 Click **Unmix**.

12 Select the directory to which the unmixed FCS files are exported or leave the default. Click **OK**.

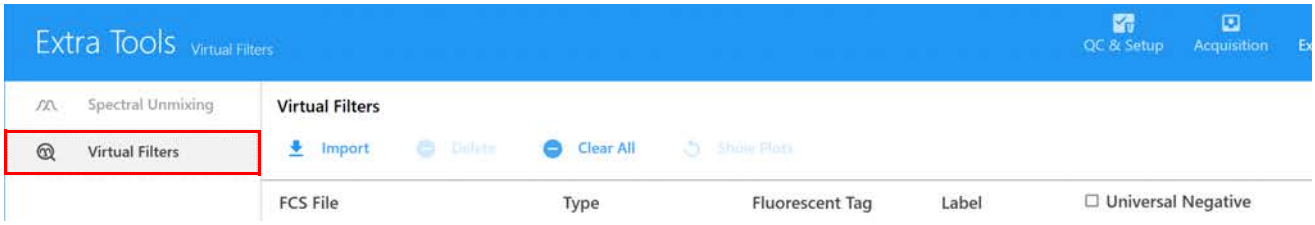


These FCS files can then be imported to an experiment for analysis or analyzed using third-party software.

Virtual Filters

The Virtual Filters option in the Extra Tools module allows you to compensate raw FCS data using conventional compensation methods.

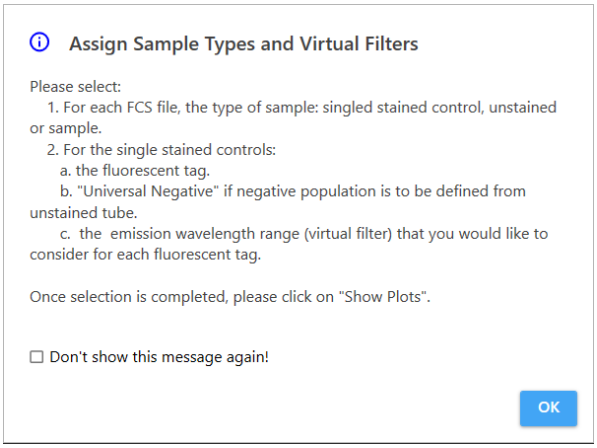
1 Click the **Virtual Filters** tab in the Extra Tools module.



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

These FCS files can be single-stained reference controls, unstained controls, and/or sample files. However, you must include an unstained control FCS file.

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. You can manually modify these if the automatic designation is incorrect.

5 FCS files designated as single stained require a fluorescent tag designation. Select the fluorescent tag for each single-stained sample.

If there is no negative population in the single-stained FCS file(s), select **Universal Negative**, and the unstained control will be used for the negative population.

The virtual filter is automatically assigned by the software based upon the fluorescent tag designation. (Optional) To increase the bandwidth of the virtual filter, use the channel pull-down menus to select the desired range. See the following table for wavelength ranges.

The following table shows the system's filter bandwidths.

Table 1: Detector Bandwidths Specifications

Laser	Channel	Center Wavelength (nm)	Bandwidth (nm)	Wavelength Start (nm)	Wavelength End (nm)
Ultraviolet	UV1	373	15	365	380
	UV2	388	15	380	395
	UV3	428	15	420	435
	UV4	443	15	436	451
	UV5	458	15	451	466
	UV6	473	15	466	481
	UV7	514	28	500	528
	UV8	542	28	528	556
	UV9	582	31	566	597
	UV10	613	31	597	628
	UV11	664	27	651	678
	UV12	692	28	678	706
	UV13	720	29	706	735
	UV14	750	30	735	765
	UV15	780	30	765	795
	UV16	812	34	795	829

Table 1: Detector Bandwidths Specifications

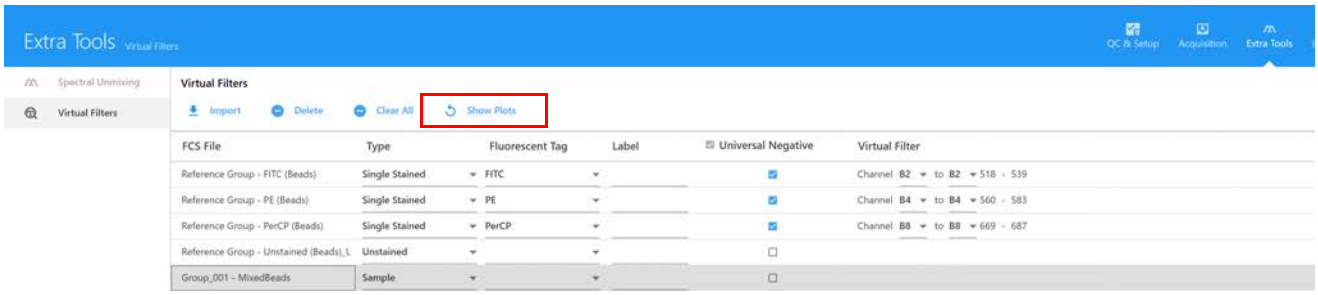
Laser	Channel	Center Wavelength (nm)	Bandwidth (nm)	Wavelength Start (nm)	Wavelength End (nm)
Violet	V1	428	15	420	435
	V2	443	15	436	451
	V3	458	15	451	466
	V4	473	15	466	481
	V5	508	20	498	518
	V6	525	17	516	533
	V7	542	17	533	550
	V8	581	19	571	590
	V9	598	20	588	608
	V10	615	20	605	625
	V11	664	27	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	525	17	516	533
	B3	542	17	533	550
	B4	581	19	571	590
	B5	598	20	588	608
	B6	615	20	605	625
	B7	661	17	653	670
	B8	679	18	670	688
	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
Yellow Green	YG1	577	20	567	587
	YG2	598	20	588	608
	YG3	615	20	605	625
	YG4	661	17	653	670
	YG5	679	18	670	688
	YG6	697	19	688	707
	YG7	720	29	706	735
	YG8	750	30	735	765
	YG9	780	30	765	795
	YG10	812	34	795	829

Table 1: Detector Bandwidths Specifications

Laser	Channel	Center Wavelength (nm)	Bandwidth (nm)	Wavelength Start (nm)	Wavelength End (nm)
Red	R1	661	17	653	670
	R2	679	18	670	688
	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

6 (Optional) Select a label for the single-stained controls and samples.

7 Click **Show Plots** to display the plots.



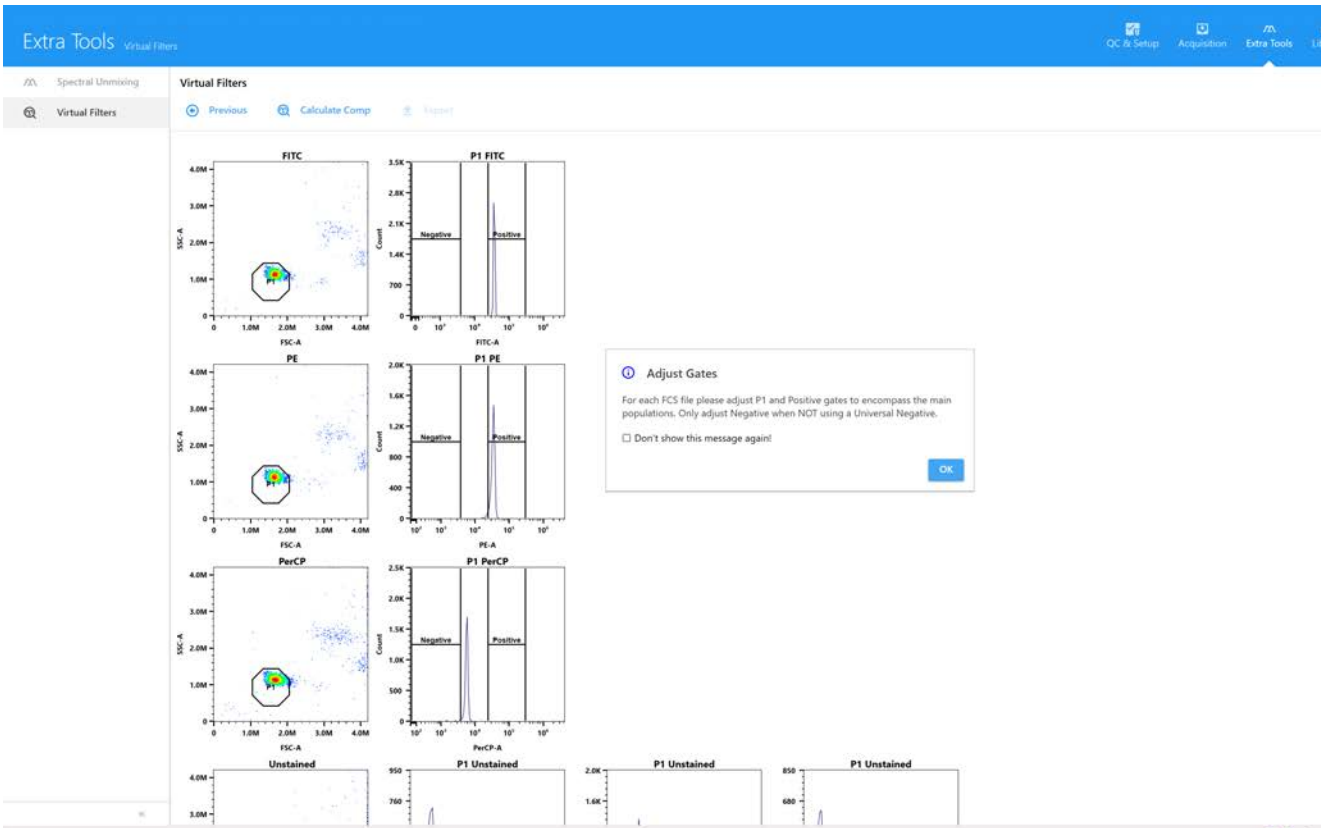
The data is displayed in the FSC vs SSC plot and fluorescent tag histogram plot.

8 The positive and negative populations need to be identified through the appropriate placement of the gates. Click **OK** to adjust the gates.

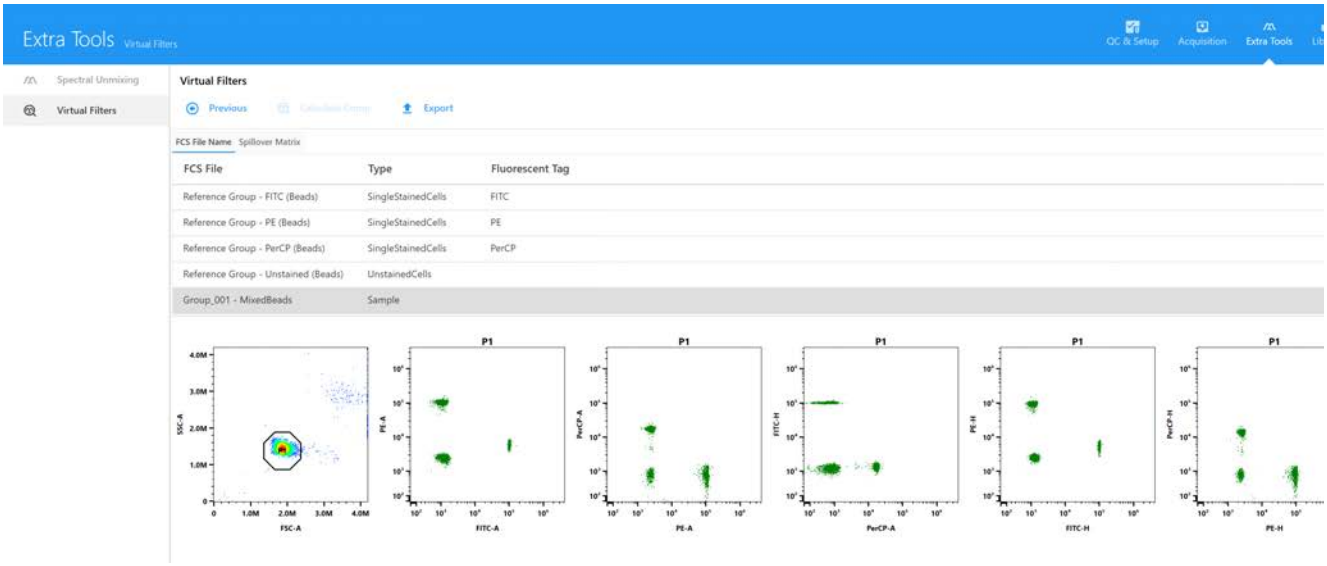
a. Move the polygon gate in the FSC vs SSC plot to include the singlet population. Hold down Ctrl to move all the polygon gates at once.

b. Move the interval gate 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.

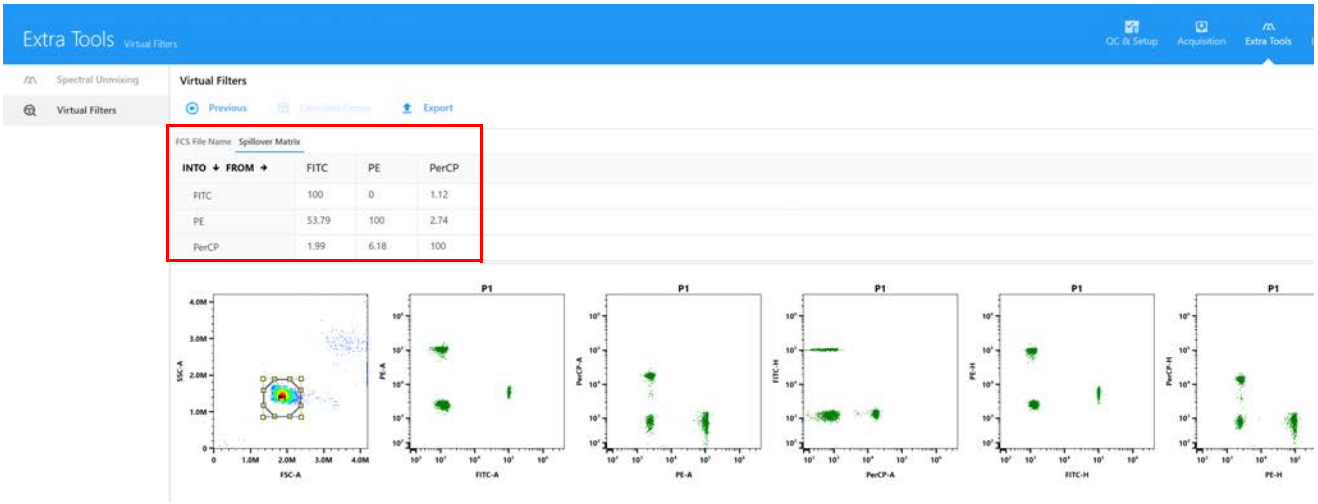
The histogram x-axes are labeled with the fluorescent tag instead of the channel/detector.



9 Click Calculate Comp once gates have been set correctly. The conventionally compensated data is displayed. To view the data for a specific FCS files, select the file.



The spillover matrix is also calculated. Click Spillover Matrix to view the spillover values.



10 Click Export and select the location where you wish to export the conventionally compensated data. The files are exported to a folder named Compensated followed by the current date and time. Files can then be imported back into an experiment to analyze in SpectroFlo CS software.

7

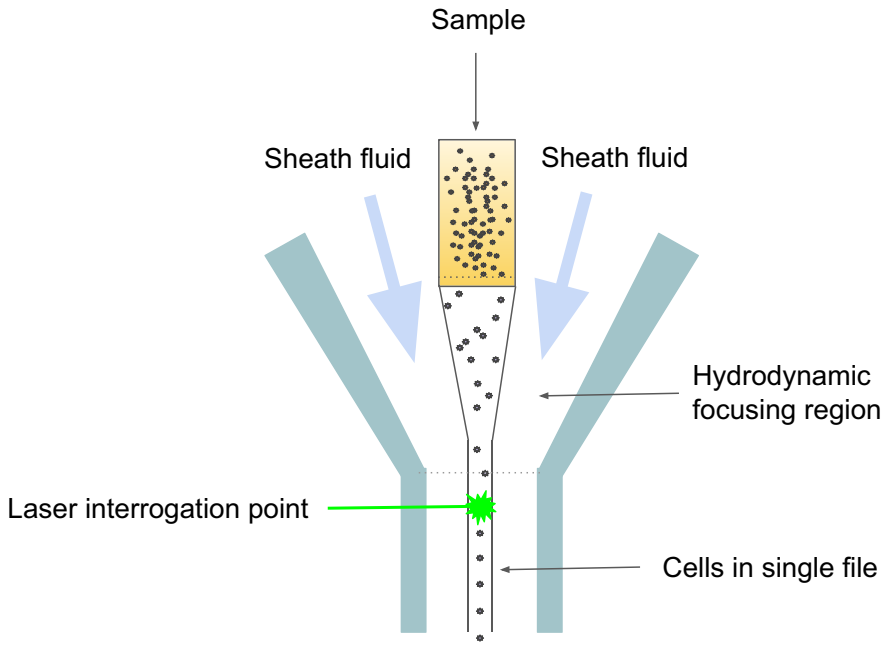
Sorting Theory

Sorting Theory Overview

In order to use the Aurora CS, it is important to have a basic understanding of the principles used in identifying and sorting target cell populations. This chapter explains the technology, the hardware, and software required to identify and to isolate particles based on their characteristics to use in further research. In droplet-based or electrostatic cell sorters, particles are interrogated by the lasers, then fluorescence is measured and used to analyze and identify which cells are to be sorted.

Sample Injection and Hydrodynamic Focusing

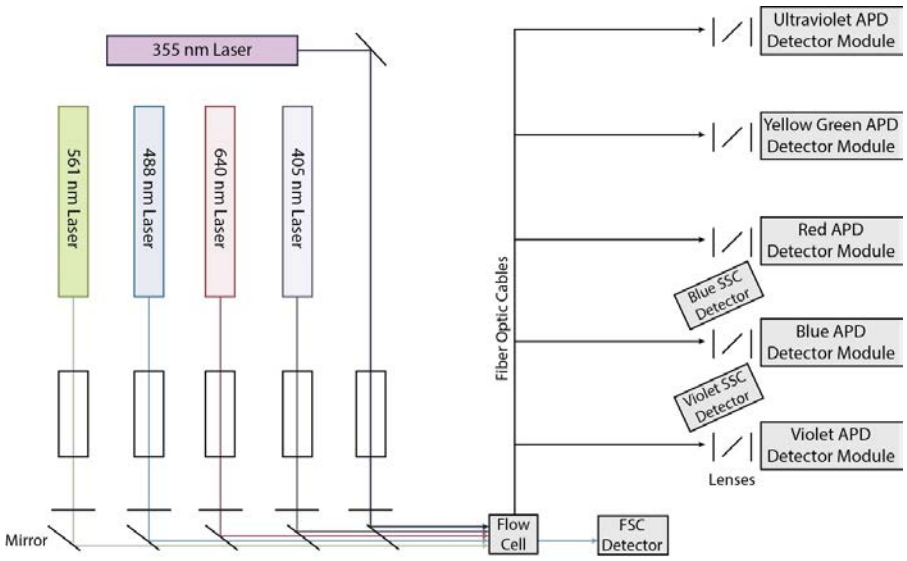
When acquisition starts, the sample chamber is pressurized, and the sample travels through the sample line and into the flow cell. Sheath fluid surrounds the sample and focuses cells into a narrow core stream before they pass single-file through the laser interrogation point within the flow cell. The core stream is smaller at lower sample pressures, which allows the cells to pass through the most focused portion of the laser. This provides consistent illumination and low CVs. Higher sample pressures provide for higher event rates and faster sorting, but can cause higher variance, so careful consideration should be used when choosing the sample pressure, which is regulated by the flow rate.



Laser Interrogation, Signal Generation, and Signal Detection

As cells pass through the laser beams, light that is physically scattered by cells and light that is emitted by dyes bound to or contained in cells is collected. Laser light that is scattered by the cells at 180° incident to the laser beam is called forward scatter (FSC), and refracted at 90° incident to the laser beam is called side scatter (SSC). Forward scatter is proportional to the size of cells, while SSC can give information about the internal complexity of cells.

The Aurora CS uses an APD detector array for each laser. APDs are highly sensitive semiconductor chips that provide more sensitivity and resolution than photomultiplier tubes (PMTs).



Electronic Pulses and Signal Processing

As light from each cell (or particle) is captured, the photons are converted to electrons by the APD, which generates a pulse. This pulse is measured for each cell individually, from each detector. The area under the pulse is calculated, and represents the total fluorescence, while the height of the pulse is the maximum fluorescence.

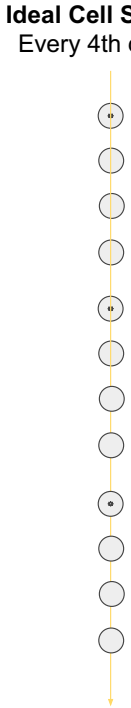
In a multi-laser system, each cell passes through each laser beam independently. The lasers are spatially separated and carefully aligned so that during the QC process, the time required for each cell to travel between the beams is determined. This time value, called the laser delay, is critical for the software to assign the correct fluorescent signal to each cell as it passes through each laser.

Droplet Generation and Charging

After cells targeted for sorting leave the flow cell and pass through the nozzle, they are ready to be directed to the collection vessels. After interrogation in the flow cell, the cells enter the nozzle. The nozzle is user-selectable, based on the size and fragility of the cells being sorted. Generally, the nozzle size should be five times the size of the cells being sorted. (Reference: Current Protocols in Cytometry, *Practical Issues in High-Speed Cell Sorting*, Larry W. Arnold[1] and Joanne Lannigan[2] [[1]The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, [2]The University of Virginia, Charlottesville, Virginia.]) The cells are accelerated through the nozzle into the stream where droplets begin to generate.

To capture cells and direct them to the vessels, the stream is broken up into droplets, which are formed by applying acoustic energy to the stream using the drop drive, which is turned on as soon as the stream is started. This acoustic energy is produced by a piezoelectric device driven by the drop drive that vibrates the stream at a known frequency and amplitude. The frequency, also known as the drop drive frequency (DDF), determines the number of droplets per second, and the amplitude is the magnitude of the force applied to the piezoelectric device that vibrates the stream. These values can be adjusted to optimize the sort by creating a stable droplet break-off and stable sort streams.

Cells enter the droplets relative to the cell concentration. Ideally, one cell would be contained in every fourth droplet to minimize the chance of a target and non-target cell being in the same drop. The higher the sample concentration, the higher the chance that a droplet will contain more than one cell, or that two droplets in proximity will both contain cells.

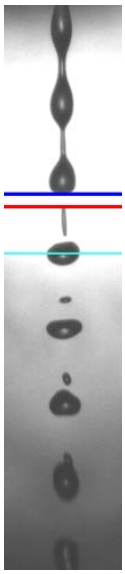


The stability of the stream is critical for optimal cell recovery and sort purity. The stream is monitored during the sort to ensure that the last connected droplet remains in a constant position. An image of the stream is captured by the break-off camera, and allows you to visualize the last connected droplet and subsequent droplets that have broken off. The image of the last connected droplet and first disconnected droplet are also used by the Sort Monitoring function to monitor the location of the last connected droplet, and if necessary, make adjustments to keep the location constant. Movement of this droplet could result in decreased purity and yield of your target population by capturing undesired populations as well as targeted populations.

The Sort Monitoring function for the Aurora CS uses the following indicator lines to maintain a constant droplet break-off location (see following figure):

- The blue line in the droplet break-off window is positioned at the bottom of the last connected drop.
- The red line is positioned at the top of the first disconnected drop.
- The space between the blue and red lines is the drop interval.
- The teal line is positioned in the center of the first disconnected drop.

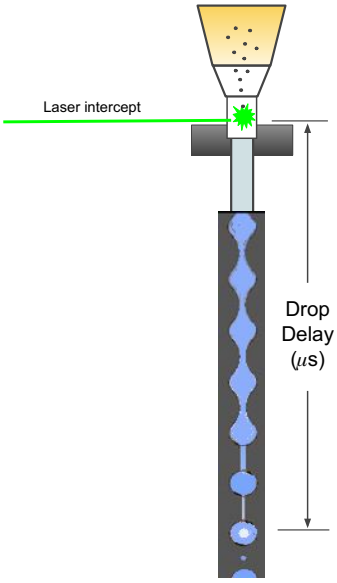
The Sort Monitoring function automatically adjusts the amplitude to ensure a constant droplet break-off location. This ensures that the stream is charged at exactly the time that the cell of interest enters the droplet, immediately before the droplet breaks from the stream.



Drop Delay

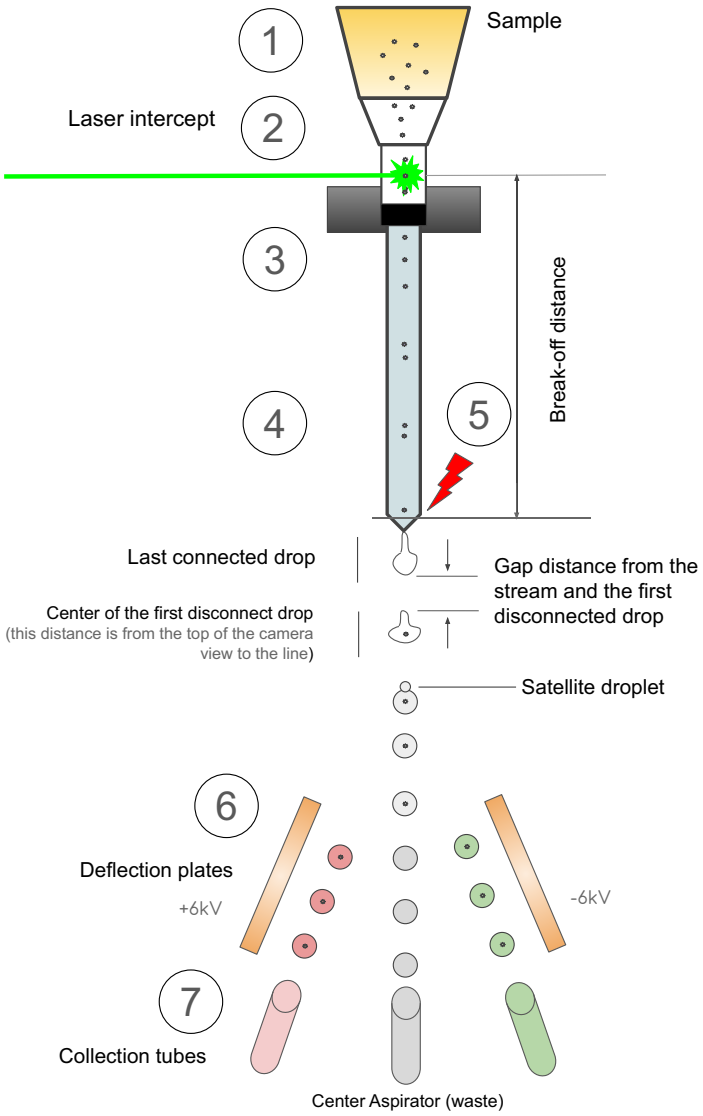
The entire stream is charged at the instant the droplet containing the desired cell is breaking off. This is accomplished with careful timing using the *drop delay*. The drop delay is the time, measured in microseconds, it takes for a particle of interest to travel from detection by the laser to the break-off point. It is calculated during the sort setup. Note: This calculated time will need to be re-calculated if there are changes to the break-ff profile, drop center or drop interval.

Just as the particle reaches the droplet break-off point, the entire stream is charged positively or negatively, depending on the cell identified to be sorted.



As the droplet breaks from the stream, it carries the negative or positive charge and travels into an electric field generated by two charged deflection plates. The droplet is deflected according to its charge and collected into the selected vessel. The amount of charge applied to each droplet allows for multiple streams to be directed to each side. The stream returns to an uncharged state until the

next droplet to be sorted arrives at the break-off point. This process repeats for every particle selected for sorting.



1	Flow cell
2	Hydrodynamic Focusing
3	Laser intercept
4	Cells in single file
5	Charge applied just before droplet break-off
6	Deflection plates deflect charged droplets into the appropriate collection tube
7	Collection of target cells. Non-targets go to Center Stream Aspirator (waste).

The drop delay is calculated using fluorescent beads. Precise calculation of the drop delay is critical to sort purity, yield, and recovery. If the delay is incorrectly calculated, the wrong droplet will be sorted, resulting in a droplet with a non-target particle or an empty droplet.

The drop delay is calculated by deflecting beads over a range of values (in tenths of microseconds) and calculating the precise time when all the beads are contained in a single stream sorted to the right. Once this value is calculated, the sort monitoring function is used to maintain the drop delay at this value.

Sorted Sample Collection

Several different collection devices are available for sorting into tubes or plates. These collection devices allow for tubes or plates to be easily inserted and removed while maintaining aseptic conditions for sorting. The sort chamber can be temperature controlled, so that the sorted sample can be kept at 4 to 37 °C during the sort.

The user can enter a defined volume of buffer or media, and the software monitors the volume in each collection vessel as it fills. Precise aiming of the streams is done using a camera.

Sort Decisions, Masks, and Modes

Sort Decisions

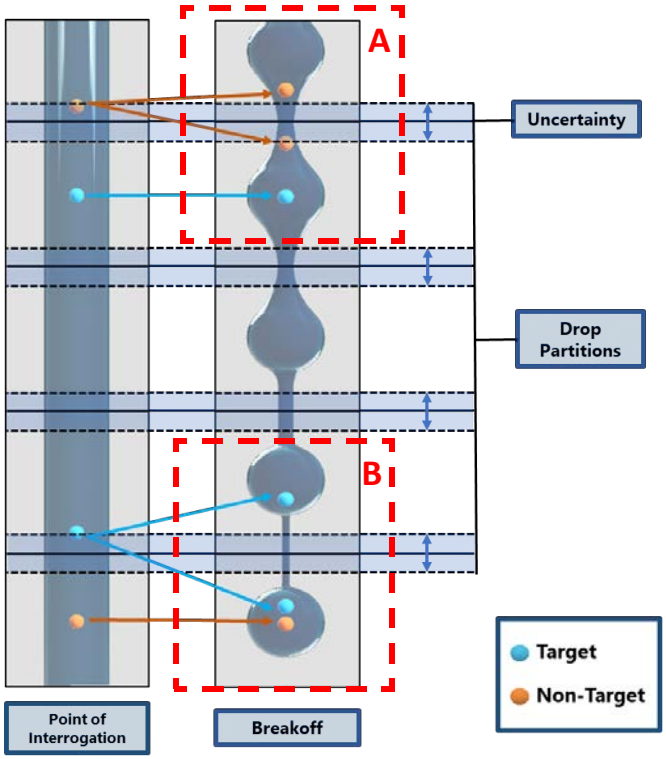
In between the interrogation point and the droplet break-off, predictions must be made on how the particles will be distributed in the droplet as they approach the break-off point.

- **Sort Decisions** dictate which droplets will be deflected based on those predictions. However, there is a level of uncertainty in these predictions due to factors including particle size and fluid dynamics.
- **Sort Modes**, such as Yield or Purity, provide a set of criteria that drive the sort decisions and are based on the desired outcome of the sorted material.
- Sort modes use a combination of **Sort Masks**, or theoretical zones, to inform the sort criteria.

In the following illustration, we can see the position of target cells (blue) and non-target cells (orange) in the stream, just past the interrogation point. As these particles travel through the stream, there is uncertainty about which droplet they will fall into once they reach the break-off point. There are two scenarios depicted in the illustration:

- In the upper portion of the stream (A) there is a target cell located in the center of a droplet with a non-target cell located near the edge causing a high level of uncertainty on whether it will fall into the same droplet as the target cell.

- In the lower portion of the stream (B), the opposite scenario is depicted.



Sort Masks in the SpectroFlo CS Software

There are three types of masks in the SpectroFlo CS Software:

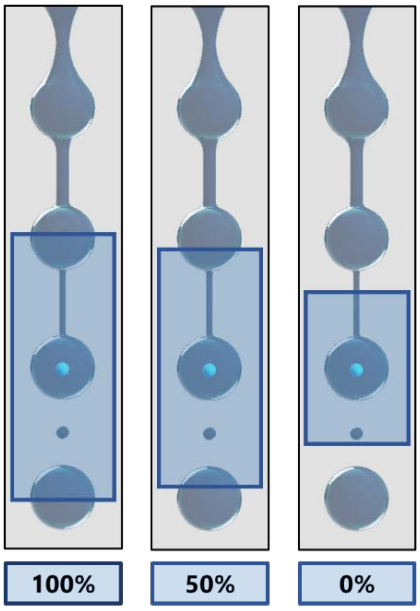
- “Purity Mask” on page 92
- “Yield Mask” on page 93
- “Phase Mask” on page 93

The purity and yield masks may be set between 0 and 100% and the phase mask is fixed at 25%. For purity and yield masks, the percentage is divided between the leading and trailing droplets. The interrogated droplet is always included in the mask.

The following illustration shows mask settings of 100, 50, and 0%. The mask setting is the sum of the portion of the leading and trailing droplet that is included in the sort decision. For example:

- A sort mask of 100% includes 50% of the leading droplet and 50% of the trailing droplet (left).
- A sort mask of 50% includes 25% of the leading droplet and 25% of the trailing droplet (middle).

- A sort mask of 0% only considers the position of a target particle in the interrogated droplet (right).

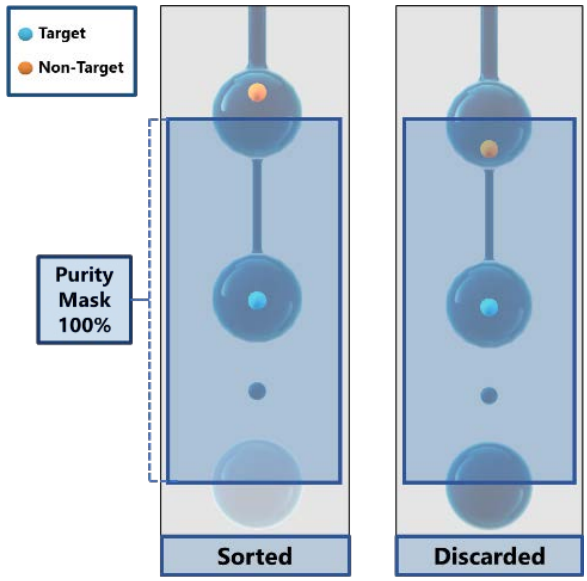


Purity Mask

The purity mask is an exclusion zone for non-target particles. If a non-target particle falls into the purity mask along with the target particle, the interrogated droplet is discarded.

In the following illustration, the purity mask is set to 100%.

- Therefore, if a non-target cell falls outside the purity mask (not located within the last 50% of the leading droplet and the first 50% of the trailing drop), the target cell will be sorted (left).
- Conversely, if a non-target cell falls within the purity mask, the target will not be sorted (right).



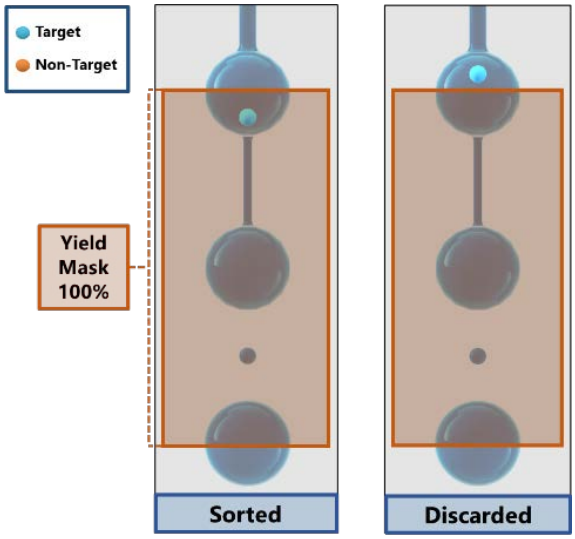
- If another target particle falls within the purity mask, then both target particles will be sorted.

Yield Mask

The yield mask increases the chances of capturing the target particle of interest, especially if the particle is located at the edge of the droplet, where there is more uncertainty.

With a yield mask of 100%:

- If the target particle falls within the last 50% of the leading droplet, the leading droplet is sorted along with the interrogated drop.
- If the target particle falls within the first 50% of a trailing droplet, the trailing droplet is sorted along with the interrogated droplet (left).
- If the target particle falls outside the yield mask, no droplets are sorted for this interrogation (right).



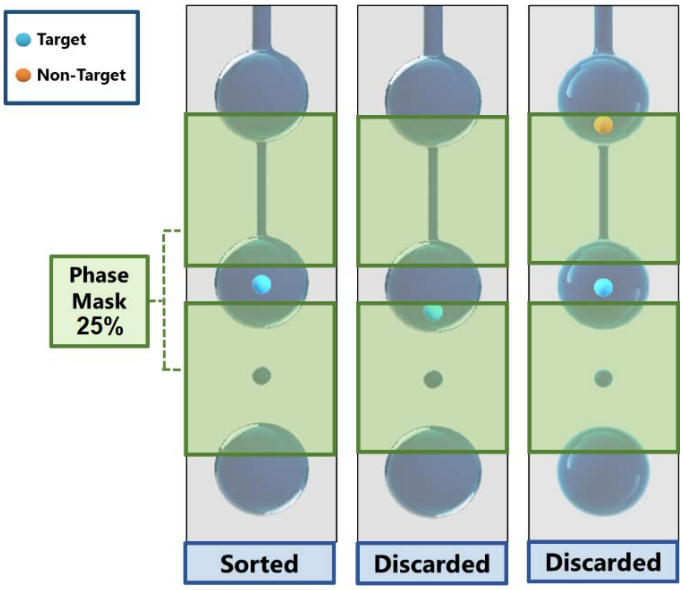
Phase Mask

The phase mask is the most restrictive mask and used in special cases (i.e., single cell sorting) to set criteria for the position of the target particle within the interrogated droplet by using exclusion zones that extend equally to the adjacent droplets.

The target particle must be located between these zones in the interrogated drop. This maximizes the chance that the target particle will remain in the droplet as it approaches the break-off point.

- When the phase mask is enabled, the value is set to 25%.

- If there is any particle (target or non-target) in the adjacent 12.5% of the leading or trailing droplet, the interrogated droplet will be discarded.



Sort Modes

Default sort modes are provided in the SpectroFlo CS software as shown in the following table. The default sort modes cannot be edited or deleted. Custom sort modes can also be created.

Mode	Purity %	Yield %	Phase	Purpose
Purity	100	25	False	High purity, lower yield.
Enrich	0	100	False	High yield, lower purity. All target particles are sorted along with non-target particles that are present in the sorted droplets.
Mixed	25	50	False	Increases yield for rare events.
Multi-Way	100	0	False	Minimizes fanning with one droplet envelope. Ideal for 4 to 6-way sorting.
Single Cell	100	0	True	One droplet envelope for plate sorting. Ensures only one target per well, and ensures accurate cell count.
Gross Adjust	0	100	False	Required for drop delay calculation.
Fine Tune	0	0	False	Required for drop delay calculation.

8

Preparing for Sorting

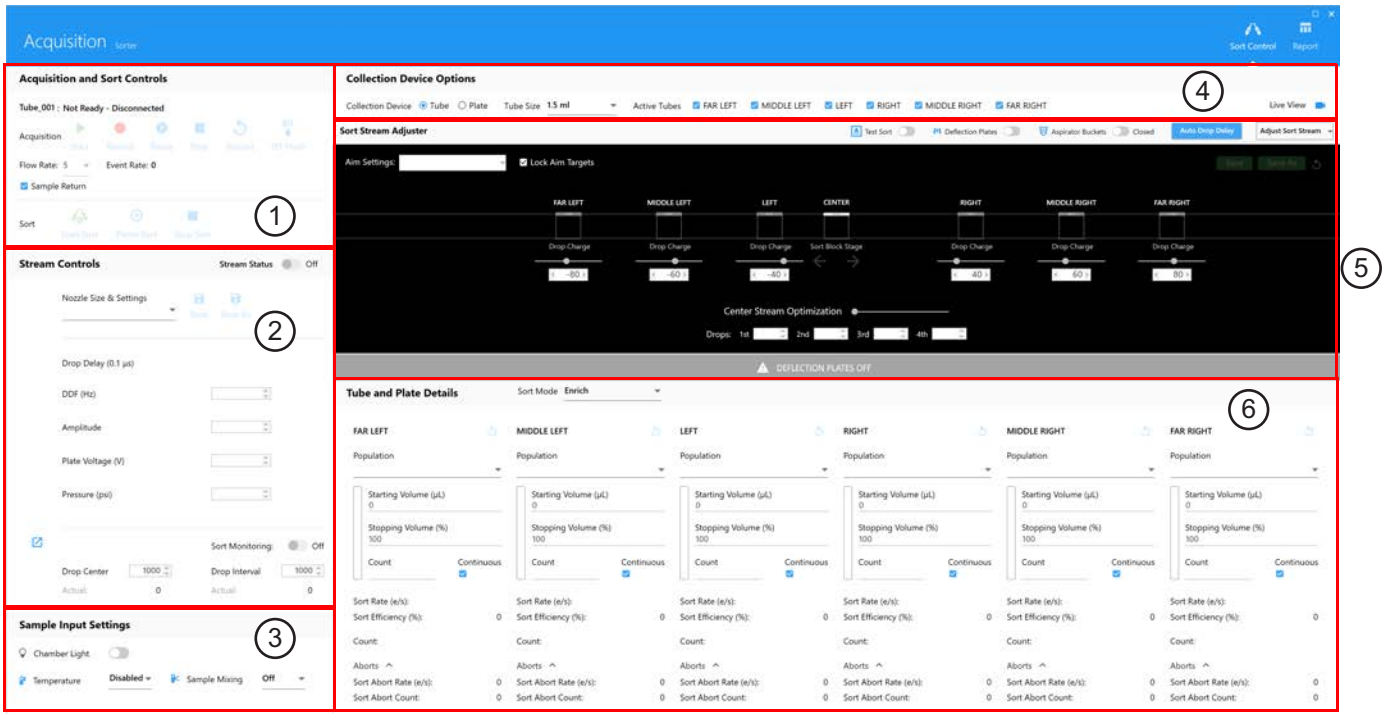
Sorter Controls Overview

The Sorter Control window provides the necessary elements for preparing for a sort and sorting samples. To access the sorting controls, open an experiment, then click Sorter Control at the bottom of the experiment control panel.

For information on preparing for a sort, proceed to "Sorting Workflow" on page 101.

The Sorter Control tab consists of Acquisition and Sort Controls, Stream Controls, Sample Input Settings, Collection Device Options, Sort Stream Adjuster or Drop Delay Adjuster (depending on what is selected to view), and Tube and Plate Details.

■ **NOTE:** The Tube and Plate Details area of the screen changes when you select Plate for the Collection Device.



The following table lists the sections of the Acquisition Sorter screen.

Section	See...
1	“Acquisition and Sort Controls” on page 96
2	“Stream Controls” on page 97
3	“Sample Input Settings” on page 99
4	“Collection Device Options” on page 99
5	“Sort Stream Adjuster” on page 99 and
6	“Tube and Plate Details” on page 100

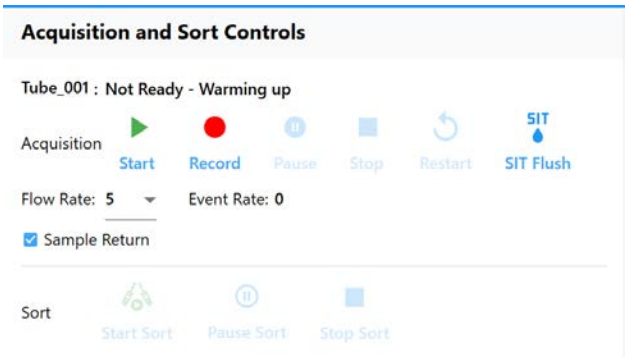
Acquisition and Sort Controls

The Acquisition and Sort Controls pane allows you to start, stop, and pause acquisition, record data, and restart acquisition counters. You can also run a SIT flush, view the event rate, and select the flow rate (from 1-10, 1 = 10 µL/min, 10 = 80 µL/min).

Use the Sample Return feature to recover sample remaining in the sample line. When selected, the sample remaining in the sample line is returned to the sample tube once acquisition is complete.

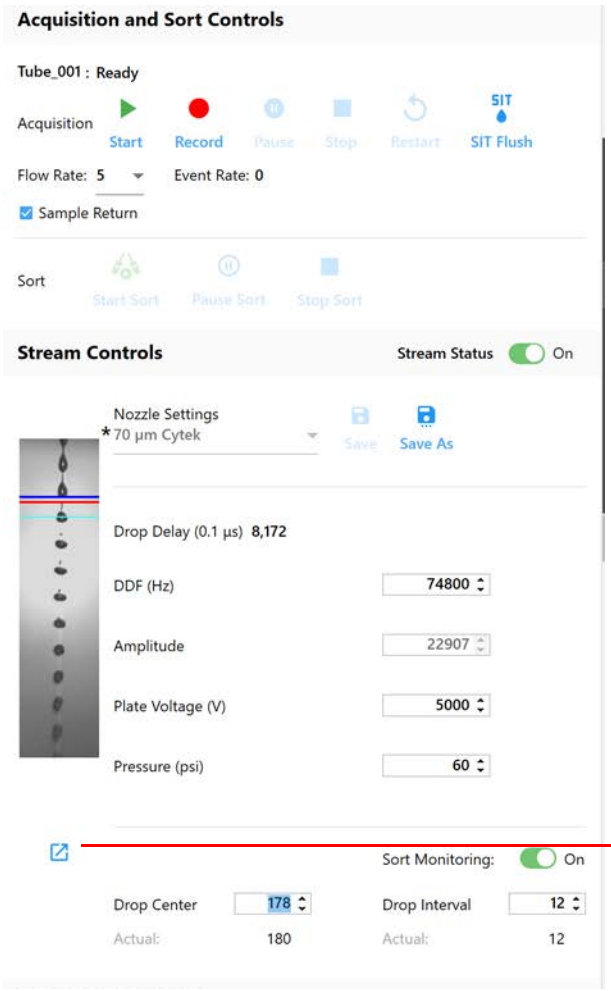
The acquisition controls are enabled when the stream is turned on. Once acquisition starts, the **Start Sort** button is enabled. You can pause/restart or stop sorting at any time. When a plate is selected for the collection device, an option to eject the plate appears.

SIT Flush	Allows you to flush the line from the sample input to help remove any residual left from sample acquisition.
Sample Return	Select this to return sample left in the SIT back into the sample tube.



Stream Controls

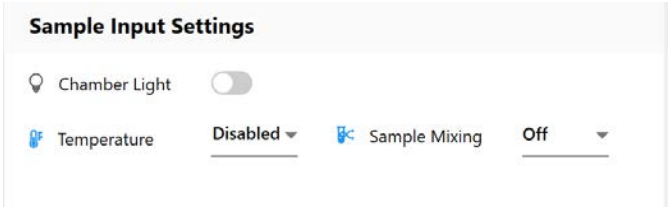
Stream Controls	Use to turn the stream on/off and select the nozzle size and settings for the experiment. The Stream Controls allow you to optimize the droplet break-off profile, and they can be saved as nozzle settings and stored in the Library for future use.
Sort Monitoring	Sort Monitoring is used to maintain a consistent drop interval once a satisfactory break-off profile is established. When Sort Monitoring is turned on, the software will adjust the amplitude so that the distance between the last connected droplet and the first disconnected droplet is carefully maintained.



click to magnify the drop-profile image

Sample Input Settings

Chamber Light	Use to turn on a light in the sample loading station.
Temperature	If temperature control is needed for the input sample and sorted samples, select the temperature. You can select a temperature from 4 to 37°C.
Sample Mixing	The sample mixing feature can be used to agitate the sample tube in a circular motion. Three mixing speeds (low, med, and high) are available.



Collection Device Options

The Collection Device Options allows you to select tubes or a plate for collection. Two tube sizes are supported for collection, 1.5 mL and 5 mL. If tubes are selected, choose the size that will be used for collecting the sorted samples, as well as the tube locations within the collection device receptacle. You can sort into 1–6 tubes (up to three on each side of the center stream). This determines the number of side streams generated. For a six-way sort, 1.5-mL collection tubes are required. See "Sort Collection Devices" on page 23 for more information.

Use Live View to see a real-time image of the side streams as they enter the collection tubes. This facilitates the adjustments you make when aiming the streams into the centers of the tubes. Live view can also be used to monitor the sort volume in the tubes during a sort.

Sort Stream Adjuster

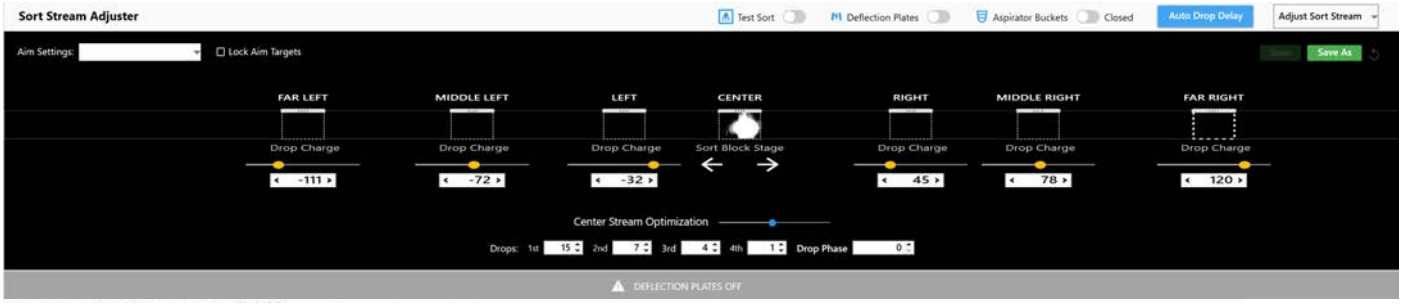
The Sort Stream Adjuster allows you to visualize and adjust the tightness and position of the center and side streams. Modification of the tightness of the center streams is done using the center stream optimization control, as well as the Drop 1st, 2nd, 3rd, and 4th controls. The Lock Aim Targets checkbox is used to lock (or fix) the boxes that mark the position of the streams. The boxes serve as reference locations for future side stream adjustments. If the checkbox is not selected, the boxes can be adjusted individually to ensure that the streams are centered within the boxes.

The Test Sort button allows you to turn on/off the side streams and deflection plates. The deflection plates automatically turn on when you turn on Test Sort. The Aspirator Buckets button allows you to open and close the aspirator buckets. The buckets open (move back to allow the side streams to enter the collection vessels) when sorting starts. They close (move forward to capture the side streams as waste) when sorting ends or a clog is detected. Opening the buckets along with the live view functionality, while the stream is on, allows you to visualize and aim the side streams into the center of the collection tubes.

This area of the sorter controls allows you to calculate the drop delay, either manually or automatically. By selecting Adjust Drop Delay from the menu in the upper-right corner, you can

manually adjust the drop delay. You can select Auto Drop Delay to open a new window allowing the system to automatically set the drop delay with the use of SpectroSort™ beads.

Once these stream settings are established for your experiment, they can be saved and stored in the Library as Aim Settings. We recommend including the nozzle size and information on the collection device(s) in the aim settings file name, for example, “4 way 70 µm.”

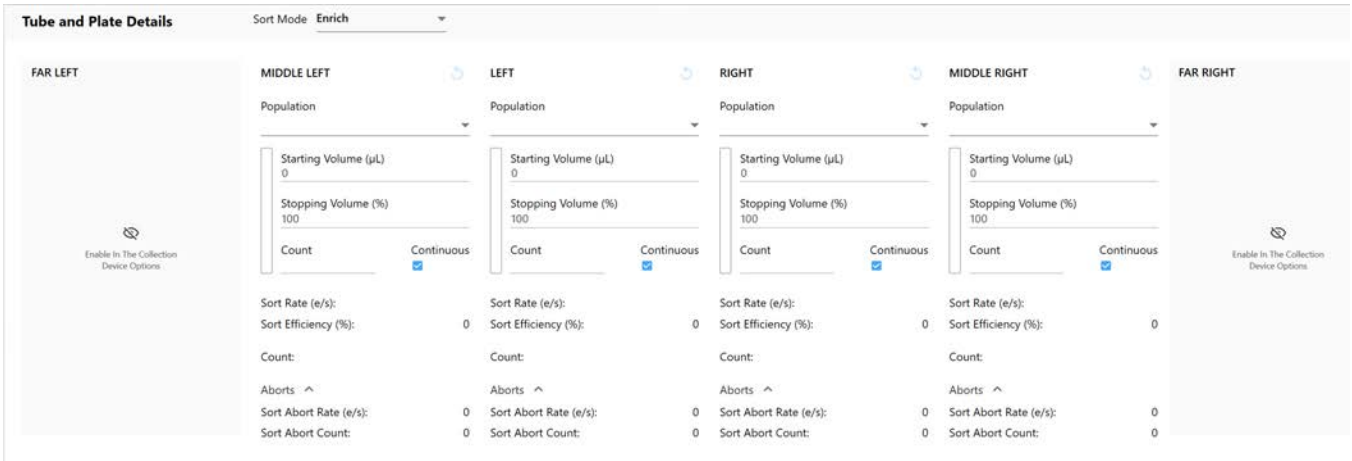


Tube and Plate Details

The Tube and Plate Details allow you to select the sort mode, as well as the populations to be sorted as defined by the gates set in the worksheet and assigned to the tube. You can set the starting volume of buffer in the collection tubes and set the sort stopping criteria—a count or the stopping volume, which takes priority. The system uses the starting volume and selected tube size to calculate the total volume in the tube.

An indicator displays the current volume in each collection tube. You can also sort continuously and the sort will stop when the collection tubes are filled to the stopping volume % you defined.

The sort rate, sort efficiency, and count for the collected samples, as well as information for the aborted sample is displayed in real time.



Sorting Workflow

Perform the following workflow steps in the order presented. Always start the day with the Fluidics Startup procedure. If you plan to sort, perform the instrument setup steps with your nozzle of choice then proceed to "Daily QC" on page 45.

Sorting Workflow Overview

Step	Task	See...
1	**Instrument Setup** Perform instrument setup to select a nozzle, adjust the stream and droplet profile, and run daily QC.	"Instrument Setup" on page 101
2	**Aseptic Cleaning** Aseptic cleaning is done when sorting particles requiring aseptic technique.	"Aseptic Cleaning" on page 115
3	**Experiment Setup** Create or open an experiment; acquire samples and set gates on the population(s) you wish to sort.	"Creating an Experiment" on page 59 and "Worksheets" on page 55
4	**Sort Setup** Set up for a sort and sort samples.	"Sorting Setup for Tubes" on page 105 and "Sorting Setup for Plates" on page 110

Instrument Setup

Before preparing for a sort, start up the instrument and run the fluidics startup procedure as usual. See "Instrument Startup" on page 33.

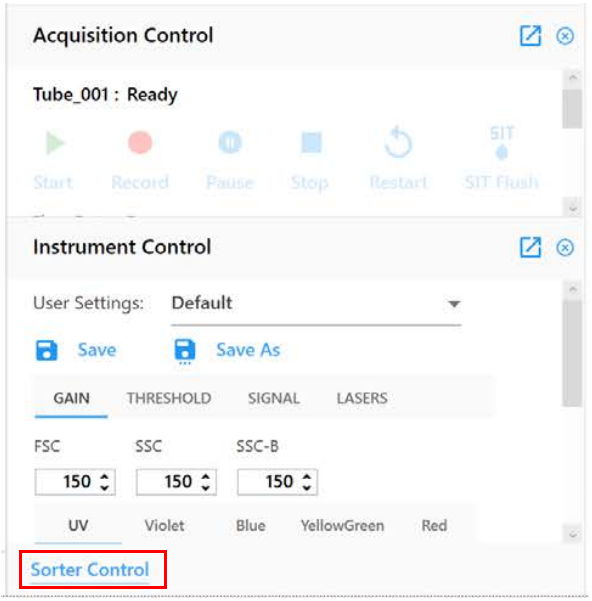
In the instrument setup workflow you will select a nozzle, optimize the stream, adjust the droplet profile, then run daily QC. The instrument can warm up while you optimize the stream and droplet profile. However, the instrument must be warmed up for at least 30 minutes before you can run daily QC.

1 Select Acquisition from the Get started menu, or click the Acquisition tab.

2 Open an experiment to get access to the sort controls.

You can open a default or existing experiment, or create a new experiment. If you create a new experiment, follow the steps in the wizard to define all the experiment parameters. See "Creating a New Experiment" on page 60.

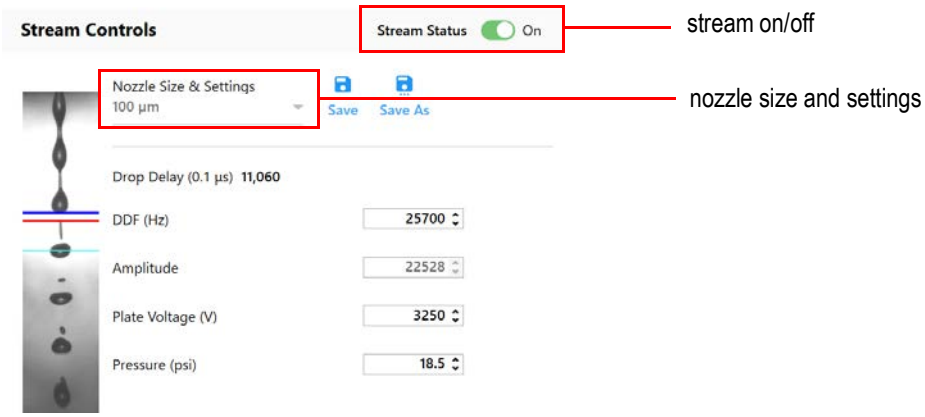
3 Click Sorter Control at the bottom of the experiment panel to display the sorter controls.



4 Select the nozzle size from the Nozzle Size & Settings menu. Install the selected nozzle. See "Installing a Nozzle" on page 18 for information.

The stream controls update with the settings associated with that nozzle.

5 Turn on the stream with the Stream Status control.



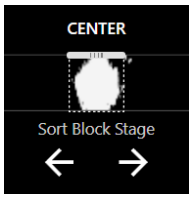
6 Optimize the stream, if necessary, so it is centered in the deflection plates and in the center stream aspirator:

a Allow the stream to settle for 10 minutes.

b If necessary, center the stream between the deflection plates.

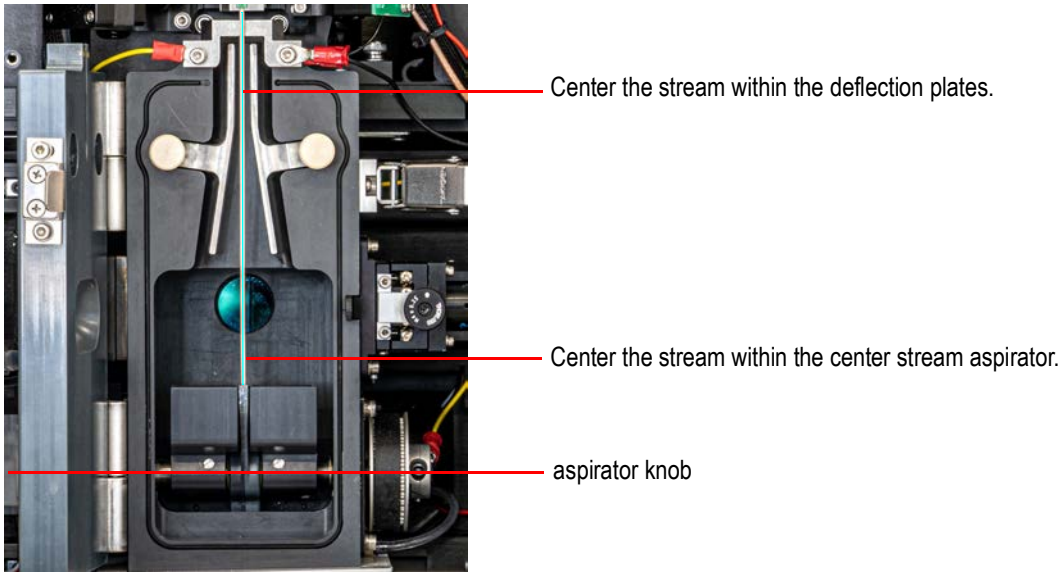
Open the sort chamber door and view the stream as it passes through the deflection plates. If the stream is not centered between the plates, use the CENTER Sort Block Stage arrows to

move the sort block right/left. For example, if the stream appears to the right you will need to move the sort block to the right so that the stream is centered.



c If necessary, center the stream in the center stream aspirator.

Check the center stream in the center stream aspirator. If it is not centered, use the aspirator knob (to the left of the sort chamber) to move the aspirator assembly left or right so that the stream is centered in the center stream aspirator.

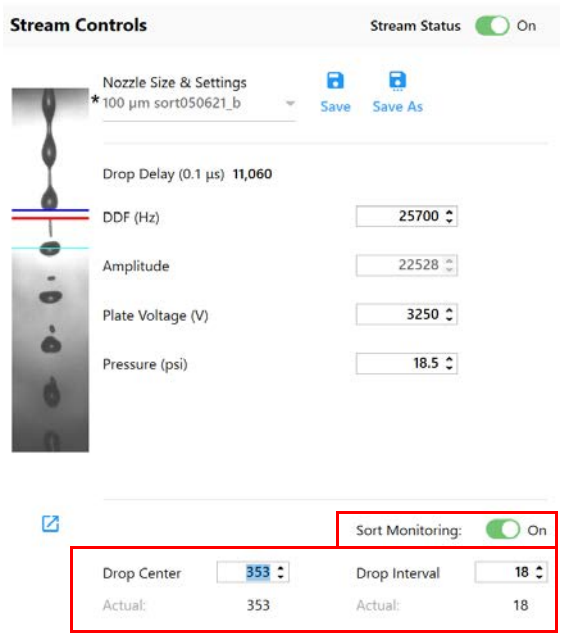


d Once the stream is centered in the deflection plates and aspirator, left-click the CENTER box to move the box left/right so that the stream image appears centered in the box.

7 If necessary, adjust the droplet break-off profile. If the profile is not satisfactory, make the following adjustments to improve the profile.

a While monitoring the droplet break-off, adjust the Amplitude and the DDF (drop-drive frequency) so that there are 2 to 3 (may be fewer depending on the nozzle size, ensure you see at least 1-2 at a minimum) connected drops at the top of the droplet profile and the satellite drops merge with the droplets within 5 drops or less.

■ **NOTE:** The blue line in the droplet break-off window is positioned at the bottom of the last connected drop. The red line is positioned at the top of the first disconnected drop. The space between the blue and red lines is the drop interval. The teal line is positioned in the center of the first disconnected drop. These lines are used by the Sort Monitoring function to maintain a consistent break-off.



b Enter the Actual values into the Drop Center and Drop Interval fields, then turn on **Sort Monitoring**. Ensure the Actual Drop Interval value is within the correct range for the selected nozzle. Note: Optimal interval results in tighter side streams and less fanning. See the default nozzle settings table on page 18.

c These nozzle settings can be saved and stored in the Library for future use. Click **Save As** to save the settings.

8 Once a stable droplet break-off profile is achieved, perform daily QC. See "Performing Daily QC" on page 45. Before running daily QC the instrument must be warmed up for at least 30 minutes as indicated by the timer at the bottom of the window.

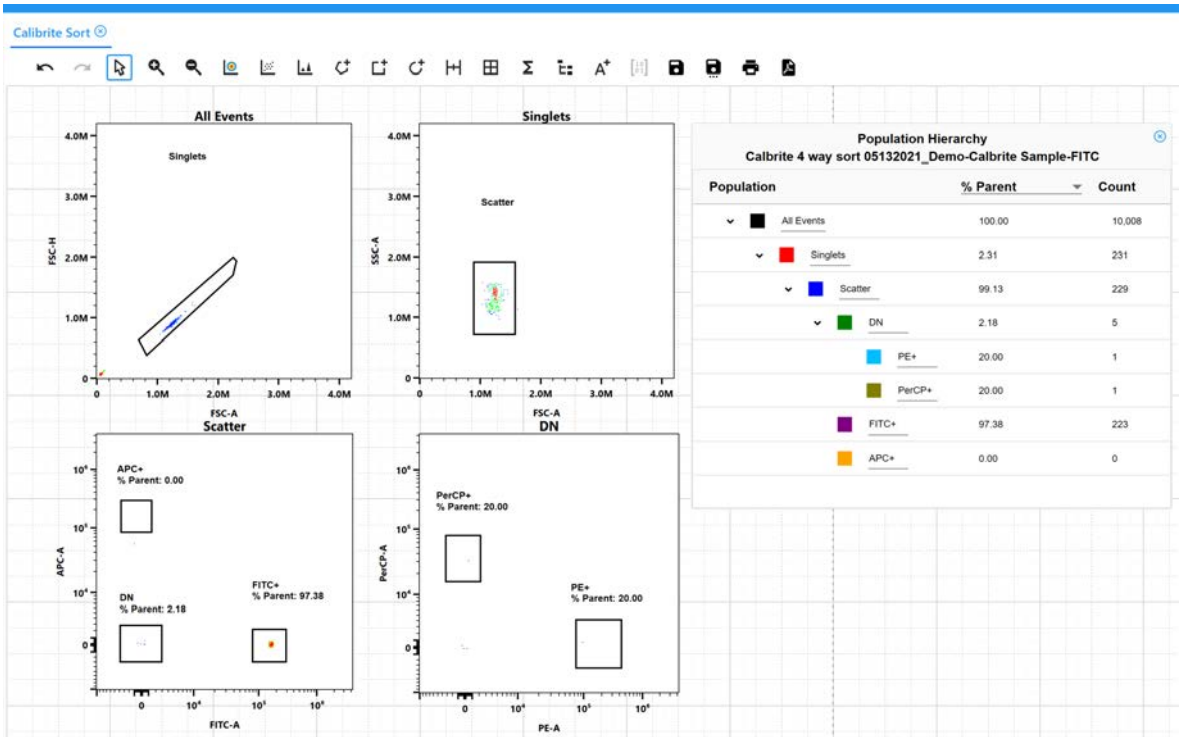
9 Proceed to "Experiment Setup" in the following section.

Experiment Setup

You can create a new experiment, open an existing experiment or an experiment template, or start with the default experiment. You can also import and open an experiment created on the Aurora or Northern Lights system using SpectroFlo software, v2.2 or lower. See "Creating an Experiment" on page 59. Set gates on the population(s) you wish to sort. See "Worksheets" on page 55.

Acquire sufficient events of a multicolor sample to set gates for sorting.

Run your experiment and set gates on the sample population to be sorted. During sort setup, you will select the gate for the samples to collect. Make sure there are no conflicts in the gating hierarchy between different populations to be sorted.



10 Proceed to "Sorting Setup for Tubes" in the following section.

Sorting Setup for Tubes

The following procedure guides you through the steps to sort into tubes.

1 Select Tube for the Collection Device, then select the collection tube size.

2 Select the locations for the Active Tubes. Place the corresponding collection tubes into the respective receptacle and install it in the DDU chamber.

3 If you have saved aim settings, you can select them (nozzle size, voltage, and stream location) for each of the related streams. For each of the tube sizes and plate sort, saved aim settings are available for reuse and can be applied. Note: There are factory optimized aim settings as guidelines to start from. There are settings for 4 way with 5ml tubes, and 6 way with 1.5ml tubes.

   The aim settings you select include the nozzle size, sample tube size, side stream target box locations, drop charge settings, and collection tube number and locations.

4 Turn on Test Sort. This turns on the deflection plates.

WARNING: The deflection plates can be on even when the sort door is open. A red LED illuminates when the plates are on.

5 Optimize the center and side streams.

The center and side streams must be optimized to achieve the “tightest” appearance possible, then adjusted to get the side streams aimed at the centers of the collection tubes.

a Use the Center Stream Optimization slider to achieve the tightest possible center stream profile in the side stream window. If necessary, the streams can be further optimized by adjusting Drops 1st, 2nd, 3rd, and 4th until the center and side streams are as tight as possible.

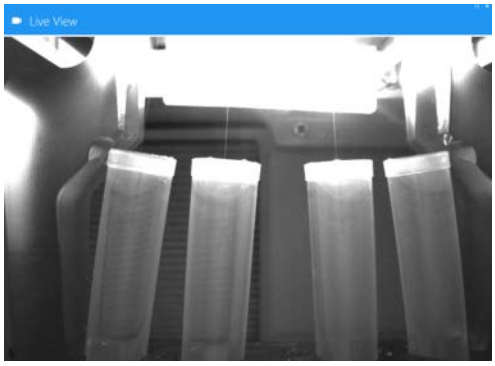
b Turn on the Live View to see the stream images in the collection devices.

c Adjust the drop charge to make sure that side streams are inside the box under the sort stream adjuster. The position of the boxes is saved and provides a place to start for the location of side streams.

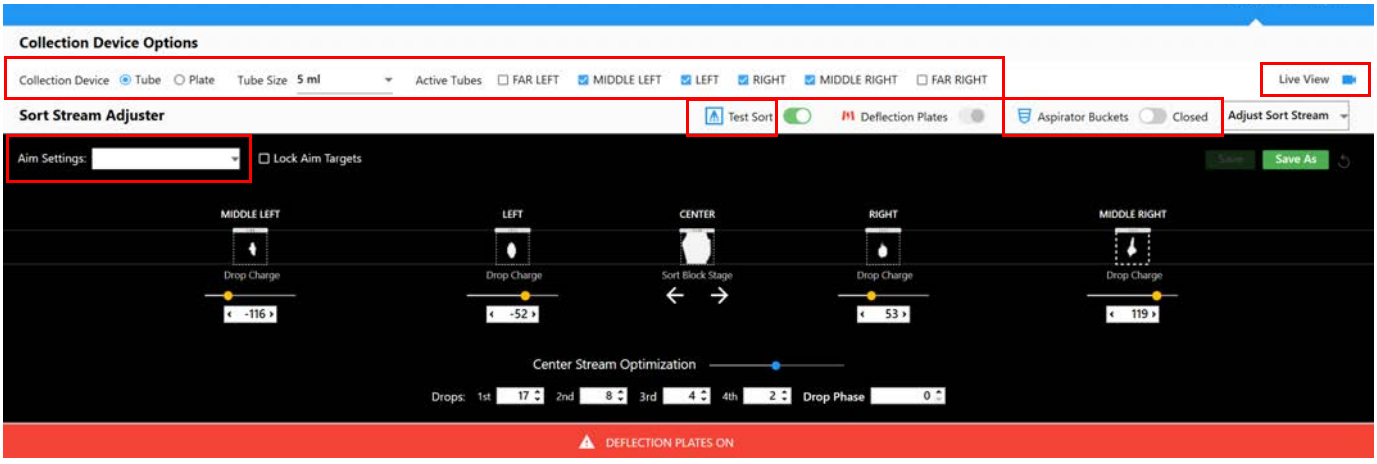
d Open the Aspirator Buckets so you can see the streams entering the collection tubes. If necessary, adjust the side stream droplet charges so that each stream is aimed at the center of its corresponding tube.

Use the arrows and/or the sliders to make adjustments.

■ **NOTE:** The 2nd droplet should be half of the 1st. The 3rd droplet should be half of the 2nd. The 4th droplet should be half of the 3rd.



e Uncheck Lock Aim Targets to unlock the targets. then drag the side stream boxes over the corresponding stream images. Lock the targets when the streams are centered.



f These aim settings can be saved and stored in the Library for future use. Click Save As in the Sort Stream Adjuster to save the settings. We recommend including the nozzle size and number of collection tubes in the name.

6 Turn off Test Sort to turn off the side streams and deflection plates.

7 Close the Aspirator Buckets and turn off Live View.

8 Run Auto Drop Delay.

a Click Auto Drop Delay.

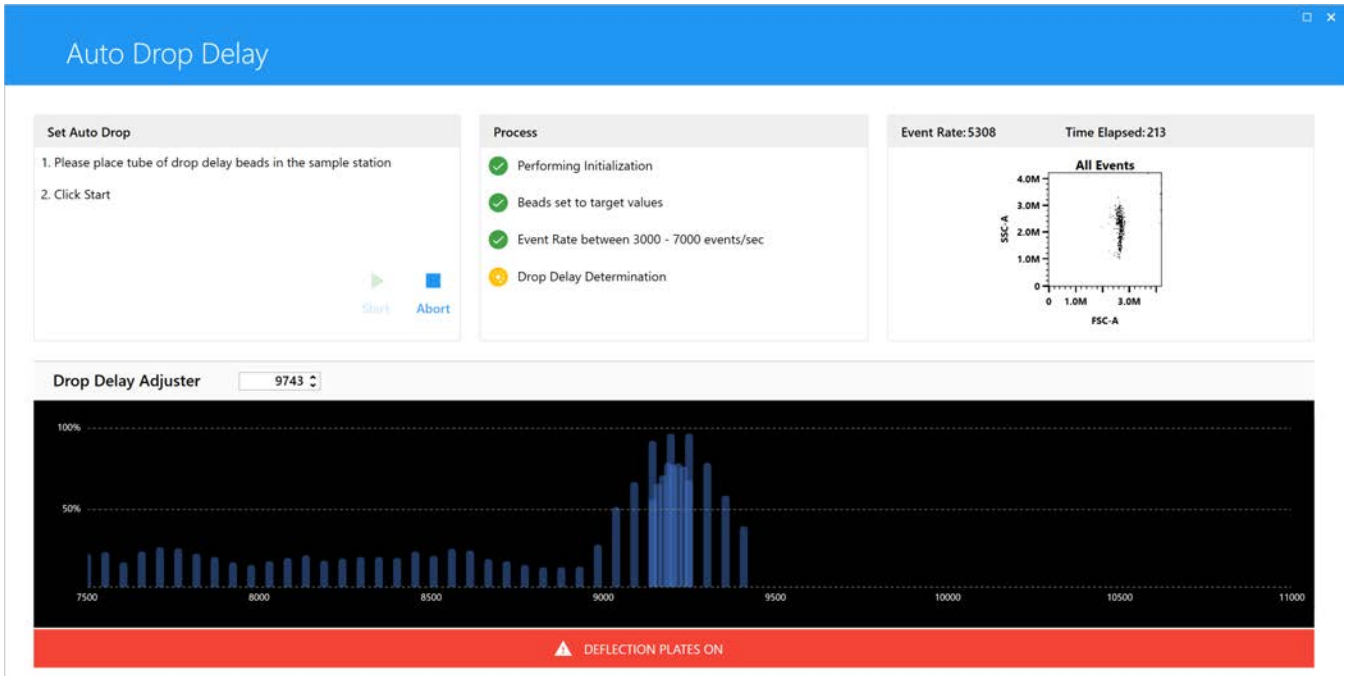
b Prepare SpectroSort beads and load them in the sample loading station.

To prepare the beads add 2-4 drops of beads to 500 µL of filtered sheath solution.

c Click Start in the Auto Drop Delay window.

This turns on the deflection plates, activates a SIT flush, and starts sample acquisition.

The graph displays the drop delay on the x axis and % sorted on the y axis. The system tests a range of drop delays until the optimal drop delay for the current stream setup is achieved.

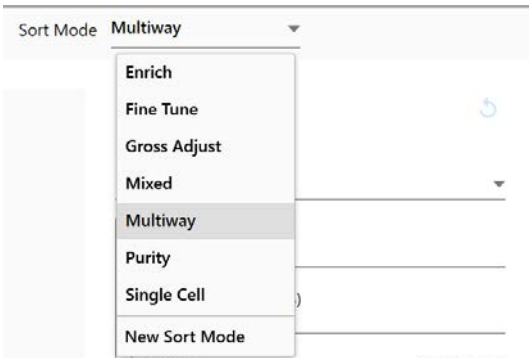


A message is displayed that Auto Drop Delay was successfully calculated. The drop delay values in Stream Controls are updated with the newly calculated values.

If Auto Drop Delay does not complete successfully, the SpectroSort beads may not be at the correct concentration or the stream may not be stable. Refer to "Troubleshooting" on page 153 for information.

d Click OK when the Auto Drop calculation completes successfully.

9 Select the Sort Mode in Tube and Plate Details.



10 Select the sort gates for each collection tube from the Population menus.

The gates listed in the Population menus are the gates assigned to the tube in the active or associated worksheet.

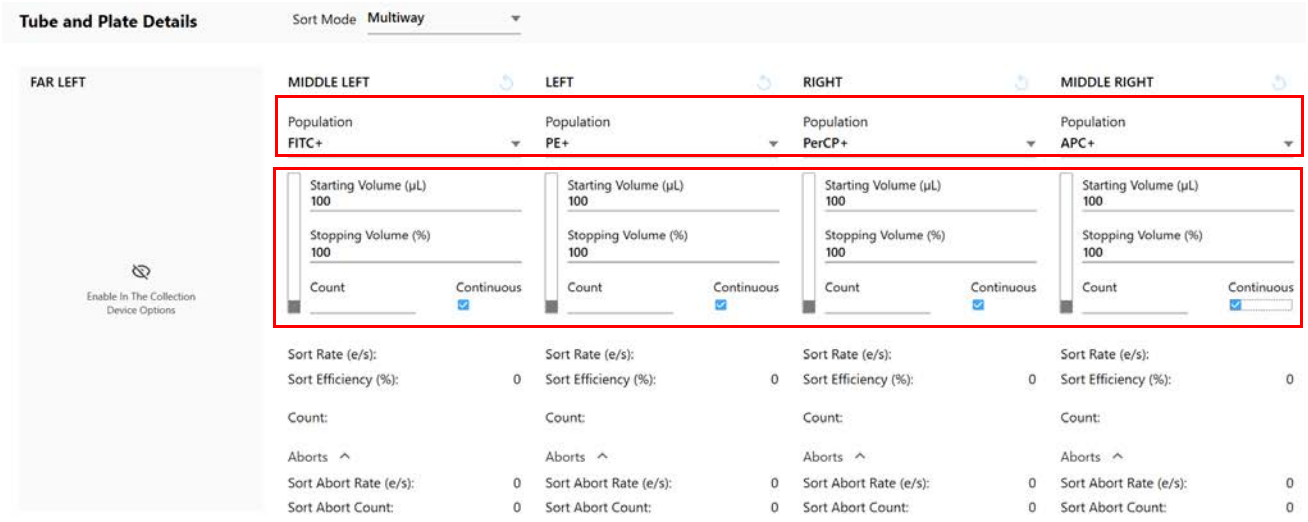
11 Select the starting volume in each tube. (Optional)

Enter the amount of collection buffer (in µL) in each tube to define the starting volume. An indicator displays the volume for each collection tube.

12 Select the stopping criteria—stopping volume (%) or count. Or select Continuous.

Stopping volume takes priority over count.

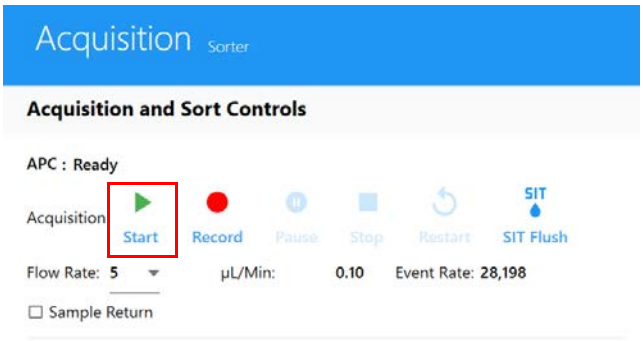
Selecting Continuous allows the sort to continue until the collection tubes are filled to the % that you defined. You can stop the sort at any time by clicking Stop Sort in the Acquisition and Sort Controls.



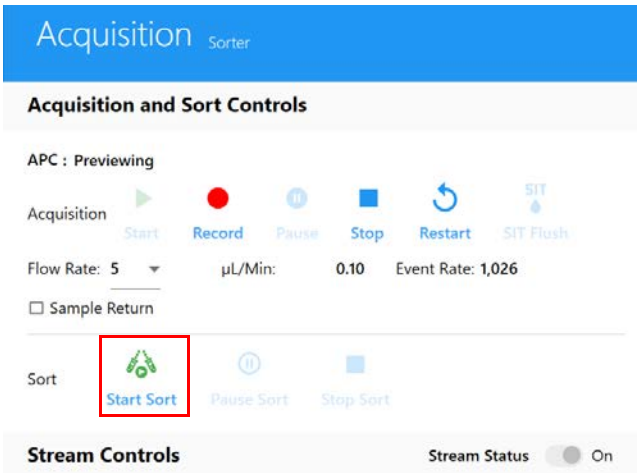
13 Load the collection tubes in the DDU chamber.

If applicable, add cell media to the collection tubes. The volume of media you add is the starting volume you will input when defining the starting and stopping volumes.

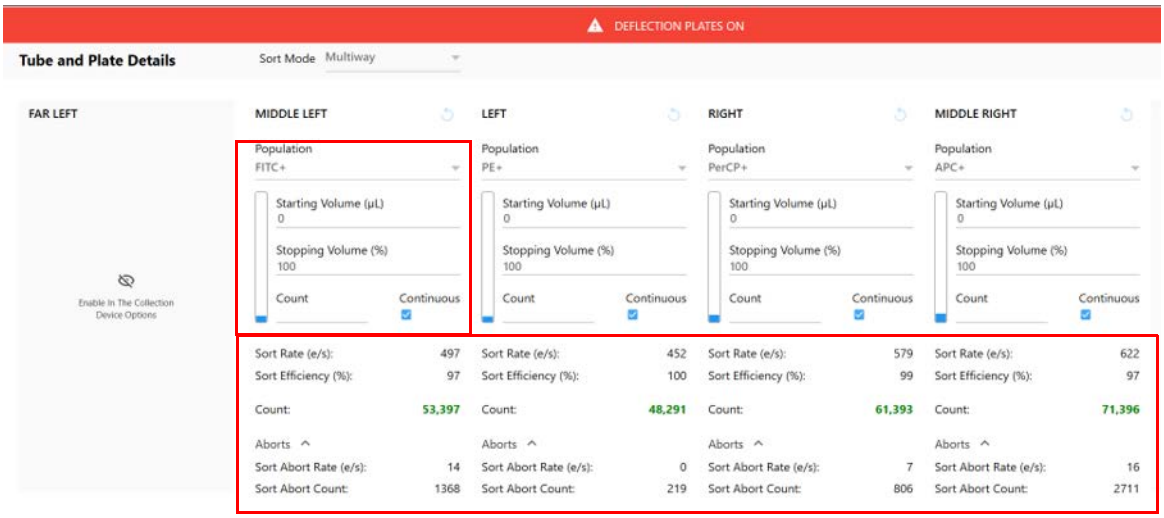
14 Load the sample tube and click **Start** to begin acquisition. Ensure that all gates are set properly.



15 Select **Start Sort** to begin sorting.



The deflection plates turn on, the aspirator buckets open, and the sort counters begin populating. The volume collected in each tube is updated real time.



Once the sorting criteria are met, the sort stops. If **Continuous** is selected, the sort stops once the tubes are filled to the defined volume or if you click **Stop Sort**. The deflection plates turn off automatically. Note the following things to watch while sorting:

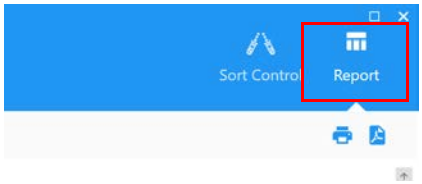
- Monitor your counts in each tube to make sure you are collecting cells.

- Make sure your populations are not drifting out of their gates. This can indicate a fluidic issue.
- Monitor the Sort Rate, Sort Efficiency, and Aborts to make sure your sort is going smoothly. These rates should remain fairly constant. Any major changes can indicate an issue with the sort.

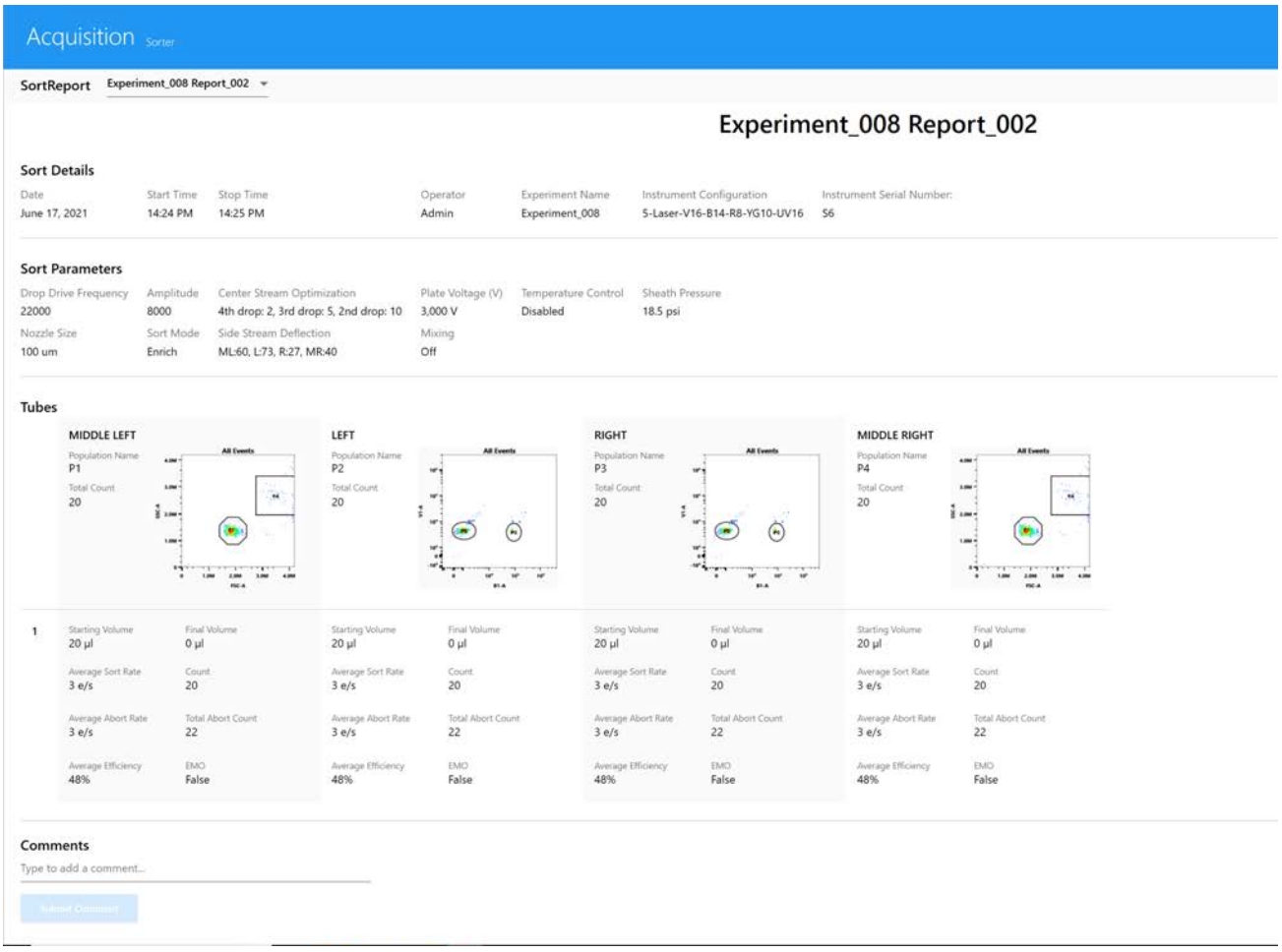
16 Click **Stop** to stop acquisition.

17 Click the Report tab to view the sort report.

The report includes experiment information, plots including the sorting gates, stream and nozzle settings, counts, volume, and stopping criteria.



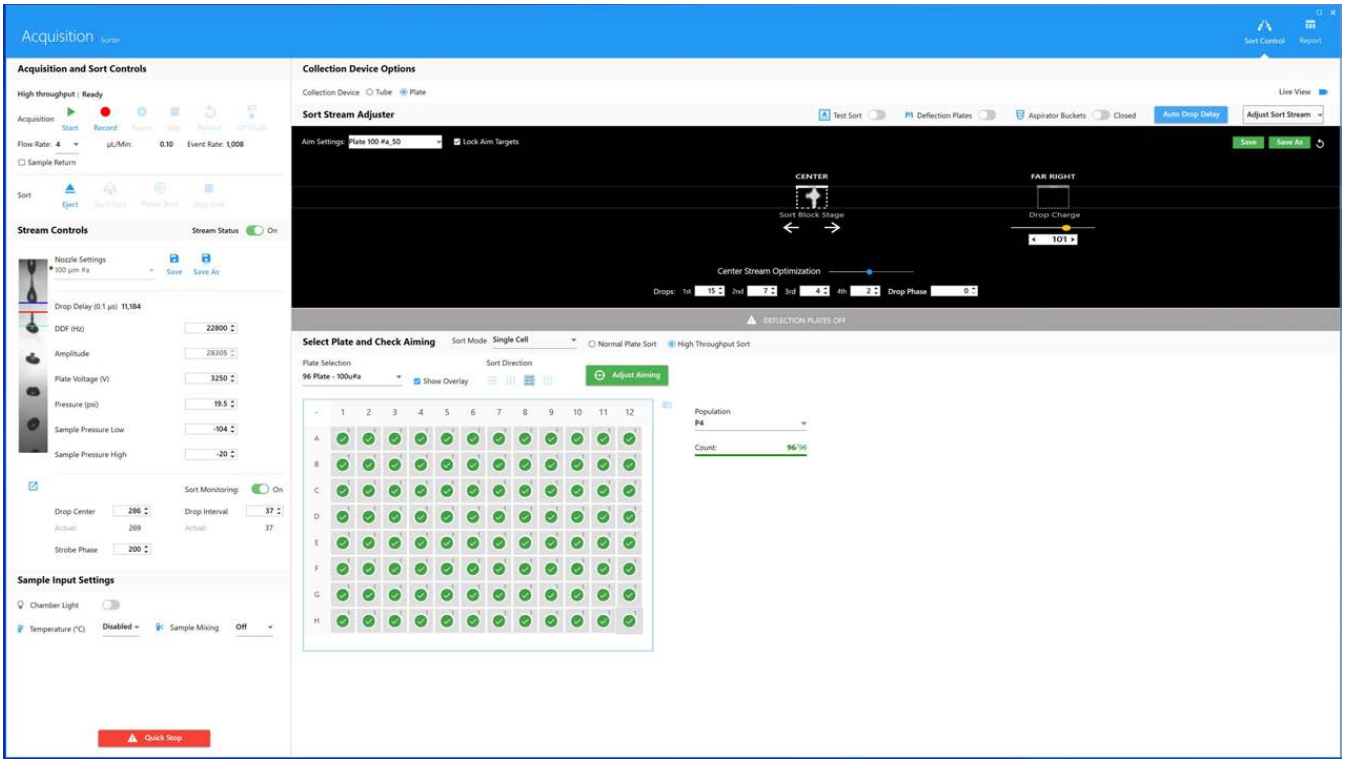
Example report



Sorting Setup for Plates

- "Preliminary Steps" on page 111

- “Start the Sort” on page 115



Preliminary Steps

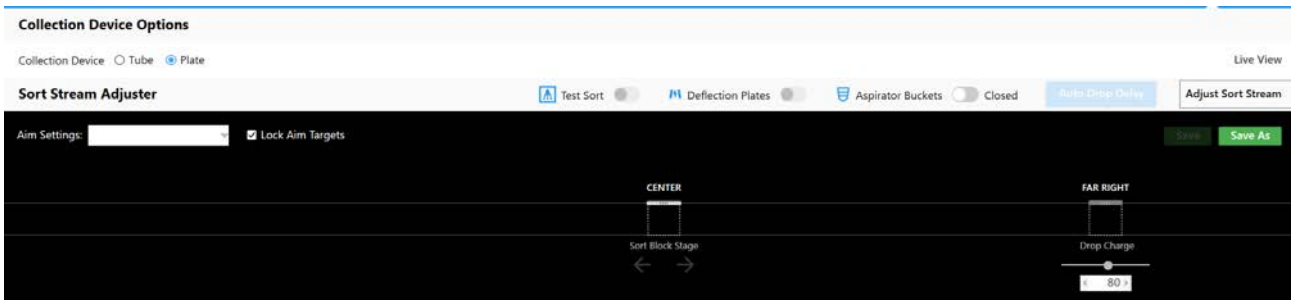
1 Set up experiment for your sample. Run samples and unmix if needed.

a Set up the gating on the population(s) you wish to sort.
Note: it is important to link the worksheet being used to the tube or group in the experiment. This tells the system what gates are available to select.

b Acquire a presort file to ensure the gating is set on the population of interest.
Tip: you can sort on raw and unmixed worksheets.

2 Ensure the stream is stable and drop delay has been performed.

3 Adjust the stream in the stream window.



For the first time adjusting the side stream:

a Select Plate in Collection Device.

b Turn on Test Sort to view the streams. The streams should fall into the 2 boxes in the stream window, one for the center stream and one for the far-right stream.

c To adjust the boxes, uncheck the Lock Aim Targets box. Move the Center box to the center stream.

d Put the Drop Charge value at 80 for the Far Right box and move the box to the location of the side stream. Check Lock Aim Targets box.

e Turn off Test Sort.

f Click Save As to save the settings for the first time. A suggestion is to put the nozzle size in the file name. for example: 100 µm, the file name will automatically append 96_ to the name. So the resulting name will be 96_100µm.

To reuse settings:

a Select Plate in the Collection Device.

b Go to the dropdown of Aim Settings and select the file being used for your sort.

c Turn on Test Sort and ensure the center stream is located in the center box and the side stream is in the Far Right box. If the side stream is not in the Far Right box, adjust the Drop Charge to bring the stream into the box.

d Turn off Test Sort.

e Click Save to update the settings.

4 In the sort window select the collection device and sort mode.

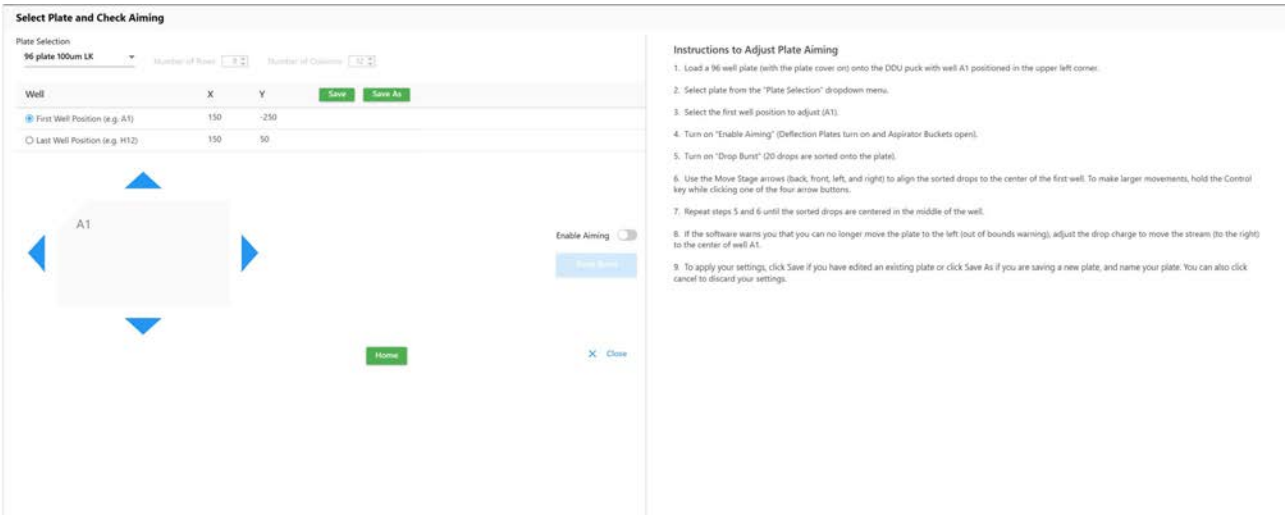
The following table shows plate sort modes and descriptions of what the selection combination contains.

Selection	Index sort	Description
Single Cell, Normal plate sort	Yes	- Ability to select different acquisition directions (e.g., serpentine, row by row) - Can manually select multiple wells to sort into - Can have more than one cell per well selected - Can have different populations selected for each group of wells
Single Cell, High throughput sort	Yes	- All wells automatically selected - Can only sort 1 cell per well - Only 1 population can be selected - Sort direction only serpentine

5 Under the sort stream adjuster, under aim settings select the 96-well plate aim settings (these are saved settings and provide a place to start). This setting contains the drop charge of the right side stream. This can be modified to move the aiming horizontally. The arrows under aim settings are used to adjust in the vertical direction.

6 Place plate with lid on the puck and place in sort chamber on the landing lights.

7 Click adjust aiming. This will open the plate puck adjustment function.



a In the Select Plate and Check Aiming section, select plate from the dropdown.

b If you haven't set up a plate with saved x,y coordinates, choose the default selection to customize if needed. Or if you have previously saved settings, choose those and verify that aim settings are correct.

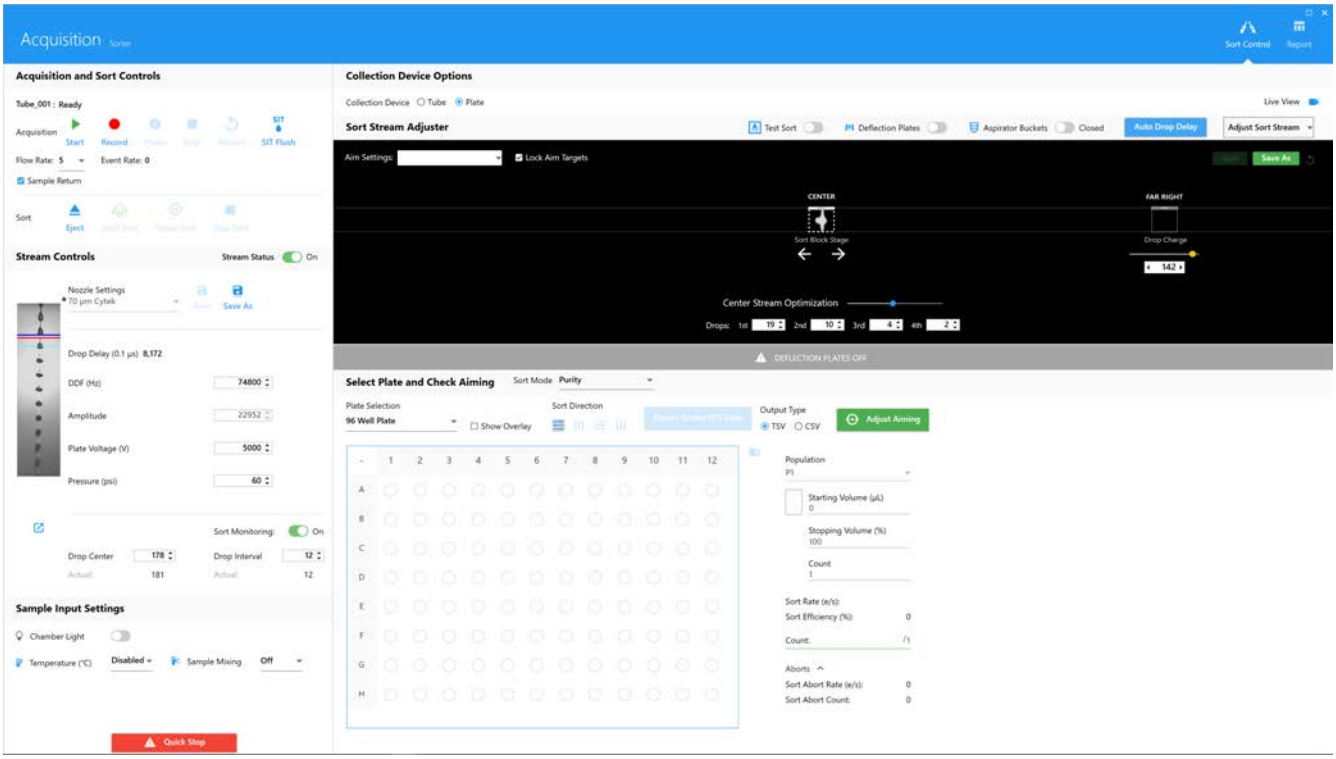
c Click on the Enable Aiming button to toggle on and activate the Drop Burst button. Enabling this button allows you to aim the drops into the center of well A1.

d (Optional) Click on Live View to use the light from the camera view above the Auto Drop delay. This will open a camera view and turn on a light in the collection chamber. This makes it easier to see the sort droplet on the plate cover when aiming the drops into well A1.

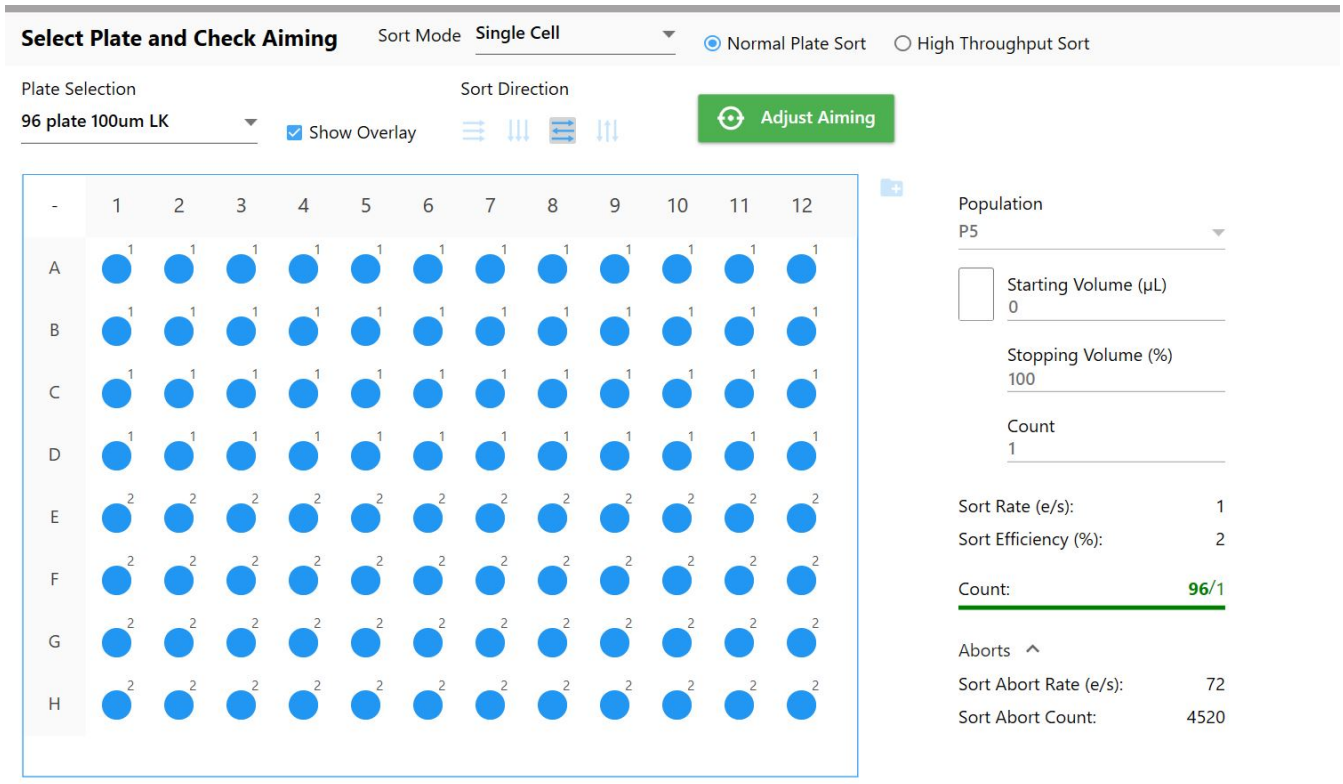
e Click Burst to sort 50 drops on the lid of a plate.

i. If the stream is not in the middle of well A1, adjust using the arrows to move the puck in the direction you will need to center the stream on A1.
ii. Click Save As once the stream is adjusted. It is a good idea to include the nozzle size. Plate type is automatically saved in the name.

8 Close the Check Aiming window.



9 Set up the wells for sorting. Click and drag the cursor over the number of wells you desire to sort into. Click on the group icon and select Group. This will make the wells active for customizing.



In the above example the Sort Mode is Single cell.

NOTE: Single cell with Normal Plate Sort or High Throughput Sort will both have index sorting active and will save a file when sorting.

10 Choose the sort direction and click on Show Overlay if you choose to see index sorting results. This feature can be selected after the sort is completed. The file output can be selected before or after as well.

11 Click on the Population dropdown to choose the gated population you wish to sort. Based on your worksheet, the gates will show in the Population dropdown.

Note: The next two steps are only active in Normal Plate sort mode.

12 Choose starting volume if you are sorting multiple cells or have media in each well.

13 Enter the count of the desired number of events to go in each well. This is available when the Normal Plate Sort button is selected. In high throughput mode, the count is automatically set to 1.

14 Remove the lid and place the plate on the puck. Note: Keep the lid on until the last second to prevent risk of contamination.

Start the Sort

1 Place the tube on the instrument and click Start to start acquiring. Confirm the target population is in the chosen gate.

2 Click sort in the sort window. You will get a message to enter the starting volume if you didn't. You can select sort anyway or click button to enter starting volume. Once the sort is done, you can create a plot on a worksheet to show the events that were sorted by well location.

Single cell sorting using High Throughput Sorting

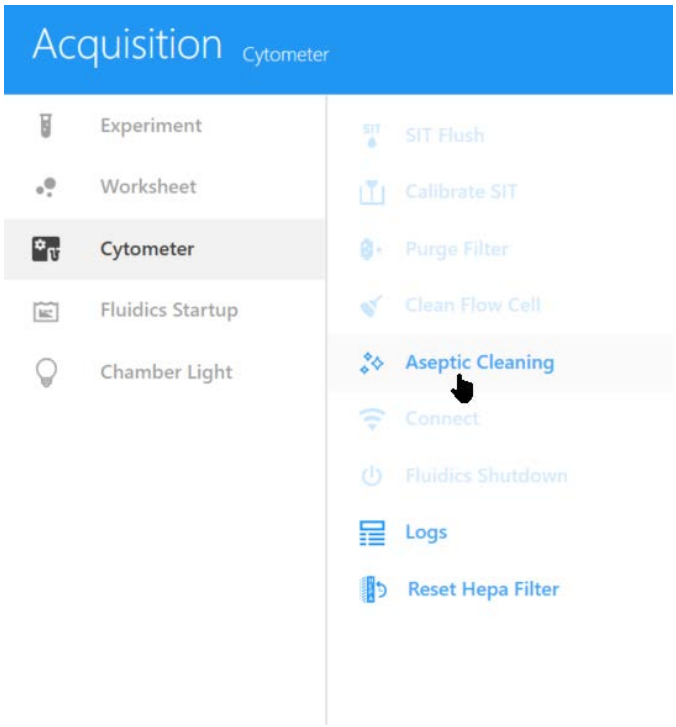
When the Single Cell Mode is selected and the High Throughput Sort button is selected, the following items happen. Note: This mode is also used when you have a higher percent target population and a higher event rate. Enable a combined sort rate of 200 e/s when you want to sort fast (complete a plate in ~1 min):

- All 96 wells are selected.
- Can only select 1 target population to sort per plate in this mode.
- Can sort only 1 cell per well.
- The sort direction is only Serpentine.
- Index sorting is active and a file is saved with the sort locations.

Aseptic Cleaning

Aseptic cleaning is done when sorting particles requiring aseptic technique. Perform this procedure when you are preparing for a sort that requires aseptic conditions. This procedure is performed by using the wizard built into the Acquisition tab of the software. This process will take approximately 1 hour.

1 Go to Cytometer and click on Aseptic Cleaning.



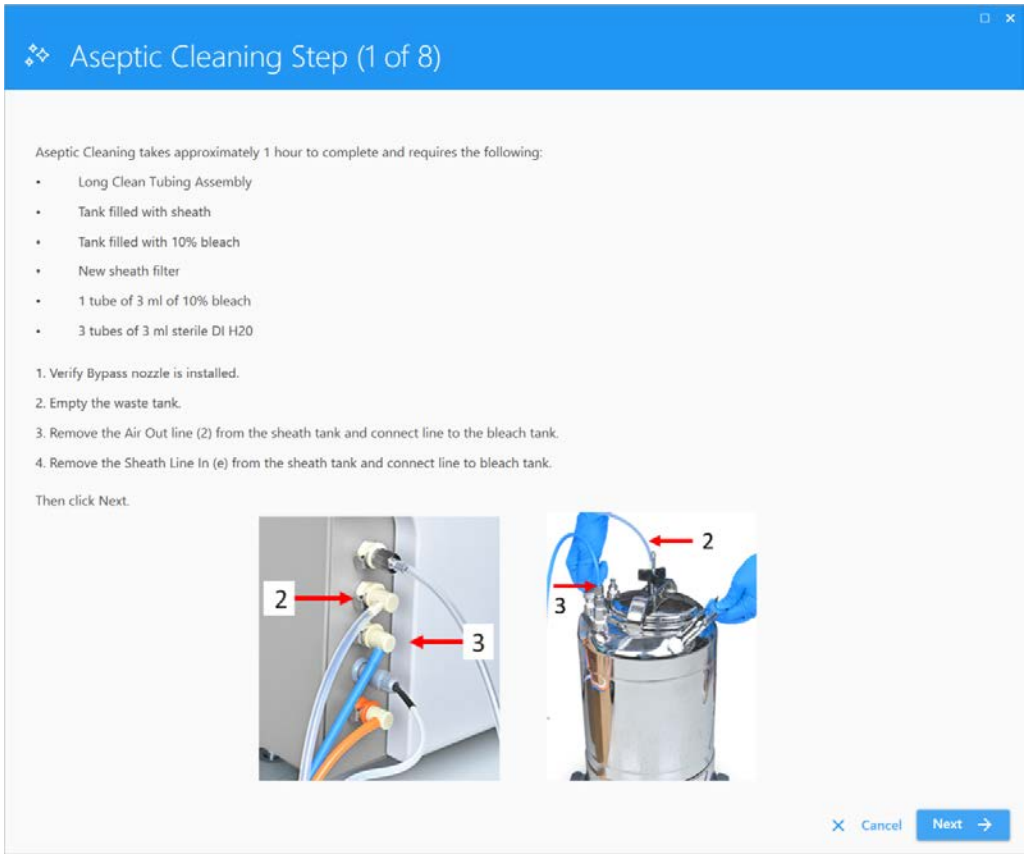
2 To perform the Aseptic Cleaning, you will need to have the items listed below ready to use when prompted:

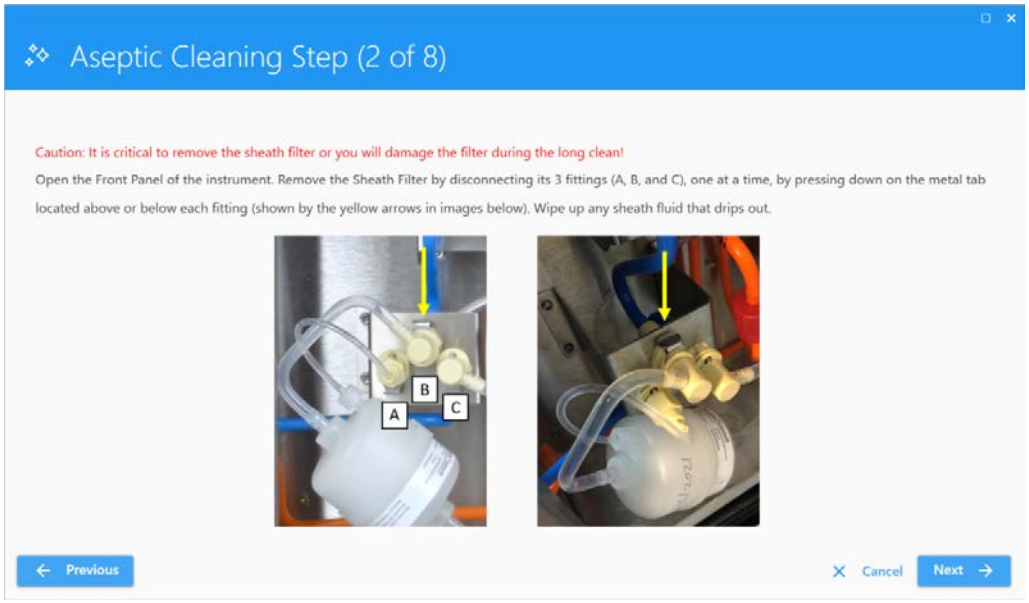
- Long Clean Tubing Assembly
- Tank filled with sheath (use sterile sheath fluid)
- New sheath filter
- 1 tube of 3 ml of 10% bleach
- 3 tubes each with 3 ml of DI water

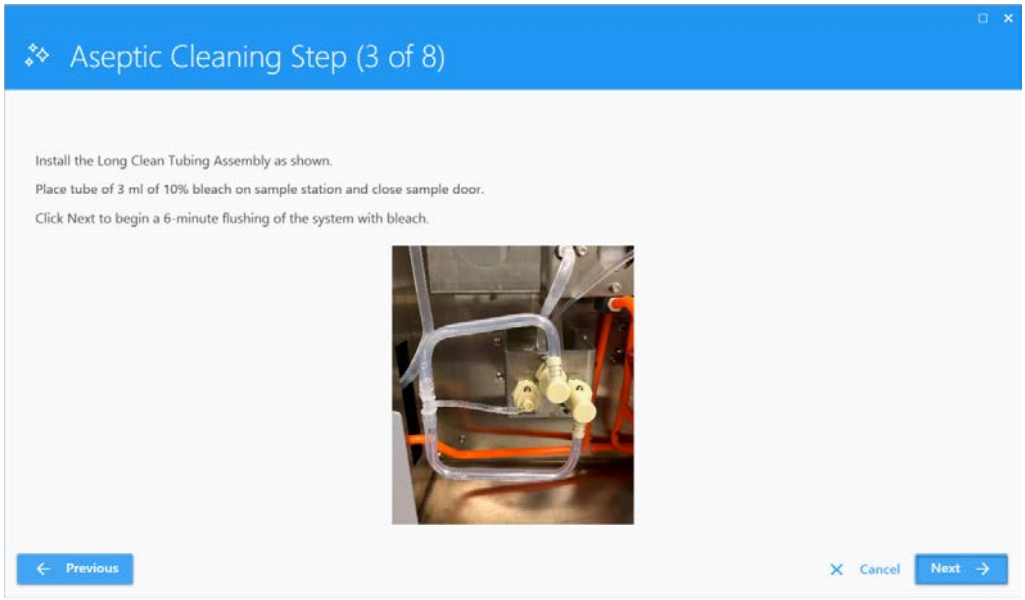
3 Click Next. The next several steps provide instructions with pictures to perform the next actions.

a Remove the sheath filter and connect the Long Clean Tubing Assembly in it's place.

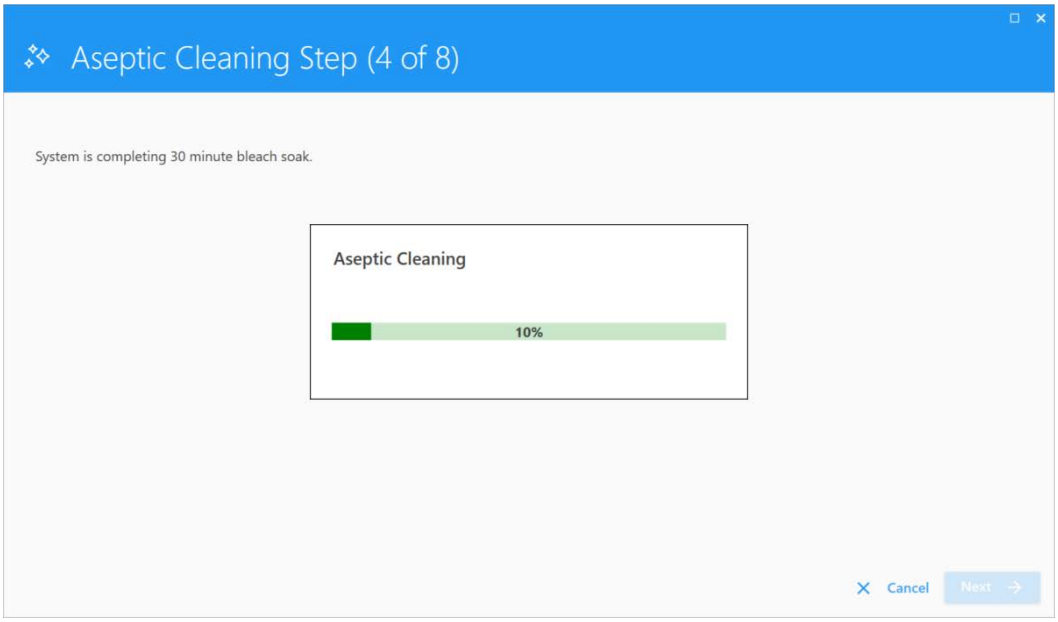
b Empty the waste tank and disconnect the fluid and air lines from the sheath tank (if not already done). Connect the fluid and air lines to the tank containing 10% bleach.



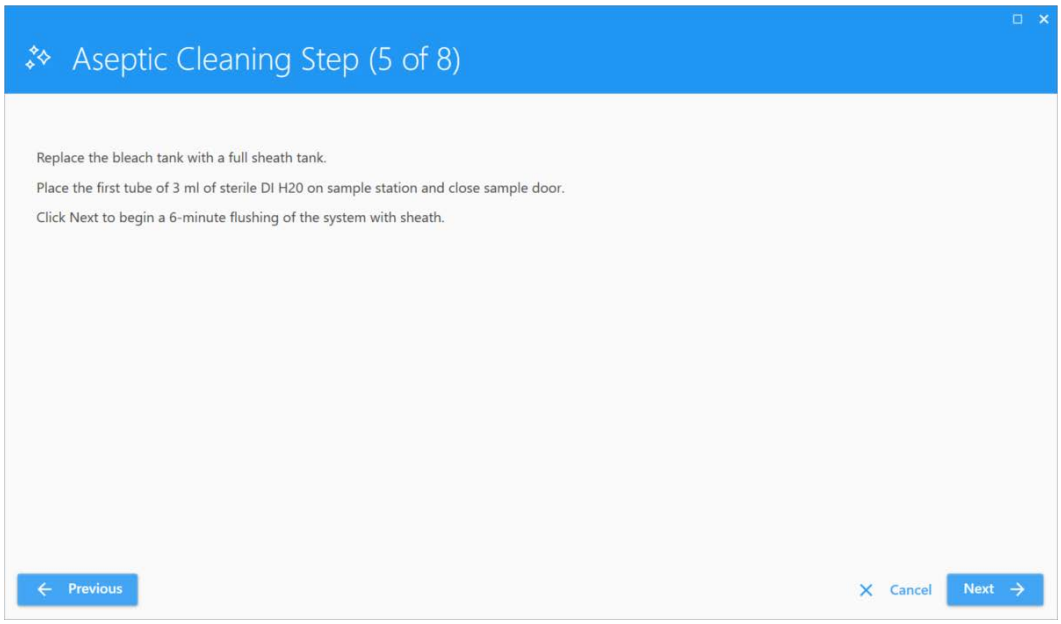




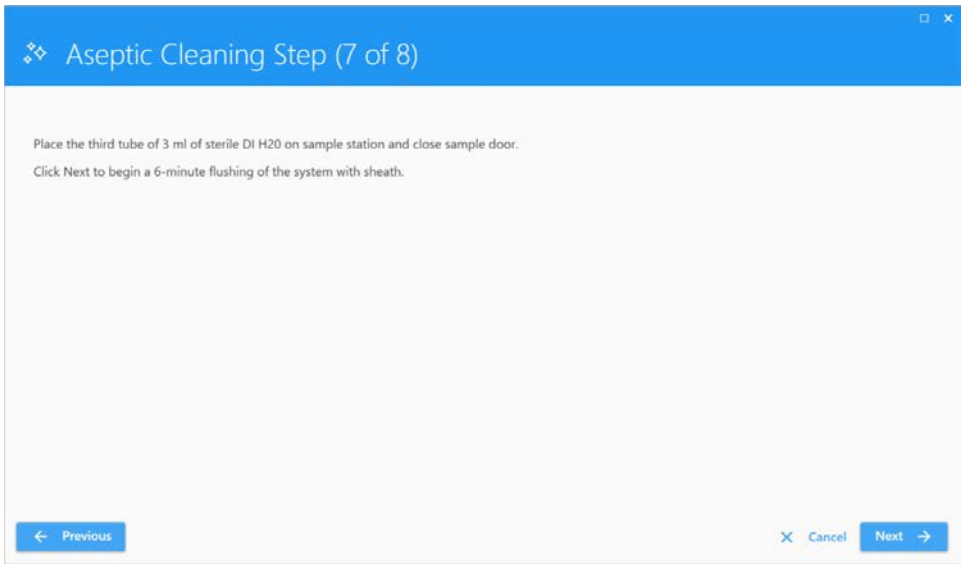
4 Click Next on the wizard step 3 of 8. The system will now run the bleach into the system and do a 30 minute bleach soak.



5 When the 30 minute soak is finished the next window will prompt you to disconnect the bleach tank and connect the sheath tank. Place the first tube of DI on the sample station.

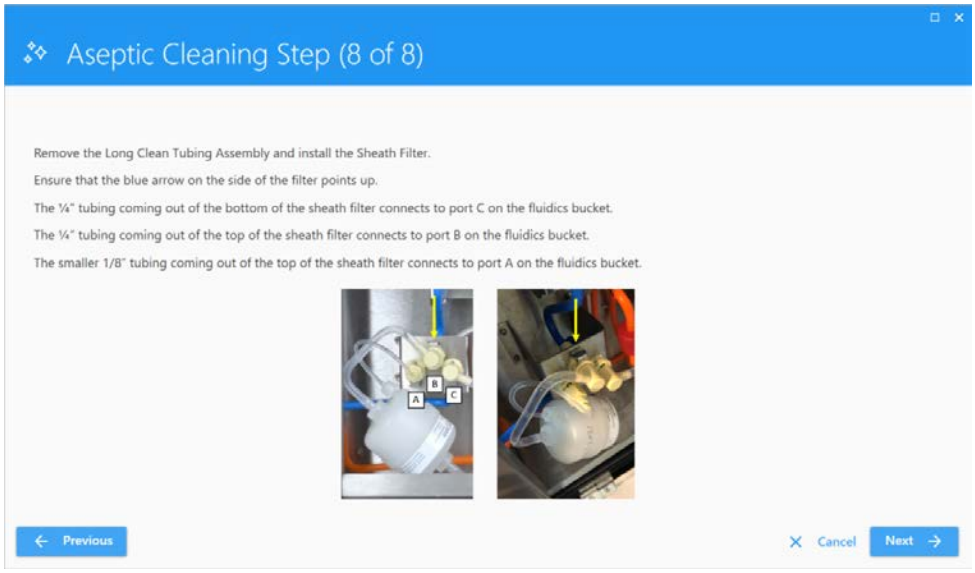


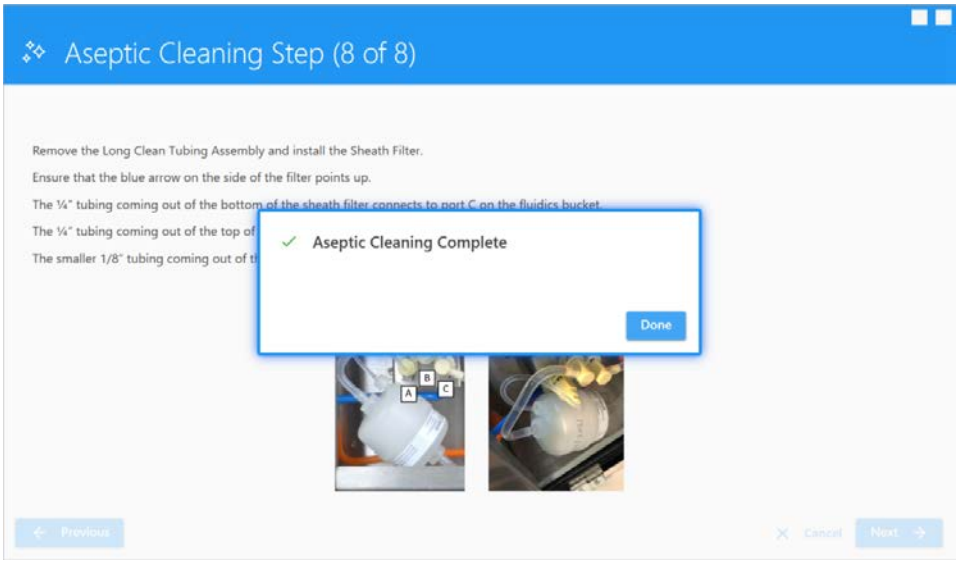
6 Click Next. Continue following the prompts for loading the remaining tubes.



7 Click Next.

8 The next steps have you connecting the new sheath filter to complete the Aseptic cleaning procedure.





9

Library, Preferences, and Users

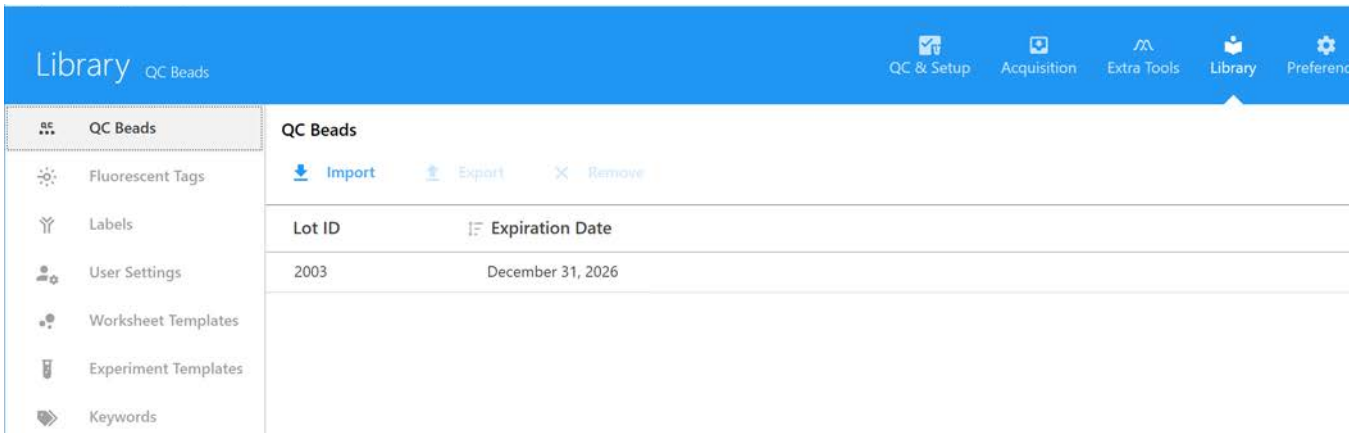
Library

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, experiment templates, keywords, and Loader settings. Information stored in the Library can be saved, exported, and imported for reuse.

Users can see their own Library settings. Administrator's can see the settings for all users.

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.

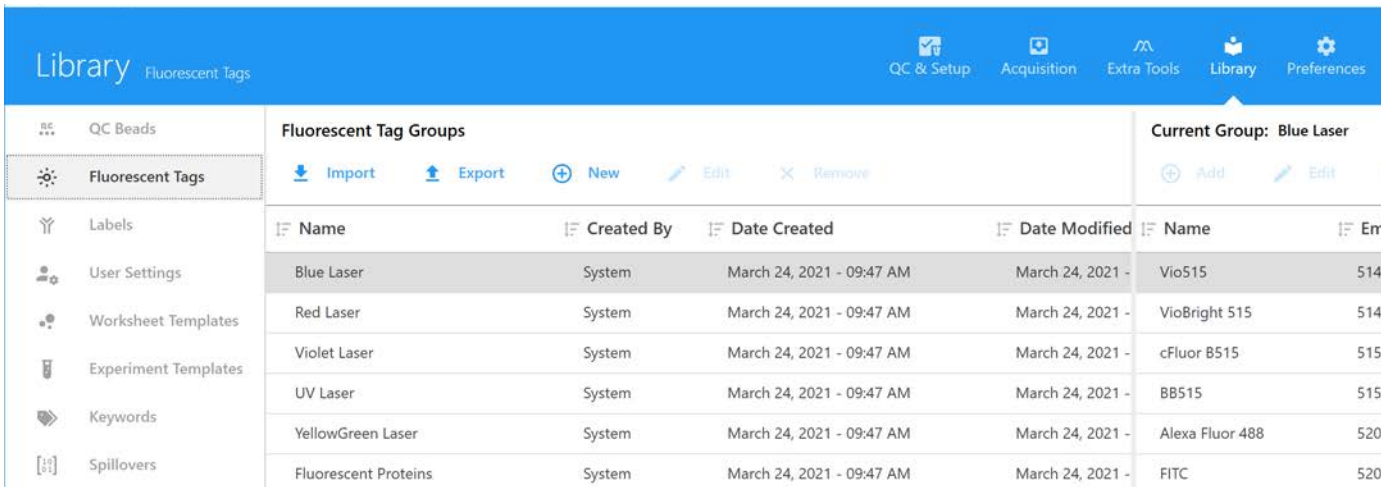


Fluorescent Tags

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, several groups of fluorescent tags are pre-installed with the software—Blue Laser, Red Laser, Violet Laser, Yellow Green Laser, Ultraviolet Laser, Fluorescent Proteins, and Viability Dyes.

These groups contain the most commonly used fluorophores excited by the system's on-board lasers. Additional tags can be added to these groups.



You can create groups or individual fluorescent tags by selecting **New**. Both groups and individual fluorescent tags can be imported or exported.

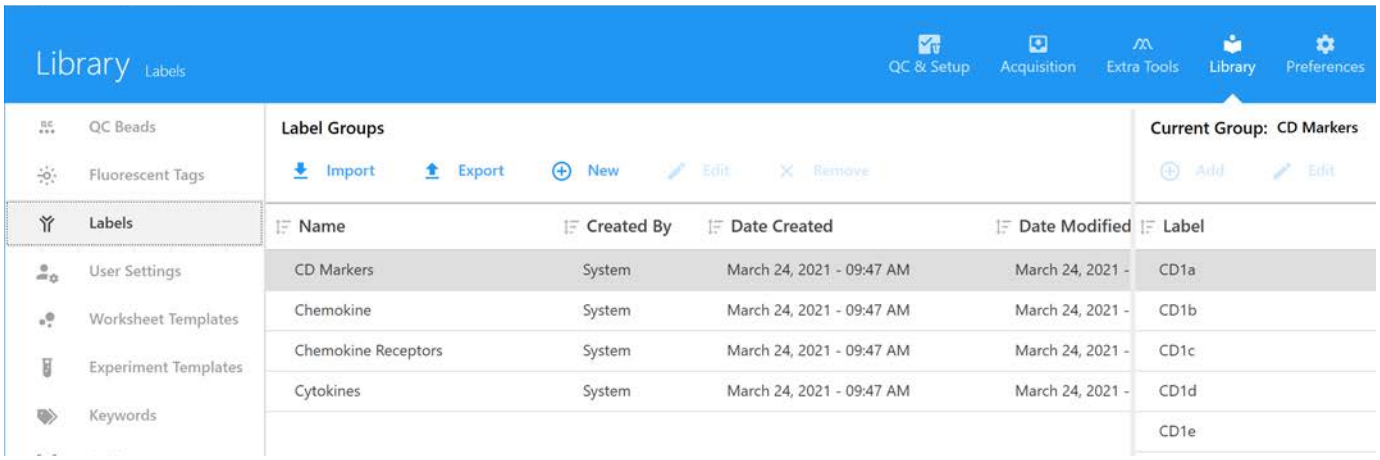
To edit the properties of a fluorescent tag that you added, select the fluorescent tag of interest and select **Edit**. Properties that can be edited include fluorescent tag name, laser excitation wavelength, emission wavelength, and display name. The default tags that are included with the software cannot be edited or deleted. However, they can be exported and imported across different systems running SpectroFlo software, v2.2 or lower.

If the fluorophore is known by another name or identified by a different spelling, those additional names or spellings can be added as synonyms.

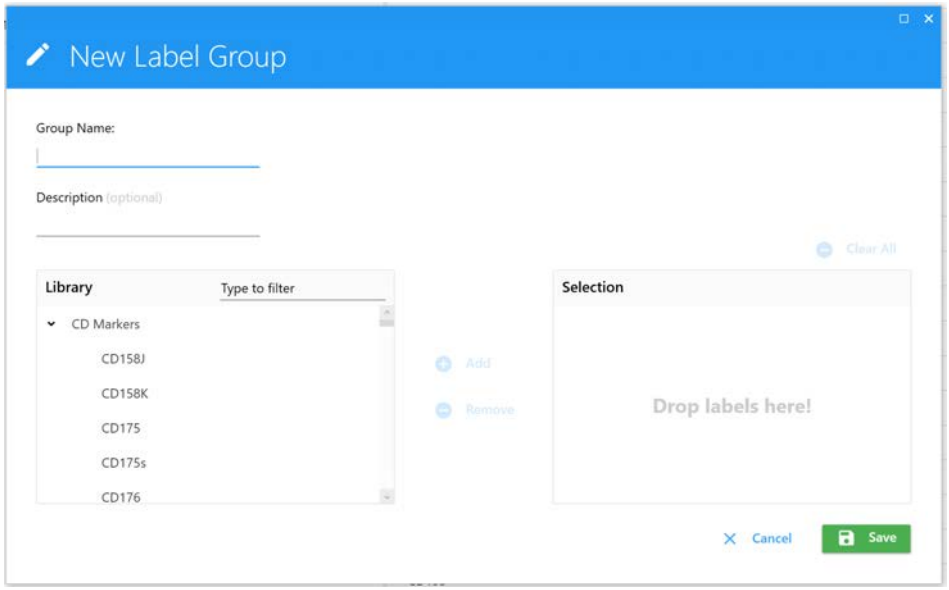
Any groups that you created can also be edited in this window. The default groups cannot be edited or deleted.

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.



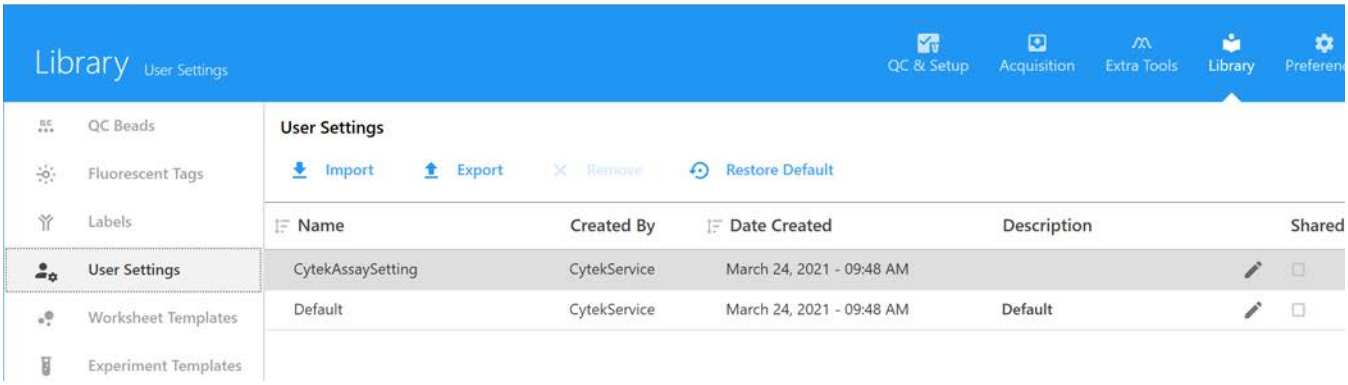
New label groups and individual labels can be created by clicking New. Label groups can also be imported and exported for use on other systems. The default labels cannot be edited or deleted.



User Settings

User Settings are the set of gain settings, threshold, and signal type for all detector channels. The date when it was created, as well as the name of user who created it are also saved. The settings are saved from the Instrument Control Pane in the Acquisition module. The name and description can be modified in this tab. Administrators can check the Shared checkbox to allow all user to see and use the settings and worksheets created by other users. These settings can be saved from the Acquisition screen.

If you wish to reset the system-defined Default user settings to their original settings, select the Default user setting, then click Restore Default.



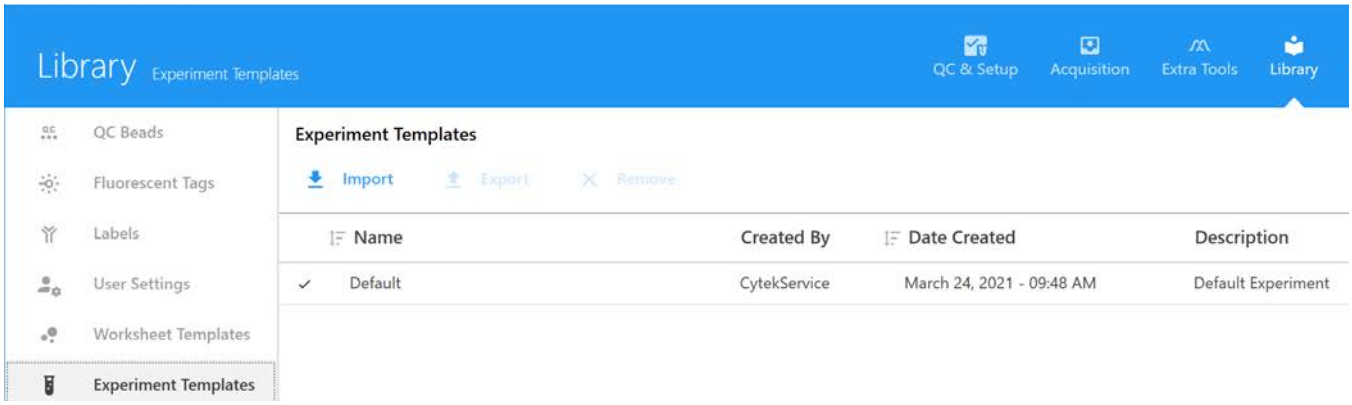
Worksheet Templates

All worksheets created in the Acquisition module 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. For more information on worksheets, see “About Worksheets” on page 30.

To view a worksheet, select it, then click **View**. To remove a worksheet that is no longer needed, select it, then click **Remove**. You can also set the default raw and unmixed worksheets back to their original defaults by selecting the worksheet, then clicking **Restore Default**.

Experiment Templates

Experiment templates contain the key elements of an experiment without the data. They can be saved and stored in the Library for future use. The name, creation date and time, description, and creator information is displayed. Experiment templates can also be imported and exported from this tab. For more information on experiments, see “About Experiments” on page 27.

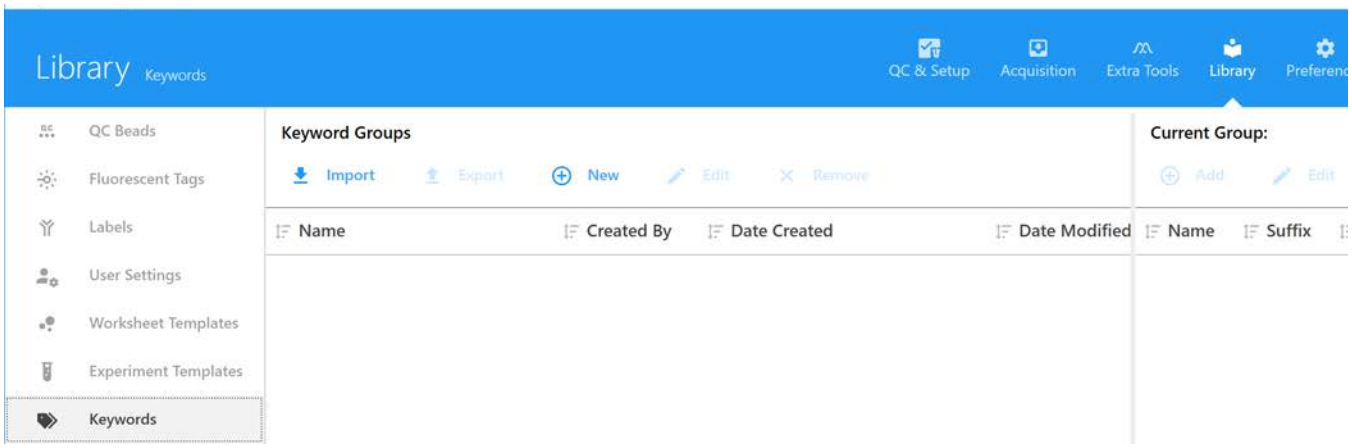


Keywords

Keywords allows you to type, import, or export user-defined, custom FCS keywords by group. These keywords can be assigned in an experiment at the experiment, group, or tube level and are exported with the FCS files.

The following types of keywords can be created:

- Numeric
- String
- Boolean
- Selectable numeric
- Selectable string



Standard Keywords

The following table describes the standard FCS 3.1 keywords found in SpectroFlo FCS files.

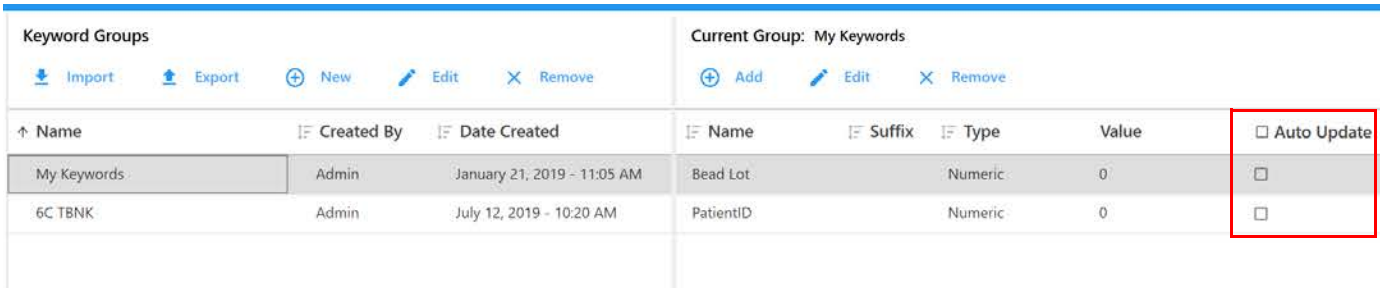
Keyword	Description
$FIL	Tube name
$VOL	Total sample volume (microliters)
$DATE	Date that the FCS data set was acquired
$BTIM	Beginning time of data acquisition
$ETIM	End time of data acquisition
$OP	Operator name
$INST	Institution name
$CYT	Name of cytometer (Aurora)
$CYTSN	Cytometer serial number
$SPILLOVER	Spillover matrix

Custom Keywords

The following table describes the custom keywords found in SpectroFlo FCS files. Custom keywords are not required as part of the FCS 3.1 standard.

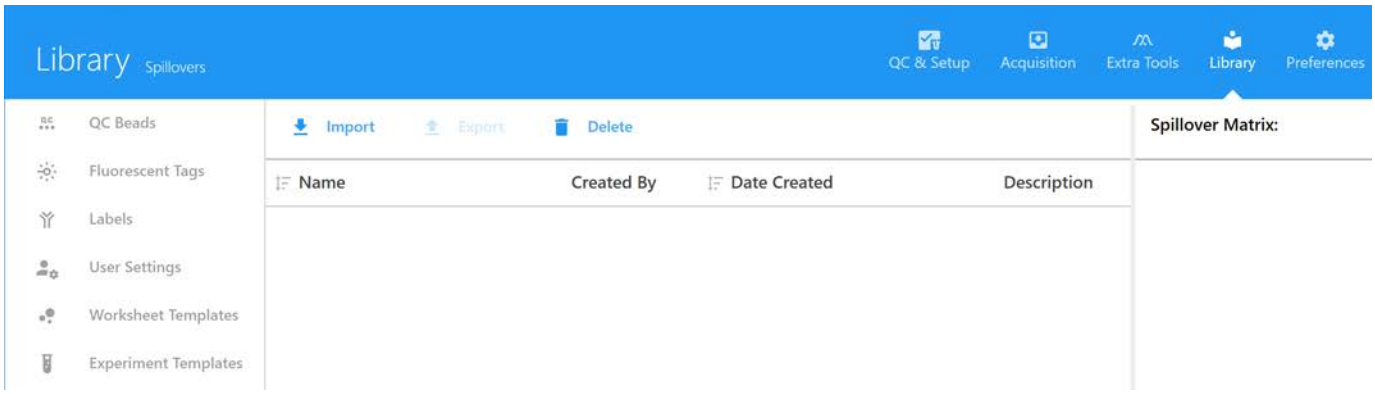
Keyword	Description
APPLY COMPENSATION	True or False to indicate whether compensation is enabled when FCS file is loaded into SpectroFlo
CHARSET	Encoding. UTF8.
CREATOR	Software name and version
FSC ASF	FSC area scaling factor
LASER{x}DELAY	Laser delay for each laser
LASER{x}ASF	Area scaling factor for each laser
LASER{x}NAME	Name for each laser
P{x}DISPLAY	Preferred display scale
THRESHOLD	Threshold channel and value
TUBENAME	Name of sample tube
WINDOW EXTENSION	Window extension used to record the tube
USERSETTINGNAME	The user setting name used to record the tube

Users can change the value of a custom keyword, then select Auto Update for that keyword to update the keyword in subsequent experiments.



Spillovers

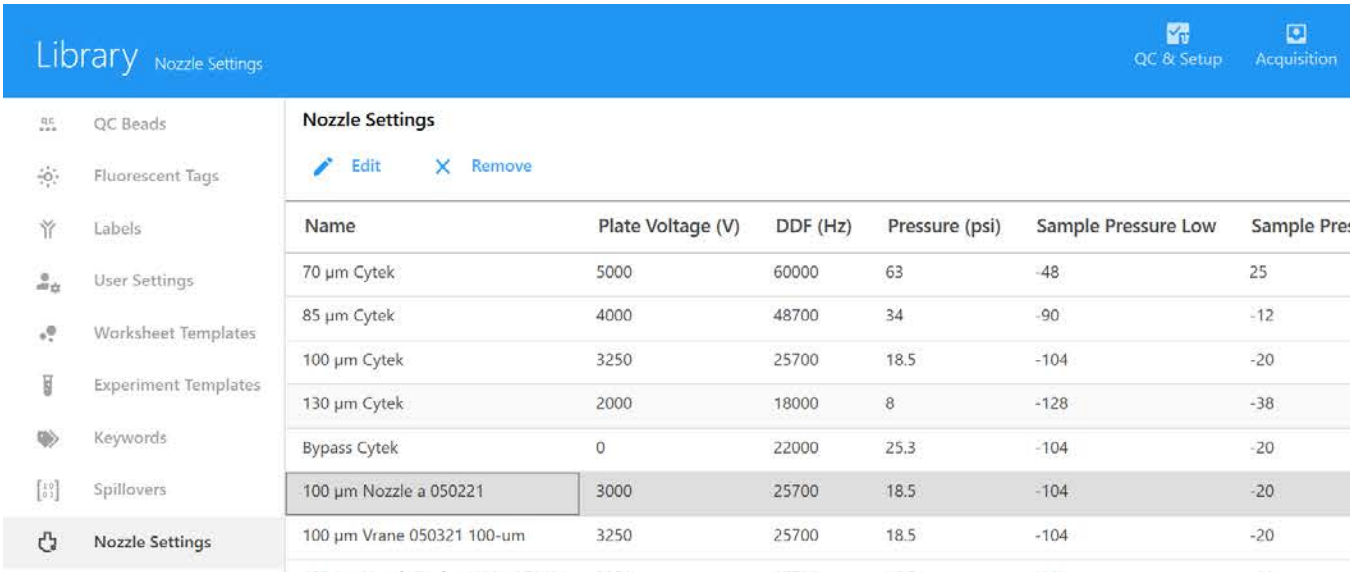
Spillovers shows the spillover matrices applied during unmixing. These saved matrices can be applied to individual tubes and wells. For more information on the spillover matrix, see "Adjusting Spillover" on page 58.



Nozzle Settings

Nozzle Settings display the default nozzles and their associated default parameters. These nozzle settings cannot be edited or deleted. However, you can create new nozzle settings with custom parameters based on a selected nozzle.

You can also create a custom nozzle in Sorter Settings based on a selected nozzle, then Save As. All nozzles, default and custom, appear in the Library displaying their associated parameters.



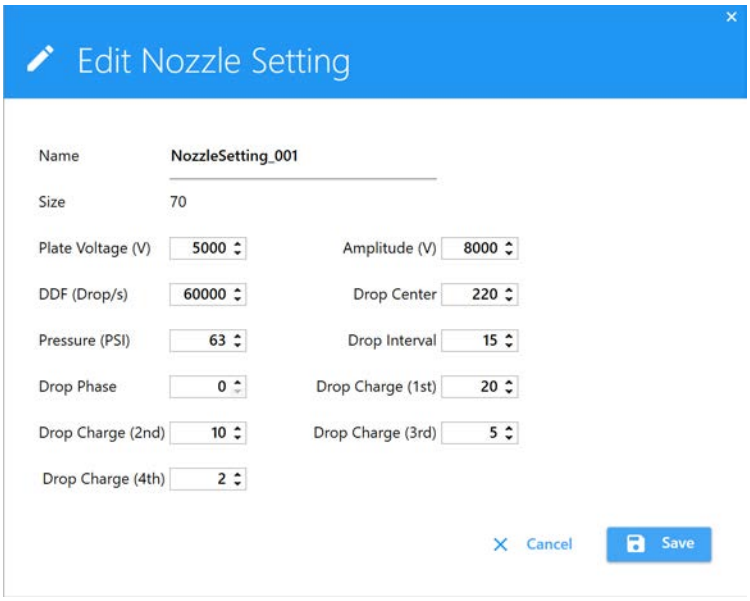
Creating Custom Nozzle Settings

1 Click the + at the end of the row for the nozzle you wish to base the new nozzle on.

A new nozzle appears in the list with a default name.

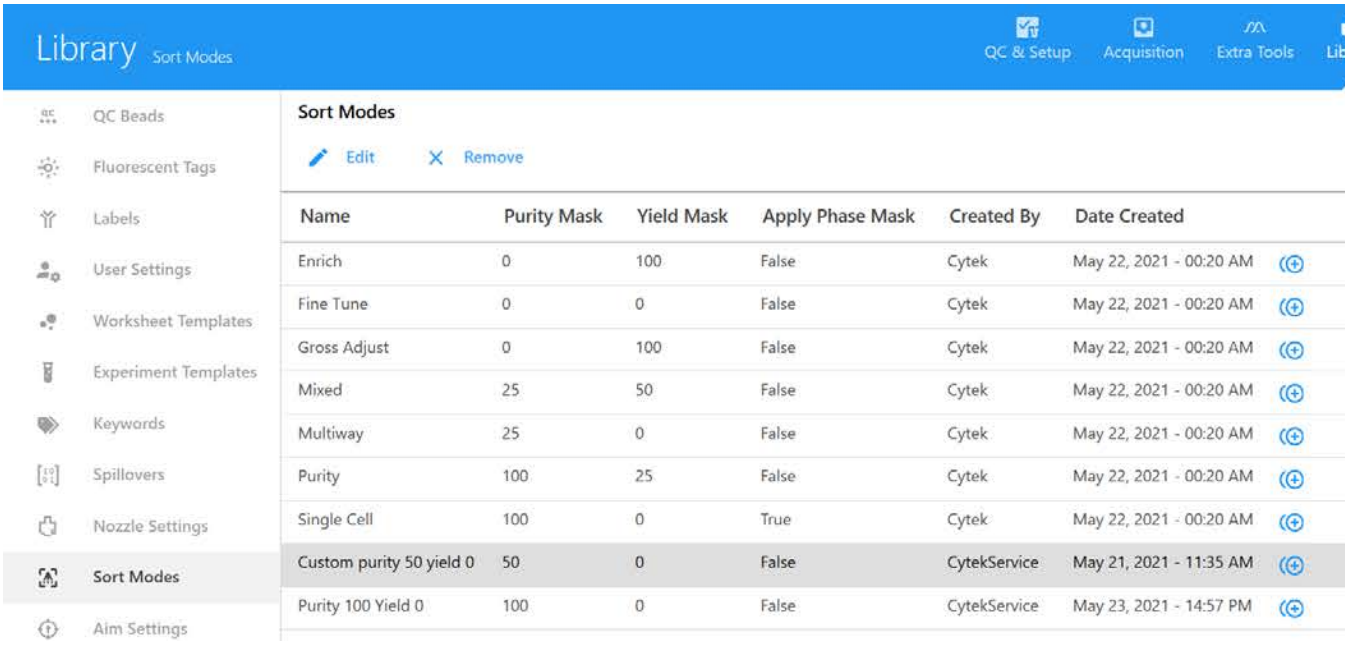
2 Select the new nozzle, then click **Edit**.

3 Enter the parameters for the new nozzle and click **Save**.



Sort Modes

Sort modes lists the sort modes provided by Cytek. These default sort modes cannot be edited or deleted. However, you can create custom sort modes based on a selected mode.

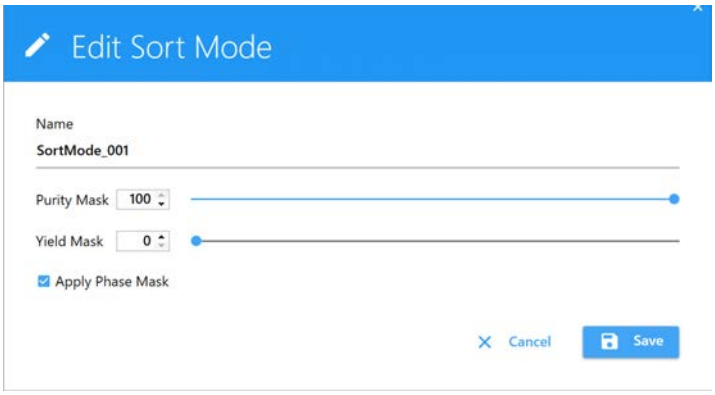


Creating a Custom Sort Mode

1 Click the + at the end of the row for the sort mode you wish to base the new mode on.

2 Select the new sort mode, then click **Edit**.

3 Enter a name for the sort mode, then use the sliders to select the purity and yield masks, and select whether you want to apply a phase mask.



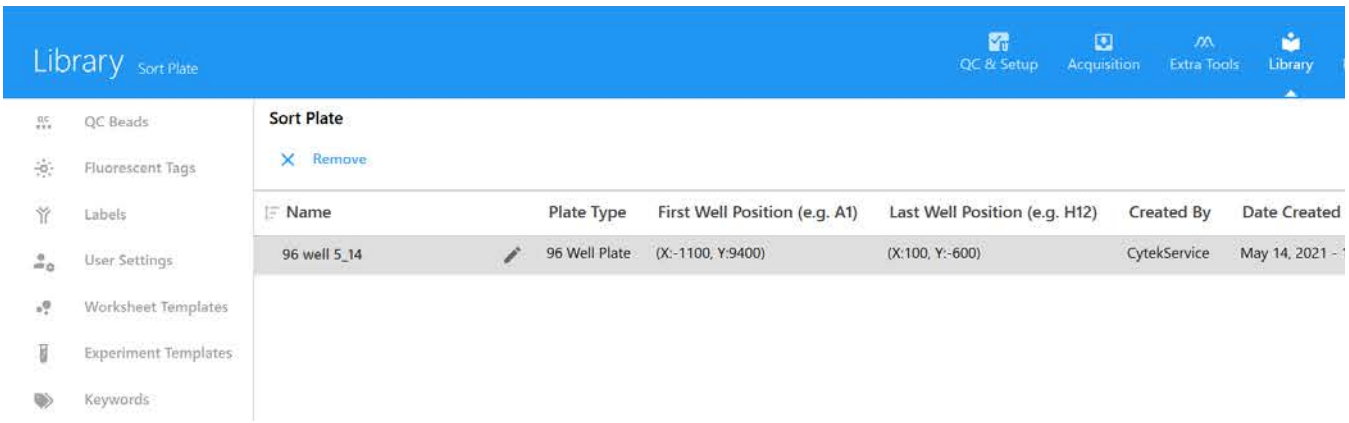
4 Click Save.

Aim Settings

During sorting, when you finish aiming the streams to their selected tubes, the exact voltages that were required to aim the streams can be saved and reused. Library Aim Settings displays the list of saved settings and allows you to edit them.

Sort Plate

You can save the settings for a sort plate after defining the aiming parameters (X and Y locations of well A1). Library Sort Plate settings displays the list of saved settings.

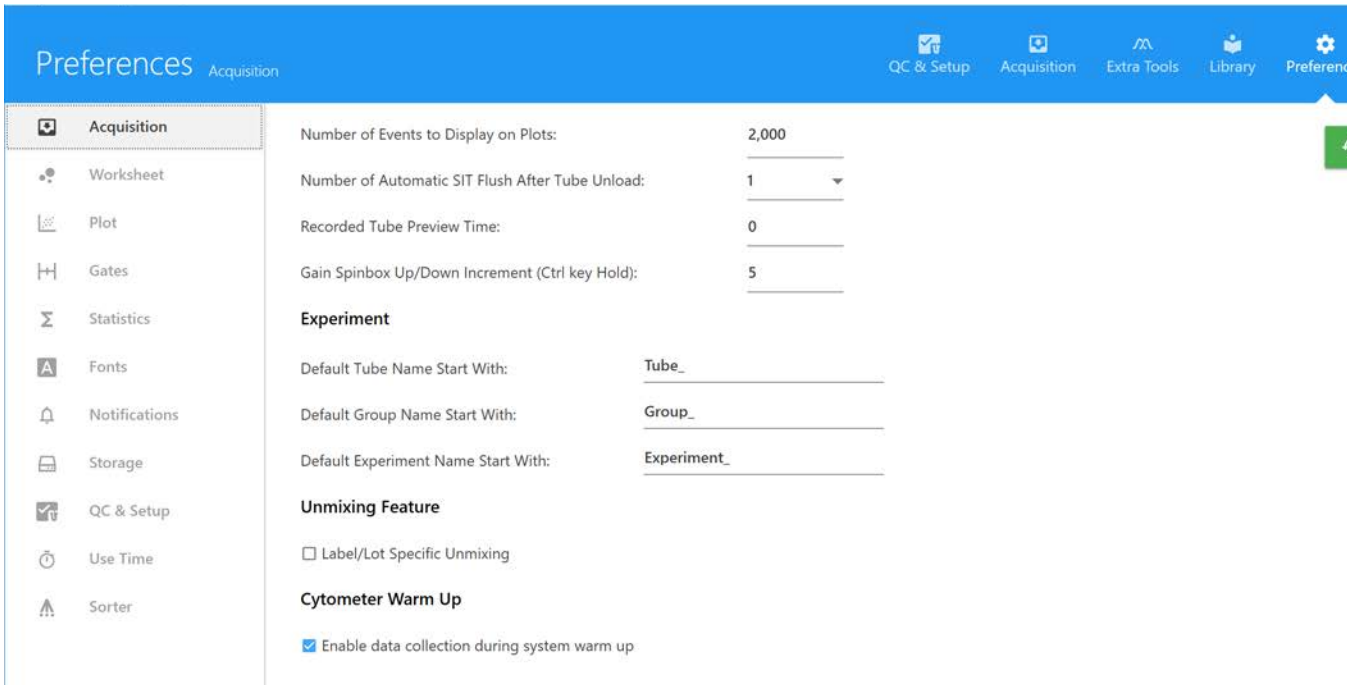


Preferences

The Preferences module 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 module. Each section within the Preferences can be restored to its default settings by selecting Restore Default Preferences.

Acquisition Preferences

In the Acquisition tab, you can change the number of events displayed on plots during acquisition, the number of SIT flushes, the preview time before recording begins, the value that the gain settings increment when you click the spin-box arrow during acquisition, and the SIT Lift Distance. You can also enter default prefixes for tube, group, and experiment names. A label/lot specific unmixing feature allows you to choose specific reference controls for unmixing.



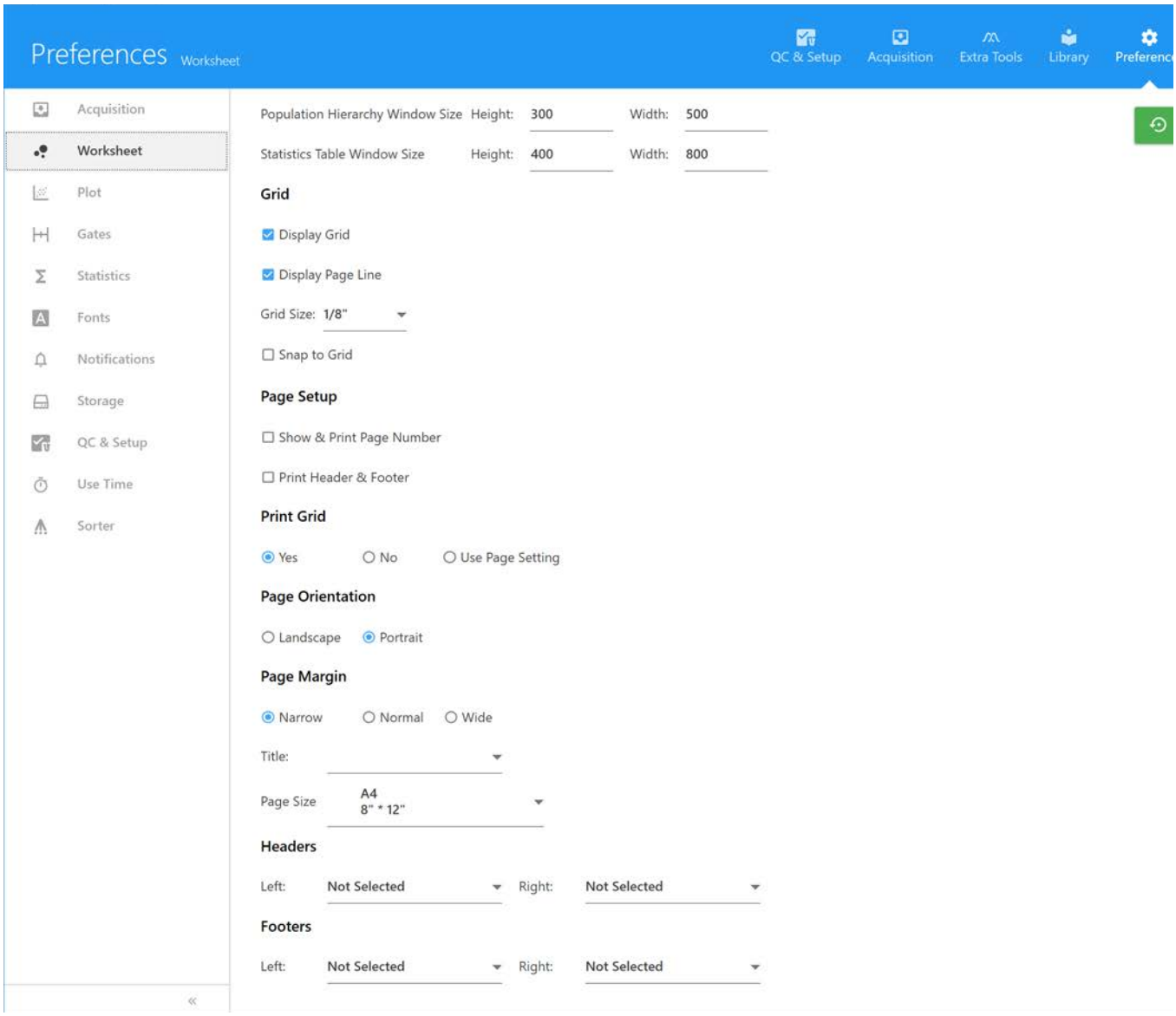
The following table describes the options in the Acquisition preferences.

Item	Description
Number of Events to Display on Plots	The number of events displayed in the pseudocolor plots, dot plots, and histograms. The default is 2,000 events.
Number of Automatic SIT Flush After Tube Unload	Choose the number (0–2) of SIT Flushes that will be performed once a tube is removed from the sample loading station.
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.

Item	Description
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.
Experiment (Default Names)	Enter the default name for tubes, groups, and experiments. Edit the default name and/or append to the names.
Label/Lot Specific Unmixing	Allows you to use lot-specific reference controls for unmixing when appropriate. If this option is selected, the software will search the experiment reference group for reference controls that have the same fluorochrome, label, and lot information in order to use the corresponding control for unmixing.
Enable data collection during system warm up	Allows you to begin data acquisition before the recommended 30-minute warm-up time has elapsed.

Worksheet Preferences

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.



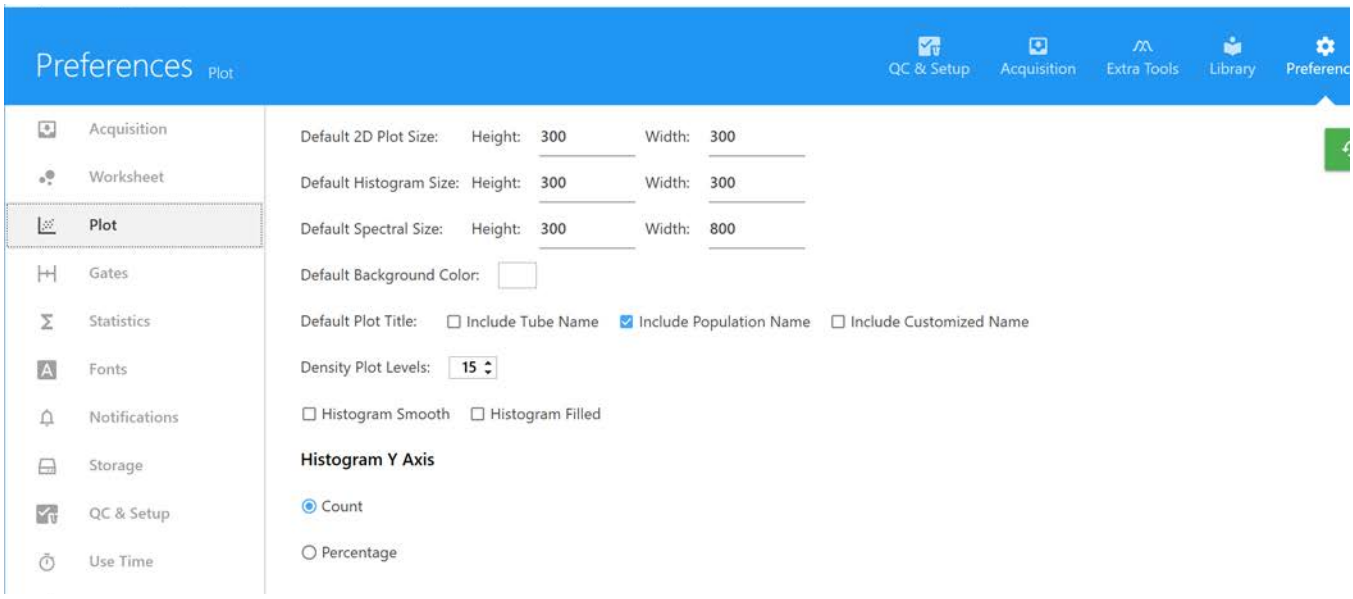
The following table describes the options in the Worksheet preferences.

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.

Item	Description
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.
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. Select the page size from a list of standard paper sizes.
Headers	Sets the text that is displayed in the left and right headers.
Footers	Sets the text that is displayed in the left and right footers.

Plot Preferences

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

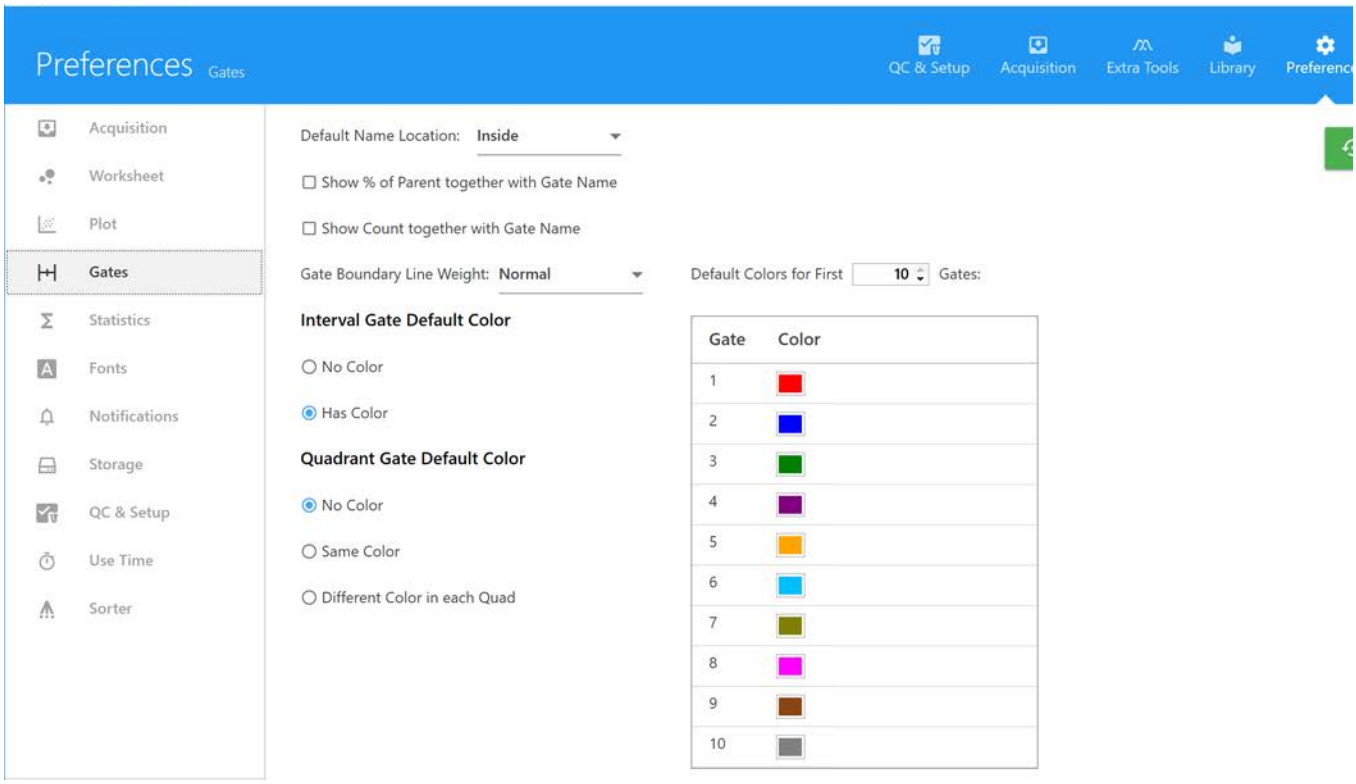


The following table describes the options in the Plot preferences.

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 Spectral Size	Set the default height and width of spectrum plots in pixels.
Default Background Color	Set the default background color for all plots. Click to select from the available colors.
Default Plot Title	Customize the title of all plots to include the tube name, population name, and/or a custom name.
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 Preferences

Gate properties can be adjusted in the Gates tab.



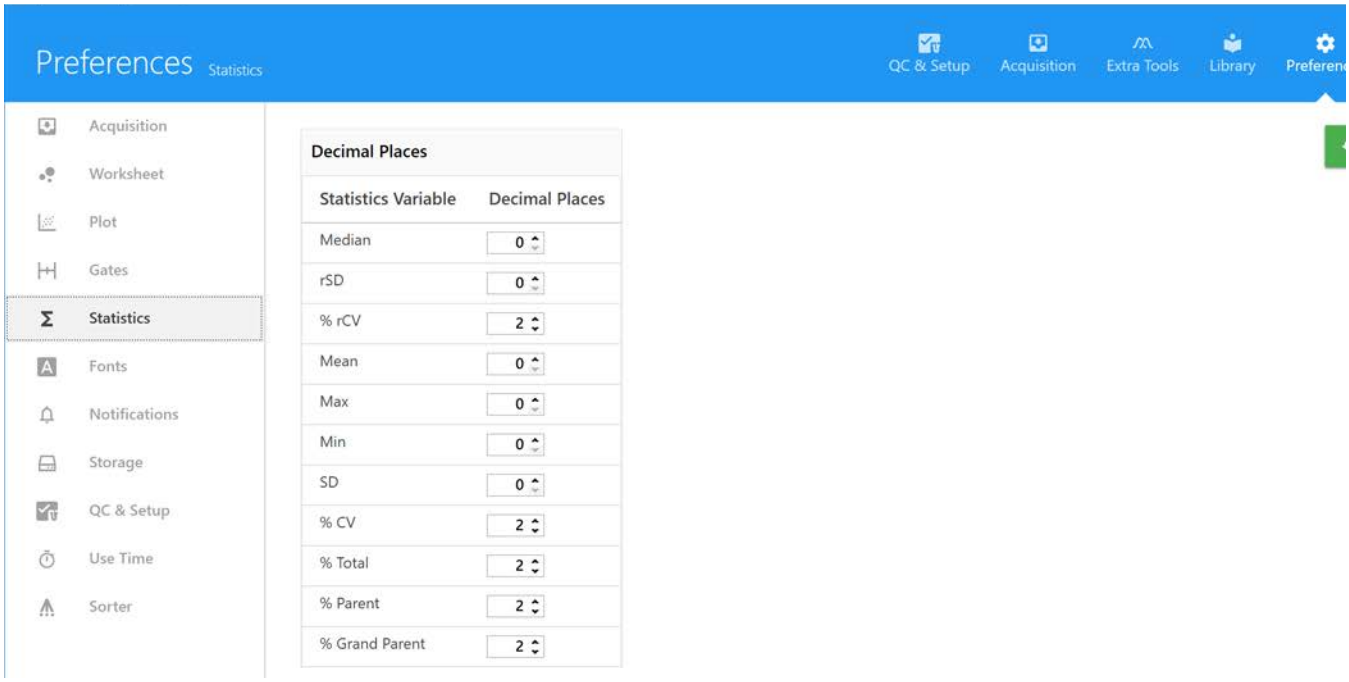
The following table describes the options in the Gates preferences.

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.
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 Preferences

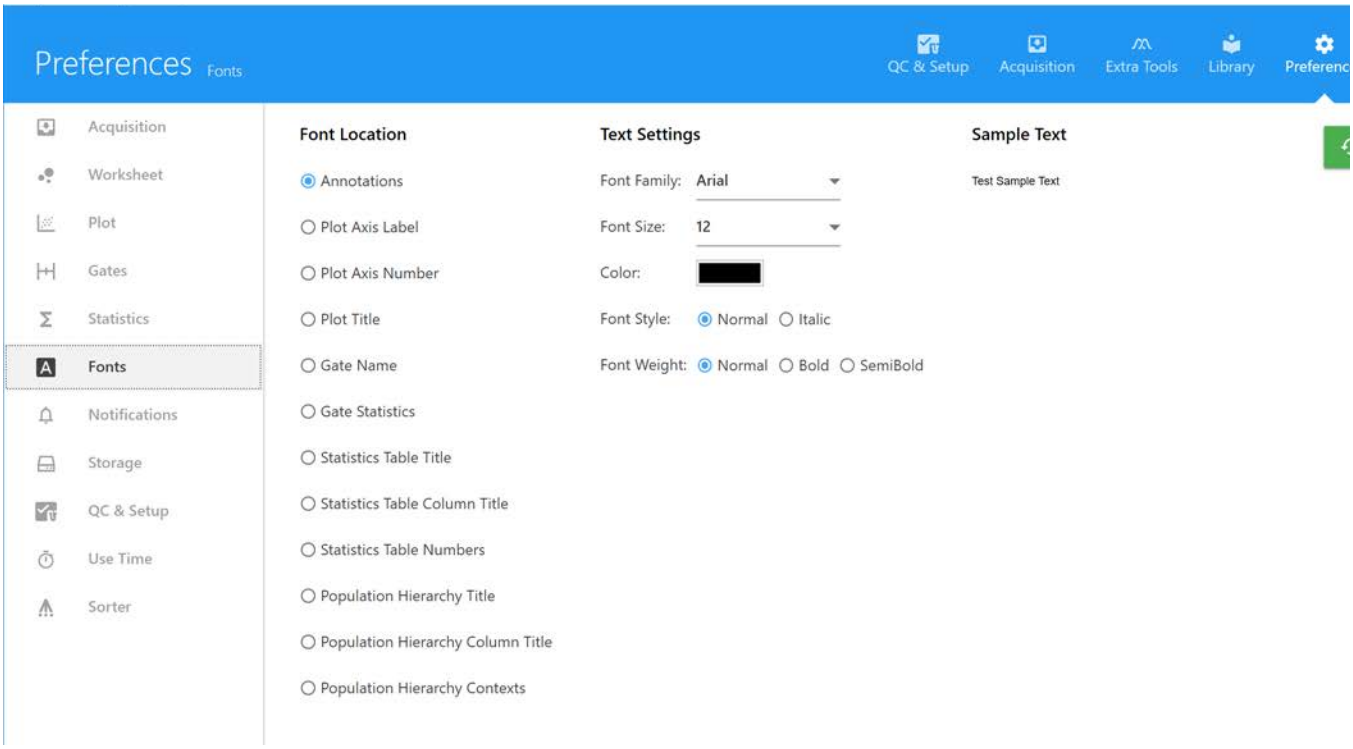
The default degree of precision (number of decimal places) of the statistics displayed in the worksheet can be modified in the Statistics tab.

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



Fonts Preferences

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

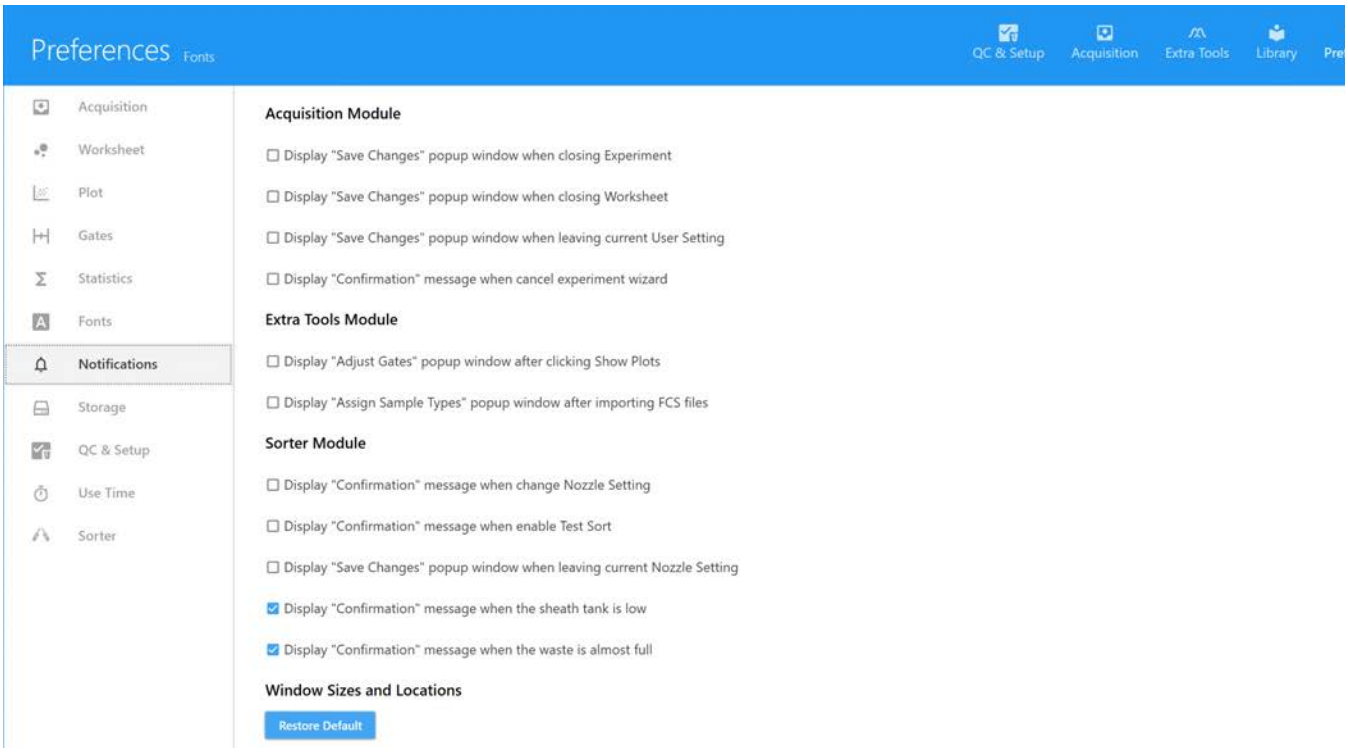


The following table describes the options in the Fonts preferences.

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 Preferences

The Notifications tab allows you to change certain notification settings in the Acquisition and Extra Tools modules.



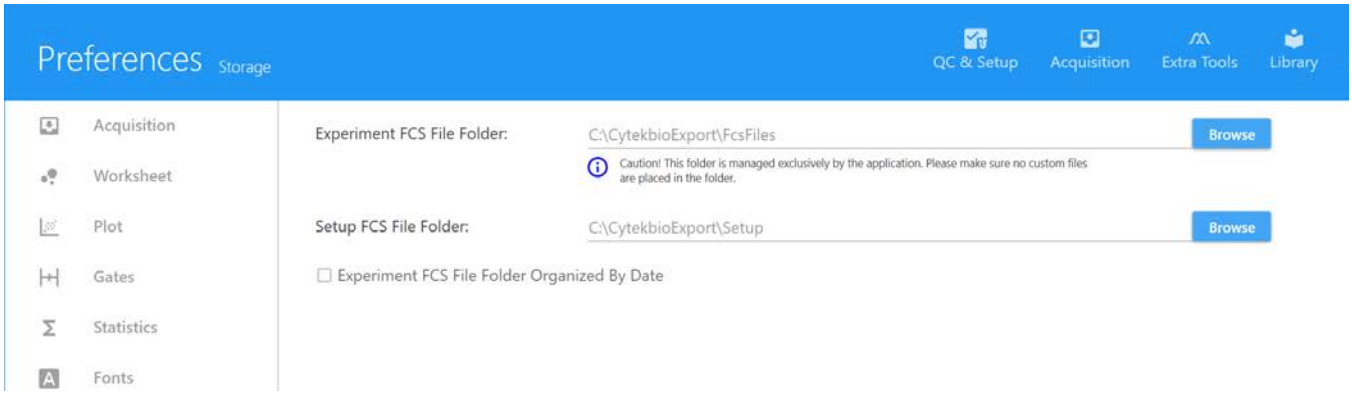
The following table describes the options in the Notifications preferences.

Item	Description
Acquisition Module	Toggles whether to display the Save Changes pop-up window when closing an Experiment, Worksheet, or User Settings, as well as whether to display a confirmation message when canceling the experiment wizard.
Extra Tools Module	Toggles whether to display the instructional dialog boxes in the Extra Tools module.
Sorter Module	Toggles whether to display a confirmation message when changing nozzle settings, enabling test sort, and when the sheath tank is low and the waste tank is almost full. You can also toggle to have a Save Changes pop-up window when leaving the current nozzle settings.
Window Sizes and Locations	Select Restore Default to set pop-up window sizes and locations to the defaults.

Storage Preferences

The Storage tab allows administrators to set the default storage locations for the experiment FCS files and setup FCS files. This option is available only for administrators.

■ **NOTE:** Do not move files stored in these folders.

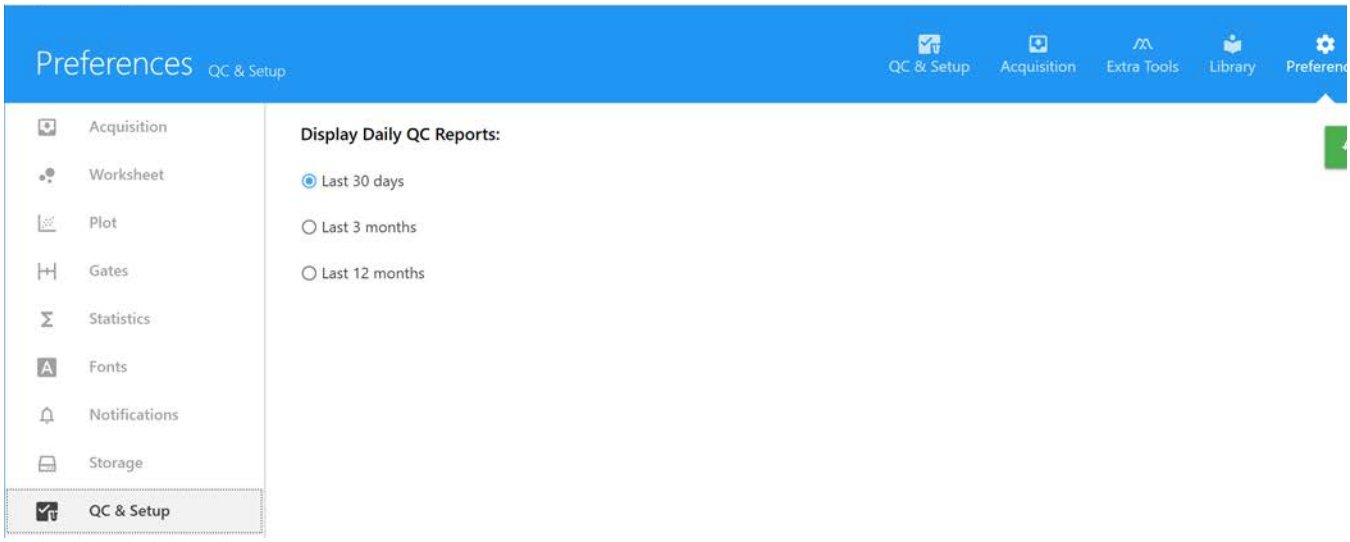


The following table describes the options in the Storage preferences.

Item	Description
Experiment FCS Files Folder	Select the folder where Experiment FCS files are saved. FCS files from experiments where only raw data was acquired, as well as FCS files from experiments where live unmixing was performed are stored here. For experiments where live unmixing is performed, both raw and unmixed FCS files are saved.
Setup FCS Files Folder	Select the folder where FCS files generated by QC & Setup procedures are saved.

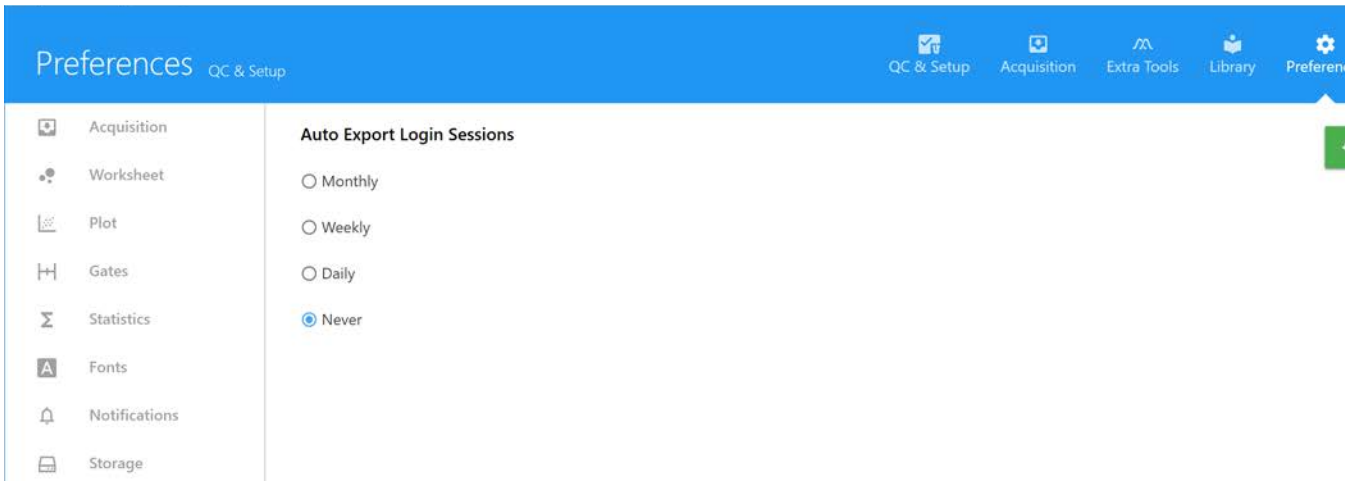
QC Setup Preferences

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



Use Time Preferences

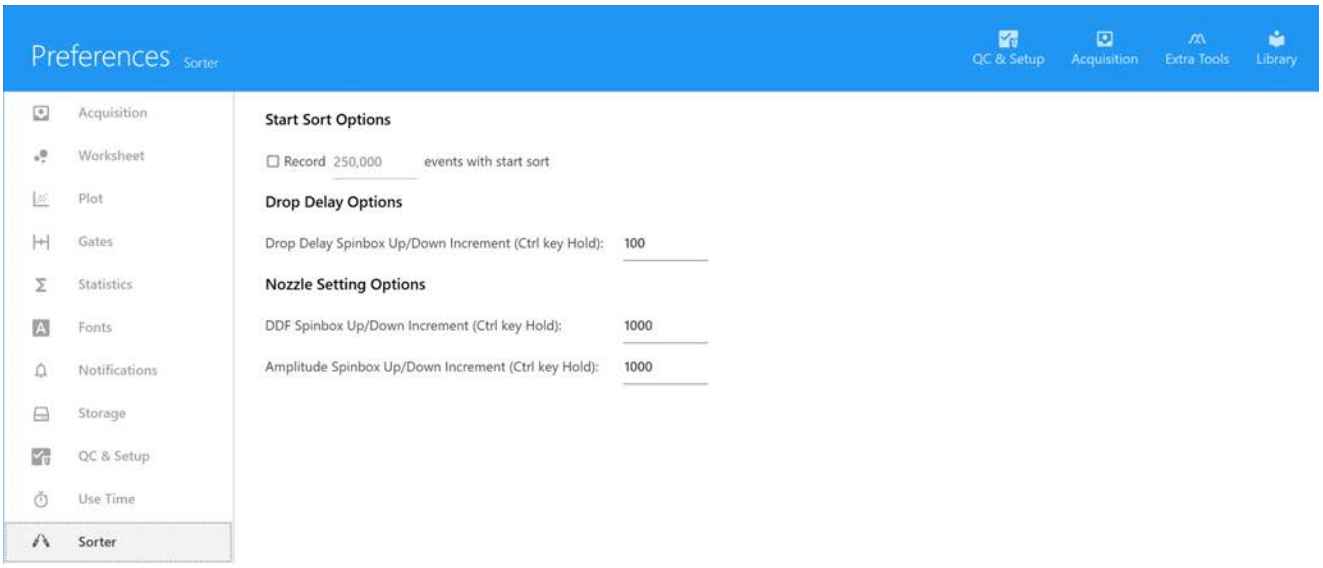
The Use Time tab allows you to select if and when (daily, weekly, monthly) to automatically export the login sessions. The auto-exported use time files are exported to C:\CytekbioExport\LoginSessions. For more information on exporting login sessions, see "Use Time" on page 144.



Sorter Preferences

Sorter preferences allow you to set the amount that the drop delay, DDF, and amplitude spinboxes increment when you hold the Ctrl key and select the up and down arrows of the spinbox. The drop delay spin box can be found in the Drop Delay Adjuster when you select Adjust Drop Delay in the Acquisition Sorter screen.

You can also use the Start Sort Options feature to automatically start acquisition and record the number of events you define (up to 250,000) when you click Start Sort during your experiment.



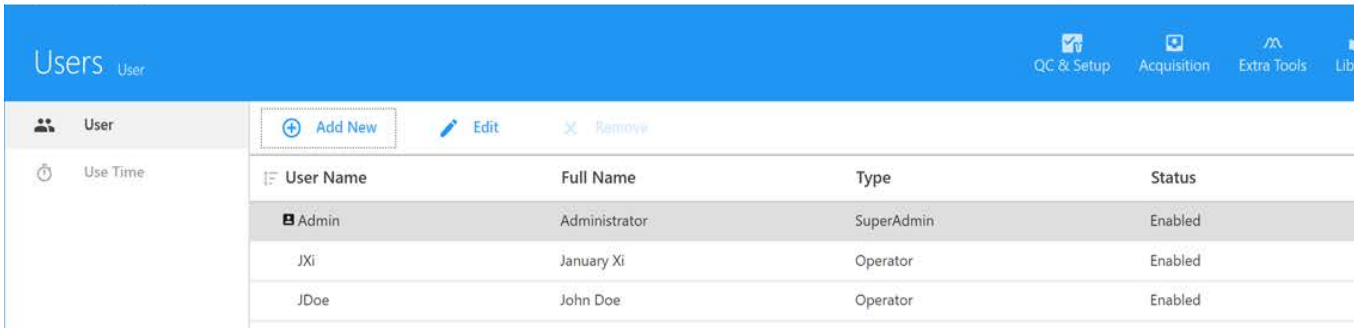
Users

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

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

Managing Users

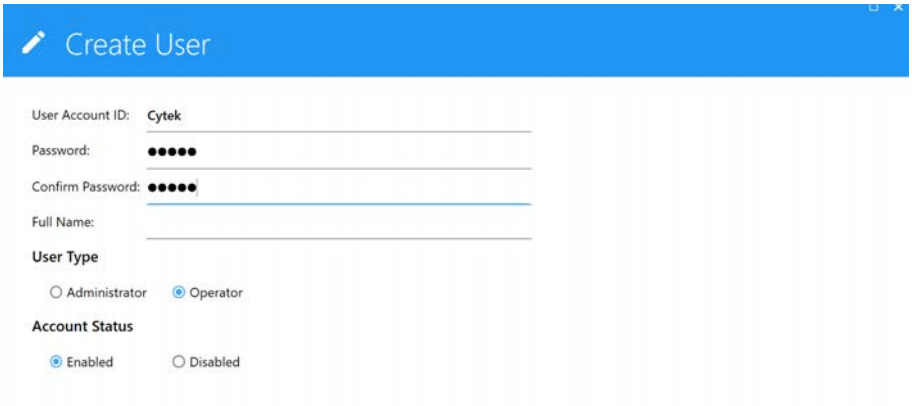
Administrators can add, remove, edit, and disable user accounts from the User tab. User passwords can also be changed/reset. The User tab lists all users and displays the type and status of each.



Adding a New User Account

1 Click ⊕ Add New in the User tab of the Users module.

This option is available only for administrators.



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

The user account ID appears in the user name filed in the User's tab.

3 (Optional) Enter the user's full name.

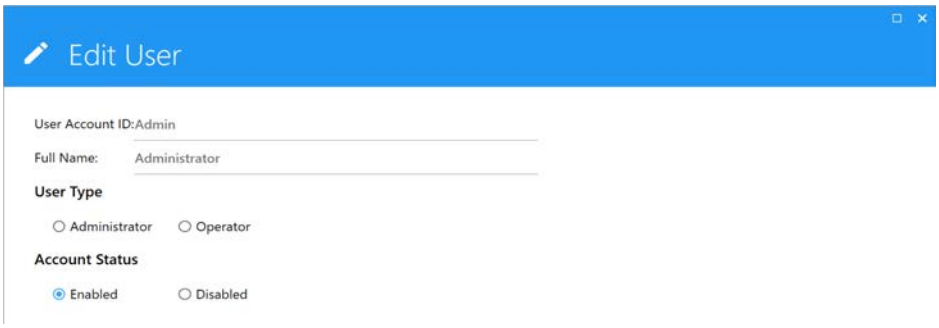
4 Select the user type—administrator or operator.

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 module, then click **Edit**.



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

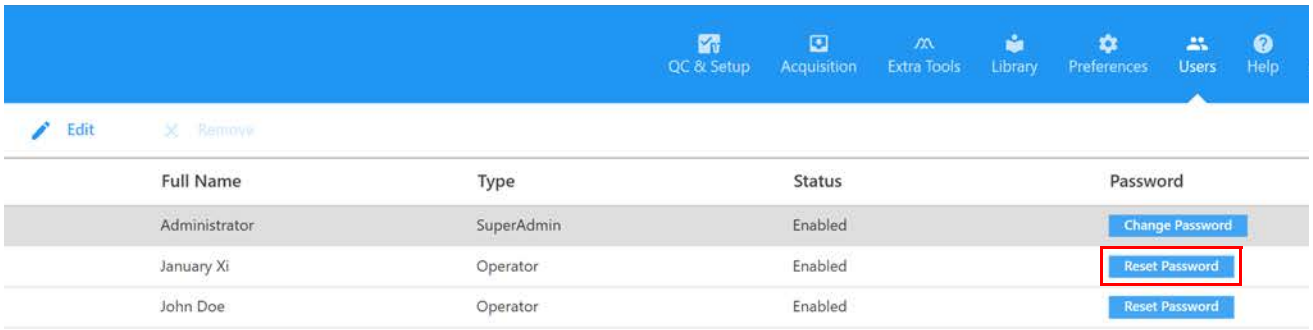
3 Click **Save**.

Resetting a User Password

An administrator can change their or another administrator's password or reset an operator's password.

1 Select the user from the User tab of the Users module.

2 Click **Reset Password**.



3 A dialog opens displaying the new password.

4 Make note of the password and click **OK**.

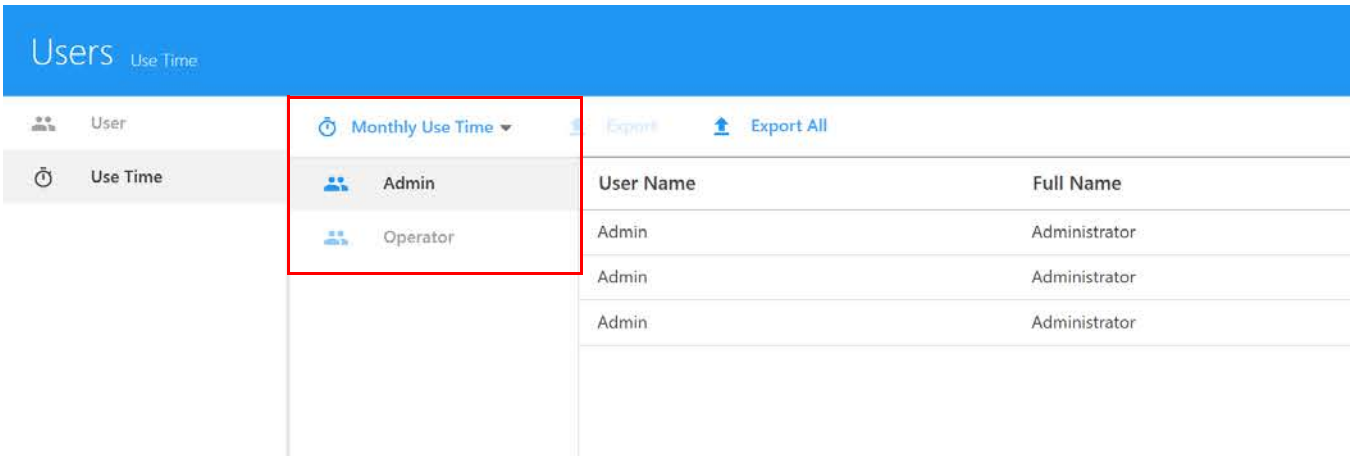
Removing a User Account

To remove a user account, select the user from the User tab of the Users module, then click **Remove**. When you remove a user account all the account information and that data acquired by that user is deleted. The FCS files saved by the user are retained.

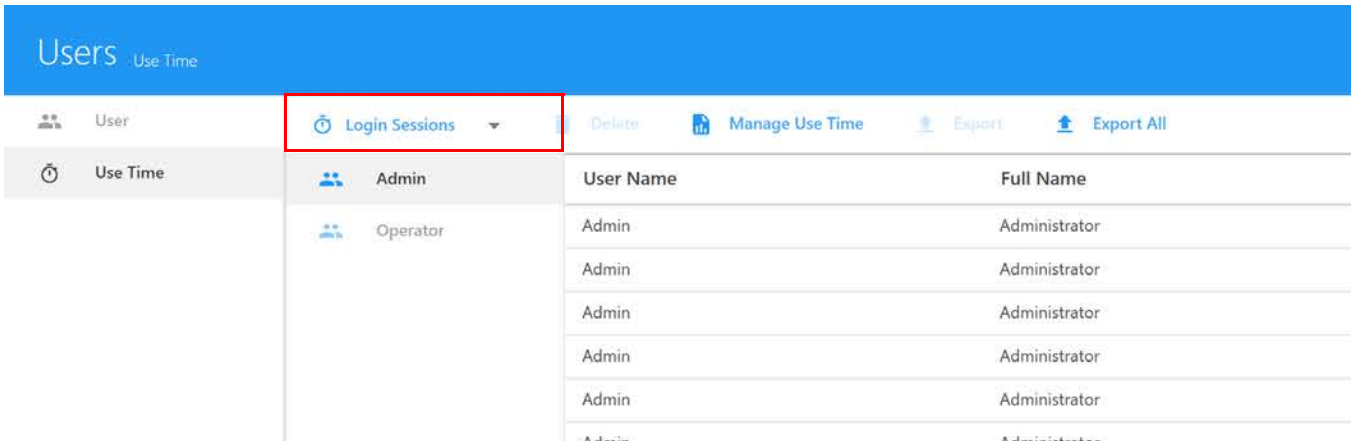
Use Time

Select the Use Time tab of the Users module.

Use Time shows the total daily, weekly, or monthly use time (duration) and number of sessions that each user is on the system. Choose Admin or an operator to view and export the use times for the respective user account.



To see the individual login sessions for each day, select **Login Sessions**. Choose Admin or Operator to view and export the login sessions for the respective user account. The log on and log off times for each session, as well as the session duration are displayed. Administrators can manage the list of login sessions by deleting all sessions before a specified date. To delete sessions, click **Manage Use Time**, select the date, and click Delete.



Administrators can export daily, weekly, and monthly use times and login session to .csv files by selecting the user(s) and session dates and clicking **Export** or selecting Admin or user(s) and clicking **Export All** to export all use times or login sessions.

10

Glossary

amplitude, drop break-off	The force applied to the nozzle to maintain the drop break-off. The higher the amplitude, the shorter the break-off. The value can be adjusted from 0–65,535. The break-off is maintained by setting a consistent frequency, and the amplitude is adjusted as needed by the sort monitoring function.
APD	Avalanche photodiode (APD) is a highly sensitive semiconductor electronic device for measuring light intensity. APDs convert light to electricity and can be thought of as photodetectors.
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.
DDF (drop drive frequency)	The speed (Hz) at which the drop drive generates droplets. The value can be adjusted from 0–100,000 Hz. The higher the frequency, the more droplets are produced. The droplets are smaller, and the sort can be faster without affecting yield. Lower frequencies generate larger, slower droplets.
deflection	The process by which charged droplets travel through an electronic field generated by high-voltage plates into collection tubes.
detector	A device that responds to a specific stimulus. Photodiodes and APDs 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.
drop delay	The amount of time between the laser interrogation point and the point at which the first droplet breaks off, measured in microseconds.
drop interval	The distance between the first satellite or the first broken-off drop, and the last attached drop (used to monitor and maintain the drop delay).
efficiency	The number of target cells sorted divided by the total number of target cells detected.
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.
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 chemical compound that absorbs light and re-emit 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.
mask	A sort decision zone set to select or reject a droplet based on the user defined priority of purity and yield.
optical filter	An optical device that blocks the passage of part of the incident light, allowing the rest to pass virtually unchanged.
photodiode	A device for measuring light intensity. A photodiode generates an output current proportional to the incident light intensity.
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.
rSD	Robust standard deviation. The robust SD is based on the deviation of individual data points to the median of the population.
sensitivity	A measure of a cytometer’s ability to distinguish particles from background noise. It is often expressed in terms of a minimum number of fluorescent molecules per particle required to distinguish a stained particle from an unstained particle. Sensitivity depends on the instrument, the dye, and the preparation method.
sample loading station	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.
sort coincidence	The arrival of a target event and a non-target event, or two target events with a phase mask, that signals the sorter to abort the sort decision.
sort mode	A combination of masks, which allow you to prioritize a combination of purity and yield decisions.

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.
spectral unmixing	The mathematical method of deconvolving signals from multiple fluorophores/dyes to differentiate them.
spillover	Emitted light from a fluorophore entering the detector of another fluorophore.
strobe	An LED device that generates a fixed stream image.
voltage	Measure of electric potential. The voltage applied to a detector affects its amplification gain.
yield	The number of target cells in the collection tube, divided by the number of target cells in the original sample.

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Specifications

Cytometer

Optics

Item	Specification
Optical platform	Fixed optical assembly configured with up to five spatially separated laser beams. Laser delays are automatically adjusted during instrument QC.
Lasers (up to five lasers are available)	355 nm: 20 mW (ultraviolet) 405 nm: 100 mW (violet) 488 nm: 50 mW (blue) 561 nm: 50 mW (yellow green) 640 nm: 80 mW (red) Base model three-laser configuration includes 405-, 488-, and 640-nm lasers. Available upgrade includes 355- and 561-nm lasers.
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
Side scatter detectors and filters	Two high-performance semiconductor detectors with 405- and 488-nm bandpass filters
Fluorescence detectors	High-sensitivity Coarse Wavelength Division Multiplexing (CWDM), 16-channel semiconductor detector array per laser, enabling more efficient spectrum capture for dyes emitting in the 365-nm to 829-nm range. No filter changes required for any fluorophore excited by the on-board lasers. **NOTE:** See "Detector Bandwidths Specifications" on page 80.

Fluidics

Item	Specification
General operation	Pressurized fluidics with the following fluidics modes: Long Clean, SIT Flush, Purge Filter, Clean Flow Cell, Fluidics Shutdown
Compatible tubes	12 x 75-mm polystyrene and polypropylene tubes
Fluidic reservoirs	10-L fluid tanks with level-sensing provided. Compatible with 20-L sheath and waste cubitainers
Sample flow rates	Ten preset settings measured by the flow meter, correspond to the following approximate flow rates: 1 – 10 µL/min 2 – 17 µL/min 3 – 24 µL/min 4 – 31 µL/min 5 – 38 µL/min 6 – 45 µL/min 7 – 52 µL/min 8 – 59 µL/min 9 – 66 µL/min 10 – 73 µL/min
Data acquisition rate	25,000 events/s (for five-laser systems)
Carryover	≤0.1%

Fluorescence and Scatter Sensitivity

Item	Specification
Fluorescence linearity	FITC R^2 ≥0.995 / PE R^2 ≥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
Side scatter resolution	Capable of resolving 0.1-µm polystyrene beads from noise

Workstation

Workstation specifications may vary among laser configurations. The following table provides specs for the

Item	Specification
Operating system	Microsoft® Windows® 10 Pro, 64-bit
Processor	Intel® Core™ i7 processor, 3.6 GHz
RAM	64 GB
Hard drive	500 GB SSD/1TB SATA
Video processor	530 NVIDIA® GeForce
Monitor	Two 24-in UHD 4K monitors
Minimum requirements for SpectroFlo CS software	Windows 10 OS, Intel Core i7 processor, 16 GB RAM, and 1 TB hard drive

Installation Requirements

Item	Specification
Dimensions (W x D x H)	75 x 57 x 65 cm (29.5 x 22.4 x 25.6 in)
Weight	105 kg (231.5 lb)
Workstation	3.5 x 18.3 x 17.9 cm (1.4 x 7.2 x 7.0 in)
Recommended workspace	183 x 81 x 94 cm (72.1 x 31.9 x 37in)
Power	100–140 VAC, 15A 200–250 VAC, 10A
Fuse rating	250 VAC, 5 A, size 5 x 20-mm
Heat dissipation	1,000 W with all solid-state lasers
Temperature	18°C – 28 °C outside the biosafety cabinet 18°C – 26 °C inside the biosafety cabinet
Air supply	551.5–586 kPa (80–85 psi) clean, dry air
Environmental conditions	Indoor use
Humidity	20% to 85% relative non-condensing
Air filtering	No excessive dust or smoke
Lighting	No special requirements

Sorting

Item	Specification
Nozzles	70-, 100-, and 130-µm nozzles with predefined and customizable settings
Sort collection	• Up to 4-way sorting in 5-mL and 1.5-mL polystyrene and polypropylene tubes • Up to 6-way sorting in 1.5-mL polystyrene and polypropylene tubes • 96-well plate with index sorting (deposit 1 cell/well into 96 wells in <2 minutes)
Sort modes	Enrich, Fine Tune, Gross Adjust, Mixed, Multiway, Purity, and Single Cell; plus user-definable sort modes
Sort purity	1%–2% population of lymphocytes using a 70-µm nozzle, mixed sort mode, and a system threshold rate of 20,000 events/s Sort purity ≥95% and sort yield ≥90% to theoretical yield
Sort gates	Sort up to 6 populations up to 64 levels deep in the gating hierarchy

12

Troubleshooting

This chapter 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.

Electronics Troubleshooting

Observation	Possible Causes	Recommended Solutions
Instrument does not power on	Power switch at the back of instrument is off	Check power switch on back.
	Loose power plug on the back of the instrument or at the wall socket	Check both ends of the power cable to make sure it is firmly seated.
Connectivity issue: Software fails to connect to cytometer	Software initialization failed	Close and re-open software. If still no connection, power cycle the instrument and computer.
	Loose USB connection	Reseat USB connection to both the computer and the instrument.
		Replace USB cable.
Connectivity issue: No connection, software does not attempt to connect to the instrument	Loose USB connection	Reseat USB connection to both the computer and the instrument.
		Cycle power for the instrument and the computer.
		Replace USB cable.

Fluidics Troubleshooting

Observation	Possible Causes	Recommended Solutions
Sheath level not reported	Scale not connected or not correctly connected	Reconnect scale at both ends.
Sheath tank reported empty when it is not	Sheath level too low	Refill tank.
	Scale not connected or incorrectly connected	Reconnect scale at both ends. If error persists, and must run samples, ignore the error. Keep track of the sheath level and contact Technical Support.

Observation	Possible Causes	Recommended Solutions
Sheath tank does not pressurize	Air source not turned on or not connected to tank	Check connections, and ensure air source is turned on.
	Tank lid not properly sealed	Re-position and close lid.
		Check for leaks on the tank, and connections.
		Check O-ring seal.
	Tank lid gasket is damaged	Replace gasket.
Waste tank shows to be full when empty	Waste tank float is stuck	Clean sensor on lid with DI water.
		Replace waste lid.
	Sensor wire broken	Replace sensor.

Stream Troubleshooting

Observation	Possible Causes	Recommended Solutions
Stream does not turn on	System is not pressurized	Check that air source is turned on.
		Check connections on the sheath tank and verify fluid/air connections on the front of the instrument.
		Check the sheath filter connection.
		Check that tank lid is properly sealed.
		Check for bubbles in the sheath filter. Run a purge filter to help remove bubbles.
	The nozzle is clogged	Sonicate the nozzle.
		Try a different nozzle.
	No sheath in the sheath tank	Fill tank with sheath and make sure to remove all air from system.
Center stream not centered in Center Stream Aspirator	Nozzle performance	If you have changed nozzles, adjust stream using Sort Stream Adjuster and Center Stream Aspirator Adjuster.
		Remove the nozzle and sonicate.

Observation	Possible Causes	Recommended Solutions
No stream, or dripping in sort block	The nozzle is clogged, or the O-ring is damaged.	Remove the nozzle and sonicate.
		View the nozzle under a microscope and check for damage to the nozzle or O-ring. Replace the O-ring, if needed.
	Nozzle locking lever not engaged	Ensure the nozzle locking lever is fully engaged.
The nozzle is wet when removed Note: The nozzle insertion area must be dried before re-inserting.	Defective O-ring	Replace O-ring.
	The nozzle is missing an O-ring when removed, O-ring is in flow cell	Remove the nozzle and try to remove O-ring from the flow cell.
	The nozzle is not correctly inserted	Remove the nozzle, dry with lens paper, and re-insert.
	Incorrect pressure settings applied	Check that the nozzle settings are correct for current nozzle.
Stream coming out of nozzle at an angle	The nozzle is not correctly inserted	Reseat the nozzle.
	The nozzle is clogged, or the O-ring damaged	Sonicate the nozzle, inspect O-ring, replace, if needed.
	Defective O-ring	Inspect O-ring under microscope, replace if needed.
Aspirator full and overflowing/foaming	Stream not aimed at center	Adjust aspirator aim slightly to the left or right.
	Aspirator clogged	Run 10% bleach into aspirator. Check for debris, such as the nozzle O-ring, in the center aspirator.

Stream Break-off Troubleshooting

Observation	Possible Causes	Recommended Solutions
No break-off profile	Break-off settings not optimized	Adjust amplitude or DDF.
		Check that the selected nozzle settings match the size of the inserted nozzle. Verify that the frequency matches the last setting for the nozzle size being used.
	The nozzle is clogged, or there is a defective O-ring	Sonicate the nozzle.
		Inspect O-ring under microscope, replace if needed.
		Replace the nozzle.
	Air in the system	Let stream run for 10 minutes then adjust the amplitude. Adjust DDF if changed from the last saved setting.
		Bring DDF down to 15,000 and increase amplitude to 35,000 for 20 seconds, and then bring back to original DDF.
		Perform 2 SIT flushes.
		Turn stream off and on several times.
	Incorrect sheath fluid	Make sure there is a PBS-type solution in sheath tank.

Observation	Possible Causes	Recommended Solutions
Unstable (jumping) stream with sort monitoring OFF	Air in the system	Let stream run for 10 minutes then adjust the amplitude. Adjust DDF if changed from the last saved setting.
		Bring DDF down to 15,000 and increase amplitude to 65,000 for 20 seconds, and then bring back to original DDF.
		Perform 2 SIT flushes.
		Turn stream off and on several times.
	Ambient light disturbs the stream lighting background which can affect sort monitoring	Close the sort block door.
	The nozzle is clogged, or there is a defective O-ring	Sonicate the nozzle, inspect O-ring, replace, if necessary.
		Replace the O-ring, if necessary.
		Replace the nozzle.
	Sheath/air lines perturbed	Check air lines and place away from obstructions.
	Air bubbles in the sheath filter	Run a Purge Filter.

Observation	Possible Causes	Recommended Solutions
Unstable (jumping) stream with sort monitoring ON	Air in the system	Turn stream off and on several times and observe stream.
		Bring DDF down to 15,000 and increase amplitude to 65,000 for 20 seconds, then back to original DDF.
		Adjust DDF/Amplitude.
	Ambient light disturbs the stream lighting background which can affect sort monitoring	Close the sort block door.
	The nozzle is clogged, or there is a defective O-ring	Sonicate the nozzle, inspect O-ring, replace, if necessary.
		Replace with another nozzle.
	Sheath/air lines perturbed	Check air lines and place away from obstructions.
	Break-off settings not optimized	Turn Stream Monitoring off and on.
		Check drop interval and make sure it is correct for the nozzle size. See nozzle settings chart in the User Guide.
	Dirty stream camera	Clean camera window (use lens paper) or mirror.

Sorting Troubleshooting

Observation	Possible Causes	Recommended Solutions
Test Sort: No side streams observed in the Side Stream Adjuster view	Door open	Close chamber door.
	Side stream laser not hitting streams	Adjust side stream laser.
	The drop charge settings are incorrect	Check Drop Charge settings.
	Deflection Plate voltage not optimized	Ensure that the Deflection Plate voltage is correct for the nozzle size. See the nozzle settings chart in the User Guide. Change Deflection Plate voltage, if necessary. ⚠ **WARNING:** Make sure the Deflection Plate voltage is turned off before performing this step!
		Turn off stream and reseat the charge plates, if necessary.
	Side stream camera window dirty	Use lens paper to clean Side Stream Window.
	Air in the system	Turn test streams on and off.
	Incorrect drop charge	Check Drop Charge settings.
	Software issue	Reboot instrument and software.
	Dirty/wet Deflection Plates	Clean plates and inspect post plug area for salt buildup.
	Deflection Plate voltage not optimized	⚠ Check Deflection Plate voltage. Turn off stream and reseat the charge plates.

Observation	Possible Causes	Recommended Solutions
Side streams unstable	The nozzle is clogged	Sonicate the nozzle.
		Check break-off.
		Clean any extra residue from aerosols/spray.
	Airflow disturbs the streams	Ensure all covers are closed.
	Incorrect settings for the nozzle	Check the nozzle settings.
	Dirty plates	Clean plates. **WARNING:** Make sure the Deflection Plate voltage is turned off before performing this step!
	Salt buildup in chamber or on plates	Clean sort chamber (use DI water).
	Sort chamber doors not closed	Close chamber doors.
Side streams do not have enough deflection	The nozzle settings are incorrect	Check the nozzle settings (pressure/ DDF and Amplitude).
	Wet tube collection device	Clean and dry device using DI water and lens paper.
	Incorrect Deflection Plate voltage	Check Deflection Plate voltage.
	The nozzle is clogged, or the Deflection Plates are dirty	Sonicate the nozzle and clean the deflection plates. **WARNING:** Make sure the Deflection Plate voltage is turned off before performing this step!
Additional side streams or fanning	The nozzle is clogged	Sonicate the nozzle.
	Break-off not optimized	Check break-off settings.
	Center stream not optimized	Optimize the Center Stream (1st, 2nd, 3rd drop...).
	Dirty/wet plates	Clean/dry plates.
Center stream is showing spread	Center stream not optimized	Optimize the Center Stream (1st, 2nd, 3rd drop...).
Excessive aerosols around center stream aspirator/ buckets	Stream hitting edge of center aspirator or aspirator buckets	Adjust drop charge to not hit aspirator or bucket sides.

Observation	Possible Causes	Recommended Solutions
Aerosols observed in Sort Stream Adjuster view when the aspirator buckets are in the open position	Stream is hitting front/back of collection tube(s) or center aspirator or aspirator buckets	Adjust the tube holder so that the side streams are directed to the center of the corresponding collection tube. If the stream is hitting the back of the tubes, contact Technical Support.
Cannot see farthest streams	Drop charge too high	Decrease drop charge to get all side streams in view.
	Side stream laser not optimized	Adjust side stream laser.
	Side stream laser not adjusted correctly	Sweep side stream laser to fully center on side streams.
Cannot complete Drop Delay	Bead concentration incorrect	Remake beads and acquire tube under an Experiment to confirm concentration is between 3,000-7,000 events/second.
	Side stream laser not focused on the streams	Adjust laser by sweeping back and forth to get the brightest signal on camera.
	Break-off not stable	Redo break-off setup to achieve a stable stream.
		Ensure that Sort Monitoring is turned on.
	Auto Drop Delay does not complete	Try setting Drop Delay manually (refer to the user's guide for instructions).
	Camera window dirty	Clean camera window (use lens paper) or mirror.
	Aerosols block viewing of stream	Adjust center stream using the Stream Adjuster in the Stream Viewing window.
	The nozzle settings are incorrect	Recheck the nozzle settings to stabilize break-off.
While sorting, no side streams seen	Sort settings are incorrect	Check sort event rate to see if events are being sorted.
	Sort event rate is too low	Sort event rate needs to be above approximately 200 events /sec to have enough illumination to be seen.

Observation	Possible Causes	Recommended Solutions
No events in collection tube(s)	Streams missing collection device	Check aiming/holder positioning.
	Incorrect gating	Check gating strategy.
		Ensure collected events go into the buffer and are not sticking to the sides of the collection tube.
Poor purity	Sort mode not ideal	Change sort mode.
	Break-off not stable	Recheck the nozzle settings to stabilize break-off.
		Ensure that Sort Monitoring is turned on.
	Drop Delay incorrect	Redo Drop Delay (manual or automatic).
	Gating conflicts	Verify the gates make biological sense, the gating hierarchy is correct, and there are no common parents gates that have conflicting logic for the sorted populations.
	Threshold too high	Lower threshold to better identify all biological events for sorting.
	Doublet discrimination not used	Set up gating to eliminate doublets (aggregates).
	Sample preparation issue	Check sample prep protocol and check samples.
	Shifting populations	Possible air in system. Follow recommendations above for removing air.
		Sample too concentrated, dilute sample.
	Event rate too high and high aborts	Lower flow rate or dilute samples.
	Flow rate too high	Lower flow rate.

Observation	Possible Causes	Recommended Solutions
Poor yield	Poor stream trajectory	Check side streams to make sure they are centered in the collection tube.
		Check the holder position to make sure it is completely pushed in.
	Not enough or incorrect buffer in collection tubes	Test to find the correct type or ideal amount of buffer to maintain viability and support the number of cells sorted into collection device.
	Abort rate high	Check flow rate.
		Dilute sample.
		Check gating.
	Population shift/gating	Possible air in system. Follow recommendations above for removing air.
		Check to make sure gates are fully around your population of interest.
	Event rate vs. DDF	Recommended event rate should be 1:5 of DDF.
	Not ideal sort mode	Change sort mode to ensure better yield.

Plate Sorting Troubleshooting

Observation	Possible Causes	Recommended Solutions
DDU: Puck does not move smoothly	Dirty separation plate	Clean plate area using DI water.
	Dirty or stuck puck wheels	Clean wheels using DI water.
		Sonicate puck to clean wheels.
Strange puck movement	Puck alignment issue	Check alignment with landing lights.
	Plate lid hit the live view camera	Remove lid for sorting if it is partially on. Do not use the lid to partially cover the plate while sorting.

Observation	Possible Causes	Recommended Solutions
Missing wells	Plate not installed on puck correctly	Ensure plate is properly installed on the puck.
	Stream to well alignment incorrect	Adjust drop charge.
		Re-verify alignment of the stream to the plate well.

Acquisition Troubleshooting

Observation	Possible Causes	Recommended Solutions
High electronic abort rate (greater than 10% of event rate)	Threshold too low	Increase Threshold.
	Event rate too high	Decrease flow rate.
	Sample aggregated	Filter sample.
	Sample too concentrated	Dilute sample.
Daily QC does not complete	Wrong QC bead sample	Ensure you are running SpectroFlo QC beads.
	Wrong nozzle setting selected	Select the correct nozzle setting before running Daily QC.
	Bead sample not properly mixed	Mix the bead sample.
	Bead sample too dilute	Concentrate the bead sample or prepare a fresh bead sample.
	Air bubble in sample line	Run a SIT Flush.
	Degraded beads	Prepare fresh beads.
Daily QC failed	Air bubble in fluidics	Run a Purge Filter.
	Dirty flow cell	Run a Clean Flow Cell. If the problem persists, run a Clean Flow Cell using 25%–50% Contrad 70, followed by DI water.
	Wrong nozzle setting selected	Select the correct nozzle setting before running Daily QC.
	QC beads not prepared properly	Verify the 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.

Observation	Possible Causes	Recommended Solutions
No events detected when acquiring sample	Leak in sample chamber preventing proper pressurization	Lubricate sample chamber seal on tube platform.
	Sample line clogged	Perform SIT flush. Replace sample line.
	Sample line height position wrong	Adjust sample line to correct position.
	Air Pressure issue	Check to make sure you are using the proper air pressure setting for your nozzle. Try default pressure if using custom setup.
	Sample ran dry or no sample loaded	Check for presence of tube and cells.
	Flow Cell door open	Close door.
	Flow rate too low	Increase flow rate.
	Threshold too high	Lower the threshold.
	Threshold set to incorrect parameter	Set the threshold to the appropriate parameter for the application.
	No data in gate	Check gating strategy, move gate.
	Sample not properly mixed	Mix the sample to suspend cells/ particles.
		Turn on mixing to resuspend sample while sorting.
Event rate dropping over time	Sample settling over time	Mix sample before installation, and turn on mixing to resuspend the sample while sorting.
	Partial clog of sample line	Perform SIT flush, run 10% Bleach.
		Replace sample line.

Observation	Possible Causes	Recommended Solutions
Erratic event rate	Partial clog of sample line	Perform SIT flush, run 10% Bleach.
		Replace sample line.
		Run a Clean Flow Cell.
	Sample clumping	Remove sample and filter, vortex or disaggregate sample.
		Adjust sample buffer to minimize clumping.
	Air in sheath fluid not removed by degasser	Check sheath fluid in sheath tank and replace, or degas, as needed.
	Sample is contaminated	Prepare a new sample and ensure the tube is clean.
High event rate	Threshold is too low	Adjust Threshold.
	Sample is too concentrated	Dilute the sample.
	Flow rate is too high	Decrease flow rate. Recommended event rate should be 1:5 of DDF.
	Bubbles in flow cell	Turn stream off and on.
Low event rate	Sample not mixed well	Turn on mixing to resuspend sample while sorting.
	Threshold too high	Adjust Threshold.
	Sample too dilute	Concentrate sample.
	Sample line is clogged or kinked	Perform SIT flush, run 10% Bleach followed by DI water.
		Replace sample line.
Distorted data in scatter plots	Air in sheath filter	Run a Purge Filter.
	Air in flow cell	Remove sample and turn stream off and on.
	Dirty flow cell	Run a Clean Flow Cell.
	Air in fluidics	Run a SIT flush.
		Check degasser.
	Area scaling incorrect	Verify area scaling.

Observation	Possible Causes	Recommended Solutions
High CVs	Air bubble in fluidics	Run a SIT Flush and a Purge Filter.
	Sample flow rate set too high	Lower flow rate and monitor electronic abort rate, gates, and conflict rate.
	Dirty flow cell	Run a Clean Flow Cell. If the problem persists, run a Clean Flow Cell using 25%–50% Contrad 70, followed by DI water.
	Questionable Sample prep	Verify the sample prep technique.
	Air in sheath filter	Run a Purge Filter.
Excessive debris in plots	Threshold too low	Adjust Threshold.
	Dead cells or debris in sample	Make sure you have a viability gate to exclude dead cells or debris. Verify sample viability and cleanliness using a hemocytometer.
	Sample is contaminated	Prepare a new sample.

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Cytek Biosciences, Inc
47215 Lakeview Blvd.
Fremont, CA 94538
1.877.92.CYTEK (1.877.922.9835)

products@cytekbio.com
cytekbio.com

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