

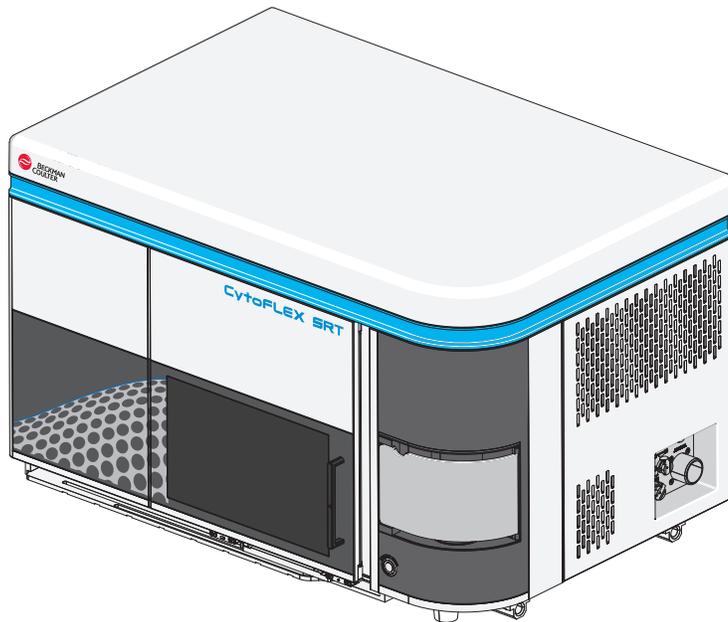


Instructions for Use

CytoFLEX SRT

Cell Sorter

For Research Use Only. Not for use in diagnostic procedures.



C37808AC
March 2022



Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821 U.S.A.



CytoFLEX SRT
Cell Sorter
PN C37808AC (March 2022)

Original Instructions

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- In Austria, call us at 0810 300484.
- In Germany, call us at 02151 333999.
- In Sweden, call us at +46(0)8 564 859 14.
- In Netherlands, call us at +31 348 799 815.
- In France, call us at 0825838306 6.
- In the UK, call us at +44 845 600 1345.
- In Ireland, call us at +353 (01) 4073082.
- In Italy, call us at +39 0295392 456.
- In other locales, contact your local Beckman Coulter Representative.

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www.beckman.com/patents



Beckman Coulter (UK) Ltd.
Oakley Court
Kingsmead Business Park, London Road
High Wycombe
United Kingdom HP11 1JU
01494 441181

Glossary of Symbols is available at beckman.com/techdocs (PN C24689).

Revision History

Initial Issue AA, 01/2021

Software Version: CytExpert SRT 1.0

Issue AB, 08/2021

Software Version: CytExpert SRT 1.0

Updates were made to the following sections:

CHAPTER 1, System Overview

- Sample Temperature Control

- Sorter

- Performance Characteristics

CHAPTER 2, Using the CytExpert SRT Software

- Acquisition/Sorting Control

- Software Menu

- Sort Mode Library

- Software Settings

CHAPTER 4, Instrument Quality Control and Standardization

- Overview

- Preparing the QC Sample

- Importing Lot-Specific Target Values

- Collecting QC Data

- Preparing the Standardization Sample

CHAPTER 5, Sorting

- Default Amplitude Setting (Optional)

- Setting Sample Flow Rate

- Setting Up Tube Sorting

- Setting Up Plate/Slide Sorting

- Calibrating the Sort Collection Device

- Manual Side Stream Calibration (Optional)

- Side Stream Monitor

- Auto Maintain

- Viewing Sorting Statistics

- Pause Sorting and Resume Sorting

CHAPTER 7, Data Review

- Calculating Sample Concentration

This document applies to the latest software listed and higher versions. When a subsequent software version affects the information in this document, a new issue will be released to the Beckman Coulter Web site. For labeling updates, go to www.beckman.com and download the latest version of the manual or system help for your instrument.

CHAPTER 9, Troubleshooting

- Laser Warning Labels
- Troubleshooting Table

CHAPTER 11, Replacement/Adjustment Procedures

- Replacing the Nozzle
- Replacing the Sample Line
- Setting Laser Delay

APPENDIX B, Consumables

- Consumables

APPENDIX C, Biosafety Cabinet Accessory

- Biosafety Cabinet Installation
- Biosafety Cabinet Specification

APPENDIX E, Good Practices for Cyber Security

- Installing the Operating System Updates

Issue AC, 03/2022

Software Version: CytExpert SRT 1.1

Updates were made to the following sections:

CHAPTER 1, System Overview

- Fluidics Cart
- Sheath Tank
- Wavelength Division Multiplexer (WDM)
- CyClone Movement System
- System Connections
- Sorter

CHAPTER 2, Using the CytExpert SRT Software

- Overview
- Start Page
- Tube Management
- Software Menu
- Acquisition and Analysis Screen Menu
- Account Menu
- Log Menu
- Backup/Restore Menu
- User Management
- Role Management
- Account Policies
- Operation Log
- Collection Device Library

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- Software Settings
- CHAPTER 3, Daily Startup
 - Logging Into the Software
 - System Startup Program
- CHAPTER 4, Instrument Quality Control and Standardization
 - Creating Levey-Jennings Charts
- CHAPTER 5, Sorting
 - Setting Drop Delay Manually (Optional)
 - Setting the Channel and Label
 - Creating Plots and Gates
 - Adjusting the Gain
 - Setting Collection Conditions
 - Setting Mixing Speed
 - Setting Up Violet Side Scatter (VSSC) Channel
 - Verifying, Selecting, Editing, and Creating Detector Configuration
 - Setting Up Tube Sorting
 - Setting Up Plate/Slide Sorting
 - Adjusting Tube Position (Optional)
- CHAPTER 8, Shutting Down the System
 - Long Term Shutdown
- CHAPTER 9, Troubleshooting
 - Overview
 - Laser Related Hazards
 - Troubleshooting Table
 - Backup and Restore
- CHAPTER 10, Cleaning Procedures
 - Aseptic Clean Program
- CHAPTER 11, Replacement/Adjustment Procedures
 - Removing Trapped Air Bubbles
 - Replacing the Sample Probe
 - Resetting the Sheath Tank Scale
- APPENDIX D, Instrument Installation
 - Overview
 - Power Source
 - Waste Disposal
 - CytExpert Software Installation Options
 - Installing the CytExpert SRT Software
 - Upgrading and Reinstalling the CytExpert SRT Software

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APPENDIX E, Good Practices for Cyber Security

Enabling Audit Object Access

Viewing Windows Security Logs

Note: Changes that are part of the most recent revisions are indicated in text by a bar in the margin of the amended page.

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Safety Notices

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate instrument. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, [contact us](#).

Beckman Coulter, Inc. urges its customers to comply with all national health and safety standards such as the use of barrier protection. This may include, but it is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory analyzer.

This manual assumes that users have basic knowledge of the Windows operating system, as well as experience working with laboratory testing technology. Users are invited to consult the appropriate documentation for such information.

Alerts for Warning, and Caution

WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

Safety Precautions

WARNING

Risk of operator injury if:

- All doors, covers and panels are not closed and secured in place prior to and during instrument operation.
- The integrity of safety interlocks and sensors is compromised.
- Instrument alarms and error messages are not acknowledged and acted upon.
- You contact moving parts.
- You mishandle broken parts.
- Doors, covers, and panels are not opened, closed, removed and/or replaced with care.
- Improper tools are used for troubleshooting.

To avoid injury:

- Keep doors, covers and panels closed and secured in place while the instrument is in use.
- Take full advantage of the safety features of the instrument. Do not defeat safety interlocks and sensors.
- Acknowledge and act upon instrument alarms and error messages.
- Keep away from moving parts.
- Report any broken parts to your Beckman Coulter Representative.
- Open/remove and close/replace doors, covers and panels with care.
- Use the proper tools when troubleshooting.

CAUTION

System integrity could be compromised and operational failures could occur if:

- This equipment is used in a manner other than specified. Operate the instrument as instructed in the Product Manuals.
- You introduce software that is not authorized by Beckman Coulter into your computer. Only operate your system's computer with software authorized by Beckman Coulter.
- You install software that is not an original copyrighted version. Only use software that is an original copyrighted version to prevent virus contamination.

 **CAUTION**

If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and it is not presently under a Beckman Coulter service maintenance agreement, Beckman Coulter cannot guarantee that the product is fitted with the most current mandatory engineering revisions or that you will receive the most current information bulletins concerning the product. If you purchased this product from a third party and would like further information concerning this topic, [contact us](#).

 **CAUTION**

Risk of instrument damage. This device is intended for indoor use only. To avoid device damage, do not install the instrument outdoors.

 **WARNING**

Risk of personal injury. Safety protection can be impaired if used in a manner not specified by the manufacturer. To avoid personal injury, use the instrument according to the manufacturer's instructions only.

Instrument Safety Precautions

The instrument has been engineered with safety as one of its primary features. Safety of the operator, field service personnel, bystanders, and valuable samples, is paramount to Beckman Coulter's commitment to high performance design and engineering.

This section explains some general safety and hazard symbols and necessary precautions operators of the instrument should follow during operation. Engineering controls have been put in place to protect the operator, and deliberate misuse of the instrument or its instructions may result in unintentional harm. Please follow all safety and hazard instructions as directed.

General Safety

To protect the health, environment, and safety of instrument sites and their users, the following information should be reviewed by all operators.

- The instruments are intended for Professional Use Only. All operators should be trained on the proper use and limitations of the instrument prior to its operation.
- Be aware that the Sort Chamber, Interrogation Chamber, and cabinet doors on the instrument present possible pinch points. While the doors are light-weight and do not have sharp edges, care should be taken when opening and closing doors.
- The view screen on the Biosafety Cabinet can be moved manually up and down thereby creating a possible pinch point. Position hands appropriately when moving the view screen on the Biosafety Cabinet.

- The sample station has electronically controlled moving parts. Do not insert your hand in the sample chamber when it is moving.
- Wear appropriately sized gloves providing good manual dexterity to reduce the likelihood of skin pinches and abrasions.
- Use proper lifting techniques or seek assistance when handling the BioSafety Cabinet, or full tanks. Take care to reduce the likelihood of back injury.
- Protect the skin and eyes whenever handling chemicals of any kind, regardless of how benign they may appear.
- Check with the site safety officer for correct disposal of waste products and for spill clean-up protocols.
- The user should rest appropriately to avoid strain due to repetitive use, awkward movements, or sitting too long.

Biohazard Safety

Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

IMPORTANT If any hazardous organism, material, or agent is used in the instrument, the site operator or Principal Investigator is responsible for informing Beckman Coulter in writing of those hazards before receiving service or repair. This includes a list of all pathogenic cell lines, hazardous reagents, radioactive material, or agents with a BSL Level II or higher. This information will be kept confidential and will be used to inform Beckman Coulter Field Service Representatives of any hazards prior to visiting any instrument site. Failure to report this information may delay service on an instrument. Safety of the user as well as safety of Beckman Coulter employees is of overriding importance. Proper decontamination procedures must be followed for all applicable returned parts.

- Gloves, a laboratory coat, and eye protection should be worn whenever handling samples including insertion and removal of sample tubes from the sample station.
- If the system loses vacuum or the waste tube becomes clogged, waste fluid could spill into the sort chamber. Immediately turn off sheath and sample flow, wear proper personal protective equipment and attend to the spill.
- Waste fluid may contain hazardous levels of biological and chemical contamination. Gloves, a laboratory coat and eye protection should be worn whenever exposure to waste fluid exists.
- To ensure inactivation of biological organisms in the waste container, an appropriate type and quantity of an EPA registered disinfectant should be placed in the tank initially upon use, and every time the waste container is emptied and reinstalled.
- The Aerosol Containment Shield, also known as the Sort Chamber door, is part of a passive aerosol containment assembly that isolates the contents of a sort from the rest of the instrument, the operator, and the laboratory. When closed, the door limits movement of air into and out of the Sort Chamber. It is optional to purchase an Aerosol Evacuation system for additional protection from aerosols. See [Aerosol Evacuation System](#).

NOTE Use of the Aerosol Evacuation system precludes the use of Biosafety Cabinet, or vice versa.

- A BSL-2, Level A2 Biosafety Cabinet is available for purchase as an optional system accessory. Refer to [APPENDIX C, Biosafety Cabinet Accessory](#).

For additional information on laboratory biosafety, please review the U.S. Department of Health and Human Services, Centers for Disease Control document, *Biosafety in Microbiological and Biomedical Laboratories*. Contact the safety officer at your site and discuss proper waste disposal precautions and practices. Consult the Original Equipment Manufacturer (OEM) manuals for the Biosafety Cabinet and the Aerosol Evacuation System for additional information.

Electrical Safety

The instrument conforms to international regulations encompassing the accessibility of high voltages by the user (IEC 61010-1) and exposure to laser emission: IEC 60825-1:2014 Safety of Laser Products - Part 1: Equipment Classification and Requirements; 21 CFR 1040 FDA/CDRH Laser Product Performance Standard. Please familiarize yourself with the following features of the instrument and their corresponding potential hazards.

Safety Interlocks

Under normal operating conditions, the instrument protects the user from exposure to high voltages and is considered a Class 1 laser product. The instrument is equipped with four safety interlocks designed to protect the operator from inadvertent exposure to high voltage, laser radiation, and pinch hazard.

- When the Sort Protection door opens, the safety interlock disables the voltage to the deflection plates.
- When the optical bench cover is opened, all the lasers turn off.
- When the sample station door is opened, the movement of the sample chamber stops and the system LED indication turns red.

DO NOT attempt to defeat these interlocks except when this document specifically instructs you to do so. Ensure that you have the proper laser safety training prior to defeating safety interlocks.

Stream Charge

When the sheath stream is charged and individual droplets are formed, the droplets retain the charge present on the stream.

- Do not defeat the safety interlock and insert any object into the charged stream.

Drop Drive Voltage

This ranges from 50-250 Vac and is used to drive the piezoelectric crystal mounted in the nozzle. The frequency can be set either by Auto Droplet Calibration or by the operator.

Deflection Plates



Risk of personal injury. Do not touch the charged plates when power is applied.

The range of voltage applied to these plates is $\pm 3800 \sim \pm 4200$ Vdc. This high voltage is present only when the plate voltage is turned on and the interlock is closed. High voltage is accessible only if the interlock is defeated, and only if the operator inserts an object between the charged plates. Once high voltage is enabled by the operator, it is constant until changed by the operator.

Deflection Plate Arcing

Arcing may occur due to buildup of sheath solution on the deflection plates. If arcing occurs, refer to deflection plate arcing in [CHAPTER 9, Troubleshooting](#).

Laser Power Supplies

Laser power supplies have dangerous amounts of energy and could be a hazard to the operator. [Contact us](#) if laser power supplies require service.

Electromagnetic Compatibility

This device complies with the emissions and immunity requirements as specified in the EN/IEC 61326 series of Product Family Standards for a “basic electromagnetic environment.” Such equipment is supplied directly at low voltage from public mains network. This equipment is not intended for residential use.

 **CAUTION**

This device generates, uses and can radiate un-intentional radio-frequency (RF) energy. If this device is not installed and operated correctly, this RF energy can cause interference with other equipment. It is the responsibility of the end user to ensure that a compatible electromagnetic environment for the device can be maintained in order that the device will operate as intended.

In addition, other equipment can radiate RF energy to which this device is sensitive. If one suspects interference between this device and other equipment, Beckman Coulter recommends the following actions to correct the interference:

1. Evaluate the electromagnetic environment before installation/operation of this device.
2. Do not operate this device close to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these can interfere with proper operation. Examples of unshielded intentional radiators would be hand-held radio transmitters, cordless phones and cellular phones.
3. Do not place this device near medical electrical equipment that can be susceptible to malfunctions caused by close-proximity to electromagnetic fields.
4. This device has been designed and tested to CISPR 11, Class A emission limits. In a domestic environment, this device may cause radio interference, in which case, you may need to take measures to mitigate the interference.

Symbol Explanations

Safety symbols alert you to potentially dangerous conditions. The symbol applies to specific procedures and appears as needed throughout this manual.

Symbol	Warning Condition	Action
	Biohazard Symbol	Consider all materials (specimens, reagents, controls, and monoclonal antibodies) and areas these materials come into contact with as being potentially infectious. Wear appropriate barrier protection and follow safe laboratory procedures when handling any material in the laboratory.
	Caution	To indicate that caution is necessary when operating the device or control close to where the symbol is placed, or to indicate that the current situation needs operator awareness or operator action in order to avoid undesirable consequences.
	Caution, Risk of Electric Shock	To indicate hazards arising from dangerous voltages.

Symbol	Warning Condition	Action
	Pinch	To warn of a closing motion of mechanical parts of equipment.
	Recycling Symbol WEEE Wheeled Bin Symbol	<p>The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates:</p> <ol style="list-style-type: none"> 1. that the device was put on the European Market after August 13, 2005 and 2. that the device is not to be disposed via the municipal waste collection system of any member state of the European Union. <p>For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take-back program which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of device.</p> <p>For the Japan market:</p> <p>This system is considered an industrial waste, subject to special controls for infectious waste. Before disposal of the system, refer to the Waste Disposal and Public Cleaning Law for compliance procedures.</p>
	Laser Radiation	The laser radiation symbol indicates that there can be laser light radiation in the area. Take precautions to prevent exposure.
	RoHS Caution Symbol	This label indicates that the electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.
	California Proposition 65 Symbol	This label indicates that this product can expose you to chemicals including phthalates, which are known to the State of California to cause cancer and birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov .

Symbol	Warning Condition	Action
 <p>CLASS 1 LASER PRODUCT PRODUIT LASER CLASSE 1 1类激光产品</p>	Laser Class I	This label indicates that this product is a Class I Laser product. Take precautions to prevent exposure.
	Consider all materials (specimens, controls, monoclonal antibodies, and so forth) as being potentially infectious.	Wear standard laboratory attire and follow safe laboratory procedures when handling any material in the laboratory.
	UKCA Mark	A "UKCA" mark indicates that a product has been assessed before being placed in UK market, and has been found to meet UK safety, health, and/or environmental protection requirements.

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Overview

This introduction contains the following information:

- [How to Use Your Manual](#)
- [About this Manual](#)
- [Conventions Used](#)
- [Graphics](#)

How to Use Your Manual

Use this **Instructions for Use** manual for information on the day-to-day operation of your CytoFLEX SRT Sorter. You can find detailed step-by-step procedures for Daily Startup and Quality Control, configuring settings, running samples, sorting, analyzing data, and performing Shutdown. This manual also contains physical and system specifications, safety and troubleshooting information, as well as procedures for cleaning and replacement.

About this Manual

The information in your Instructions for Use manual is organized as follows:

CHAPTER 1, System Overview

Provides information regarding the individual components of the CytoFLEX SRT Sorter and the corresponding functions of these components.

CHAPTER 2, Using the CytExpert SRT Software

Provides an overview of each aspect of the software's functions.

CHAPTER 3, Daily Startup

Provides instructions for starting your CytoFLEX SRT instrument and navigating to the sample testing standby state.

CHAPTER 4, Instrument Quality Control and Standardization

Provides instructions for performing daily quality control (QC) on your CytoFLEX SRT instrument to confirm the instrument is working correctly and to ensure accurate experimental data measurement. Quality control allows you to determine whether your instrument can provide adequate signal strength and precision.

CHAPTER 5, Sorting

Provides instructions for operating the CytoFLEX SRT instrument, including data acquisition, sorting, analyzing, and exporting results, and manually adjusting the compensation during the acquisition and analysis.

CHAPTER 6, Compensation

Describes how to create a compensation experiment and automatically calculate compensation values after acquiring the single color data. It also explains how to use these calculations for other experiments.

CHAPTER 7, Data Review

Describes how to use the Analysis screen to analyze data that has already been acquired.

CHAPTER 8, Shutting Down the System

Describes how to keep the instrument in optimal condition through daily cleaning during the shutdown procedure.

CHAPTER 9, Troubleshooting

Describes some common problems and their solutions in a basic troubleshooting matrix.

CHAPTER 10, Cleaning Procedures

Describes how to carry out certain routine and nonscheduled cleaning procedures.

CHAPTER 11, Replacement/Adjustment Procedures

Describes how to carry out certain routine and nonscheduled replacement and adjustment procedures.

APPENDIX A, Approved Cleaners and Disinfectants

Contains a list of Cleaners and disinfectants that can be used on the instrument.

APPENDIX B, Consumables

Contains a list of consumables to be used with the instrument.

APPENDIX C, Biosafety Cabinet

Defines the cautions and warnings for the optional Biosafety Cabinet.

APPENDIX D, Instrument Installation

Provides instructions for installing CytExpert software.

APPENDIX E, Good Practices for Cyber Security

Provides a list of good practices for cyber security.

APPENDIX F, Table of Hazardous Substances

Provides the table of hazardous substances with the hazardous substance name and concentration.

Conventions Used

This document uses the following conventions:

- **Bold face** font indicates buttons or selections that appear on the workstation screen.

- The term “select” is used to indicate the following action:
 - To click with a mouse.

NOTE The verb “press” is reserved for mechanical buttons, such as keys on the keyboard.

- Sections that contain entirely new content are flagged with a New Section icon  at the end of the section title.
- The software path to a specific function or screen appears with the greater than (>) symbol between screen options.
- Links to information in another part of the document for additional information are in blue and are underlined. To access the linked information, select the blue, underlined text.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the IMPORTANT adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during use, or maintenance of this equipment.

Graphics

All graphics, including screens and printouts, are for illustration purposes only and must not be used for any other purpose. For example, software screens that show the CytoFLEX SRT system in the background may not depict the latest production version of the system.

Overview

This chapter describes the individual components of the CytoFLEX SRT instrument and the corresponding functions of these components.

This chapter contains information on:

- [Product Description](#)
- [Main Components](#)
- [Fluidics System](#)
- [Optical Components](#)
- [Cell Sorting](#)
- [System Connections](#)
- [Instrument Specifications](#)
- [Performance Characteristics](#)
- [Material Safety Data Sheets \(SDS/MSDS\)](#)

Product Description

For Research Use Only. Not for use in diagnostics procedures. The use of data generated by this instrument depends upon the regulatory status of the reagents used.

The CytoFLEX SRT Cell Sorter is a research instrument that analyzes and sorts cellular suspensions and other similarly sized particle suspensions.

The system can be ordered in various configurations from 2 lasers, 5 colors, to a maximum of 4 lasers, 15 colors.

Operation Principles

The CytoFLEX SRT Sorter examines individual particles that are propelled in saline sheath through a flow cell (1), then through one to four spatially separated laser beams (2) of differing wavelengths. If the properties of the particle, or fluorescent dye added to the particle, are excited by the wavelength of laser light, the particle emits broadband fluorescence and scattered light. The emitted light is collected, focused, reflected, and filtered, so that discrete wavelengths of light are detectable by the fiber array photo detectors (FAPD). The FAPD converts the light signals to electronic signals that are sent to the instrument electronics. Data is then acquired by CytExpert SRT software according to the parameters set by the operator.

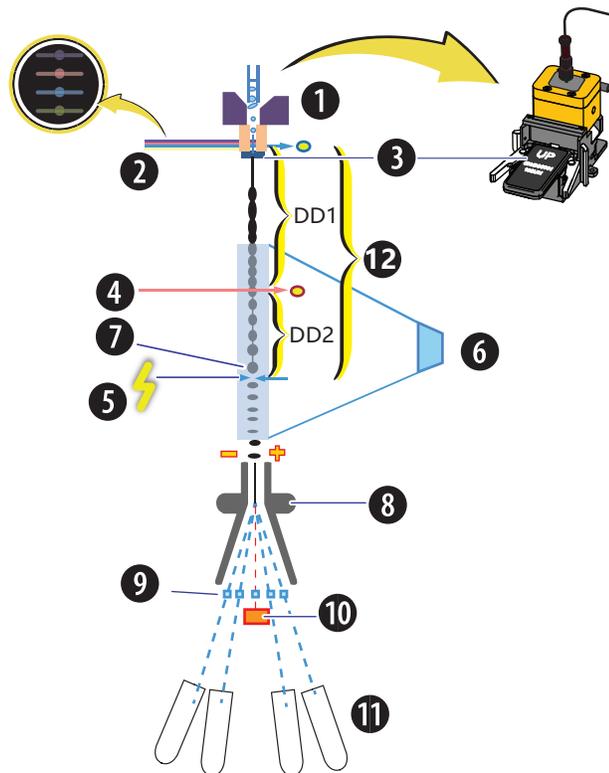
During sorting, drop drive energy (5) is applied to the piezoelectric crystal in the flow cell. The piezoelectric continually vibrates to break the stream into droplets (7). Droplets detach from the stream a few millimeters downstream from the nozzle (3). When a particle is detected and meets the predefined sorting criteria, an electrical charge is applied to the stream just as the droplet containing that particle breaks off from the stream. Once broken off from the stream, the droplet which retains its charge, passes through the strongly charged deflection plates (8). Electrostatic attraction and repulsion cause each charged droplet to be deflected into the appropriate collection device (11). Several side stream detectors (9) inside the instrument are used to monitor the deflection angle. Uncharged droplets go down into the waste catcher (10) in the default mode.

Drop delay (12) is defined as the amount of time it takes for a particle to travel from the interrogation point of the primary 488-nm laser to the point the droplet breaks off from the stream. To ensure the accuracy of drop delay calculation, a second 638-nm laser (4) is introduced to calculate the first part of the drop delay (referred to as DD1). DD1 is calculated during the QC. The second part of drop delay (referred to as DD2) is the time for a particle to travel from the interrogation point of the second 638-nm laser to the last attached drop. DD2 can be determined automatically by the sort calibration. During the sort calibration, a strobe LED and a camera (6) capture the real-time break-off images and feed them back to the software for algorithm. Refer to [Figure 1.1](#).

During a sort setup, IntelliSort makes use of the stream camera and software to automatically optimize droplets, and to determine DD2 without the use of calibration particles. When a sort is in process, IntelliSort monitors the droplet stream for instability. Several factors can alter droplet stream stability including ambient temperature, fluid temperature, and pressure changes. If IntelliSort detects instability, it modifies control parameters to ensure that the sort continues uninterrupted and without operator intervention. In the event that IntelliSort detects a dramatic drop-formation failure, sample flow is stopped, and the waste catcher moves into place to protect the sorted sample.

The CytoFLEX SRT Sorter can acquire and analyze up to 15 fluorescence, and two light-scatter parameters for each particle. Additional computed parameters can be created based on collected data.

Figure 1.1 Sort Overview Diagram



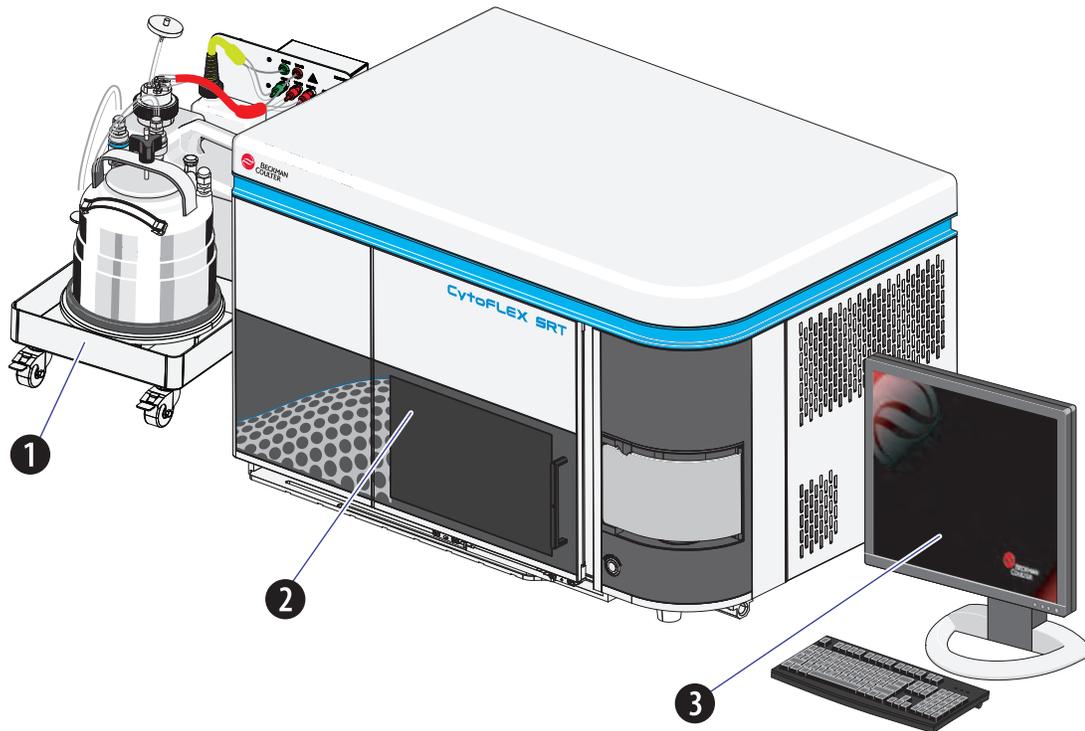
Main Components

CAUTION

Risk of instrument damage and/or instrument stability. Do not place any unnecessary objects on top of the instrument, as this could cause warping of the top cover or affect the stability of the optical path. However, airfoil is an exception if the instrument is used inside a Biosafety Cabinet.

The instrument consists of three main components: fluidics cart, Sorter, and the workstation. The fluidics cart houses a sheath tank, a shutdown fluid container, and a waste container. Refer to [Figure 1.2](#).

Figure 1.2 Main Components

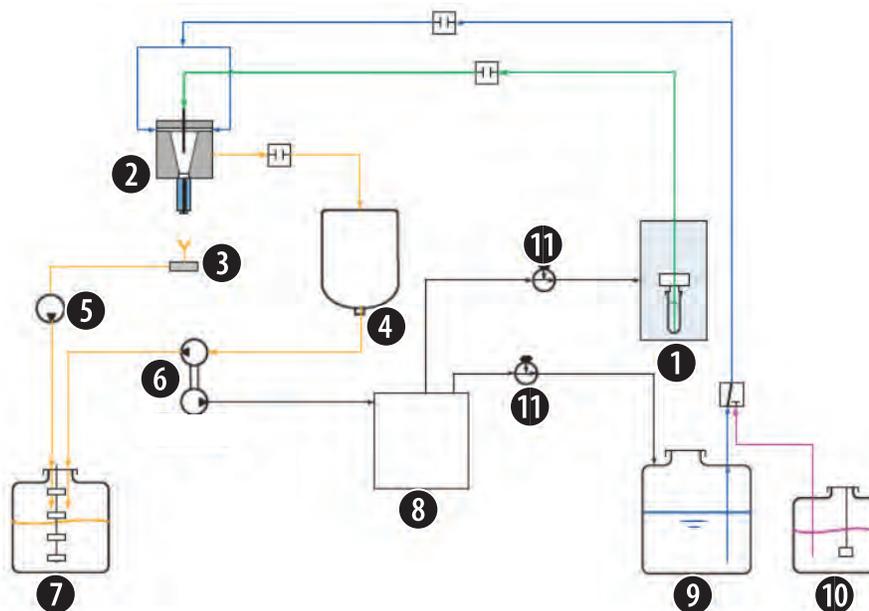


1. **Fluidics cart.** Accommodates sheath fluid and shutdown fluid as required for operation of the instrument, and collects the waste fluid from the Sorter.
2. **Sorter.** Provides signal generation and sorting.
3. **Workstation.** Acquires and analyzes data using the CytExpert SRT software, and displays data generated by the Sorter.

Fluidics System

The fluidics system helps to transmit the sheath fluid at a stable rate into the flow cell, forming a laminar flow to ensure that the tested particles go through the detection area sequentially. The fluidics system consists of the Sample Station, the fluidics module, and the fluidics cart. The following figure shows the whole fluidic flow.

Figure 1.3 Fluidic Flow



1. Sample Chamber
2. Flow cell
3. Waste catcher
4. Vacuum chamber
5. Waste pump
6. Internal air pump
7. Waste container
8. Pressure chamber
9. Sheath tank
10. Shutdown fluid container
11. Regulator

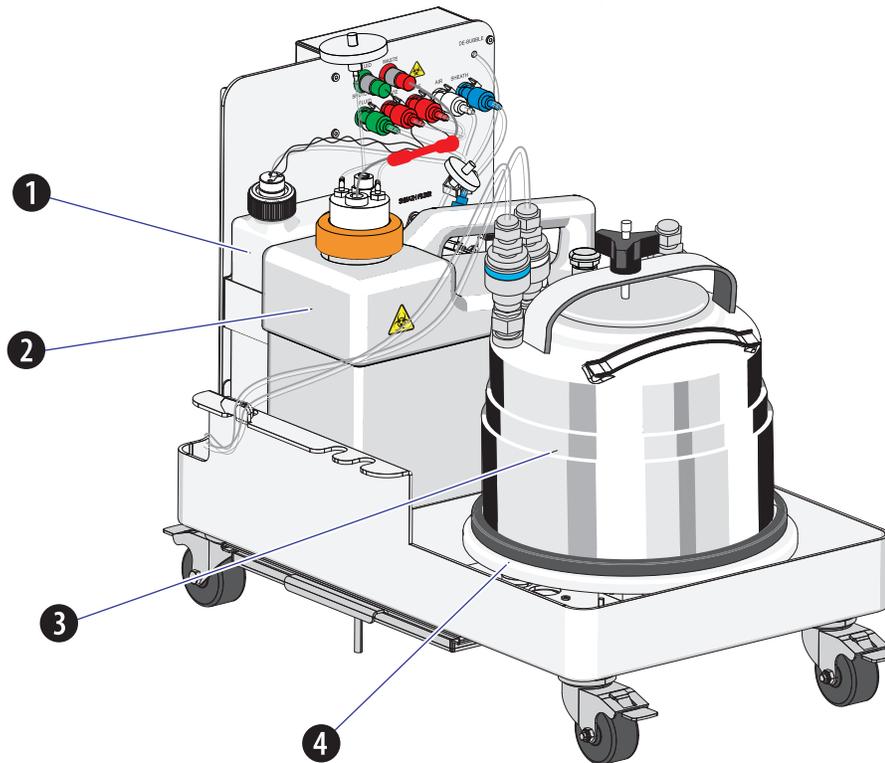
Fluidics Cart

IMPORTANT We recommend placing the fluidics cart on the floor for easy accessibility. However, you can place the fluidics cart on the same level as the instrument depending on your workspace.

If you want to relocate the fluidics cart from a table to the floor or conversely, from the floor to the table, no user intervention is required for the pressure calibration.

The fluidics cart (Refer to [Figure 1.4](#)) contains three fluidic containers, some quick connectors, fluid harness, and a sheath tank scale. Take all necessary biosafety precautions and use proper personal protective equipment when handling the fluidics containers.

Figure 1.4 Fluidics Cart



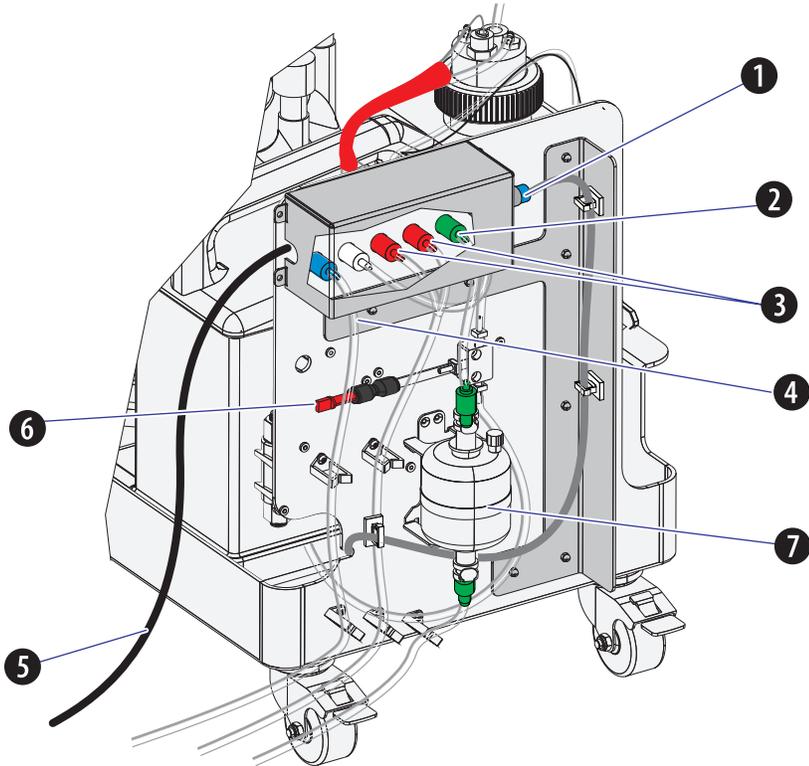
1. Shutdown fluid container
2. Waste container
3. Sheath tank
4. Sheath tank scale

NOTE The system accommodates both the rubber pads and the wheels for the fluidics cart. However, the stability of the fluidics cart is critical to the sorting performance. Beckman Coulter recommends using the rubber pads for the fluidics cart on a solid and stable surface.

If you prefer to use the scroll-wheels, activate the lock pins of the scroll-wheels to avoid any unexpected movement of the fluidics cart.

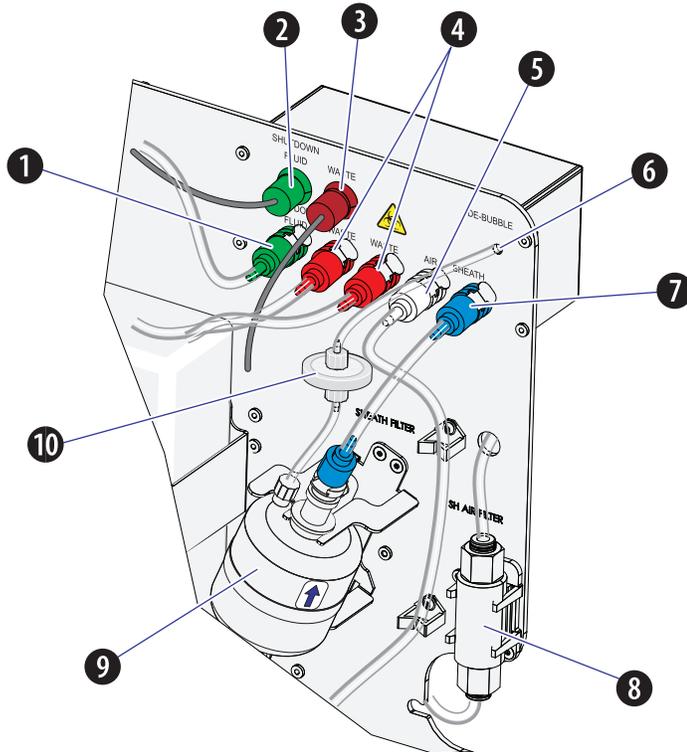
NOTE You can put the waste container on the right side of the Sorter inside a Biosafety Cabinet. In this case, you need an extension cable to connect the waste level sensor cable from the waste container to the quick connector on the fluidics cart. The extension cable is an optional accessory that can be purchased separately from Beckman Coulter. For instructions on the connection, refer to [Figure 1.35](#).

Figure 1.5 Fluidics Cart (Back)



1. Signal cable of sheath tank scale
2. Shutdown fluid tubing
3. Waste fluid tubing
4. Sheath fluid tubing
5. Fluidics cart signal cable
6. Sheath pressure check point (for service)
7. Shutdown Fluid filter

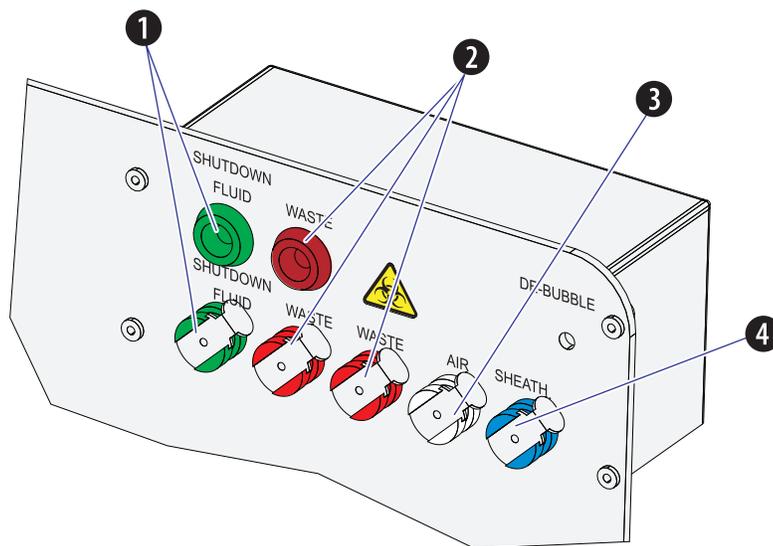
Figure 1.6 Fluidics Cart Connection Panel



1. **Shutdown fluid in.** Connects the shutdown fluid tubing to the shutdown fluid container.
2. **Shutdown fluid level sensor connector.** Connects the shutdown fluid sensor cable to the shutdown fluid container.
3. **Waste level sensor connector.** Connects the waste fluid sensor cable to the waste container.
4. **Waste out.** Connects the waste tubing to the waste container.
5. **Air inlet.** Connects the air tubing to the sheath tank.
6. **Debubble connector.** Connects the sheath filter debubble tubing to the debubble filter.
7. **Sheath fluid in.** Connects the sheath fluid tubing to the sheath fluid filter from the sheath tank.
8. **Air filter.** Filters particles larger than 0.01 μm .
9. **Sheath fluid filter.** Filters particles larger than 0.2 μm .

NOTE Beckman Coulter recommends replacing the sheath fluid filter every six months or sooner to ensure system performance. Using unfiltered sheath fluid can shorten the service life of the flow cell and/or nozzle, and increase noise and debris detection.

10. **Debubble filter.** Filters particles larger than 50 μm .

Figure 1.7 Connector Color Convention

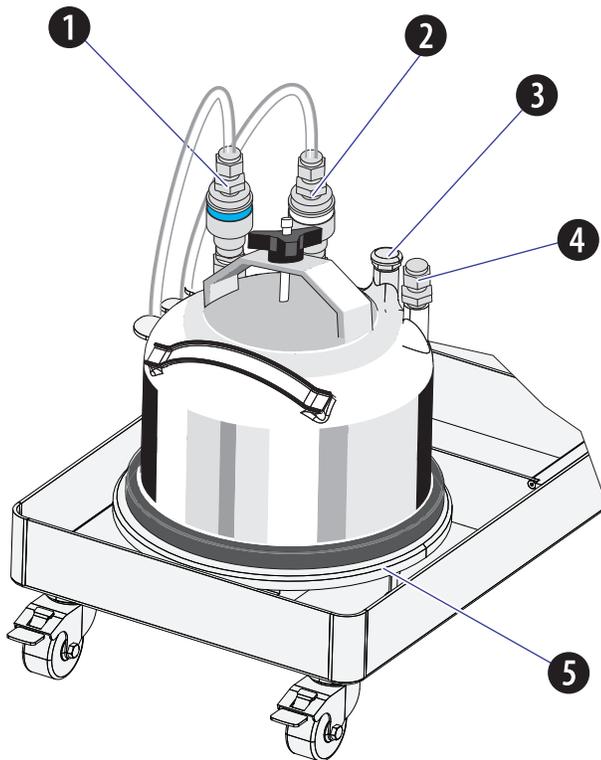
1. Green: shutdown fluid
2. Orange or red: waste fluid
3. White: air tubing
4. Blue: sheath fluid

Sheath Tank

The sheath tank is an autoclavable, electroplated and stainless-steel tank, with 4-L capacity. Refer to [Figure 1.8](#). The system accommodates the 7.5 L sheath tank as an optional accessory that can be purchased separately.

Sheath fluid is transported to the flow cell through a clear sheath tubing. Sheath flow is controlled through the sheath pressure. The sheath status icon  is shown on the right corner of the CytExpert SRT software. When the sheath tank is near empty, a warning notice is transmitted to the instrument and audible signal sounds as a warning. For the sheath status information, refer to [Table 3.1](#).

Figure 1.8 Sheath Tank



1. **Sheath outlet.** Connects the sheath tubing to the sheath filter.
2. **Air inlet.** Provides pressure to convey the sheath to the flow cell or wash station.
3. **Safety valve.** Releases the pressure from the tank in case of emergency.
4. **Pressure relief valve.** Releases the pressure from the tank manually.
5. **Sheath tank scale.** Monitors the volume of sheath remaining in the sheath tank.

NOTE If you need to switch between the 4 L sheath tank and the 7 L sheath tank, reset the sheath tank scale first. Refer to [Resetting the Sheath Tank Scale](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

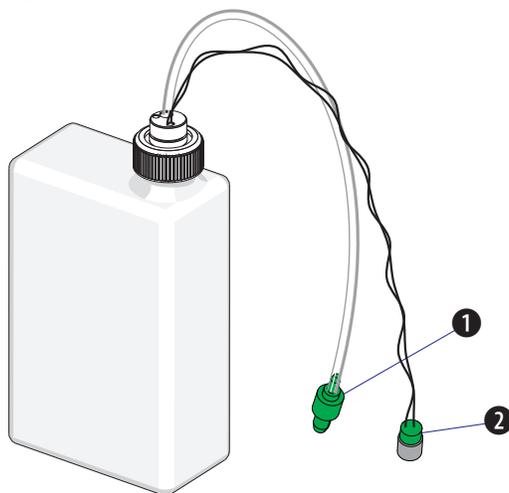
Shutdown Fluid Container

The shutdown fluid container is a polypropylene container with 1-L capacity. Refer to [Figure 1.9](#). It accommodates the shutdown fluid for decontaminating the fluid lines. The container does not require pressurization.

The shutdown fluid icon **Normal** is shown on the right corner of the CytExpert SRT software. When the shutdown fluid container is near empty, a warning notice is transmitted to the instrument and audible signal sounds as a warning. For the shutdown fluid status information, refer to [Table 3.3](#).

NOTE Beckman Coulter recommends using CytoFLEX SRT Shutdown fluid to ensure system performance. For instructions on filling the Shutdown Fluid container, refer to [Filling the Shutdown Fluid Container](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

Figure 1.9 Shutdown Fluid Container



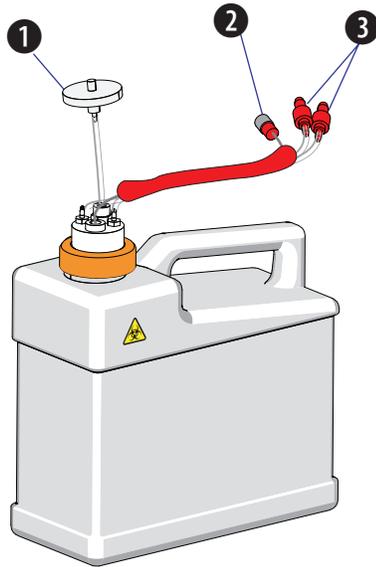
1. **Fluid tubing.** Connects the container to the fluidics cart.
2. **Level sensor cable.** Transfers the fluid level signal from the container to the fluidics cart.

Waste Container

The waste container holds 7-L of waste fluid. Refer to [Figure 1.10](#). The waste from the sample station, flow cell or the waste catcher are transported to the waste container by a dual-head pump and a waste pump. The waste status icon **25%** is shown on the CytExpert SRT software screen. When the waste container is near full, a warning notice is transmitted to the instrument and audible signal sounds as a warning. For the waste status information, refer to [Table 3.2](#).

Attention to biosafety and waste labeling is required.

Figure 1.10 Waste Container

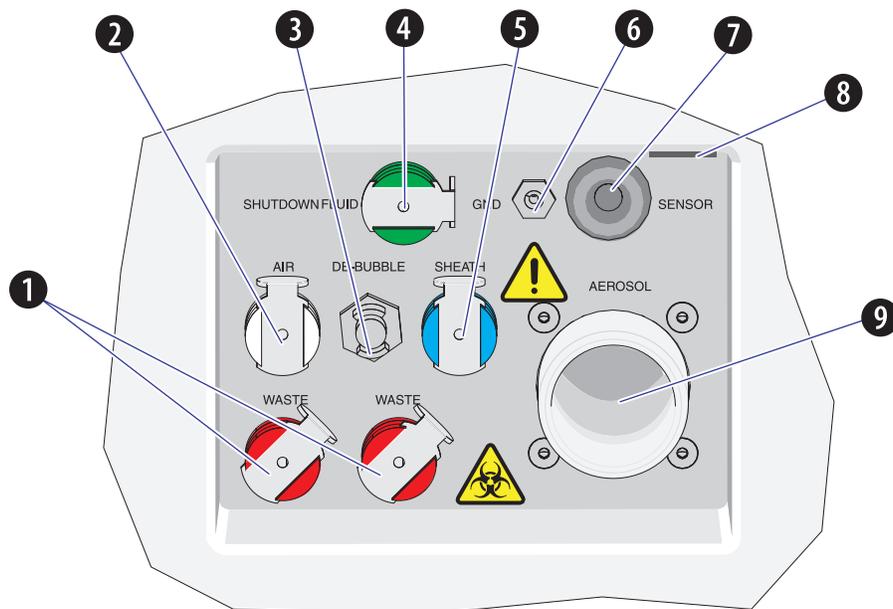


1. **Waste air filter.** Filters the contaminated aerosol.
2. **Waste level sensor.** Monitors the volume of waste in the waste container.
3. **Waste inlets.** Carries the waste fluid from the Sorter to the waste container.

Fluidics Module

The Fluidics module is on the right side of the Sorter. Inside the module, in addition to working pumps, valves, regulators, tubing, several sensors, and fluid filters, there is a pressure chamber, a vacuum chamber.

Figure 1.11 Interconnections on the Right Side of Sorter



1. **Waste out.** Connects the waste tubing from the Sorter to the fluidics cart.
2. **Air outlet.** Connects the sheath air tubing from the Sorter to the fluidics cart.
3. **Debubble tubing.** Connects to the debubble tubing from the fluidics cart to the Sorter.
4. **Shutdown fluid tubing.** Connects the shutdown fluid tubing from the fluidics cart to the Sorter.
5. **Sheath fluid in.** Connects the sheath fluid tubing from the fluidics cart to the Sorter.
6. **Grounding cable.** For system earthing protective.
7. **Fluidics sensor cable.** Connects the fluidics signal cable from the fluidics cart to the Sorter.
8. **Camera USB cable.** Connects the camera USB cable to the workstation.
9. **Aerosol connector.** Connects to the Aerosol Evacuation system.

Sample Station



Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

WARNING

Risk of biohazardous contamination and/or instrument damage. When running samples, it is important to insert the sample tube all the way down into the sample tube holder, until the bottom of the sample tube touches the base of the holder. Failing to do this could cause the sample probe to bend or break on entry.

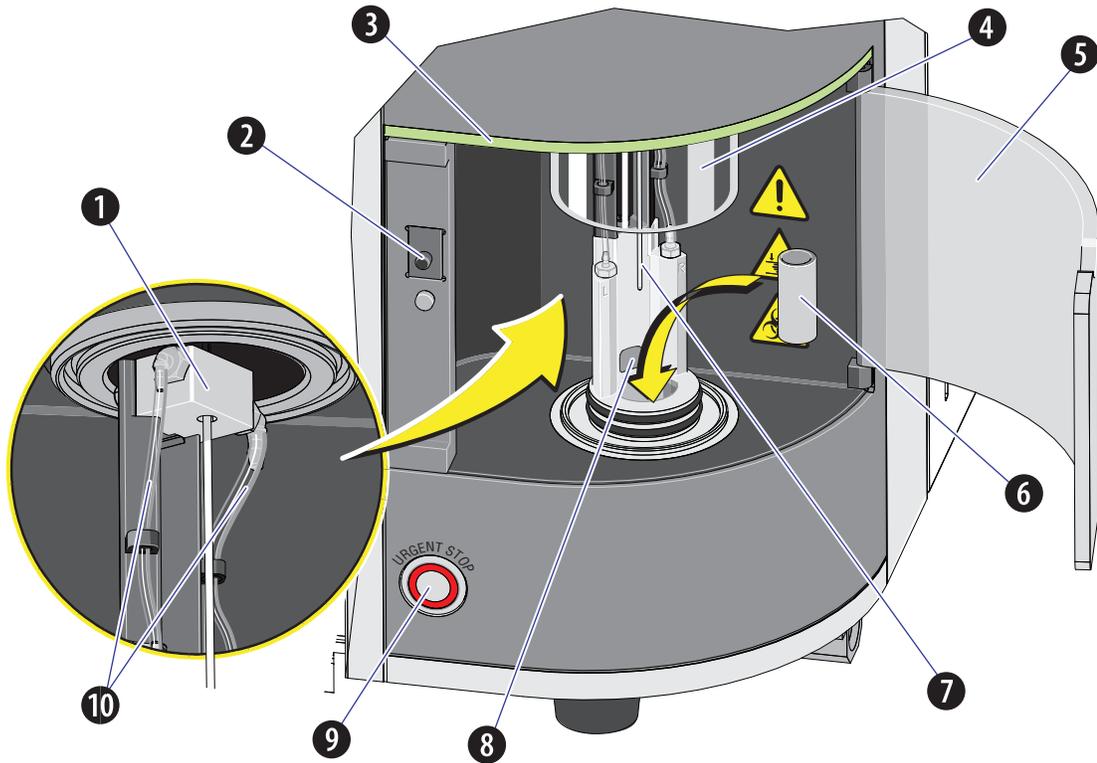
Sample tubes must not exceed 80 mm in height and the outside diameter must not exceed 12 mm.

⚠ WARNING

Risk of personal injury. Keep your hands off the sample station when the sample chamber is moving to avoid pinching your hand.

The Sample Station is where to load a tube and introduce a sample to the instrument. During acquisition, the sample chamber is enclosed and pressurized to force the sample toward the flow cell. The sample chamber supports sample temperature control and automatic mixing.

Figure 1.12 Sample Station



1. **Wash station.** Automatically cleans the sample probe when the instrument performs a backflush.
2. **Safety interlock.** Stops the sample chamber movement when the sample station door is opened.
3. **LED indicator.** Indicates the working status of the Sorter.
 - Green: Sorter is in either idle, or ready, or standby state.
 - Red: A critical error has occurred
 - Yellow: The system requires the operator's attention.
 - Flashing green: Certain program is in process, for example, loading sample, QC, etc.
4. **Sample chamber.** During the sampling process, the sample chamber cover moves down to form a closed and pressurized chamber.

NOTE Do not open the sample station door when the sample chamber cover is moving. Otherwise, the system prompts an error.

5. **Sample station door.** Engages or disengages the safety interlock of the sample chamber movement.
6. **Sample tube holder.** Supports 12 x 75 mm tubes for sample acquisition.
7. **Sample probe.** Draws and transfers sample into the flow cell.
8. **Tube light.** Illuminates the sample tube.



Risk of saline buildup in the fluidics system. Do not use the Urgent Stop button for routine shutdown. Urgent stop skips the important cleaning steps and may cause high carryover.

9. **Urgent Stop.** Puts the system into the idle state in urgent cases.

NOTE Long-pressing this button puts the instrument in the following states:

- Sample loading stopped
- Sheath flow off
- Waste catcher extended
- Deflection plate voltage off
- Lasers off.

NOTE After using the Urgent Stop button, follow the Daily Startup procedures to resume use. Refer to [CHAPTER 3, Daily Startup](#). Beckman Coulter recommends running Shutdown Program prior to perform the acquisition or sorting.

10. **Backflush tubing.** Provides fluid to clean the sample probe, and conveys the waste liquid to the waste container.

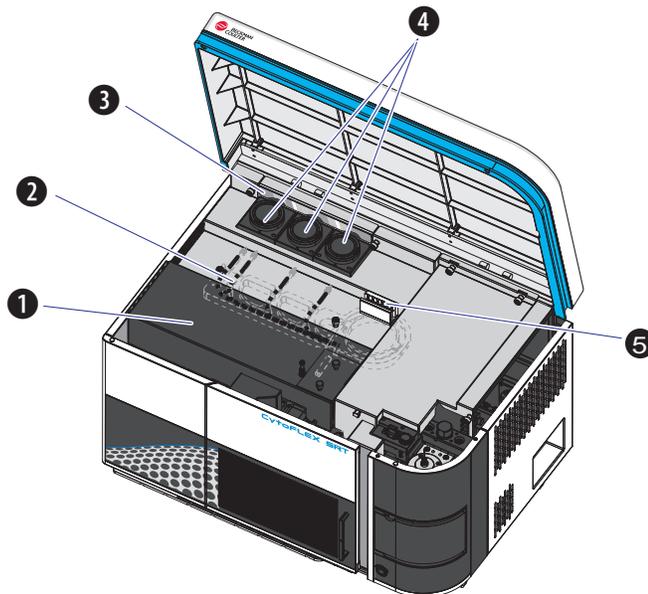
Optical Components

CAUTION

Risk of operator injury. When operating the instrument, keep the top cover in the closed position to prevent the top cover from falling. When opening the top cover, be cautious to avoid any possible pinch points.

The optical components are located in the upper portion of the Sorter and are visible when the top cover is open. Three parts are included: an optical bench, detector arrays also known as wavelength division multiplexers (WDMs), and optical fibers. Optical components include equipment such as lasers and signal detectors that are used to excite, transmit, and collect optical signals. Refer to [Figure 1.13](#).

Figure 1.13 Optical Components



1. **Optical bench.** Includes laser light sources, optical beam combiners, and an integrated optics flow cell assembly. The optical bench cover is equipped with a laser interlock that turns the lasers off unless the cover is tightly closed.
2. **Optical fiber.** Transmits emitted fluorescence to the specific WDM.
3. **Optical filter mirror.** Indicates the positions of optical filters inside the WDM when the WDM cap is removed.
4. **Wavelength division multiplexer (WDM).** Each WDM is a unique detector array that corresponds to a different laser, or in some cases two lasers. Each WDM contains optical filters and detectors for detecting channel fluorescence or scatter from a particular laser. It is necessary to ensure that the filter and software settings match for each channel. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in [CHAPTER 5, Sorting](#).

CAUTION

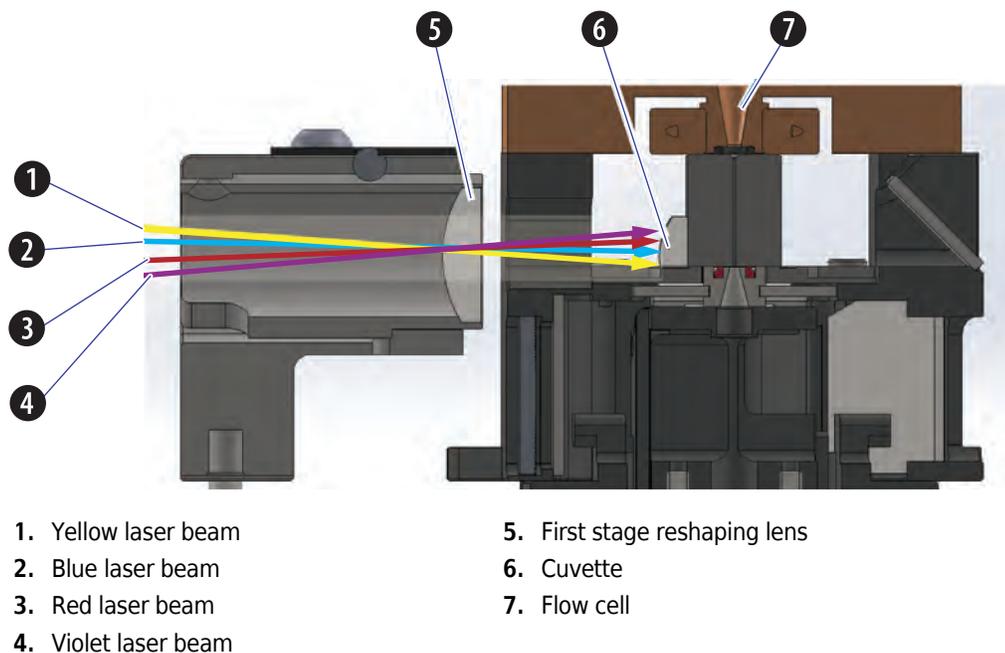
Risk of instrument damage. Do not place sample tubes in the optical filter holder. Liquid spills can damage instrument components. Use a tube rack to hold any sample tubes.

5. **Optical filter holder.** Securely holds additional CytoFLEX SRT optical filters.

Laser Beam Shaping

Before the laser beam reaches the sample stream, lenses focus the beam (refer to [Figure 1.14](#)). Focusing keeps the beam perpendicular to the sample stream flow while making the beam small enough to illuminate only one cell at a time.

Figure 1.14 Laser Beam Shaping



Cell Illumination

As cells in the sample stream go through the sensing area of the flow cell, the elliptical beam illuminates them. The cells scatter the laser light and emit fluorescent light from autofluorescence and the fluorescent dyes attached to them.

Forward Scatter

The amount of laser light scattered at narrow angles to the axis of the laser beam is called forward scatter (FSC). The amount of FSC is proportional to the size of the cell that scattered the laser light.

Side Scatter and Fluorescent Light

The amount of laser light scattered at about a 90° angle to the axis of the laser beam is called side scatter (SSC). The amount of SSC is proportional to the granularity of the cell that scattered the laser light. For example, SSC is used to differentiate between lymphocytes, monocytes, and granulocytes.

In addition to the SSC, the cells emit fluorescent light (FL) at all angles to the axis of the laser beam. The instrument measures the amount of FL emitted by cells depending on the reagents used. For example, FL above the background FL is used to identify molecules, such as cell surface antigens.

Forward Scatter Collection

The Forward Angle Light Scatter (FALS) detector collects scattered light from a particle that intersects with a laser and delivers information roughly proportional to the size of the particle. The forward angle light is filtered with a 488 nm band pass before it reaches the FS sensor which generates voltage pulse signals. These signals are proportional to the amount of light the sensor receives.

Side Scatter and Fluorescent Light Collection

Both side scatter and fluorescence are measured 90 degrees from the laser axis.

Side Scatter

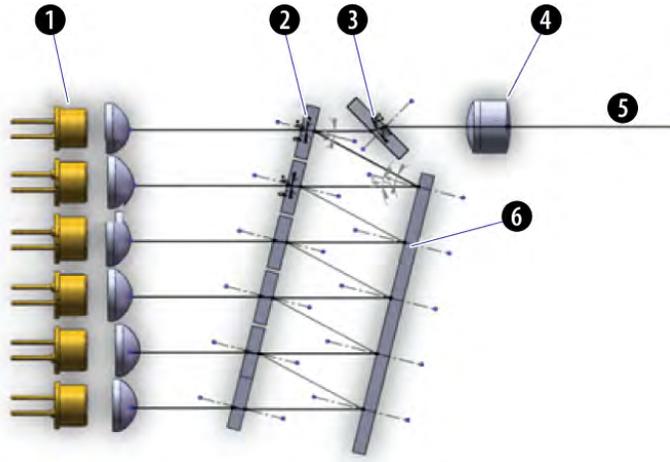
The wavelength of SS is 488 nm. It is much more intense than FL.

Side scatter light collected by the objective lens is delivered by fiber optics to a patent-pending design with high performance, solid-state, high efficiency, and low-noise detector arrays.

Fluorescent Light

Fluorescence and scattered light are transmitted by optical fibers to the Wavelength division multiplexer (WDM). Each WDM is a unique detector array that corresponds to a different laser. Refer to [Wavelength Division Multiplexer \(WDM\)](#) in [CHAPTER 1, System Overview](#). Each WDM contains optical filters and detectors for detecting channel fluorescence or scatter from a particular laser. It is necessary to ensure that the filter and software settings match for each channel.

Figure 1.15 Light Path through the WDM with a Single Port

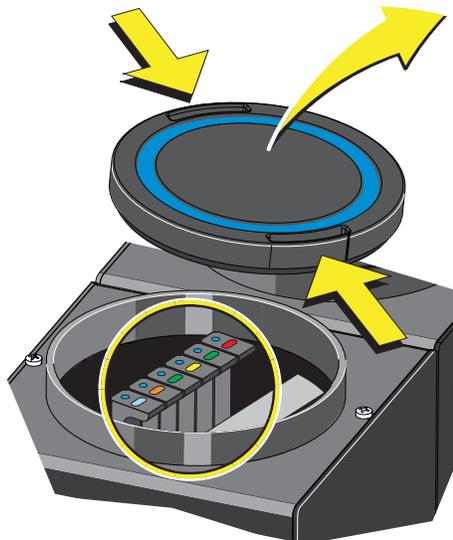


1. Fiber array photo detectors (FAPD)
2. Filter
3. 45-degree reflector
4. Doublet lens
5. Light path
6. Mirror

Wavelength Division Multiplexer (WDM)

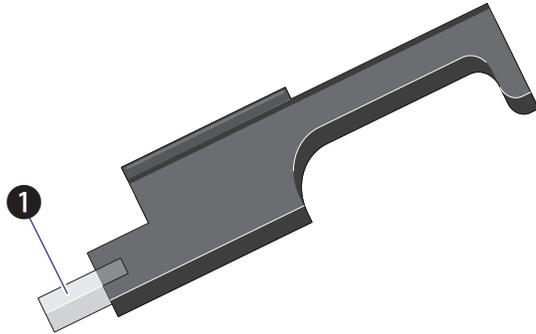
Each WDM corresponds to a different laser, or in some cases two lasers. The color of the ring on each cap corresponds to the color of the respective laser. Pressing the two release buttons on opposite edges of the cap allows you to open the WDM and replace the filters inside. Refer to [Figure 1.16](#). All optical filters are designed to be interchangeable. Refer to [Replacing the Optical Filter](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#) to replace an optical filter.

Figure 1.16 Optical Filter Mounts



Each optical filter mount has an optical filter glass piece. Refer to [Figure 1.17](#).

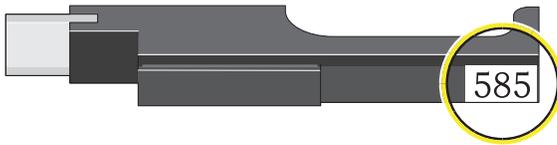
Figure 1.17 Optical Filter Mount with Optical Filter



1. Optical filter glass piece

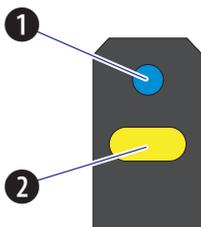
Each optical filter mount is labeled with the corresponding laser and band-pass information. Refer to [Figure 1.18](#).

Figure 1.18 Optical Filter Mount Labeled with the Band-Pass Information



The top of each optical filter mount has two marks. The color of the dot (1) indicates the color of the laser. Refer to [Figure 1.19](#). The color of the line (2) indicates the wavelength range of the optical band-pass filter. Refer to [Figure 1.19](#).

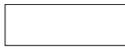
Figure 1.19 Optical Filter Mount (Top)



1.  . Indicates corresponding laser color: Blue indicates a 488 nm laser; Red indicates a 638 nm laser; Violet indicates a 405 nm laser; Yellow indicates a 561 nm laser.
2.  . Indicates the band-pass wavelength ranges; the midpoint of the band-pass is indicated numerically on the lateral side of the mount.

Band-pass filters are used to transmit fluorescence within a specific range of wavelength. These ranges are designed to measure fluorescence from fluorochromes such as those listed in [Table 1.1](#). You can change the optical filters according to your detector configuration. There is no need to realign the optical system when the filters are changed.

Table 1.1 WDM Optical Filter Mount Color Codes

Lasers	Fluorescent Channel	CytoFLEX SRT Channel Names	Commonly used Fluorescent Dyes
 405 nm	 450/45 BP	V450	Pacific Blue™ dye, V450, eFluor™ 450, BV421
	 525/40 BP	V525	Krome Orange, AmCyan, V500, BV510
	 610/20 BP	V610	BV605, Qdot® 605
	 660/10 BP	V660	BV650, Qdot® 655
	 780/60 BP	V780	BV780
 488 nm	 525/40 BP	B525	FITC, Alexa Fluor™ 488, CFSE, Fluo-3
	 690/50 BP	B690	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI, DRAQ7™
 638 nm	 660/10 BP	R660	APC, Alexa Fluor™ 647, eFluor™ 660, Cy5
	 712/25 BP	R712	APC-A700, Alexa Fluor™ 700, Cy5.5
	 780/60 BP	R780	APC-A750
 561 nm	 610/20 BP	Y610	mCherry, ECD, PE-CF594
	 780/60 BP	Y780	PC7
	 585/42 BP	Y585	PE, DsRed
	 710/50 BP	Y710	PC5.5, mPlum
	 675/30 BP	Y675	PC5, PE-AF680

Optical Fiber

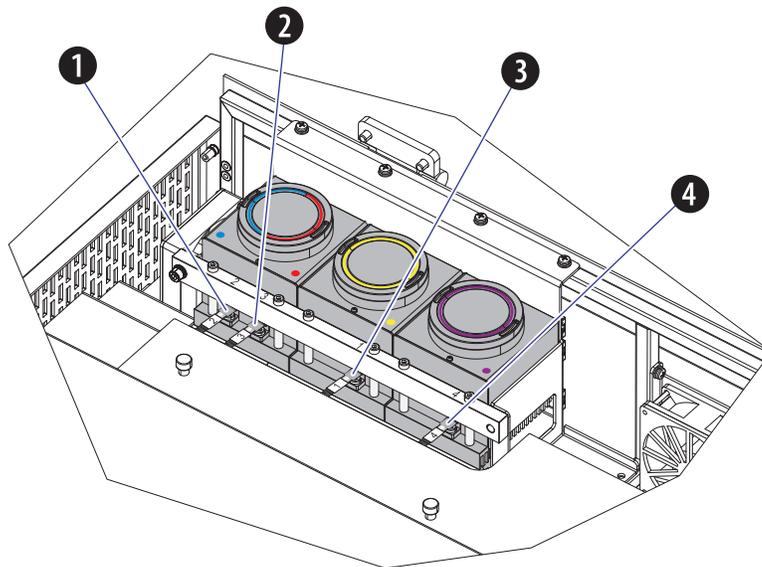


Risk of data integrity damage.

- **During use, verify that the optical fibers are securely connected to the WDM. A loose connection can alter the optical path and affect fluorescence detection.**
- **Do not disconnect the fiber as this could contaminate the tip and weaken the signal.**
- **Do not kink the optical fibers.**

Fluorescence emitted by laser-excited fluorochromes is picked up and delivered by each optical fiber to the corresponding detector module. Each optical fiber has an indicator number on the end that connects to the WDM. Ensure that the indicator number of the optical fiber matches the number of the corresponding WDM.

Figure 1.20 Optical Fiber



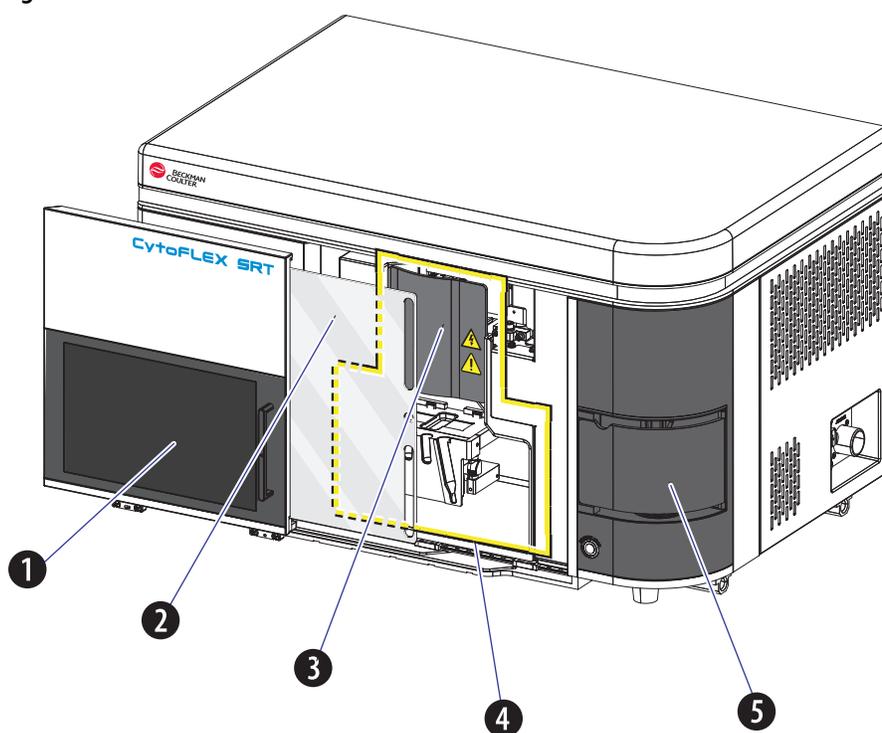
1. Blue laser fiber
2. Red laser fiber
3. Yellow laser fiber
4. Violet laser fiber

Cell Sorting

Sort Chamber

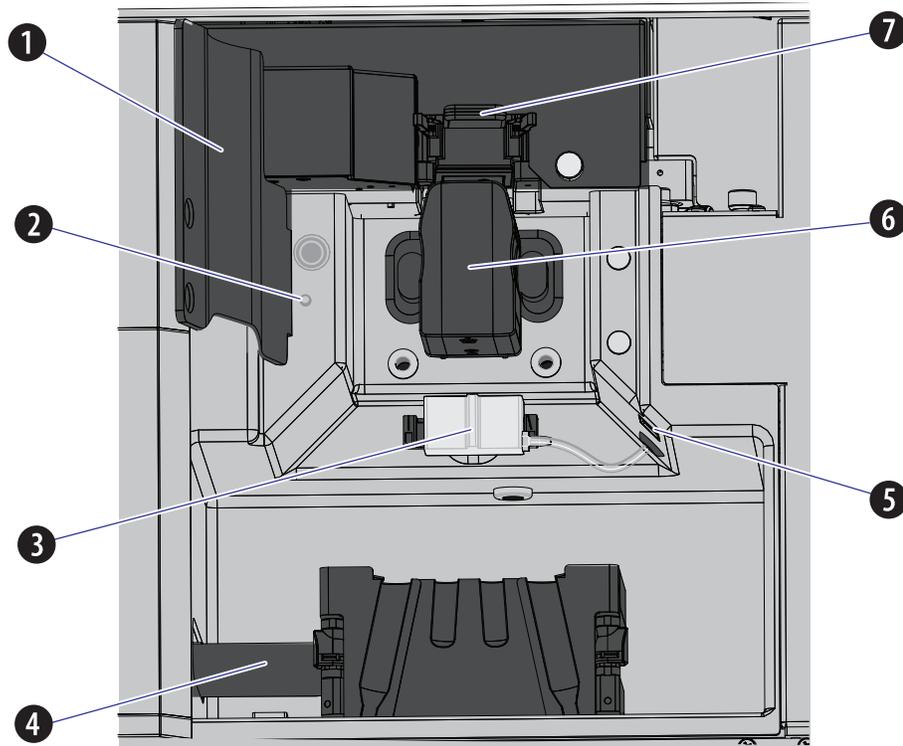
The Sort Chamber is located in the center enclosure. The Sort Chamber is well lit, and designed for easy access and cleaning. The sort chamber sliding door, is part of a passive aerosol containment assembly that isolates the contents of a sort from the rest of the instrument, the operator, and the laboratory. When closed, the door prevents movement of air in and out of the Sort Chamber. To easily clean the back side of the sort chamber sliding door, a transparent auxiliary door is designed. The auxiliary door is behind the sort chamber sliding door and can be removed completely by pulling towards right when the sort chamber sliding door is open. Refer to [Figure 1.21](#).

Figure 1.21 Sorter

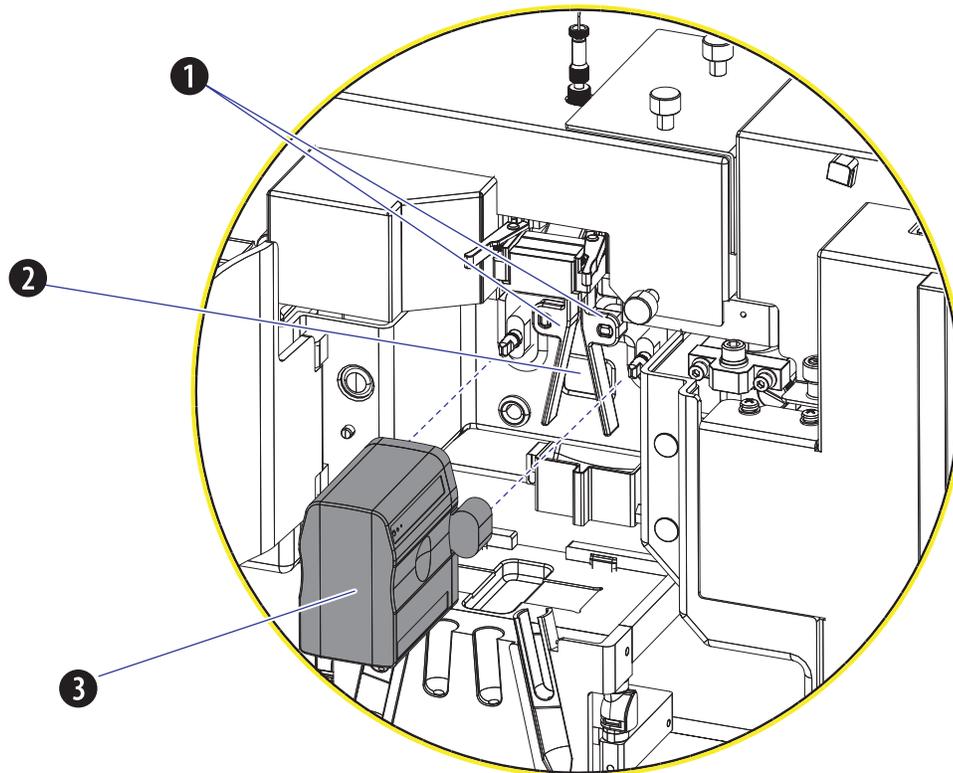


1. Sort chamber sliding door
2. Auxiliary door
3. Sort protection door
4. Sort chamber
5. Sample station

Figure 1.22 Overview of Sort Chamber



1. Sort protection door (open)
2. High voltage safety interlock
3. Waste catcher
4. CyClone movement system
5. Sort chamber illumination
6. Side stream illumination source
7. Nozzle module

Figure 1.23 Sort Chamber [with Side Stream Illumination Source Removed]

1. Deflection plates
2. Side stream detection window
3. Side stream illumination

Stream Camera and Droplet Monitor

The camera located opposite to the laser axis is not accessible by the operator. The camera is used to view the real-time break-off point, and the droplet status in the stream.

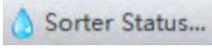
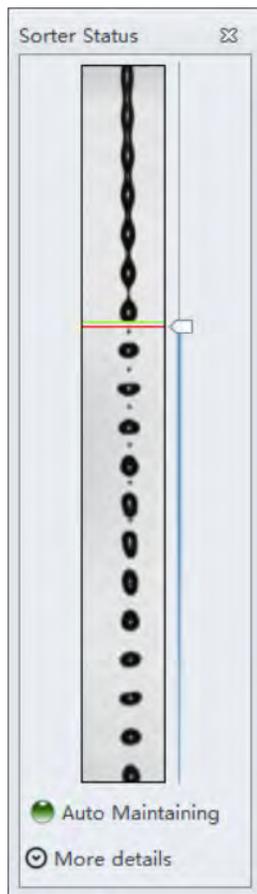
Select  from the right corner of the software status bar or select **Sorter Status** from the Sorting menu to access the Droplet Status screen. Refer to [Figure 1.24](#).

Figure 1.24 Droplet Status



NOTE For more details, refer to [Starting and Monitoring a Sort](#) in [CHAPTER 5, Sorting](#).

Deflection Plates



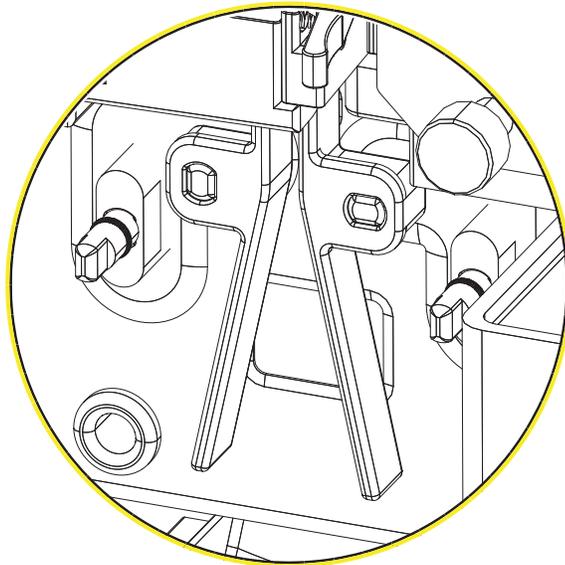
Risk of personal injury. Do not touch the deflection plates when the high-voltage power is applied.

High-voltage deflection plates deflect droplets from the mainstream and direct droplets to specific appropriate receptacles. These plates can be polarized with up to ± 4000 Vdc. Caution should be exercised when the plate voltage is enabled.

When opening the sort protection door, the high voltage will be disabled automatically by the high voltage safety interlock.

The deflection plates are designed to be easily removed and cleaned. To access the deflection plates, you need to open the sort protection door and remove the side stream illumination source. For

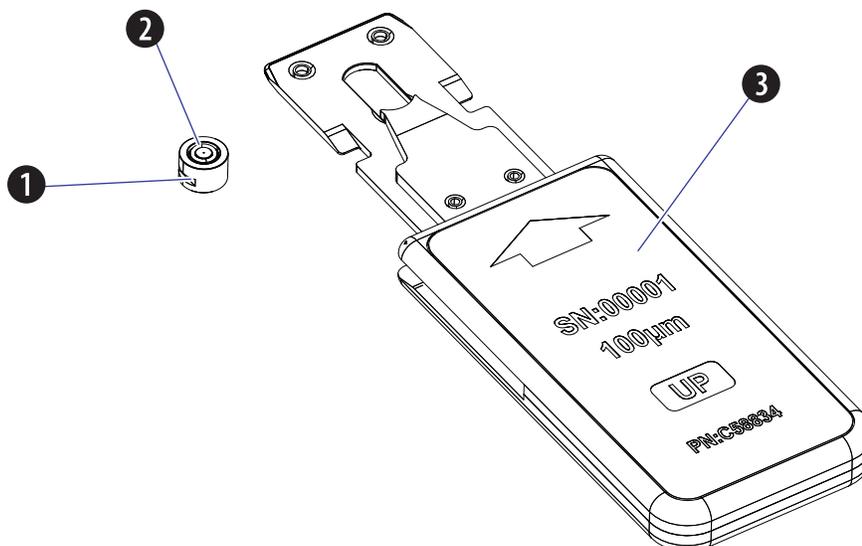
instructions on removing and cleaning the Deflection Plate, refer to [Cleaning the Side Stream Illumination Source and the Deflection Plates](#) in [CHAPTER 10, Cleaning Procedures](#).



Nozzle Module

The nozzle module (hereinafter referred to as Nozzle) contains a detachable 100- μm nozzle (1), O-ring (2) and a nozzle holder (3). The stream of sheath and sample is pressurized to go through the nozzle. This produces a tiny, fine, and fast-moving jet of sample particles. Refer to [Figure 1.25](#).

Figure 1.25 Nozzle Module



1. Nozzle
2. O-ring
3. Nozzle holder

For instructions on cleaning the nozzle, refer to [Cleaning the Nozzle](#) in [CHAPTER 10, Cleaning Procedures](#). For instruction on removing/installing the nozzle module, refer to [Removing/Installing the Nozzle Module](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

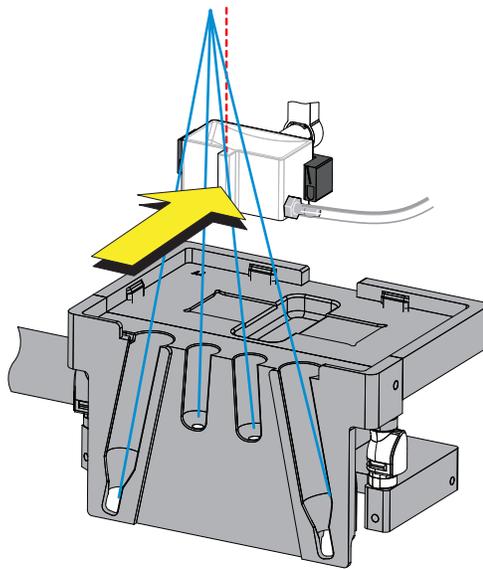
Waste Catcher

The waste catcher is located under the Side Stream Illumination Source. The waste catcher extends during the QC, and sort calibration. During normal sorting, the waste catcher is retracted so that the sorted sample can be deposited in the appropriate sort receptacles while the unwanted drops are directed to the waste catcher.

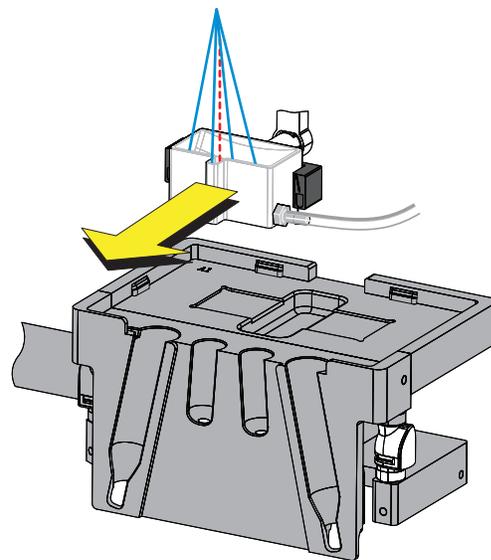
When a fault condition is detected, the waste catcher extends automatically to protect the collected sample. The fluid in the waste catcher is finally vacuumed to the waste container. Refer to [Table 1.2](#).

Table 1.2 Waste Catcher

Waste Catcher (Retracted)



Waste Catcher (Extended)



IMPORTANT Ensure that the position of the waste catcher matches the selected Stream Mode. For instruction on changing the positions of the waste catcher, refer to [Changing the Stream Mode](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

The waste catcher has two installation positions depending on the Stream Mode: the left position for Straight Down Mode, and the right position for Default Mode. The waste catcher receives the core stream as the unwanted in the Default mode while it receives the side stream L1 as the unwanted in the Straight Down mode.

CyClone Movement System

⚠ WARNING

Risk of hand pinching. Keep your hands away from the sort chamber when the CyClone Movement System is moving.

⚠ CAUTION

Risk of damaging the CyClone Movement System. Do not place objects like tube holders, unused tubes, slides, gloves inside the Sort Chamber, which might interfere with the movement of CyClone System or damage the CyClone System.

The CyClone movement system, located in the Sorter Chamber, supports X-axis and Y-axis movements. The system includes a 3-in-1 holder to hold tubes, slides or microplates. Preconfigured sort output definitions determine plate voltage and defanning to automatically direct sort streams to the appropriate receptacles.

Figure 1.26 Two 5 mL Tubes and Two 15 mL Tubes

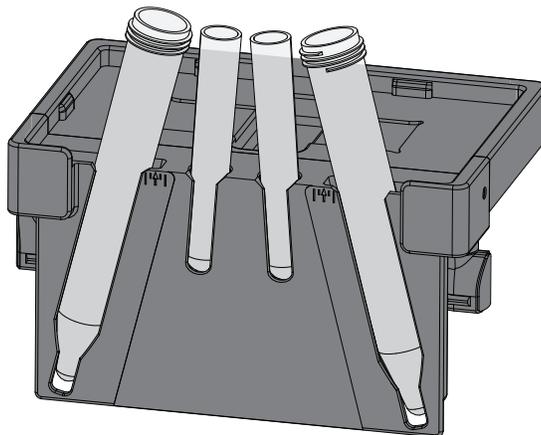
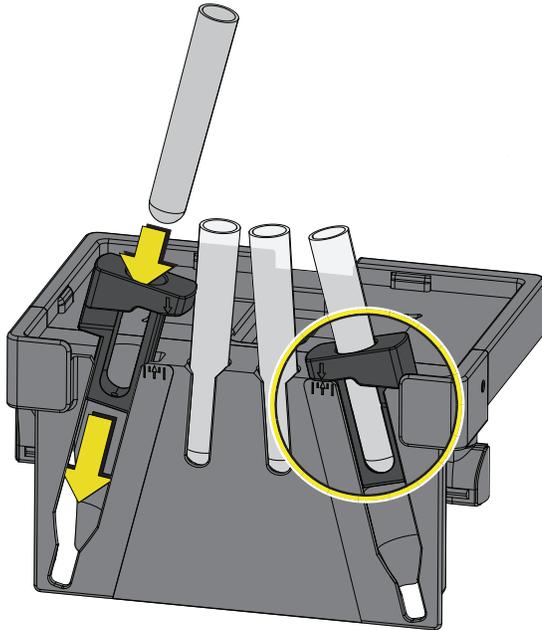


Figure 1.27 Four 5 mL Tubes



NOTE The tube adapters are required in the lateral tube slots for 5 mL tubes.

Figure 1.28 Standard 96-Well Plate

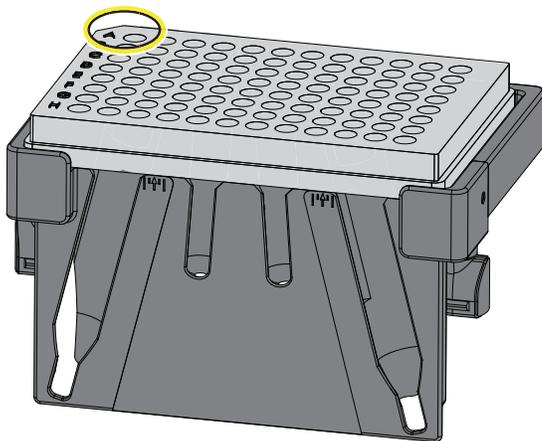
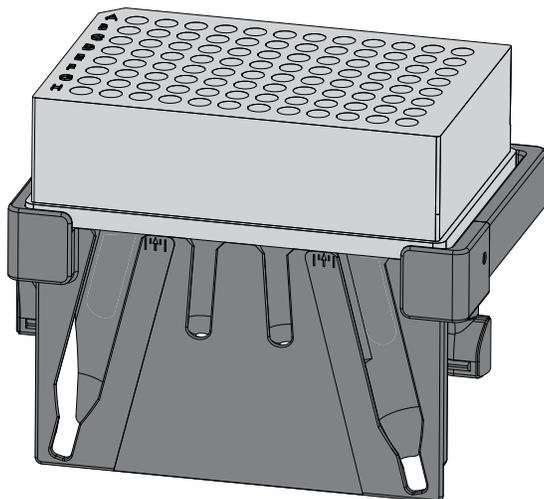
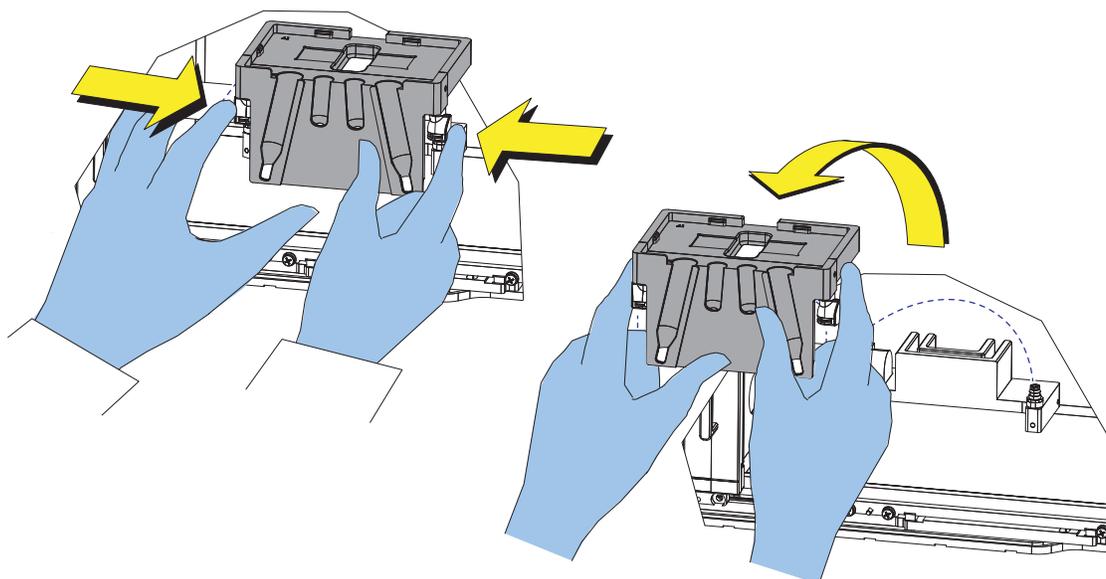


Figure 1.29 96-Well Deep Well Plate



The CyClone output holder is designed to be easily removed for cleaning. For instructions on cleaning the CyClone holder, refer to [Daily Decontamination During Shutdown](#) in [CHAPTER 10, Cleaning Procedures](#).

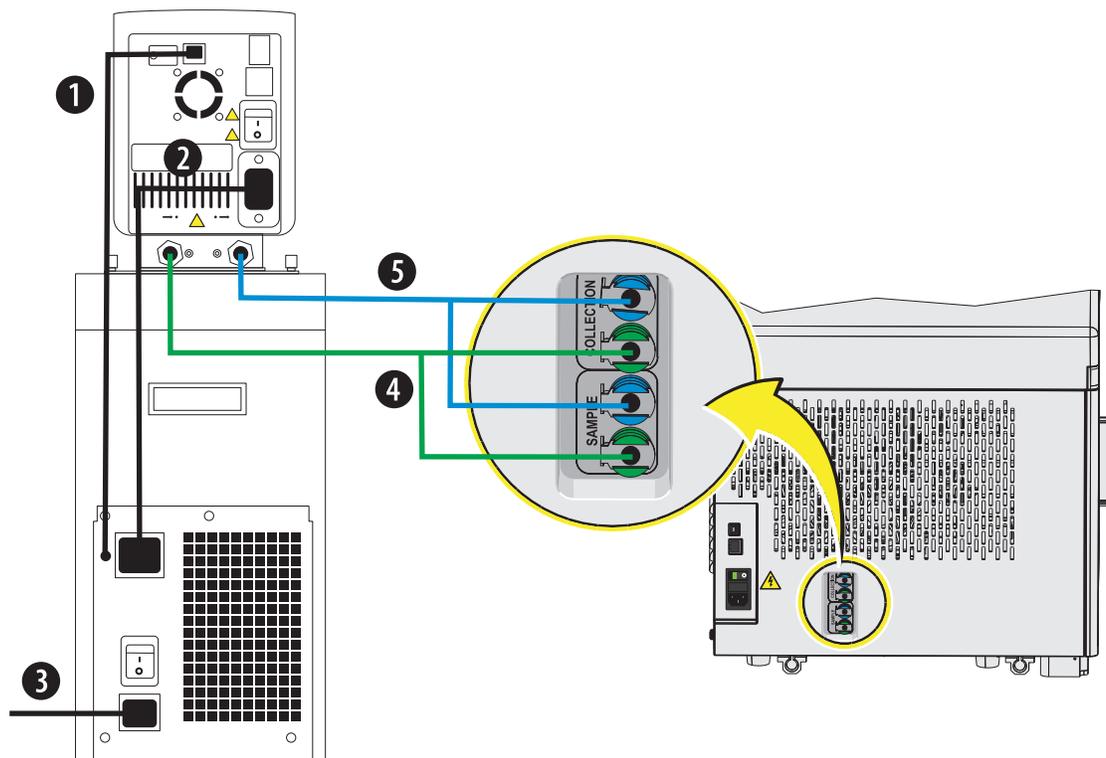


Sample Temperature Control

The system supports temperature control both for the sample tube and the sort collection receptacle. This requires a separately purchased Water Bath console which is a stand-alone unit. The operator selects a constant, regulated temperature at which to maintain samples. The Sorter transfers the refrigerant at a regulated temperature from the Water Bath through the body of the sort output holder and the sample tube holder. The Water Bath console is connected to the Sorter by two dedicated interconnection adapters (refer to [Figure 1.30](#)) which you can find in the CytoFLEX SRT system package.

Figure 1.30 Interconnection Adapters



Figure 1.31 Connecting Water Bath to the Sorter

1. Communication cable
2. Power cord
3. Power supply for Water Bath
4. Return flow tubing from Sorter to Water Bath
5. Circulating flow tubing from Water Bath to Sorter

NOTE Ensure that the color of the quick connectors on the interconnection adapters match that of the quick connectors on the Sorter.

NOTE You can use temperature control only for sample tube by connecting only the SAMPLE quick connectors on the Sorter, for sort collection receptacles by connecting only the COLLECTION quick connectors on the Sorter.

NOTE Figure 1.31 is for reference only. The back of a Water Bath can be different depending the model you use. For more details about the Water Bath, refer to the manufacturer's manual.

To ensure that the sample collection receptacle is at the desired temperature, start the Water Bath console at least 40 minutes before you start sorting.

NOTE A manual with safety instructions for the Water Bath is available directly from the manufacturer. Follow all safety and maintenance actions indicated in the manufacturer's manual.

CAUTION

Risk of instrument damage and/or erroneous results. To ensure a long service life of the Sorter, the allowable range of the temperature setting for a Water Bath is 5°C - 49 °C if using DI water, and 0°C - 49 °C if using 25% propylene glycol-Water. For the recommended temperature control setting, refer to [Table 1.3](#).

WARNING

Risk of chemical injury from propylene glycol. To avoid contact with the glycol, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire when working with the Water Bath. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

Table 1.3 Temperature Control Setting^a

Required Sample Temperature (°C)	Recommended Setting (°C)	Time Needed (min)	Refrigerant
2-8	0	60	25% Propylene Glycol-Water
35-39	39.5	8	25% Propylene Glycol-Water
8-10	5	45	DI Water
35-39	39.5	8	DI Water

a. The temperature setting and the actual time can be affected by several conditions such as, the model of your Water Bath, the volume of the refrigerant you use, and the ambient temperature in your laboratory. For the performance specification of your Water Bath, refer to the manufacturer's manual.

Aerosol Evacuation System

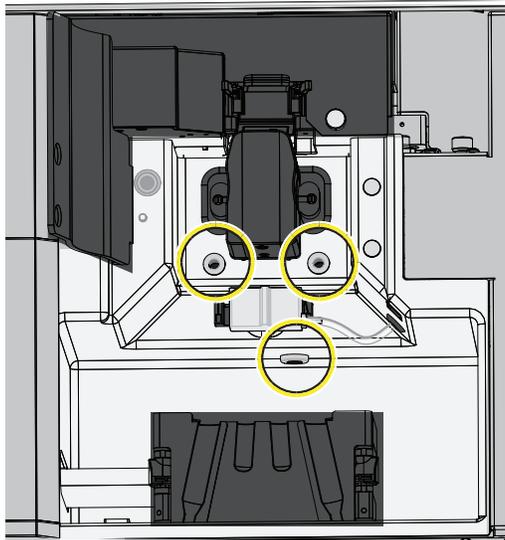
The optional Aerosol Evacuation System removes aerosols and micro droplets, which may be generated during the course of normal operation or sort failure conditions, from the sort chamber without disturbing sorting. The system features an ultra-quiet motor (40 dB Min and 69.5 dB Max) and a high-suction, high flow rate centrifugal action pump. Micro droplets and particulates greater than 0.12 µm are removed under vacuum and trapped in an Ultra-Low Penetration Air (ULPA) filter. The air flow rate of the Aerosol Evacuation System is fully user adjustable, providing clearance of the sort chamber at rates of 10 to 40 complete air exchanges per minute. A single disposable filter is used on the system to simplify installation and removal of filters. The filter is completely enclosed to protect the operator from potential contamination when changing filters. Refer to [Figure 1.32](#).

Figure 1.32 Aerosol Evacuation System

NOTE The sorting performance is sensitive to air disturbances. Beckman Coulter recommends using the 30% Suction Setting.

The aerosol connector is located at the lower right corner of the Sorter. Refer to [Figure 1.11](#).

The Aerosol Evacuation System vacuums aerosols from the port in the Sort Chamber and vents them out the right side of the instrument, trapping them in the filter on the front of the unit. Refer to [Figure 1.33](#).

Figure 1.33 Aerosol Evacuation Vents

Instrument Electronics

The instrument achieves an acquisition rate of 40,000 particles per second, and a sorting rate of 30,000 particles per second. Electronics and 64-bit software can store up to 34 million events in a single data file.

System Connections

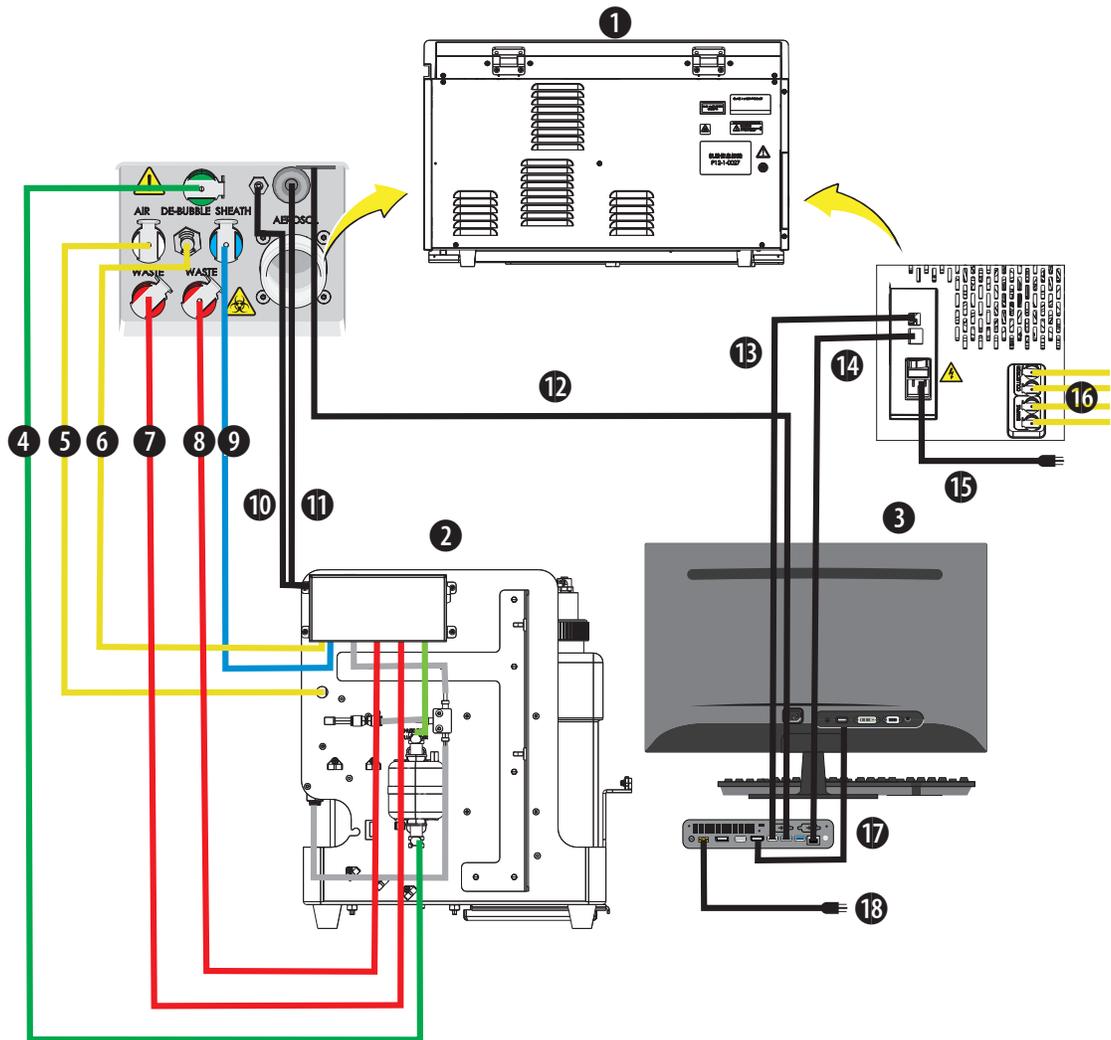


Risk of data loss and/or instrument damage. Never shut off the power or disconnect a data cable while the Sorter is in the process of performing a task. This could cause data loss or damage to the system.

IMPORTANT The sorting performance is sensitive to the vibration or airflow interference which could impact the stability of break-off point. Avoid placing the Sorter near the vibration source or the ventilation outlet.

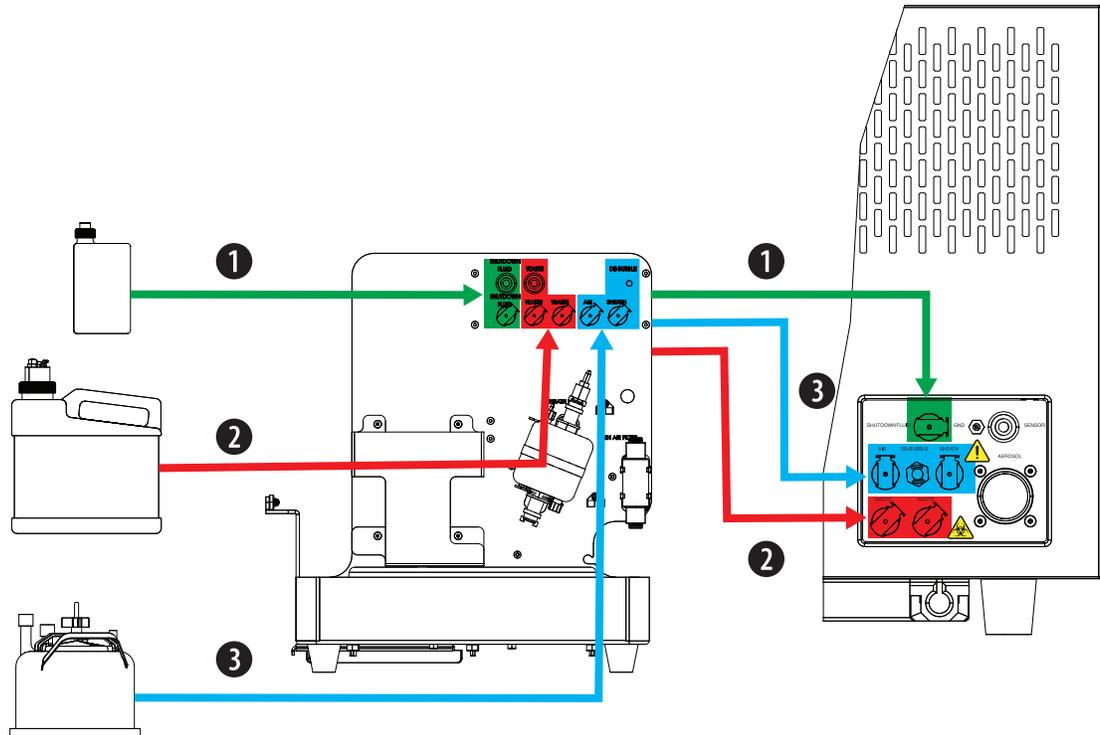
- Keep the sheath fluid tubing away from the ventilation outlets on the back of the Sorter, or other ventilation equipment.
- Ensure that the sheath fluid tubing is separated from the waste fluid tubing to avoid any proper vibration transmission.

Figure 1.34 Power and Signal Cable Connections



- | | |
|---------------------------|--------------------------------|
| 1. Sorter | 10. Earthing protective cable |
| 2. Back of fluidics cart | 11. Fluidics cart signal cable |
| 3. Workstation | 12. Camera cable (USB 3.0) |
| 4. Shutdown fluid tubing | 13. USB 2.0 cable |
| 5. Sheath air tubing | 14. Ethernet cable |
| 6. Sheath debubble tubing | 15. Sorter power supply |
| 7. Waste fluid tubing | 16. Water bath connections |
| 8. Waste fluid tubing | 17. HDMI cable |
| 9. Sheath fluid tubing | 18. Workstation power supply |

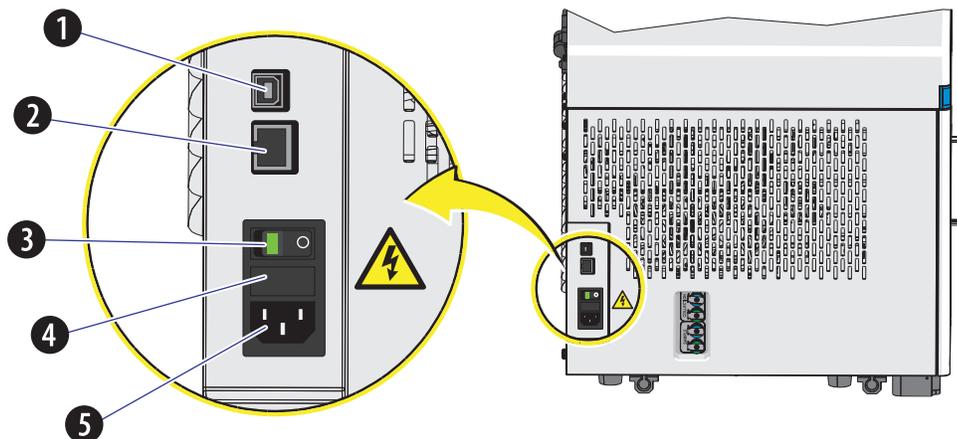
Figure 1.36 Fluid Harness Connections



1. Shutdown fluid harness cable
2. Waste harness cable
3. Sheath harness cable

NOTE For the fluidic connections on the fluidic cart, refer to [Figure 1.6](#).

Figure 1.37 Overview of Sorter Connections



1. **USB 2.0 interface.** Remote power on/off the Sorter.
2. **Ethernet socket.** Connects the Sorter to the workstation.
3. **Power switch.** Turns the Sorter on and off. An indicator light glows when the power is on.
4. **Fuse.** Protects the internal system from damage by high electrical current.
5. **Power line socket.** Supplies the power to the Sorter.

Instrument Specifications

Dimensions

Dimensions		
Instrument dimensions (Length x Width x Height)	Sorter	72.5 cm x 47.5 cm x 45 cm
	Fluidics cart	34.5 cm x 60 cm x 48.5 cm
Weight	Sorter	62 kg
	Fluidics cart (without fluid)	13.5 kg

NOTE For the Biosafety Cabinet dimensions, refer to the manufacturer's Product Specification.

Installation Category

Installation Category II^a

Maximum Altitude

IMPORTANT If operating the instrument at an altitude greater than 2000 m, you could encounter startup failure, sort calibration failure, electric spark, fluid leakage, high carryover rate, or other unknown problems. Beckman Coulter assumes no responsibility for any problem resulting from operating instrument at an altitude greater than 2000 m (6561 ft).

Do not operate at an altitude greater than 2000 m (6561 ft).

Pollution Degree

Pollution Degree 2^b

Acoustic Noise Level

Measure Level: ≤ 65 dBA (CytoFLEX SRT System with or without Biosafety Cabinet)

Electrical Ratings

Input Voltage: 100-240 VAC

Frequency: 50/60 Hz

Power: 200 VA

a Indicating the transient overvoltage of this level is < 2500 VAC. The instrument is intended for indoor use only.

b Normally, only non-conductive pollution occurs occasionally, however, a temporary conductivity caused by condensation must be expected.

Sorter

Optics																	
Excitation Optics	The CytoFLEX SRT system is configured with four spatially separated lasers. The optical system is alignment-free. The laser delays are automatically adjusted by the daily QC system, if required. No user intervention is required to ensure optimum system performance.																
Laser devices	<table border="1"> <thead> <tr> <th>Standard wavelengths and power</th> <th>Violet laser</th> </tr> </thead> <tbody> <tr> <td></td> <td> <ul style="list-style-type: none"> Wavelength: 405 nm, 90 mW </td> </tr> <tr> <td></td> <th>Blue laser</th> </tr> <tr> <td></td> <td> <ul style="list-style-type: none"> Wavelength: 488 nm, 50 mW </td> </tr> <tr> <td></td> <th>Yellow laser</th> </tr> <tr> <td></td> <td> <ul style="list-style-type: none"> Wavelength: 561 nm, 30 mW </td> </tr> <tr> <td></td> <th>Red laser</th> </tr> <tr> <td></td> <td> <ul style="list-style-type: none"> Wavelength: 638 nm, 100 mW </td> </tr> </tbody> </table>	Standard wavelengths and power	Violet laser		<ul style="list-style-type: none"> Wavelength: 405 nm, 90 mW 		Blue laser		<ul style="list-style-type: none"> Wavelength: 488 nm, 50 mW 		Yellow laser		<ul style="list-style-type: none"> Wavelength: 561 nm, 30 mW 		Red laser		<ul style="list-style-type: none"> Wavelength: 638 nm, 100 mW
Standard wavelengths and power	Violet laser																
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	<ul style="list-style-type: none"> Wavelength: 561 nm, 30 mW 																
	Red laser																
	<ul style="list-style-type: none"> Wavelength: 638 nm, 100 mW 																
Forward scatter detection	Silicon photodiode with built-in 488/15 band-pass filter.																
Fluorescence and side scatter detection	Fluorescence and side scatter light collected by the objective lens is delivered by fiber optics to a patent-pending design with high performance, solid-state, high efficiency, low-noise detector array. Reflective optics with a single transmission band-pass filter in front of each detector.																
Violet side scatter configuration (VSSC)	The system offers the ability to configure the violet laser detector to collect side scatter to better resolve nanoparticles from noise.																

Fluidics System	
Sample flow rate	10~100 µL/min
Dimensions	Nozzle: 100-µm internal diameter.
Fluid capacity	Sheath tank: 4-L, or 7.5-L Waste container: 7-L Shutdown fluid container: 1-L
Automated maintenance cycles	System Startup program, Backflush, Sheath Filter De-bubble, Flow Cell Debubble, Flow Cell Clean, Daily Clean, Aseptic Clean, System Shutdown program, Long-term shutdown
Sample input formats	5 mL (12 x 75 mm) polystyrene and polypropylene sample tubes
Sample dead volume	< 60 µL
Supported sort collection devices	1-way sorting: 6-well plates, 24-well plates, 48-well plates, standard 96-well plates, 96-well deep well plates, 384-well plates, slides 4-way sorting: 4 x 5 mL tubes, 2 x 15 mL tubes and 2 x 5 mL tubes

Electronics	
Signal processing	7-decade data display
Signal	Pulse area and height for every channel, width for two selectable channels

Data Management		
Software	CytExpert SRT software	
Language	English and Chinese	
FCS format	FCS 3.0	
Recommended Workstation/ computer requirements	Operating system	Windows® 10 Enterprise LTSC 2019 x64-bit ^a
	Processor	Intel® Core™ i7-7700 (8MB Cache, 3.6 GHz; or Intel® Core™ i7-8700T 12MB Cache, 2.4 GHz)
	Monitor	2560 x1440 32 inch
	Memory	8 GB RAM or higher
	Storage	1 TB or higher
	Ethernet	integrated 100 MB, Dual Ethernet ports
	USB interface	≥ 5 ^b interfaces, at least one USB 3.0 interface
Compensation	Full matrix compensation, manual and automatic. Novel Compensation Library for storage of spillover values of dyes to easily determine the correct compensation matrix with new gain settings.	

- a. Windows 10 (2019 LTSC) is the only operating system that has been validated to work with the CytoFLEX SRT workstation.
- b. If you need connect an external device or USB to the computer, Beckman Coulter recommends using the wireless keyboard and/or wireless mouse for the computer. Otherwise, you need at least 6 interfaces to ensure one can be used for the external device or USB when using the wired keyboard and the wired mouse.

Performance Characteristics

Performance		
Sensitivity	MESF	FITC: < 30 molecules of equivalent soluble fluorochrome (MESF-FITC) PE: < 10 molecules of equivalent soluble fluorochrome (MESF-PE) APC: < 25 molecules of equivalent soluble fluorochrome (MESF-APC)
Fluorescence resolution	rCV < 3% The CytoFLEX SRT cell sorter is capable of achieving <3% rCV. Using CytoFLEX Daily QC Fluorospheres or CytoFLEX Ready to Use Daily QC Fluorospheres for daily QC, the pass criteria is ≤ 5%.	
Side scatter resolution	< 300 nm	

Performance	
Violet Side scatter resolution	200 nm relative to polystyrene particles
Forward and side scatter resolution	$\leq 1 \mu\text{m}$ Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes as well as nanoparticles.
Carryover	$< 0.1\%$ (QC beads)
Sorting	The system shall be capable of simultaneously bulk sorting up to 4 defined populations of particles with a mode that allows $\geq 99\%$ at purity mode with meeting the criteria below: <ul style="list-style-type: none">• Target population $\leq 5\%$• Sample threshold rate $\leq 10,000$ EPS• Yield rate: $\geq 80\%$ theoretic rate An efficiency rate up to 30,000 events per second can be achieved without affecting purity but with a decrease in yield based on Poisson's statistics.

Material Safety Data Sheets (SDS/MSDS)

To obtain an SDS or MSDS for the CytoFLEX SRT reagents used on the CytoFLEX SRT systems:

- On the Internet, go to www.beckman.com:
 1. Select **Safety Data Sheets (SDS/MSDS)** from the Support menu.
 2. Follow the instructions on the screen.
 3. [Contact us](#) if you have difficulty locating the information.
- If you do not have Internet access, [contact us](#).

Using the CytExpert SRT Software

Overview

The CytExpert SRT software is a full-feature software package that controls the instrument's operation, which allows you to acquire, sort, and analyze flow cytometry data and then save the data in FCS format. This chapter will explain the software's functions and features.

This chapter contains information on:

- [Launching the Software](#)
- [Main Software Screen](#)
- [User Management](#)
- [Role Management](#)
- [Account Policies](#)
- [Operation Log](#)
- [Graphic and Gating Styles](#)
- [Collection Device Library](#)
- [Software Settings](#)

Launching the Software



Select the desktop shortcut  to launch the CytExpert SRT software.

If there is no desktop shortcut, run the “CytExpert SRT.exe” software directly from the software installation directory. The default installation path is C:/Program Files/CytExpert SRT. Or, select



> **All Programs > CytExpert SRT.**

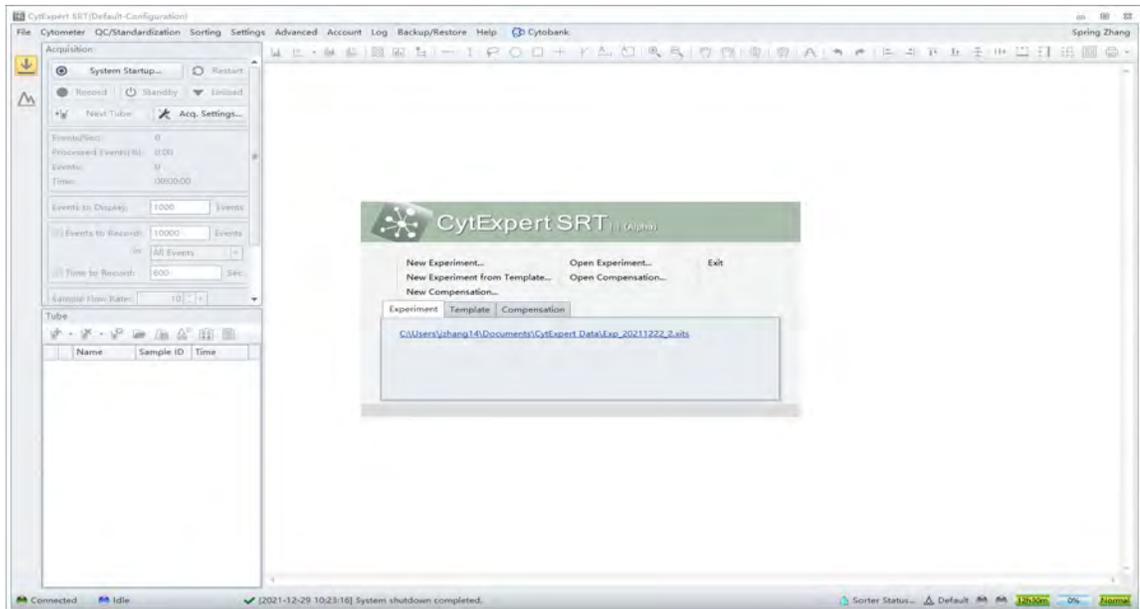
Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#), for detailed instructions on opening the software and confirming the connection status.

Main Software Screen

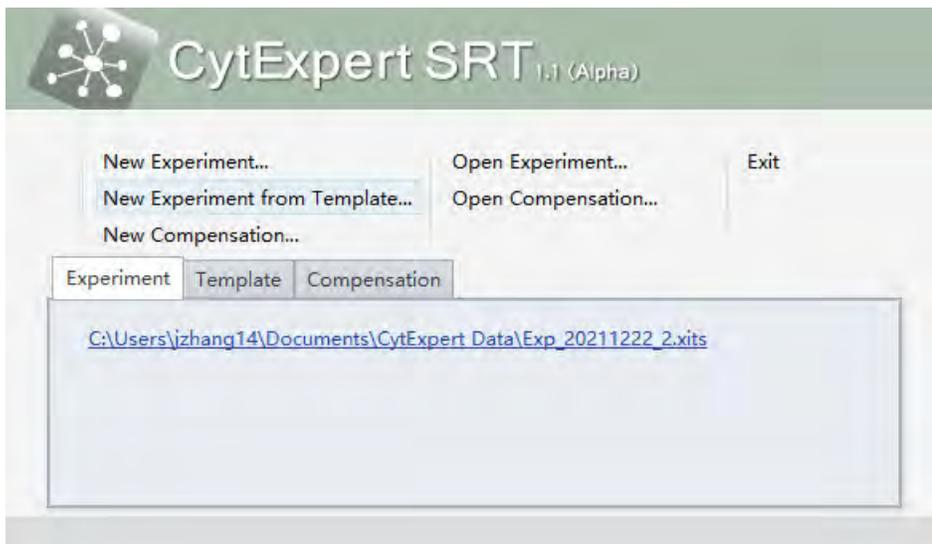
Hover your cursor over any button to display a text pop-up of the button's function.

Start Page

The start page automatically opens after logging into the software.



The following operations can be selected from the start page:



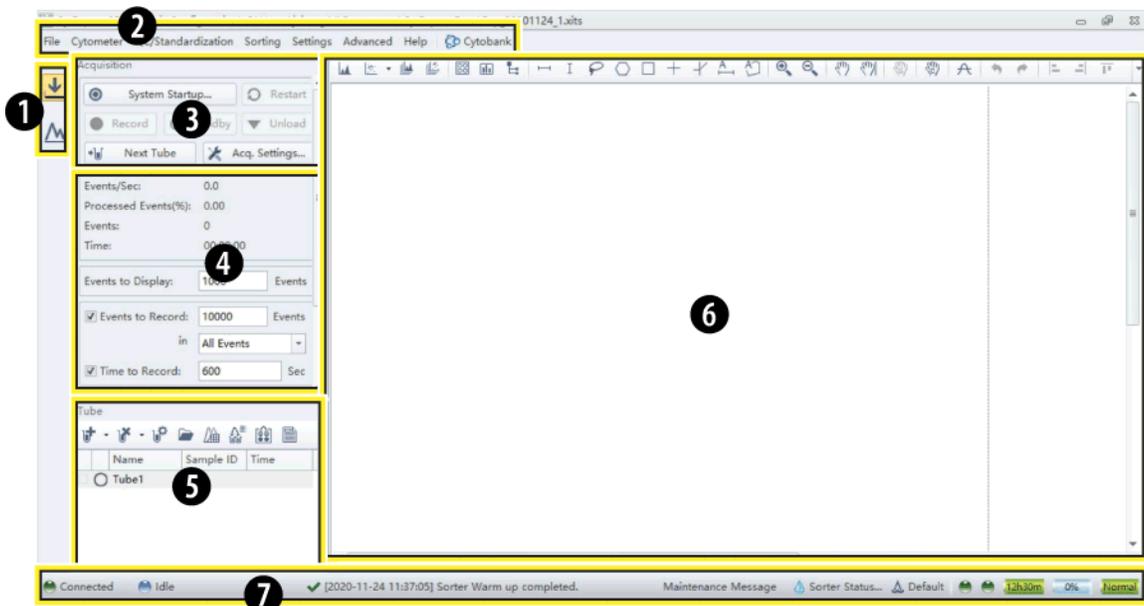
- **New Experiment.** Creates a new experiment. The process creates a file with the .xits extension and a folder with the same file name where the raw data (.fcs files) are kept.
- **New Experiment from Template.** Creates an experiment using a template saved from a previously saved experiment.
- **New Compensation.** Sets up compensation for an experiment.
- **Open Experiment.** Opens a previously created experiment.

- **Open Compensation.** Opens a previously created compensation experiment.
- **Exit.** Exits the CytExpert SRT software.

The Experiment, Template, and Compensation tabs below give you the option of opening one of the 10 most recently opened experiments.

Acquisition Screen

Selecting **New Experiment**, **New Experiment from Template**, or **Open Experiment** automatically opens the Acquisition screen. The Acquisition screen can be accessed by selecting  on the left side of the page.



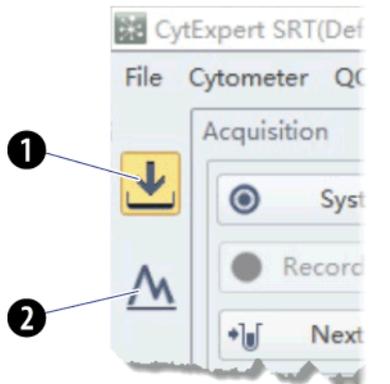
1. **Navigation.** Gives the option of accessing the Acquisition screen, or Analysis screen.
2. **Menu.** Allows you to configure settings for sample acquisition, instrument operation, and software options.
3. **Instrument operation controls.** Controls sample loading/unloading, data acquisition/sorting, and recording.
4. **Collection.** Establishes control over data recording options, displays the acquisition status, controls the sample flow rate, and sample mixing.
5. **Tube management.** Allows you to manage sample tubes, set tube attributes, manage experimental data and compensation, define sorting criteria.

NOTE The Tube section of the screen can be expanded or retracted by dragging the top border of the Tube section of the screen. Expanding this section covers other elements of the screen, including: Events/Sec, Events to Display, Sample Flow Rate and Sample Mixing Speed.

6. **Plot area.** Includes plot and gating controls, as well as an area for creating plots and generating graphs.
7. **Status bar.** Displays instrument status, system information, and fluidics container status.

Acquisition Screen Navigation

The Acquisition screens have two navigation icons, the Acquisition screen, and the Analysis screen.



1. **Acquisition screen icon.** Accesses the Acquisition screen.
2. **Analysis screen icon.** Accesses the Analysis screen.

Collection



System: idle

- Sample chamber: open
- CyClone: home position
- Lasers: off
- Sheath fluid: off

System: standby

- Sample chamber: open
- Cyclone: loading position
- Lasers: off
- Sheath fluid: off

System: ready for use

- Sample chamber: open
- CyClone: loading position
- Lasers: on
- Sheath fluid: on

1. **Acquisition control.** Controls sample loading/unloading, sorting, and access acquisition setting.
2. **Acquisition status.** Displays information such as the acquisition rate (Events/Sec), Processed Events (%), Events, and Time.

- Events/Sec: events acquired/acquisition time.

NOTE The sorting pause during the data acquisition is excluded from the acquisition time.

- Processed Events (%): events processed/total detected events. The unprocessed events go into the waste stream.

NOTE The Processed Events(%) may be less than 100% if the acquisition rate is too high or the sorting contains too many hierarchical gates, which exceed the system processing capability. If this situation persists, you need decrease the sample flow rate or optimize the gate settings.

3. **Stop conditions.** Sets the necessary conditions for recording data.

Explanation of restrictions:

Events displayed: 1-500,000

Events recorded: 1-100,000,000

Recording time: 1-28,800 seconds

4. **Sample Flow Rate.** Sets the sample flow rate. The amount of sample pressure depends on the sample flow rate you specify. The allowable range is 10-100.

NOTE High acquisition rate may increase the abort rate and measurement CVs. It is recommended to customize the acquisition rate according to the nature of your sample.

NOTE For more details, refer to [Setting Sample Flow Rate](#) in [CHAPTER 5, Sorting](#).

5. **Sample Mixing.** Sets the mixing speed.

NOTE High mixing speed may affect the viability of sorted cells.

NOTE For more details, refer to [Setting Mixing Speed](#) in [CHAPTER 5, Sorting](#).

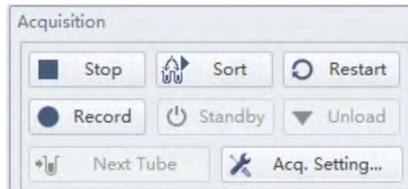
Acquisition/Sorting Control

Acquisition

Ready for acquisition:



Acquisition in process:



Sorting

Ready for sorting:



Sorting in process:



Sorting paused:



1. **Run.** Starts sample acquisition.
2. **Sort.** Starts sorting.
3. **Stop.** Stops sample acquisition or sorting and save the experiment.
4. **Unload.** Releases the sample chamber pressure and moves up the sample chamber cover.

NOTE **Unload** is only available when the sample loading is stopped or paused. Select **Unload** when you need to replace the sample tube.

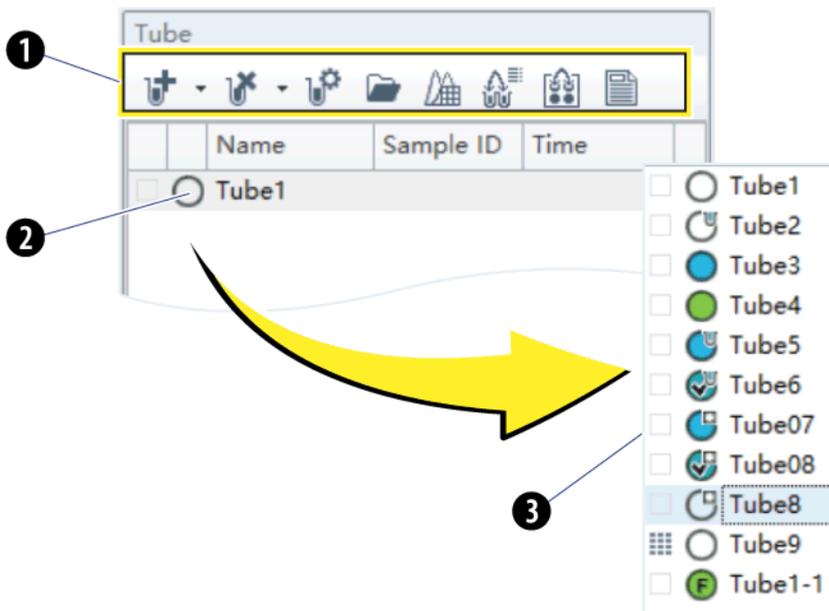
5. **Restart.** Restarts the sample acquisition.
6. **Pause.** Pauses the sorting.
7. **Resume.** Resumes the sorting.
8. **Record.** Record the data. The recorded data cannot be overwritten.
9. **UnRec.** Stop recording the data without stopping the sorting.

NOTE **UnRec** is only available when a sorting is in process. The unrecorded data is not archived in the FCS file.

10. **Standby.** Turns off the sheath and puts the system in the Standby state.

NOTE Beckman Coulter recommends re-running the sort calibration to ensure the auto drop delay is accurate if the system enters standby state during a sorting. For Sort Calibration, refer to [Sort Calibration \(Auto Drop Delay\)](#) in [CHAPTER 5, Sorting](#).

Tube Management



1. **Tube management controls.** Used to add or delete a tube, open the tube property, open the experiment folder, open the compensation matrix, define the tube/plate sorting settings, and open the sort report.
2. **Test tube status indication.** Displays a colored symbol in front of each tube indicating the status of the tube processing.

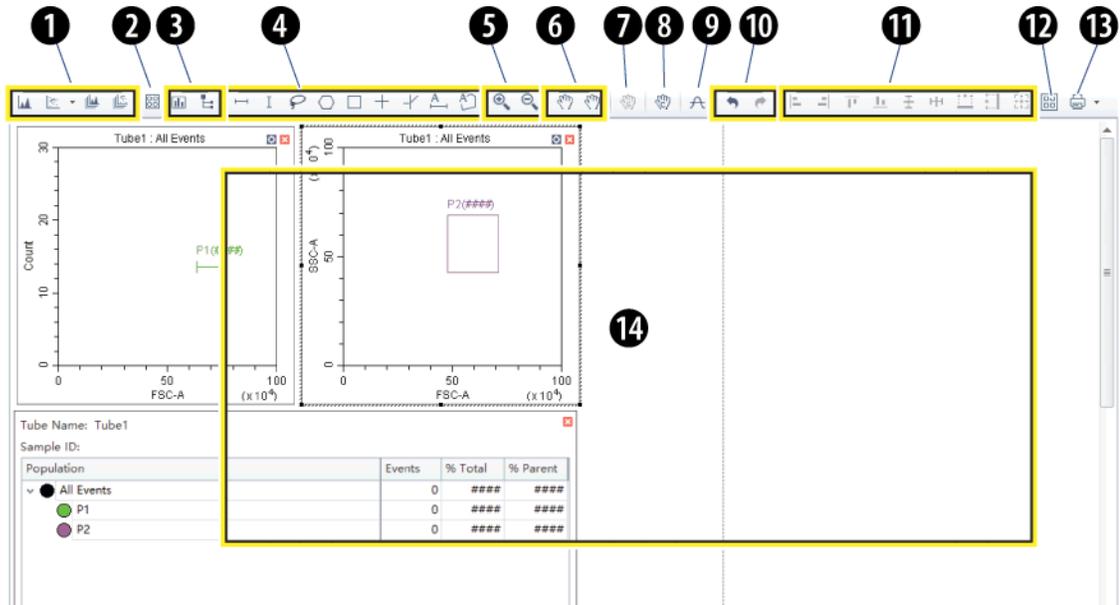
-  indicates that a tube is created, but the tube setting is not defined.
-  indicates that a tube is created, and the tube sorting setting is defined.
-  indicates that a tube is created, and the plate sorting setting is defined.
-  indicates that the tube data without sorting setting was acquired by selecting **Run**, but can be overwritten.
-  indicates that the tube data with tube sorting setting was acquired by selecting **Run**, but can be overwritten.
-  indicates that the tube data with plate sorting setting was acquired by selecting **Run**, but can be overwritten.
-  indicates that the tube data was saved by selecting **Record**, and that this data cannot be overwritten.
-  indicates that the tube data with tube sorting setting was saved by selecting **Record**, and this data cannot be overwritten.
-  indicates that the tube data with plate sorting setting was saved by selecting **Record**, and this data cannot be overwritten.
-  indicates that the tube is collecting sorting data.
-  indicates that the tube data with tube sorting setting was acquired by selecting **Sort**, but this data cannot be overwritten.
-  indicates that the tube data with plate sorting setting was acquired by selecting **Sort**, but this data cannot be overwritten.
-  indicates imported FCS data.

NOTE  to the left of the test tube status indication symbol indicates that the sample has been compensated.

-  indicates imported FCS data with sorted tube data.
-  indicates imported FCS data with sorted plate data.
-  indicates the data file is missing or there is an error in the data file.

3. **Test tube list.** Displays the sample tubes used in the experiment. Right-click a tube in the list to perform additional operations.

Plot Area



1. **Plot controls.** Creates single or multiple plots, such as dot plots, histograms, density plots, pseudo color plots, and contour plots.
2. **Index sorting.** Enables index sorting. Refer to [Index Sorting](#) in [CHAPTER 5, Sorting](#).
3. **Statistics and hierarchy controls.** Creates statistical and hierarchical charts.
4. **Graphical gating controls.** Creates graphical gates.
5. **Zoom controls.** Zooms in and out within a plot.
6. **Pan axis display controls.** Scales axis ranges in the plots.
7. **Gain adjustment control.** Increases and decreases gain adjustments on the plots.

NOTE The gain adjustment control only works when a sample is running.

8. **Adjust compensation control.** Adjusts compensation of either of the parameters on a 2D histogram.
9. **Threshold control.** Sets the minimum particle size limit, scatter value, or fluorescence intensity that acquisition will allow.
10. **Undo and redo controls.** Undoes or redoes an action in the drawing area.
11. **Display controls.** Controls how plots and tables are aligned and arranged.
12. **Rearrange.** Restores the plots to the default positions.
13. **Printing controls.** Prints and previews the plot area.
14. **Plot area.** Displays plots, statistics, and hierarchy tables.

Status Bar



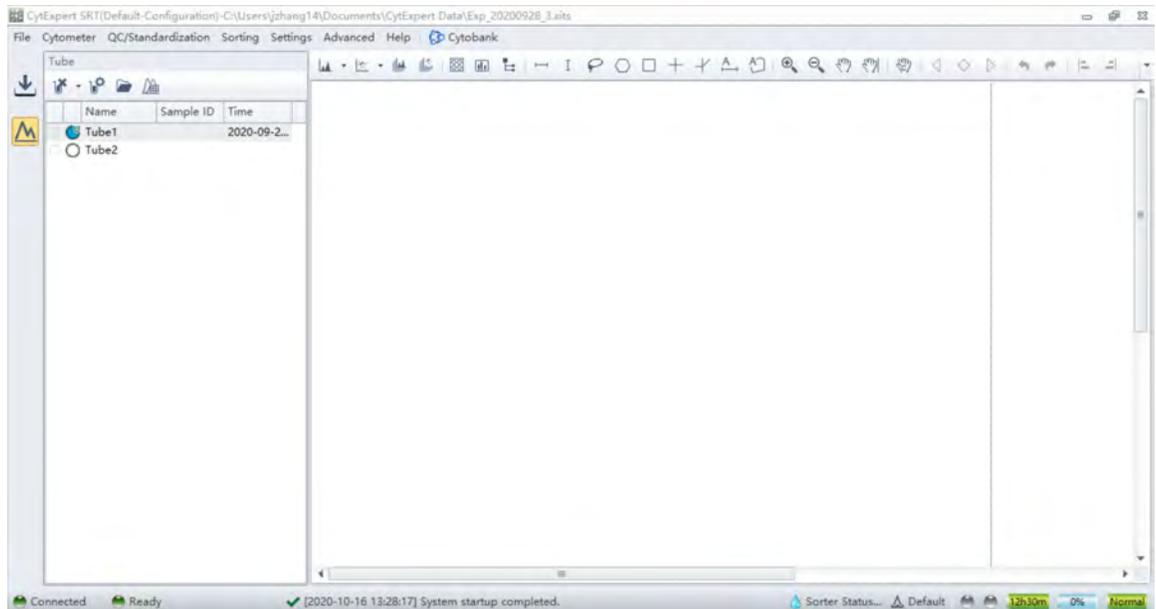
1. **Communication connection status.** Displays whether the Sorter and the Workstation are connected.
2. **Instrument state information.** Displays the working state of the instrument.
3. **System log information.** Accesses the system log window.
4. **Sorter Status.** Accesses the Sorter Status window.
5. **Stream Mode.** Displays which stream mode is used, Default or Straight Down mode.

NOTE To change the stream mode, refer to [Changing the Stream Mode](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

6. **LED indicator.** Turns the sort chamber light or the sample tube light on/off.
7. **Fluid status information.** Displays the liquid level of the fluid containers.

Analysis Screen

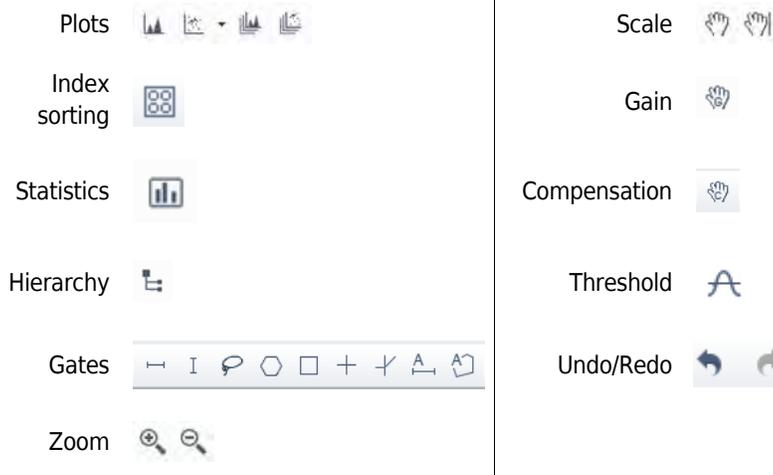
The Analysis screen is similar to the Acquisition screen, without the acquisition control modules.



Drawing controls (Refer to [Figure 2.1](#)) include the multi-data histograms and graphical display data controls.

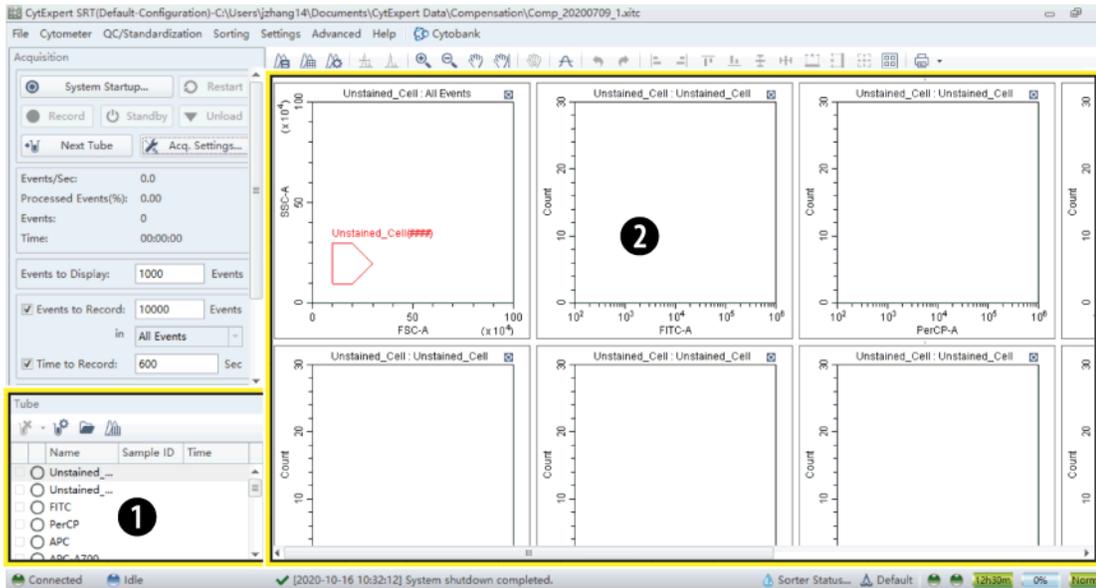
Figure 2.1 Drawing Controls Toolbar (Top of Screen)





Compensation Experiment Screen

The Compensation Experiment screen appears when you open or create a new compensation experiment.



1. **Tube management.** Displays sample tubes required for the compensation experiment.
2. **Plot area.** Displays compensation plots and gating.

The Tube management section of the screen can import saved data (.fcs) files for computational purposes.

Compensation Controls

The control options include the compensation controls, coordinate pan axis display controls, gain adjustment controls, and the undo and redo controls. The compensation controls give you the option of calculating the compensation value, displaying the compensation matrix, or changing the compensation parameters.



Compensation Calculation		Single Side Pan	
Compensation Matrix		Adjust Gain	
Compensation Setup		Undo/Redo	
Add/Delete Negative Gate		Alignment	
Zoom		Rearrange	
Pan		Print	

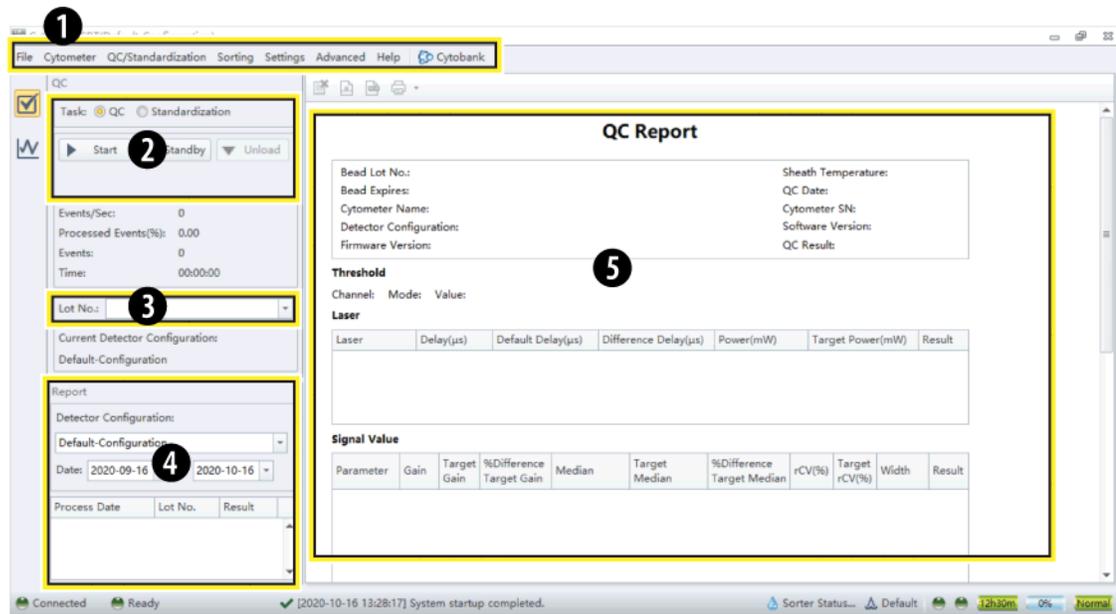
QC Experiment Screen

The Quality Control (QC) Experiment screen appears when select **Start QC/Standardization** from the QC/Standardization tab.

QC Report Screen

Before starting the QC routine, a Settings screen appears.

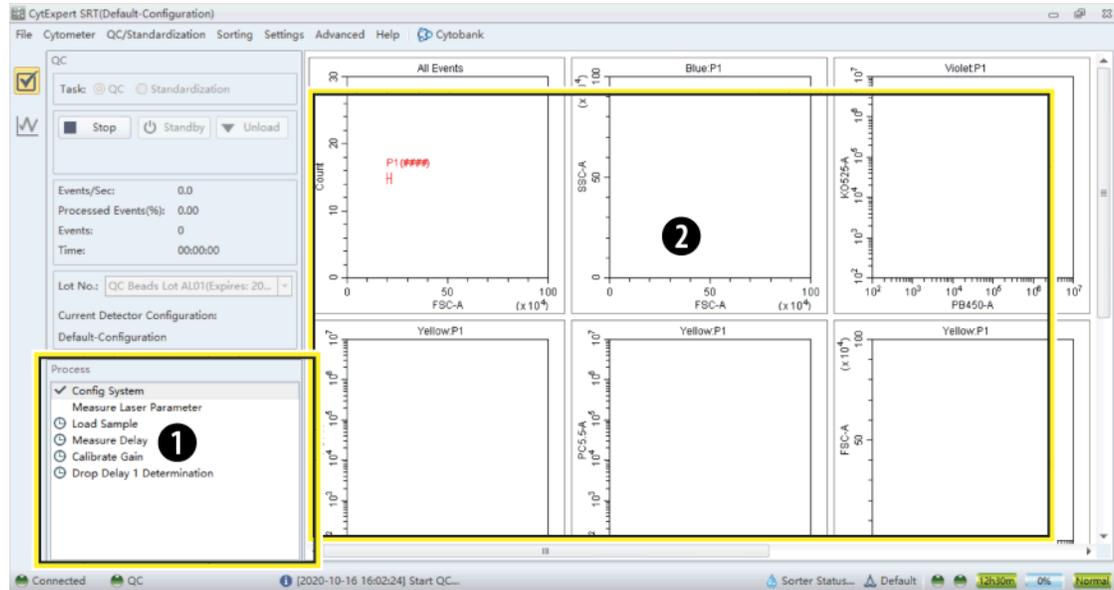
Figure 2.2 QC Report Screen



1. **Menu.** Allows you to configure settings related to QC experiments.
2. **Acquisition control.** Controls sample loading/unloading and data recording.
3. **Lot selection.** Allows you to select the lot number of the QC reagent.
4. **QC results list area.** Displays the time and results of completed QC runs.
5. **QC reports area.** Displays detailed reports for the selected QC experiment.

QC Experiment Screen

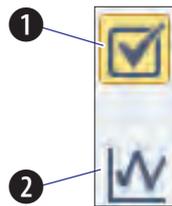
When acquiring QC samples, the software opens the QC screen.



1. **QC experiment progress indicator.** Displays the QC stage.
2. **Plot area.** Displays the QC plots.

QC Screen Navigation

The Analysis screens have two navigation icons, one for the QC screen and the other for the Levey-Jennings (LJ) charts. Refer to [Creating Levey-Jennings Charts in CHAPTER 4, Instrument Quality Control and Standardization](#).



1. **QC screen icon.** Accesses the QC screen.
2. **LJ screen icon.** Accesses the Levey-Jennings (LJ) screen.

Software Menu

IMPORTANT All menu items apply to the CytExpert SRT Default software option unless otherwise specified. Depending on the Sorter state, certain functions may not be available.

The CytExpert SRT software contains the following selectable menu items:

Figure 2.3 Software Menu Tree

File	Cytometer	QC/Standardization	Sorting	Settings	Advanced	Account*	Log*	Backup/Restore	Help	Cytobank
New Experiment...	System Startup...	Start QC/Standardization	Sort Calibration	Set Channel	Delay Setting	User Manager	Experiment Operation Log	Backup	View Help File	
New Experiment from Template...	System Shutdown...	Close QC/Standardization	Manual Drop Delay	Set Label	Laser Setting	Role Manager	System Operation Log	Restore	About	
New Experiment from FCS...	Long Term Shutdown...		Manual Side Stream Calibration	Set Customized Parameters	Event Rate Setting	Account Policies	User Management Operation Log	Log Clean-up		
New Compensation	Turn On		Sorter Status	Compensation Matrix	Sort Guardband Setting	Change Password				
Open Experiment	Turn Off		Side Stream Monitor	Compensation Library	Default Amplitude Setting					
Open Compensation	Backflush		Stream Mode Switch	Events Display Setting	Collection Device Library					
Convert CytExpert Experiment	Sheath Filter De-bubble		Reset Cyclone	Language Setting	Sort Mode Library					
Save	Flow Cell De-bubble		Tube Position Setting	Options	Maintenance					
Save As	Daily Clean...				Bubble Detector Calibration					
Save As Template	Flow Cell Clean...				Sheath Tank Scale Reset					
Import FCS File	Aseptic Clean...									
Export FCS File	Reset Sample Chamber									
Recent	Acq.Setting...									
Recent Template	Acq.Setting Catalog...									
Recent Compensation	Detector Configuration									
Exit	Sample Flow Parameter Adjustment									
	Cytometer Information...									

*Only available in the CytExpert User Management software option.

The  icon is hyper linked to the Cytobank spotlight page where you can login to the Cytobank Premium server, request a 30 day free trial and access additional information. The Cytobank platform allows you to analyze, manage and securely share flow cytometry data. FCS files can be uploaded to the platform with related attachments such as pdf files.

Figure 2.4 QC Software Menu Tree

File	Cytometer	QC/Standardization	Sorting	Settings	Advanced	Account*	Log*	Backup/Restore	Help	Cytobank
New Experiment...	System Startup...	Start QC/Standardization	Sort Calibration	QC/Standardization Setting...	Delay Setting	User Manager	Experiment Operation Log	Backup	View Help File	
New Experiment from Template...	System Shutdown...	Close QC/Standardization	Manual Drop Delay	Target Library...	Laser Setting	Role Manager	System Operation Log	Restore	About	
New Experiment from FCS...	Long Term Shutdown...		Manual Side Stream Calibration	Standardization Target Library...	Event Rate Setting	Account Policies	User Management Operation Log	Log Clean-up		
New Compensation...	Turn On		Sorter Status	Language Setting...	Sort Guardband Setting	Change Password				
Open Experiment	Turn Off		Side Stream Monitor	Options...	Default Amplitude					
Open Compensation	Backflush		Stream Mode Switch		Collection Device Library					
Convert CytExpert Experiment	Sheath Filter De-bubble		Reset Cyclone		Sort Mode Library					
Recent	Flow Cell De-bubble		Tube Position Setting		Maintenance					
Recent Template	Daily Clean...				Bubble Detector Calibration					
Recent Compensation	Flow Cell Clean...				Sheath Tank Scale Reset					
Exit	Aseptic Clean...									
	Reset Sample Chamber									
	Acq.Setting Catalog...									
	Detector Configuration									
	Sample Flow Parameter Adjustment									
	Cytometer Information...									

*Only available in the CytExpert User Management software option.

Acquisition and Analysis Screen Menu

CytExpert Default Software Option

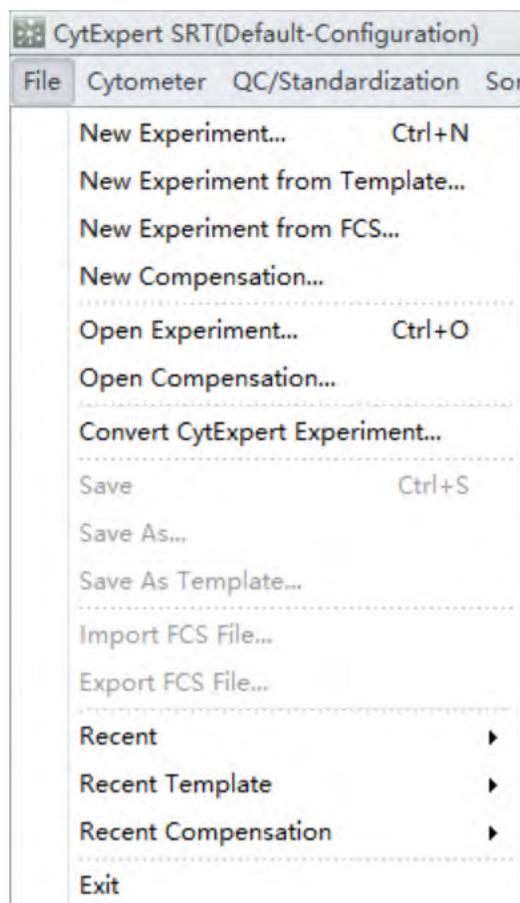
File Cytometer QC/Standardization Sorting Settings Advanced Help

CytExpert User Management Software Option

File Cytometer QC/Standardization Sorting Settings Advanced Account Log Backup/Restore

File Menu

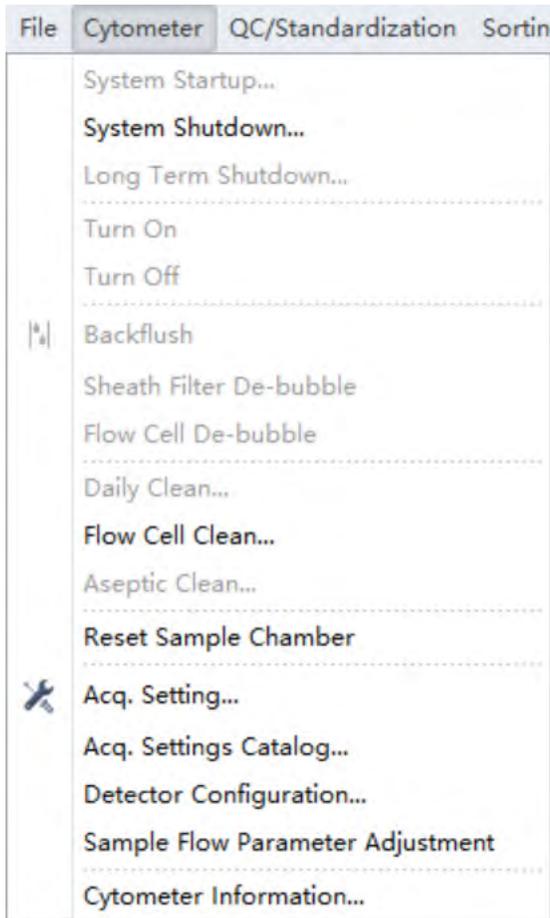
For creating new experiments, opening existing experiments, saving new experiments and data, and importing/exporting FCS data files.



Cytometer Menu

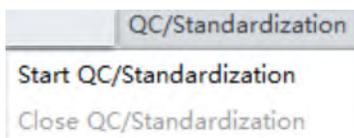
For configuring Cytometer settings and controlling Sorter functions. Depending on the Sorter state, certain functions may not be available.

[CytExpert SRT Software Option-Standby State]



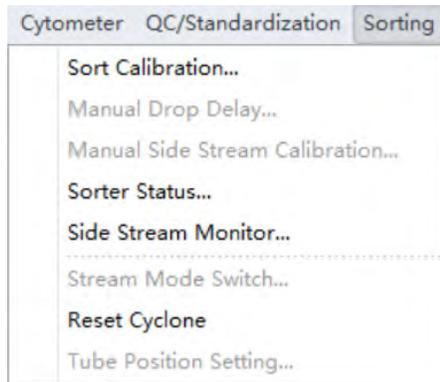
QC/Standardization Menu

Select **Start QC/Standardization** from the QC/Standardization menu to start the QC routine.



Sorting Menu

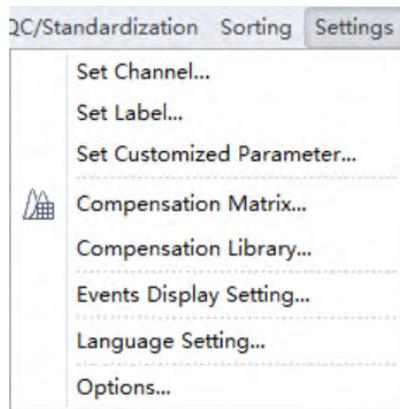
For controlling sorting options.



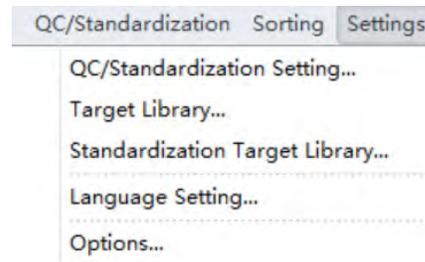
Settings Menu

Used to select and/or change software options and settings.

[Acquisition Mode]

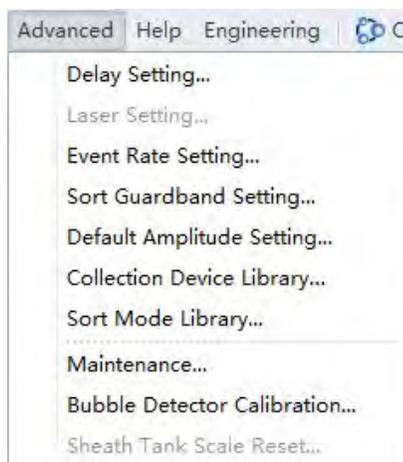


[QC/Standardization Mode]



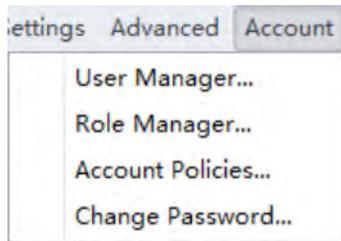
Advanced Menu

Used to access advanced settings for experienced users, including laser time delay settings, calibrations.



Account Menu

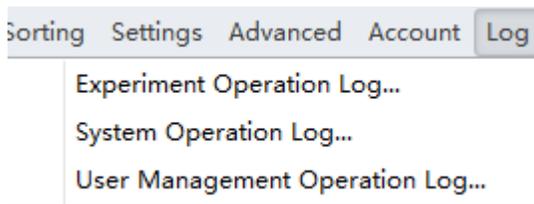
Used for user account management settings.



NOTE The Account menu is only available in the CytExpert User Management software option.

Log Menu

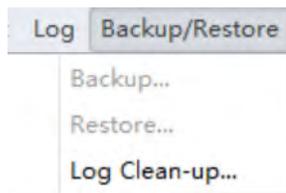
Used to access logs including the User Management Operation Log, System Operation Log, and the User Management Operation Log.



NOTE The Log menu is only available in the CytExpert User Management software option.

Backup/Restore Menu

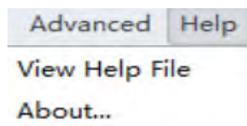
Used to backup and restore databases.



NOTE The Backup/Restore menu is only available in the CytExpert User Management software option.

Help Menu

For displaying software version information and system Instructions for Use.



User Management

IMPORTANT Only an Administrator or authorized users can manage users. You must have the CytExpert User Management software option installed to use this feature. Refer to [CytExpert Software Installation Options](#) in [APPENDIX D, Instrument Installation](#).

User Management is used to create and manage user accounts.

Select **Account > User Manager**. The User Manager window appears.

Figure 2.5 User Manager (Card View)

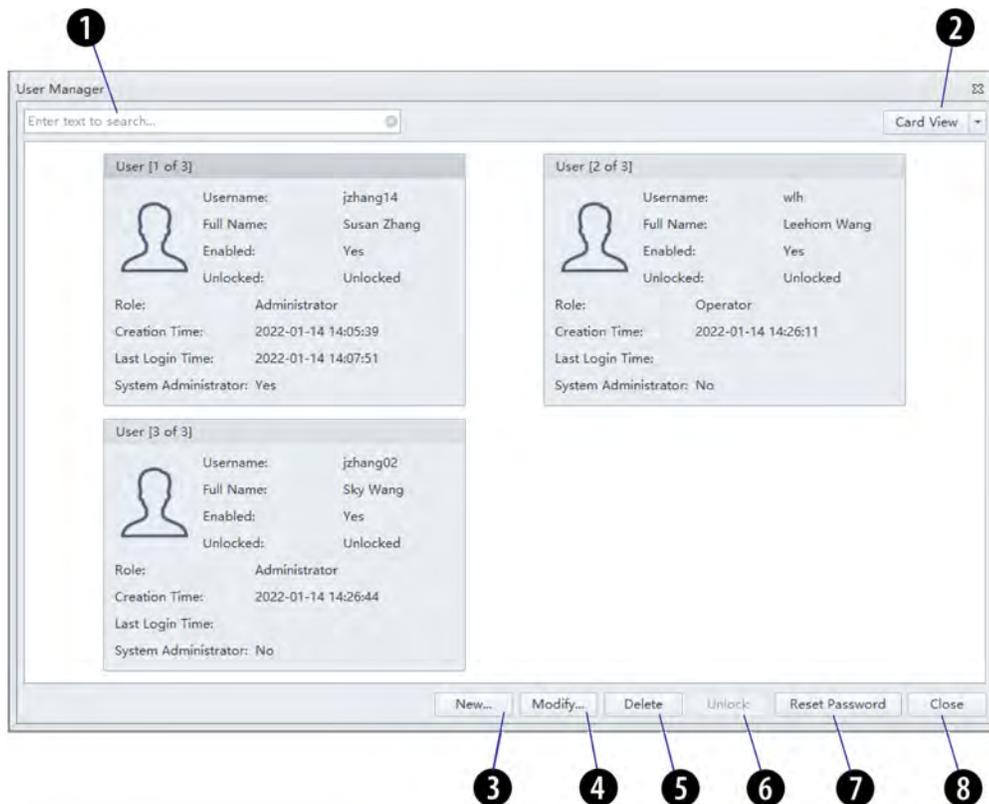
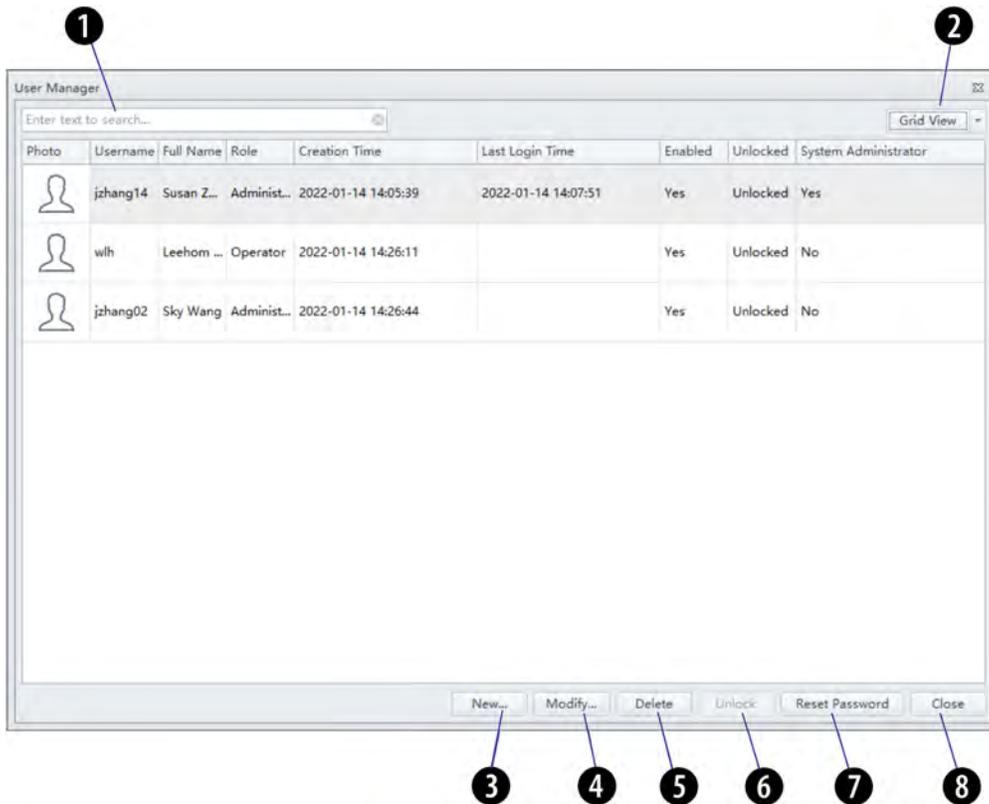


Figure 2.6 User Manager (Grid View)



- 1. Search text box:** Filters users by user name and full name.
- 2. View drop-down:** Toggles between Card View (see Figure 2.5) and Grid View (see Figure 2.6).
- 3. New:** Used to create a new user profile.
- 4. Modify:** Used to modify an existing user profile.
- 5. Delete:** Used to delete an existing user profile.
- 6. Unlock:** Used to unlock an existing account that has been locked.
- 7. Reset Password:** Used to reset an existing user password to a random password.
- 8. Close:** Closes the User Manager window.

NOTE An account locks after 3 failed password attempts by default. The number of attempts can be changed by the administrator. Refer to [Account Policies](#).

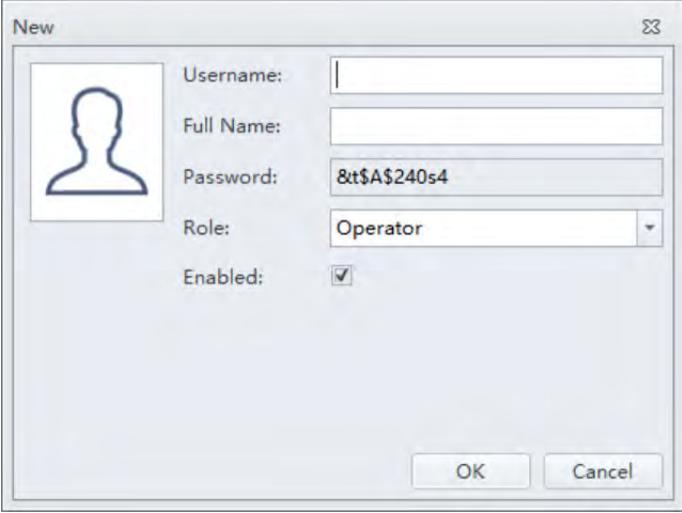
NOTE An account automatically unlocks after 30 minutes by default. The duration can be changed by the administrator. Refer to [Account Policies](#).

Creating, Deleting, and Modifying Users in User Manager

The initial System Administrator is a system default user and it cannot be deleted, modified, or disabled.

Creating a New User in User Manager

- 1 Select in the User Manager window. The New window appears.



- 2 Fill in the new user information.

- a. Enter the Username.

NOTE Username has the following naming requirements:

- The maximum number of allowable characters is 36.
- Special symbols are allowed.
- Username cannot be left blank.

- b. Enter the Full Name.

NOTE Full Name has the following naming requirements:

- The maximum number of allowable characters is 72.
- Special symbols are allowed.
- Full name cannot be left blank.

- c. Select the user Role.

- d. Select the Enabled checkbox to enable the user.

NOTE The Enabled checkbox can only be changed by an administrator.

e. **Optional:** Select the portrait to import an image.

3 Select . The new user displays in User Manager.

4 Select .

Deleting Users in User Manager

IMPORTANT If an account has been used and log information has been generated related to it, the account cannot be deleted, but it can be disabled.

1 Select the user to be deleted in the User Manager window then select .

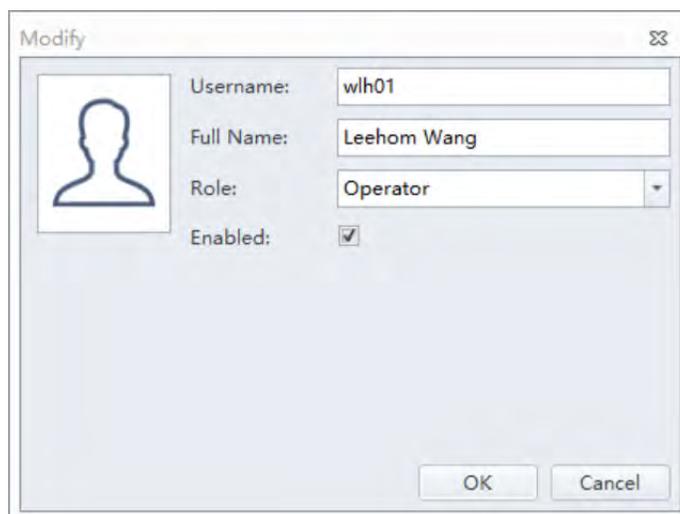
2 The following message appears: *Are you sure to you want to delete the selected user?* Select **Yes** to confirm.

3 Select .

Modifying Users in User Manager

IMPORTANT If an account has been used and log information has been generated related to it, the username cannot be modified.

1 Select in the User Manager window. The Modify window appears.



2 Modify the user information as necessary.

NOTE Uncheck the enabled box to disable a user.

3 Select  .

4 Select  .

Unlocking a User Account

Select a *Locked* user in the User Manager window and select **Unlock**.

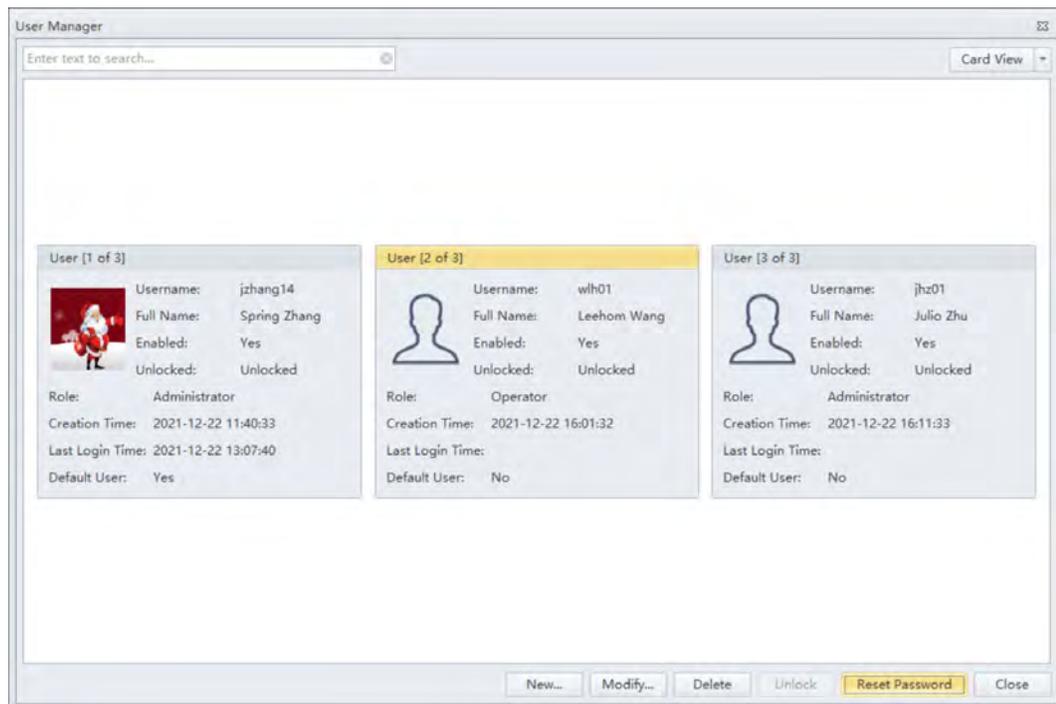
NOTE You cannot unlock an active user.

Resetting a User Password

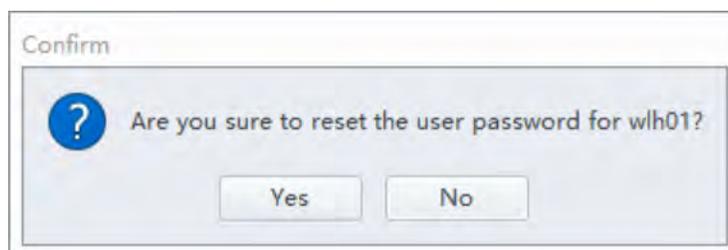
IMPORTANT Only an administrator or authorized users can reset a password for the users who forget their passwords. However, a common administrator cannot reset a password for the initial System Administrator because the initial System Administrator is a super administrator.

If you are the System Administrator and forget your password, refer to [Forgot Username or Password](#).

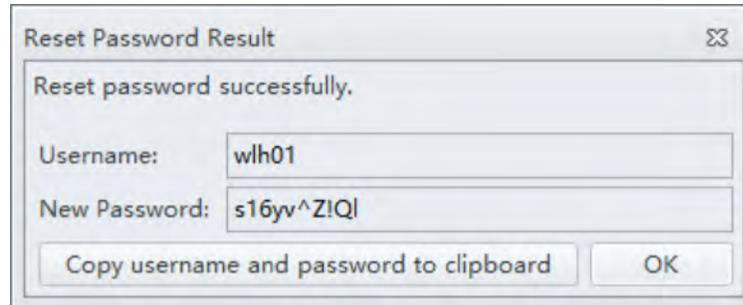
- 1 Select a user in the User Manager window then select **Reset Password**.



The confirm window appears.



- 2 Select **Yes**. A new random password is automatically generated.



NOTE Select **Copy Username and Password to Clipboard** to copy the username and password to a clipboard and inform the user of the change. The user is required to change the new random password immediately upon the initial login.

Changing a User Password

Beckman Coulter recommends changing your password on a regular basis.

- 1 Select **Account > Change Password**. The Change Password window appears.



- 2 Enter the current password, the new password, and confirm the new password.

NOTE The new password must contain at least ten digits and four of the following character types:

- letter in upper case
- letter in lower case
- numbers
- special character
- Unicode character that is categorized as an alphabetic character but is not uppercase or lowercase.

3 Select .

Forgot Username or Password

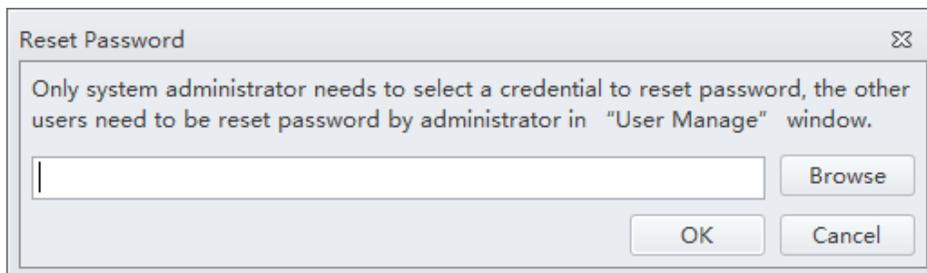
The Forgot username or password function allows only the initial System Administrator to reset a password using the system administrator credential file. If you are a common user and forget your password, contact the administrator to reset a password for you. For instructions, refer to [Resetting a User Password](#).

IMPORTANT The System Administrator password cannot be reset without the system administrator credential file. Beckman Coulter is not responsible for and will not be able to recover your system administrator account if the system administrator password is forgotten and the credential file is lost.

1 Select **Forgot username or password** on the login window.

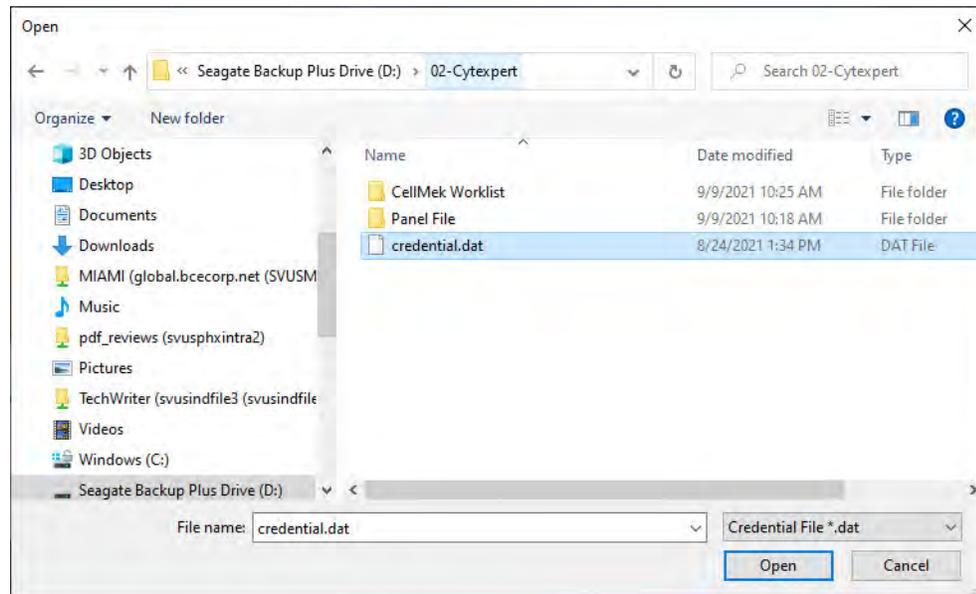


The Reset Password window appears.



IMPORTANT Find the system administrator credential file from the backup folder when installing the CytExpert software. You can also export the credential file by selecting **Export Credential File** from the Account menu if you are logged in.

2 Navigate to the system administrator credential file and select **Open**.



3 Select **OK**. The Change Password window appears.



4 Enter the new password, and confirm the new password.

NOTE The new password must contain at least ten digits and all four of the following character types by default:

- letter in upper case
- letter in lower case
- numbers
- special character
- Unicode character that is categorized as an alphabetic character but is not uppercase or lowercase.

However, the password complexity requirements can be disabled by the administrator. Refer to [Figure 2.8](#).

Role Management

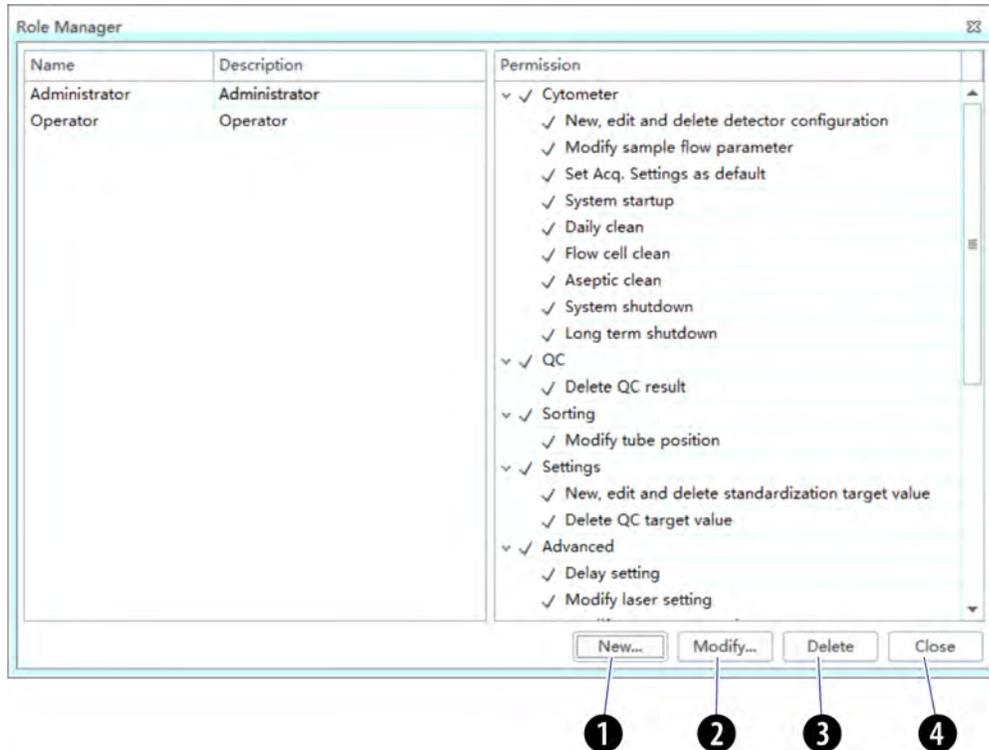
IMPORTANT Only an Administrator can manage users.

Role Management is used to manage user account permissions.

NOTE Multiple users can be applied to the same role.

Select **Account > Role Manager**. The Role Manager window appears. Refer to [Figure 2.7](#).

Figure 2.7 Role Manager

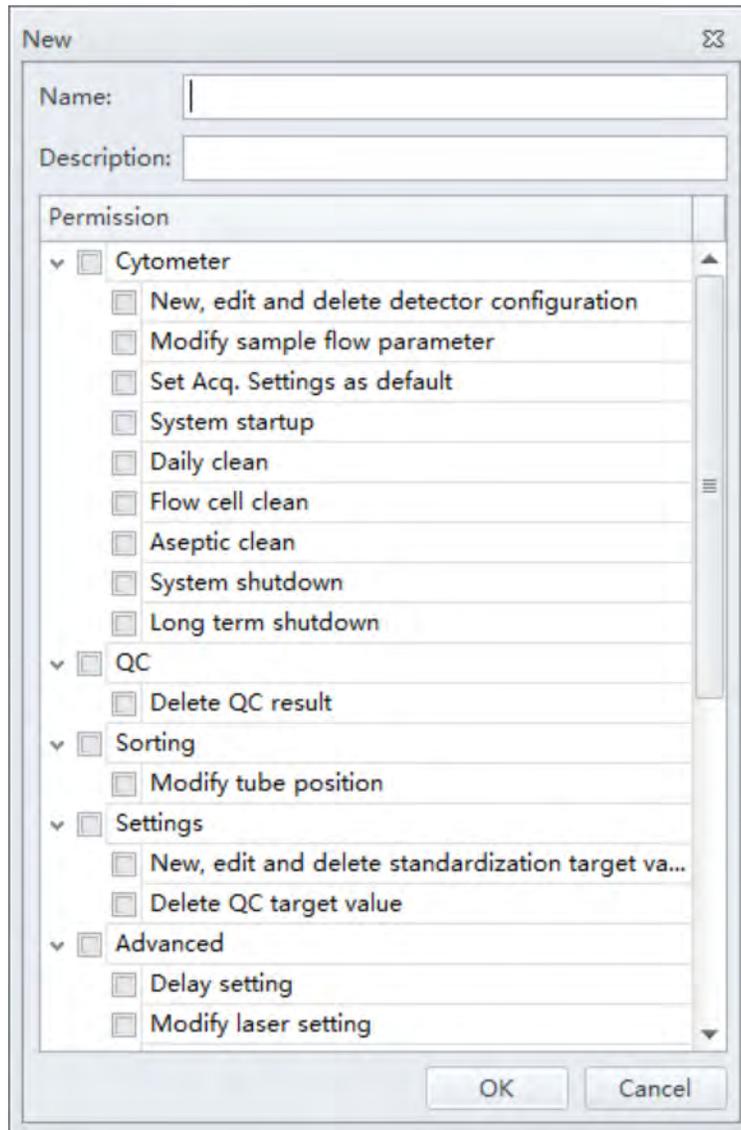


1. **New:** Used to create a new role profile.
2. **Modify:** Used to modify an existing role profile.
3. **Delete:** Used to delete an existing role profile.
4. **Close:** Closes the Role Manager window.

Creating, Deleting, and Modifying User Roles in Role Manager

Creating New User Roles in Role Manager

- 1 Select . The New window appears.



- 2 Fill in the new role information.

- a. Enter the role name.

NOTE Role Name has the following naming requirements:

- The maximum number of allowable characters is 50.
- Special symbols are allowed.
- The text box cannot be left blank.

b. Enter the role description.

NOTE Role Description has the following naming requirements:

- The maximum number of allowable characters is 100.
- Special symbols are allowed.
- The text box cannot be left blank.

c. Select the permissions applicable to the new role.

3 Select . The new role displays in the role list.

4 Select .

Deleting User Roles in Role Manager

IMPORTANT If a role has already been assigned to a user, that role cannot be deleted.

IMPORTANT The Administrator and Operator Roles are system defaults and may not be deleted.

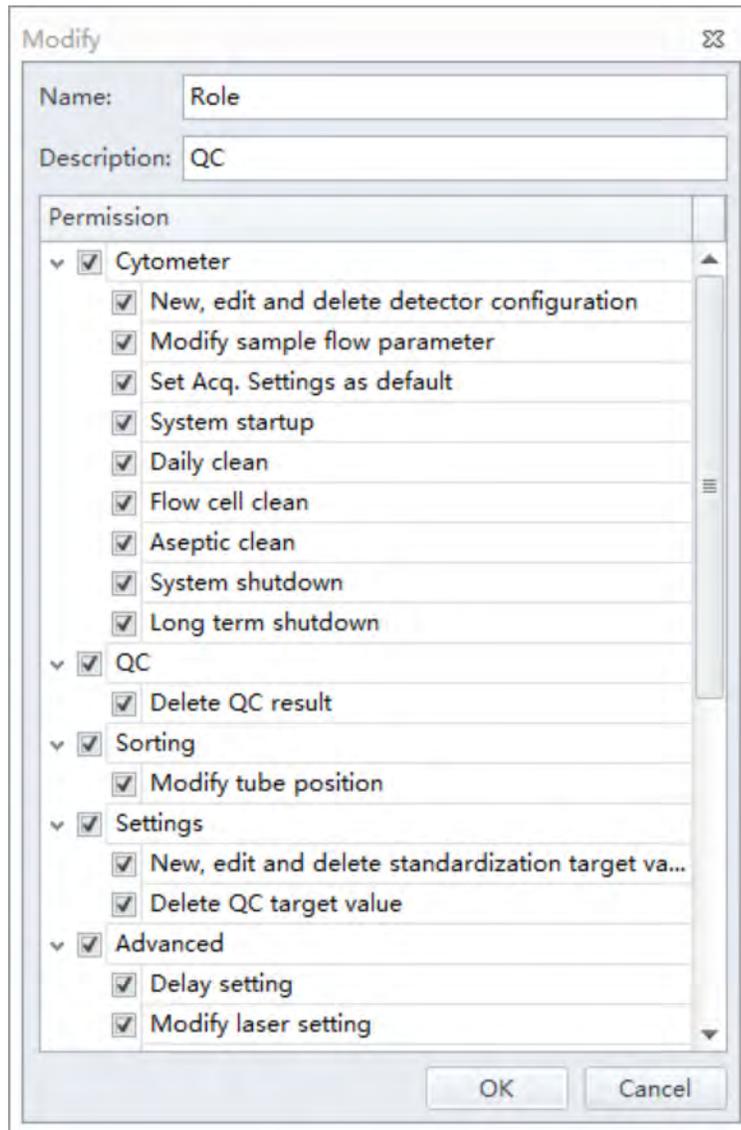
1 Select the Role to be deleted in Role Manager then select .

2 Select .

Modifying User Roles in the Role Window

IMPORTANT The Administrator and Operator Roles are system defaults and may not be modified.

- 1 Select the role to be modified and then select . The Modify window appears.



- 2 Modify the role information as necessary.

- 3 Select .

- 4 Select .

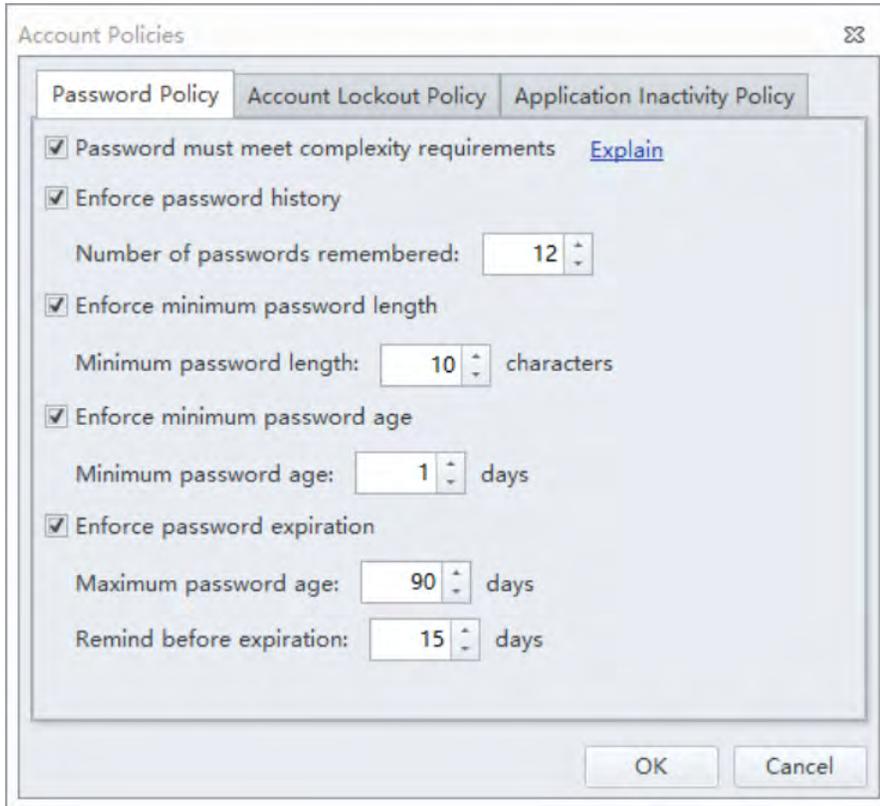
Account Policies

IMPORTANT Only an Administrator can manage users.

Account policies are used to define the default properties for the password policy, account lockout policy, and application inactivity policy.

Select **Account > Account Policies**. The Account Policies window appears.

Figure 2.8 Account Policies - Password Policy



The screenshot shows the 'Account Policies' dialog box with the 'Password Policy' tab selected. The dialog has three tabs: 'Password Policy', 'Account Lockout Policy', and 'Application Inactivity Policy'. The 'Password Policy' tab contains the following settings:

- Password must meet complexity requirements [Explain](#)
- Enforce password history
 - Number of passwords remembered: 12
- Enforce minimum password length
 - Minimum password length: 10 characters
- Enforce minimum password age
 - Minimum password age: 1 days
- Enforce password expiration
 - Maximum password age: 90 days
 - Remind before expiration: 15 days

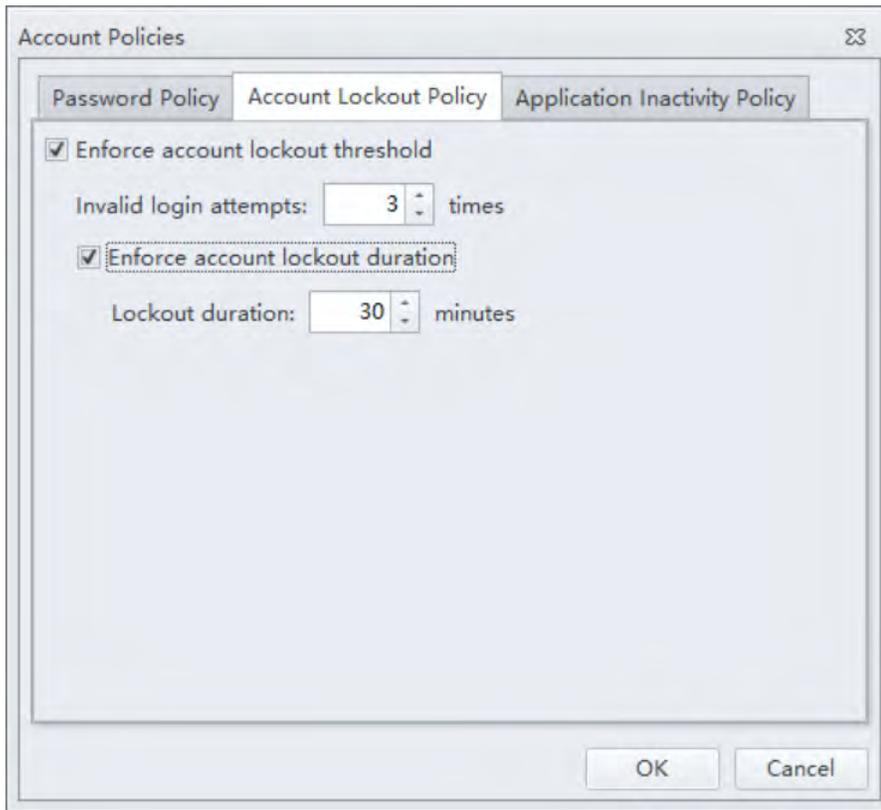
At the bottom of the dialog are 'OK' and 'Cancel' buttons.

NOTE The allowable range for each entry is as follows:

- Password Recorded: 10-12
- Minimum Password Length: 10-14 characters
- Minimum Password Age: 0-89 days
- Maximum Password Age: 1-90 days
- Reminder for Expiration: 1-90 days

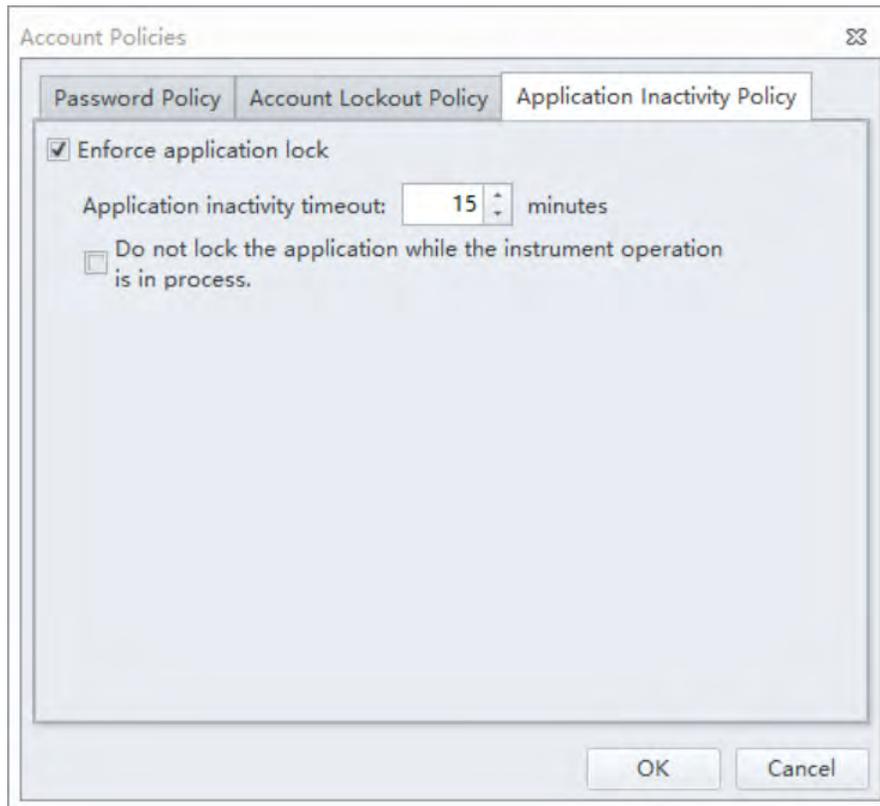
NOTE Minimum password age refers to a frozen period within which a password cannot be changed. Maximum password age refers to a date when a password expires.

Figure 2.9 Account Policies - Account Lockout Policy



NOTE The allowable range for each entry is as follows:

- Invalid Login Attempts: 3-5 times
- Lockout Time: 30-1,440 minutes

Figure 2.10 Account Policies - Application Inactivity Policy

NOTE The allowable range for each entry is as follows:

- Inactivity Duration: 1-15 minutes

Operation Log

Use the Log feature to view, export, or manage the operation logs including the experiment operation log, the system operation log, and the user management operation log.

Viewing and Exporting Experiment Operation Logs

- 1 Select **Log > Experiment Operation Log**. The Experiment Operation Log window appears.

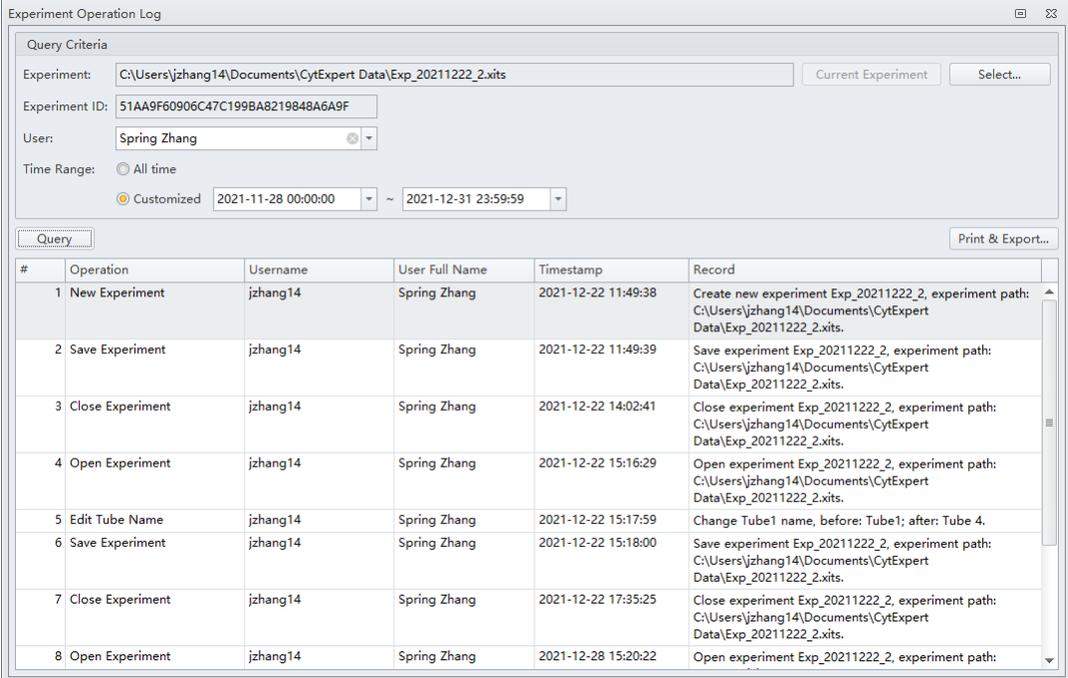
#	Operation	Username	User Full Name	Timestamp	Record
---	-----------	----------	----------------	-----------	--------

- 2 Select **Select...**. The Select Experiment Profile window appears.

Experimen...	Name	Locati...	Creator Userna...	Creator Full Na...	Creation Time	Last Modifier Userna...	Last Modifier Full...	Last Modification Time
No matches were found using the specified keyword(s).								

3 Select the experiment to be viewed.

4 Enter the filter conditions: User and Time Range, and then select . The logs appear.



The screenshot shows the "Experiment Operation Log" window. The "Query Criteria" section is filled with the following information:

- Experiment: C:\Users\jzhang14\Documents\CytExpert Data\Exp_20211222_2.xits
- Experiment ID: 51AA9F60906C47C199BA8219848A6A9F
- User: Spring Zhang
- Time Range: Customized (2021-11-28 00:00:00 ~ 2021-12-31 23:59:59)

The "Query" button is highlighted, and the "Print & Export..." button is visible in the top right of the table area.

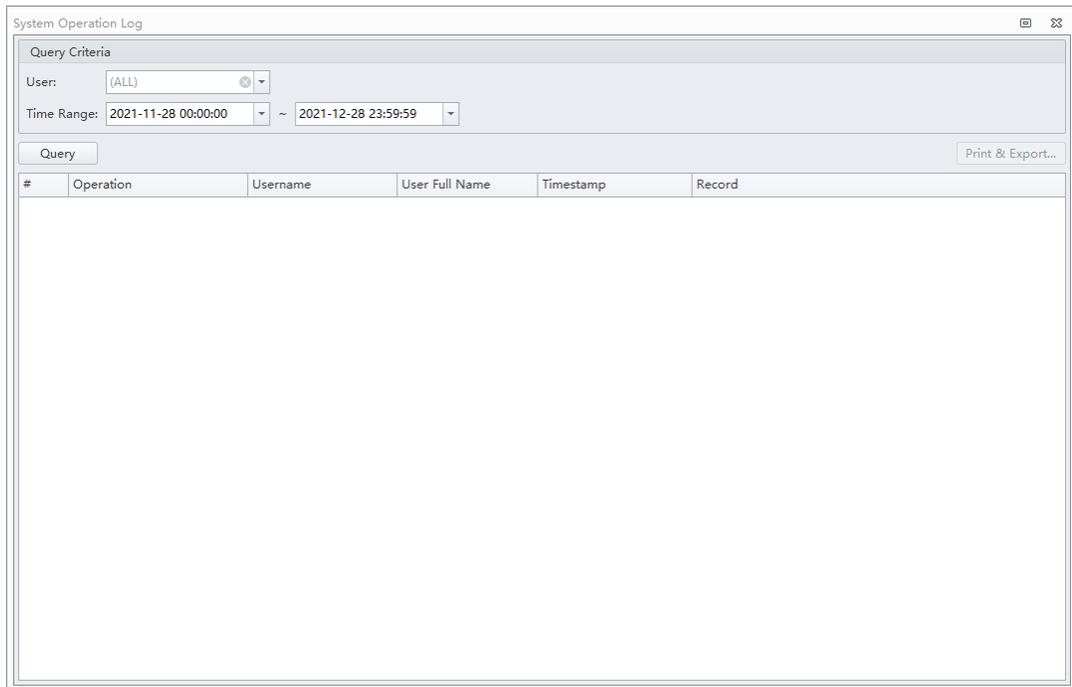
#	Operation	Username	User Full Name	Timestamp	Record
1	New Experiment	jzhang14	Spring Zhang	2021-12-22 11:49:38	Create new experiment Exp_20211222_2, experiment path: C:\Users\jzhang14\Documents\CytExpert Data\Exp_20211222_2.xits.
2	Save Experiment	jzhang14	Spring Zhang	2021-12-22 11:49:39	Save experiment Exp_20211222_2, experiment path: C:\Users\jzhang14\Documents\CytExpert Data\Exp_20211222_2.xits.
3	Close Experiment	jzhang14	Spring Zhang	2021-12-22 14:02:41	Close experiment Exp_20211222_2, experiment path: C:\Users\jzhang14\Documents\CytExpert Data\Exp_20211222_2.xits.
4	Open Experiment	jzhang14	Spring Zhang	2021-12-22 15:16:29	Open experiment Exp_20211222_2, experiment path: C:\Users\jzhang14\Documents\CytExpert Data\Exp_20211222_2.xits.
5	Edit Tube Name	jzhang14	Spring Zhang	2021-12-22 15:17:59	Change Tube1 name, before: Tube1; after: Tube 4.
6	Save Experiment	jzhang14	Spring Zhang	2021-12-22 15:18:00	Save experiment Exp_20211222_2, experiment path: C:\Users\jzhang14\Documents\CytExpert Data\Exp_20211222_2.xits.
7	Close Experiment	jzhang14	Spring Zhang	2021-12-22 17:35:25	Close experiment Exp_20211222_2, experiment path: C:\Users\jzhang14\Documents\CytExpert Data\Exp_20211222_2.xits.
8	Open Experiment	jzhang14	Spring Zhang	2021-12-28 15:20:22	Open experiment Exp_20211222_2, experiment path:

5 To export the log, select .

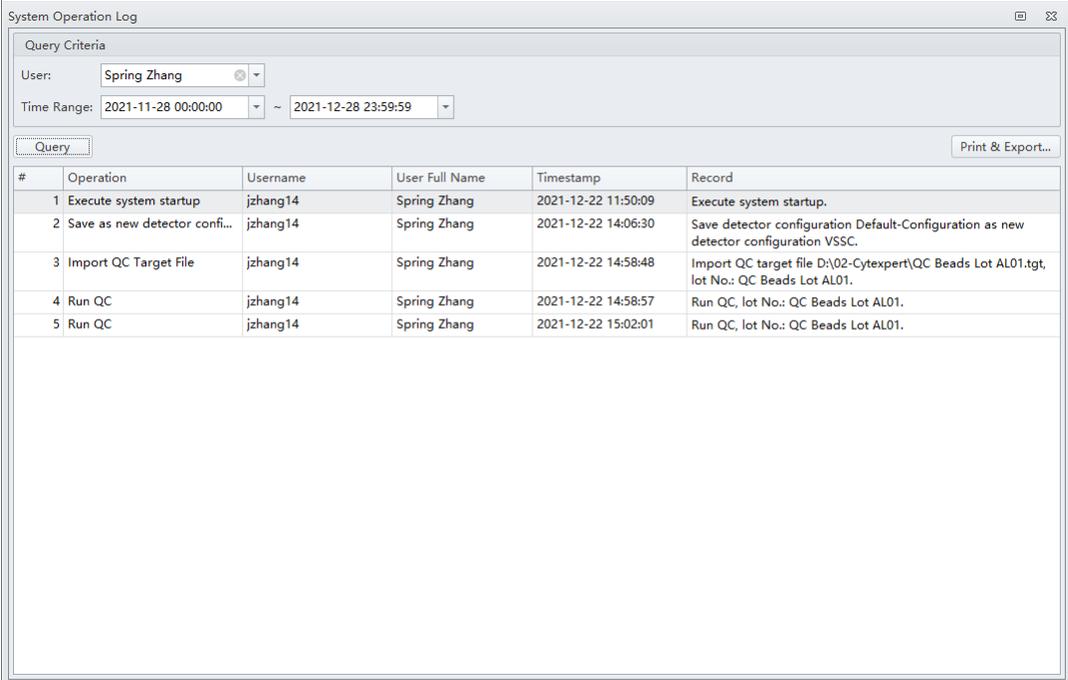
NOTE Experiment Operation logs are exported as a .pdf or .csv file.

Viewing and Exporting System Operation Logs

- 1 Select **Log > System Operation Log**. The System Operation Log window appears.



- 2 Enter the filter conditions: User and Time Range, and then select . The logs appear.



The screenshot shows the 'System Operation Log' window. At the top, there is a 'Query Criteria' section with two dropdown menus: 'User' set to 'Spring Zhang' and 'Time Range' set to '2021-11-28 00:00:00' to '2021-12-28 23:59:59'. Below this is a 'Query' button and a 'Print & Export...' button. The main area contains a table with the following data:

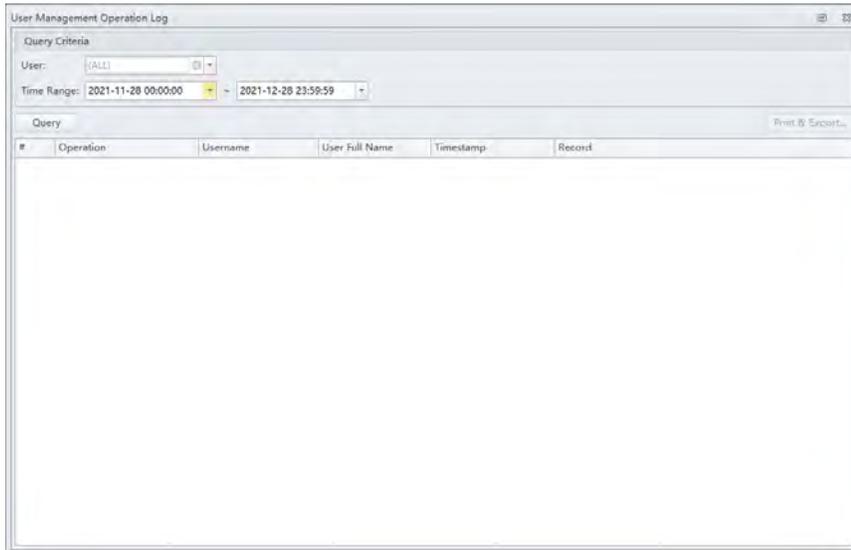
#	Operation	Username	User Full Name	Timestamp	Record
1	Execute system startup	jzhang14	Spring Zhang	2021-12-22 11:50:09	Execute system startup.
2	Save as new detector confi...	jzhang14	Spring Zhang	2021-12-22 14:06:30	Save detector configuration Default-Configuration as new detector configuration VSSC.
3	Import QC Target File	jzhang14	Spring Zhang	2021-12-22 14:58:48	Import QC target file D:\02-Cytextpert\QC Beads Lot AL01.tgt, lot No.: QC Beads Lot AL01.
4	Run QC	jzhang14	Spring Zhang	2021-12-22 14:58:57	Run QC, lot No.: QC Beads Lot AL01.
5	Run QC	jzhang14	Spring Zhang	2021-12-22 15:02:01	Run QC, lot No.: QC Beads Lot AL01.

- 3 To export the log, select .

NOTE User logs are exported as a .pdf or .csv file.

Viewing and Exporting User Management Operation Logs

- 1 Select **Log > User Management Operation Log**. The User Management Operation Log window appears.



- 2 Enter the filter conditions: User and Time Range, and then select **Query**. The logs appear.

The screenshot shows the 'User Management Operation Log' window with the 'Query' button highlighted. The 'Query Criteria' section now shows 'User' set to 'Spring Zhang' and 'Time Range' from '2021-11-28 00:00:00' to '2021-12-28 23:59:59'. The main table is populated with 15 log entries.

#	Operation	Username	User Full Name	Timestamp	Record
1	Login	jzhang14	Spring Zhang	2021-12-22 11:47:29	Login successful.
2	Logout	jzhang14	Spring Zhang	2021-12-22 11:48:43	Logout.
3	Login	jzhang14	Spring Zhang	2021-12-22 11:49:04	Login successful.
4	Login	jzhang14	Spring Zhang	2021-12-22 13:07:41	Login successful.
5	Create user	jzhang14	Spring Zhang	2021-12-22 16:01:32	Create new user, Username: wlh01, Full Name: Leehom Wang, Role: Operator, Enabled: Yes.
6	Create user	jzhang14	Spring Zhang	2021-12-22 16:02:49	Create new user, Username: jz02, Full Name: Lee SZ, Role: Administrator, Enabled: Yes.
7	Delete user	jzhang14	Spring Zhang	2021-12-22 16:10:47	Delete user (Username: jz02).
8	Create user	jzhang14	Spring Zhang	2021-12-22 16:11:33	Create new user, Username: jhz01, Full Name: Julio Zhu, Role: Administrator, Enabled: Yes.
9	Create user	jzhang14	Spring Zhang	2021-12-22 16:25:45	Create new user, Username: QWE, Full Name: Danaher, Role: Operator, Enabled: Yes.
10	Delete user	jzhang14	Spring Zhang	2021-12-22 16:26:07	Delete user (Username: QWE).
11	Reset password	jzhang14	Spring Zhang	2021-12-22 17:23:08	Reset password for user (Username: jzhang14).
12	Reset password	jzhang14	Spring Zhang	2021-12-22 17:26:36	Reset password for user (Username: wlh01).
13	Logout	jzhang14	Spring Zhang	2021-12-22 17:35:25	Logout.
14	Login	jzhang14	Spring Zhang	2021-12-28 13:35:21	Login successful.
15	Create role	jzhang14	Spring Zhang	2021-12-28 14:23:39	Create new role, Role Name: Role 1, Description: test, Assigned Permission(s): New, edit and delete detector configuration, Modify sample flow parameter, Delete QC result, Modify tube position, New, edit and delete standardization target value, Delete QC target value, Manage role, Manage user, Access account policies, View

3 To export the log, select **Print & Export...**

NOTE User logs are exported as a .pdf file.

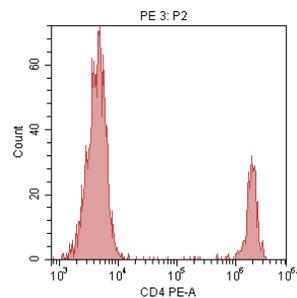
Graphic and Gating Styles

Plots

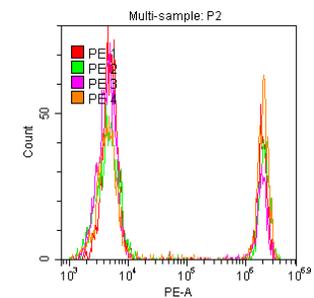
The CytExpert SRT software offers a variety of plot formats including:

- Single-parameter plots and histogram overlays
- Dual-parameter plots: dot plots, density plots, pseudo color plots, contour plots, and dot plot overlays

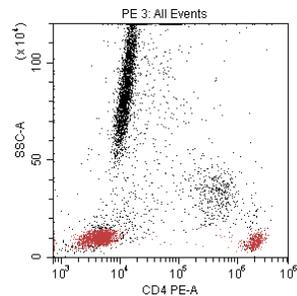
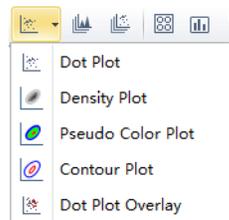
NOTE Histogram Overlays and Dot Plot Overlays can only be created from multiple samples in the Analysis screen. A maximum of 10 samples can be overlaid.



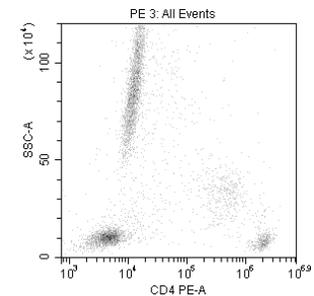
Histogram



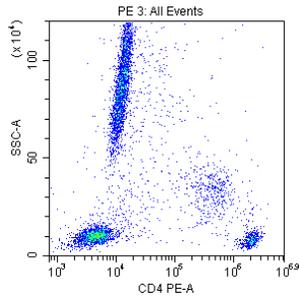
Multi-sample histogram overlays



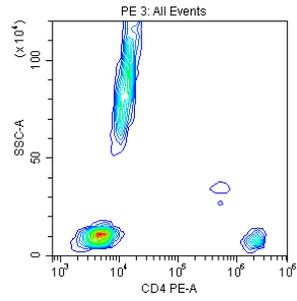
Dot plot



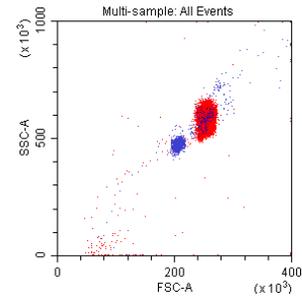
Density plot



Pseudo color plot



Contour plot



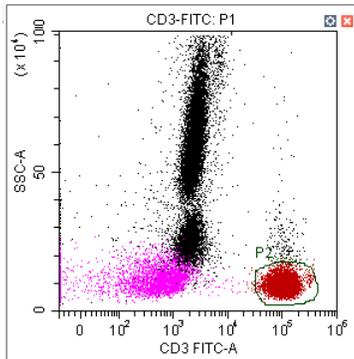
Dot plot overlay

Gates

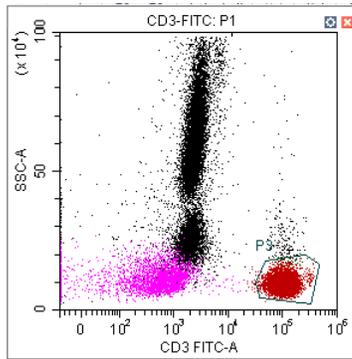
Various gating choices are available.

The software includes the following gate types:

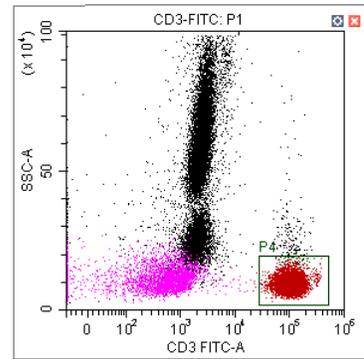
- For dual-parameter plots: lasso, polygon, rectangle, four-quadrant, hinged gates, and auto polygon
- For single-parameter plots: line-segment, vertical gates, and auto line segment



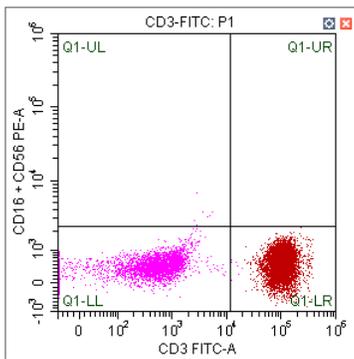
Lasso gate



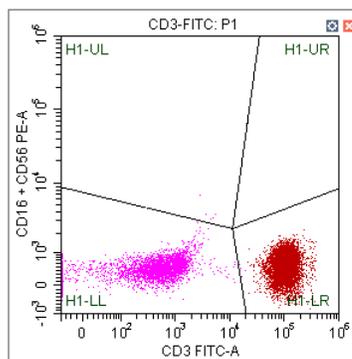
Polygon gate^a



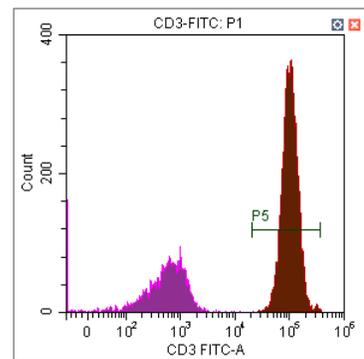
Rectangle gate



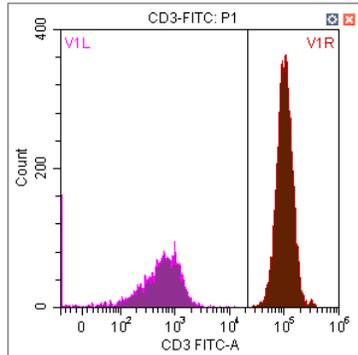
Four-Quadrant gate



Hinged gate



Line Segment gate^a



Vertical gate

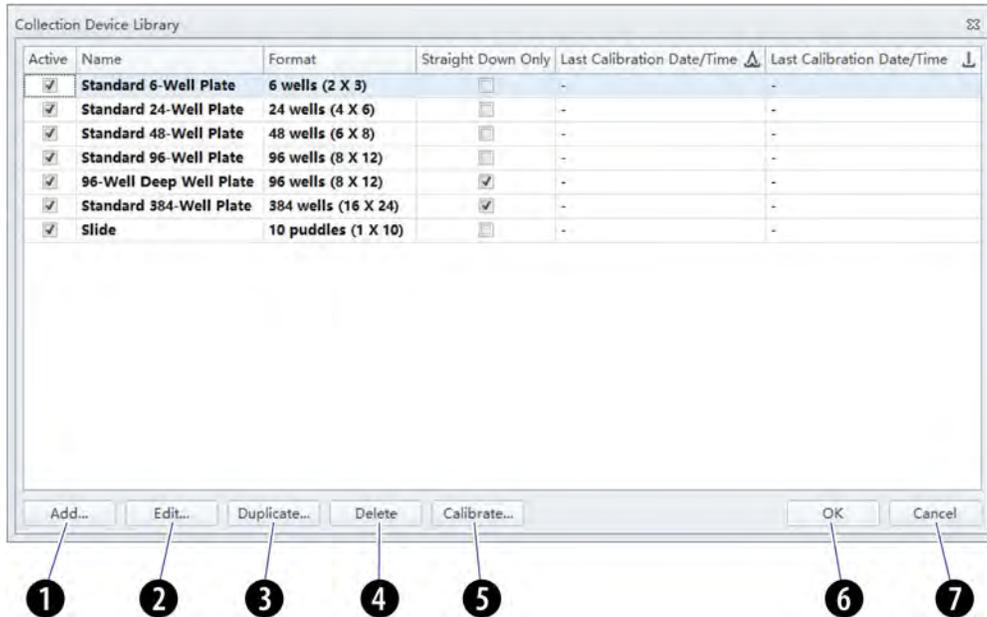
- a. This gate can be created using the autogate functionality. Refer to [Creating and Adjusting Auto Gates](#) in [CHAPTER 5, Sorting](#).

Collection Device Library

The Collection Device Library is used to manage and calibrate the position of plates or slides. To calibrate a plate or a slide, refer to [Calibrating the Sort Collection Device](#) in [CHAPTER 5, Sorting](#).

Select **Advanced > Collection Device Library** to access the Collection Device Library. Refer to [Figure 2.11](#).

Figure 2.11 Default Collection Device Library



1. **Add.** Creates a new plate or slide.
2. **Edit.** Edits a plate or slide.
3. **Duplicate.** Duplicates a plate or slide.
4. **Delete.** Deletes an existing plate or slide.
5. **Calibrate.** Calibrates the position of a plate or slide.
6. **OK.** Saves the settings.
7. **Cancel.** Cancels the settings.

NOTE The 96-well deep well plate and the standard 384-well plate support only the Straight Down mode.

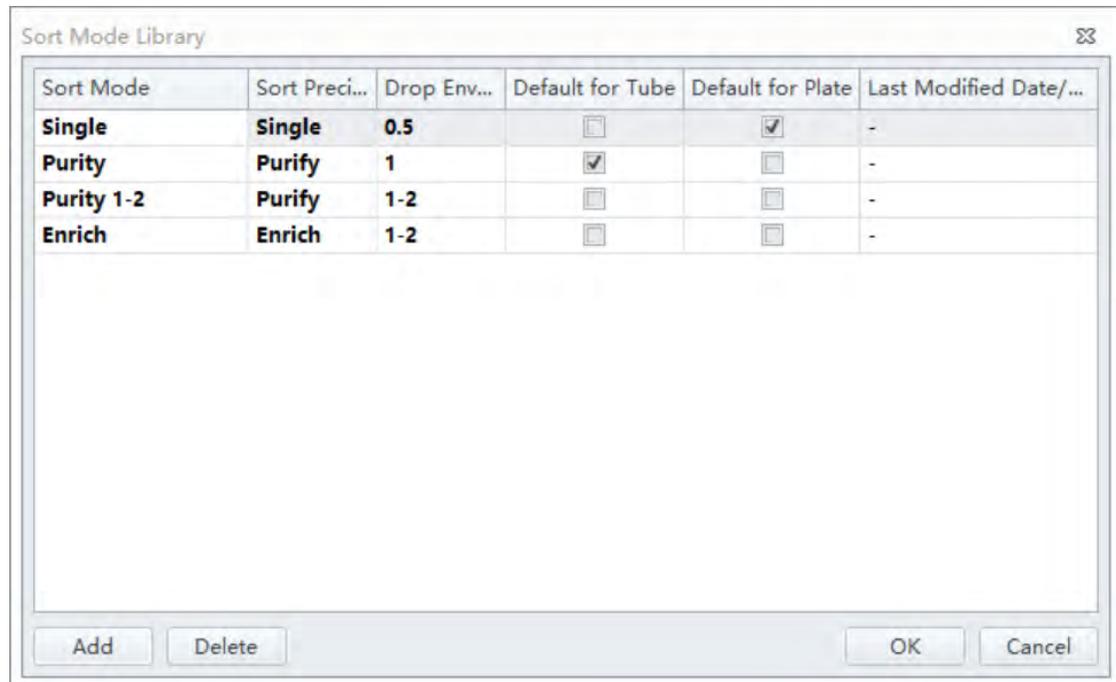
NOTE Additional collection device could be created or deleted from the library based on your needs.

Sort Mode Library

The Sort Mode Library is used to manage the sort modes, and set the default sort mode for sorting. To customize a sort mode, you have to add a sort mode first. Refer to [Figure 2.12](#).

Select **Advanced > Sort Mode Library** to access the Sort Mode Library.

Figure 2.12 Sort Mode Library



NOTE The Sort Mode Library can be edited only when an experiment is closed.

NOTE Select **Default for Tube** or **Default for Plate** to set the most frequently used Sort Mode as the default for the tube sorting or plate sorting.

Sort Mode

Sort Mode is a combination setting of Sort Precision Level and Drop Envelope. Four sort modes including **Single**, **Purity**, **Purity 1-2** and **Enrich** are available by default and cannot be deleted or changed.

The target population may be sorted using four different modes depending on the output desired. Each mode is defined by the location of the particle within the drop and the location of contaminants in either the primary drop (the central drop of interest for the sort logic) or an adjacent drop (a single drop on either side of the primary drop).

- **Single Mode:** Used when having only one event per drop is the most important aspect of the sort.
- **Purity Mode:** Used when the purity of the sort is the most important.

- **Purity1-2 Mode:** Used when the purity of the sort is the most important. The Purity 1-2 Mode is more inclusive than Purity Mode.

NOTE Beckman Coulter recommends the Purity 1-2 Mode for sorting macro-particles.

- **Enrich Mode:** Used when recovery is the most important aspect of the sort. With Enrich, all positive events (an event that falls within the sort logic) are sorted.

Sort Precision Level

Below is a detailed explanation of the three Sort Precision Levels.

Single:

- Having one positive event per droplet is the most important aspect of single mode, recovery is not as important.
- Only a single positive event and no negative events will be in the drop sorted.
- If the nearest edge of an adjacent drop contains any positive or negative events, the primary drop will be aborted. As the event rate increases, the abort rate will also increase because of the likelihood that the primary or near edge of an adjacent droplet will have either a positive or negative event.

NOTE The nearest edge depends on the setting of Sort Guard Band. Refer to [Sort Guard Band](#).

- Used most often for single cell deposition into plates/slides for cloning purposes.

Purify:

- Purity is more important than recovery.
- The drop being sorted will contain only positive events.
- If the drop contains negative events, it will be aborted.
- If the nearest edge of an adjacent drop contains any negative events, the primary drop will be aborted. As the event rate increases, the abort rate will also increase because of the likelihood that the primary or near edge of an adjacent droplet will have a negative event.

NOTE The nearest edge depends on the setting of Sort Guard Band. Refer to [Sort Guard Band](#).

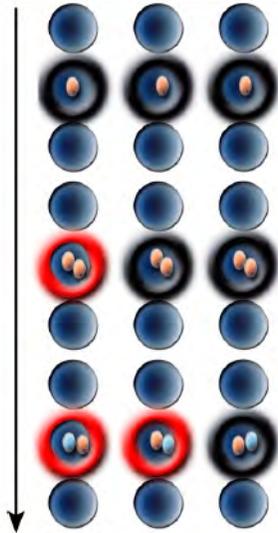
Enrich

- Recovery is more important than purity.
- All positive events are sorted, regardless of any negative events (an event that falls outside the sort logic) in or near the drop being sorted.
- Cannot be aborted due to negative events or hardware coincident events.
- Provides the greatest efficiency and recovery but least purity. Purity decreases as the event rate increases.

The Sort Precision Levels are illustrated in [Figure 2.13](#). The stream is represented by the dark blue dots flowing down the figure with either the target population (pink) or the contaminant population (blue) located within the droplet. Droplets are either sorted (black highlight) or aborted

(red highlight) depending on the sort precision level chosen. The sort precision levels are shown from left to right: **Single**, **Purify** and **Enrich**.

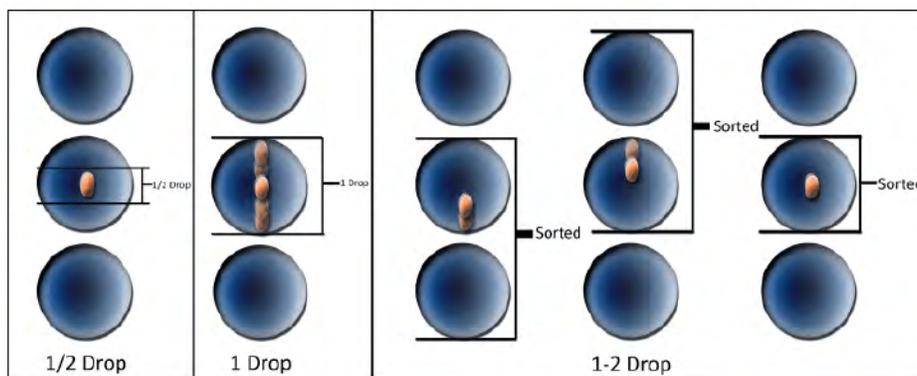
Figure 2.13 Sort Precision Modes Applied to the Same Sort Stream



Drop Envelope

The sort drop envelope defines how many drops are sorted based on the position of the positive event in the drop. Refer to [Figure 2.14](#). Depending on the required sort application, researchers may want a more stringent event location (1/2 Drop) or more inclusive (1-2 Drop). If the drop envelope is set incorrectly, recovery of the sorted population may be affected.

Figure 2.14 Drop Envelope



- **0.5 Drop:** One drop is sorted if all positive events are in the center half of the drop. This envelope typically provides the poorest yield (most aborts). This is generally used when an exact sort count is required such as for single cell deposition (Single Mode). It should never be used with the Enrich Mode because positive events will be aborted.
- **1 Drop:** One drop is sorted if the positive events fall anywhere within the drop. This envelope is used when determining drop delay. Otherwise, the only time it should be used is when sorted

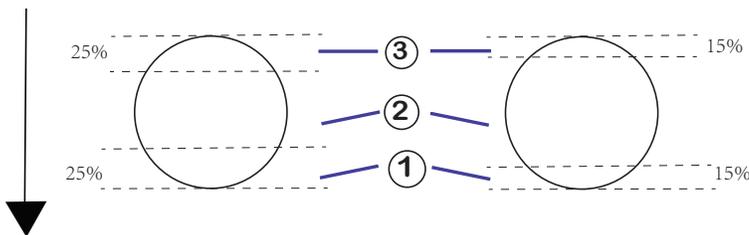
volume/dilution must be minimized. When using this envelope and the drop delay is not perfect, recovery will be reduced by 1% for each 1% error in drop delay.

- **1-2 Drop:** One drop is sorted if all positive events are in the center of the droplet. If a positive event is outside the center, then the drop adjacent to the edge containing that event is also sorted. If there is a positive event in both edges, then both adjacent drops are sorted. This envelope ensures that the positive events are always sorted. The sorted volume will be about 30% greater than the 1-drop envelope if the Sort Guard Band is set to 15%.

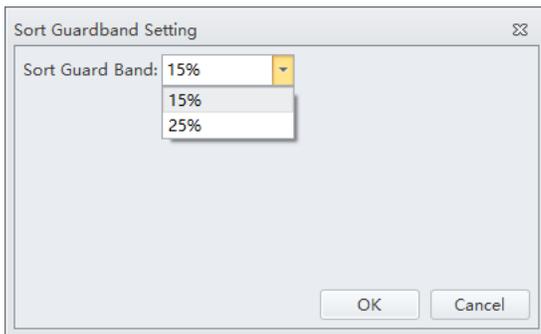
Sort Guard Band

For the accuracy of sort decision, a droplet is divided into three sections, the leading edge (1), the middle (2), and the trailing edge (3). The leading edge and the trailing edge are equal. Sort Guard Band defines the size of the two equal edges in a droplet. Refer to [Figure 2.15](#).

Figure 2.15 Sort Guard Band

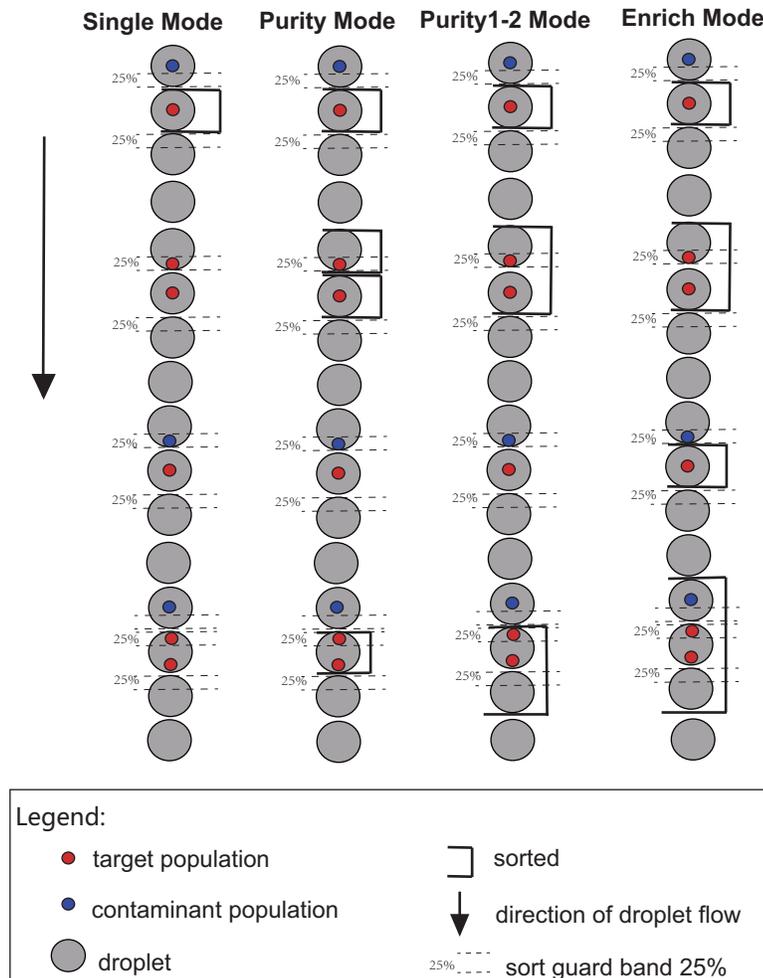


The default Sort Guard Band setting is 15%. You can set the Sort Guard Band to 25% by selecting **Sort Guard Band Setting** from the Advanced menu.



The sort decision with the 25% Sort Guard Band applied are illustrated in [Figure 2.16](#). The stream is represented by the gray dots flowing down the figure with either the target population (red) or the contaminant population (blue) located within the droplet. Droplets are either sorted (black box) or aborted depending on the sort mode chosen. The sort modes are demonstrated from left to right: **Single Mode**, **Purity Mode**, **Purity1-2 Mode**, and **Enrich Mode**.

Figure 2.16 Sort Decision with Different Modes Applied (Sort Guard Band 25%)



Sort Stream Precedence

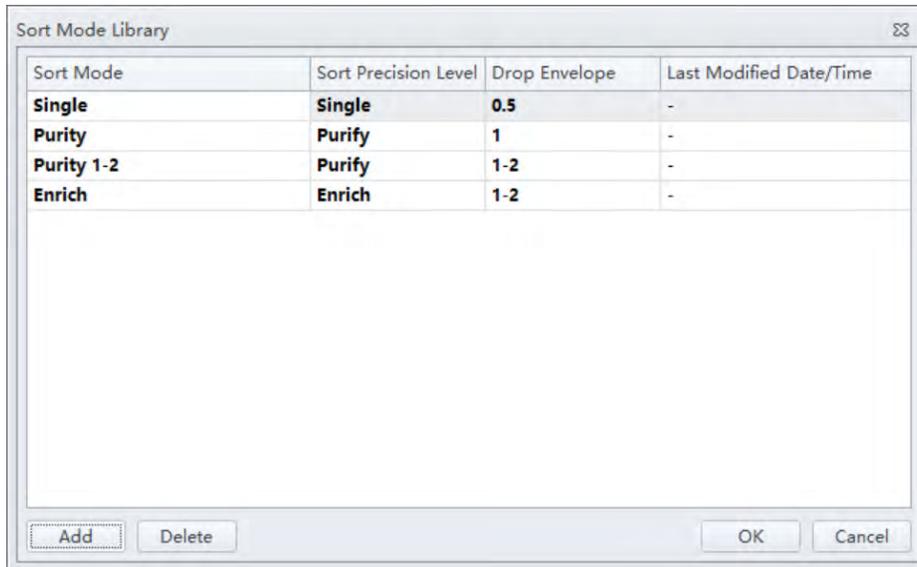
IMPORTANT When setting up a sort, place the most precious and/or rare events in the outer streams (L2/R2) for maximum purity. However, it is better to use the inner streams (L1/R1) for sorting macro-particles ($\geq 15 \mu\text{m}$).

During a sort, an analyzed cell may meet the sort logic for more than one stream. The sort logic has two precedence settings: Sort Mode and Stream Precedence. When multiple populations are being sorted simultaneously (especially at high speeds), users should choose the proper Sort Mode and Sort Stream location. Ineffective precedence decisions may affect the recovery of the desired

population. The sort mode precedence is as follows: **Single > Purify > Enrich**. The Stream Precedence is from outside to inside streams (L2>R2>L1>R1).

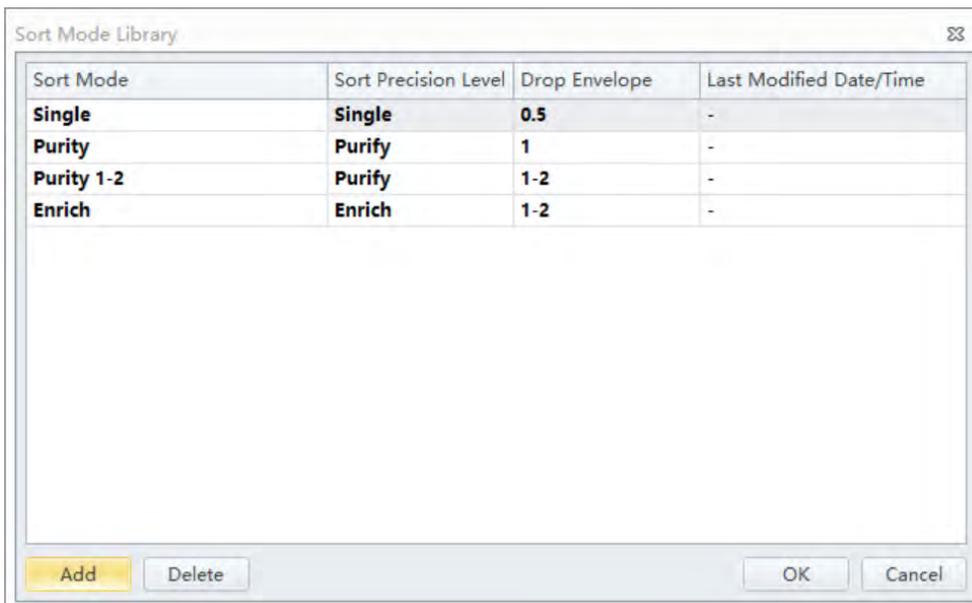
Adding a Sort Mode

- 1 Select **Sort Mode Library** from the Advanced menu. The Sort Mode Library window appears.

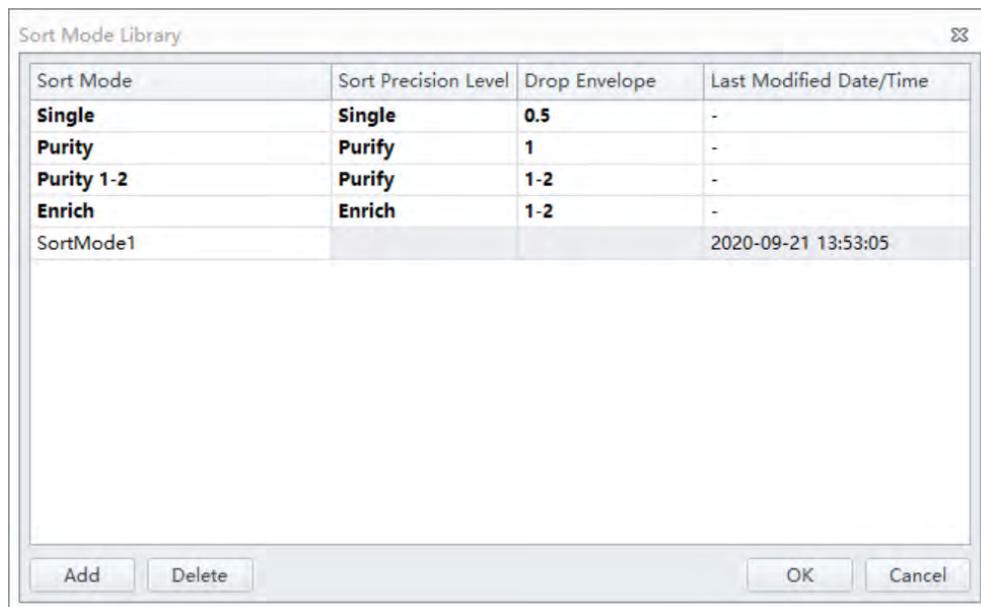


NOTE The Sort Mode Library is editable only when the experiment has been closed.

- 2 Select **Add** from the Sort Mode Library window.

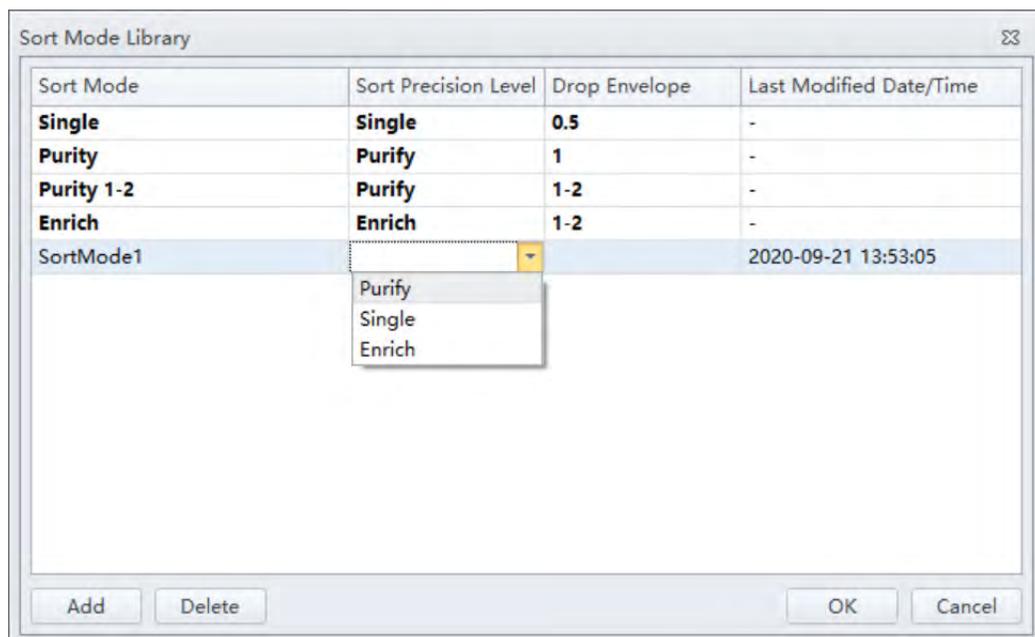


A new sort mode is added in the Sort Mode Library.

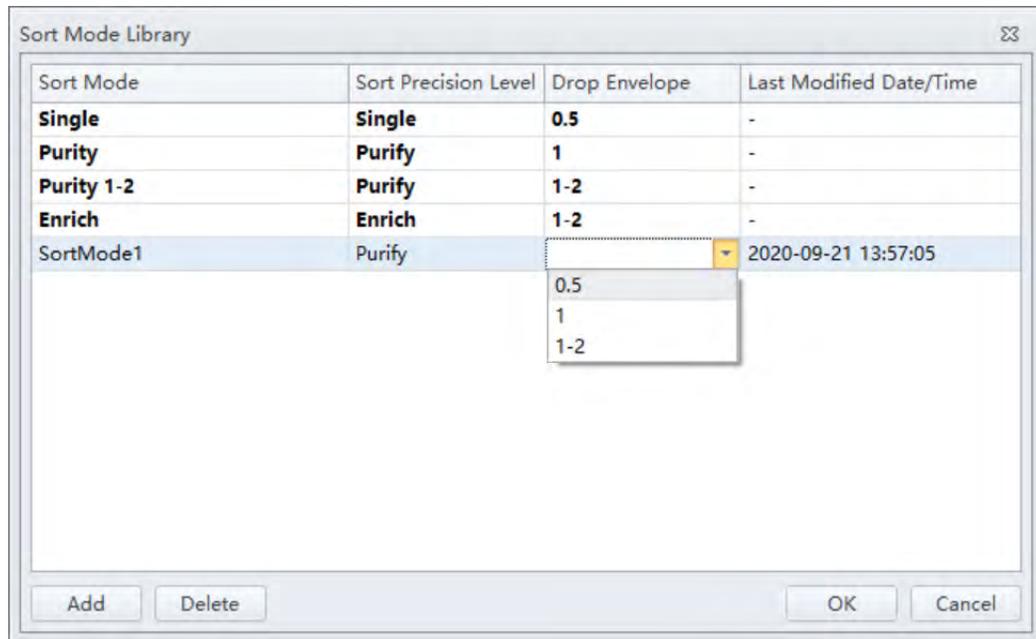


NOTE Enter a name for this new sort mode.

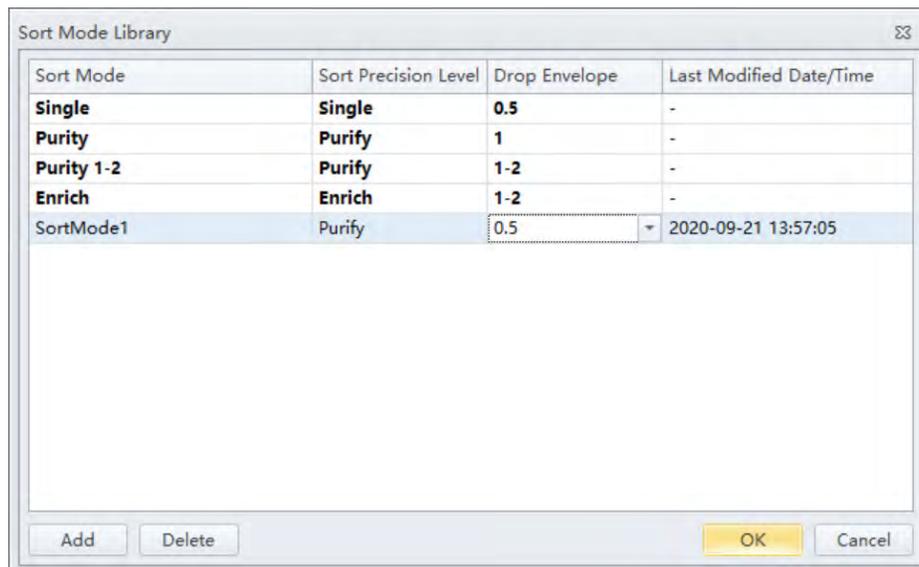
- 3 Select the desired Sort Precision Level from the dropdown menu.



- 4 Select the desired Drop Envelope from the dropdown menu.



- 5 Select OK.

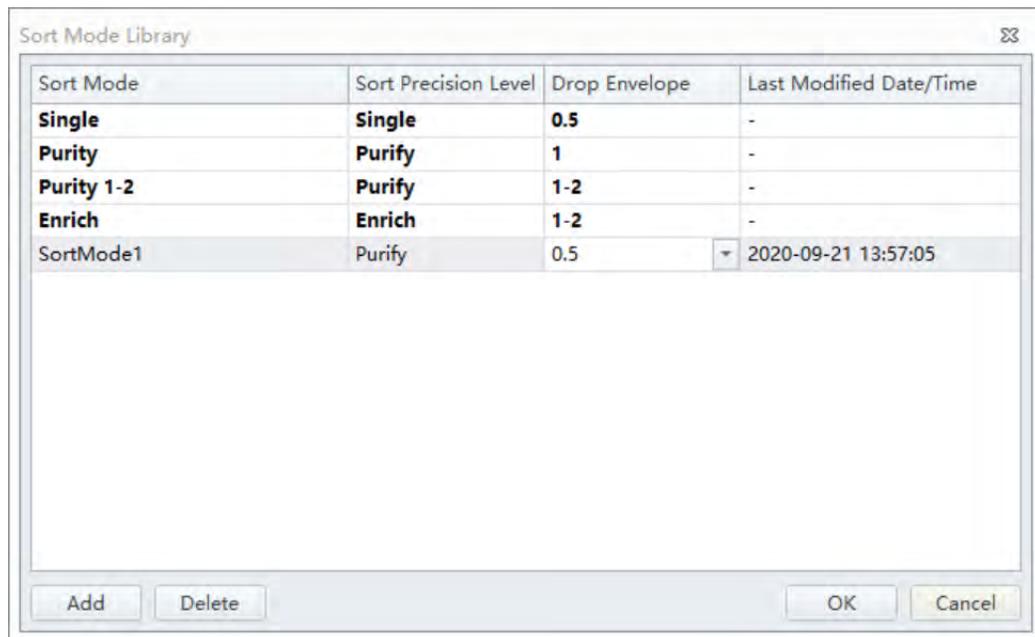


The *SortMode1* is added in the Sort Mode Library. To apply this new sort mode, refer to [Setting Up Tube Sorting](#) or [Setting Up Plate/Slide Sorting](#) in [CHAPTER 5, Sorting](#).

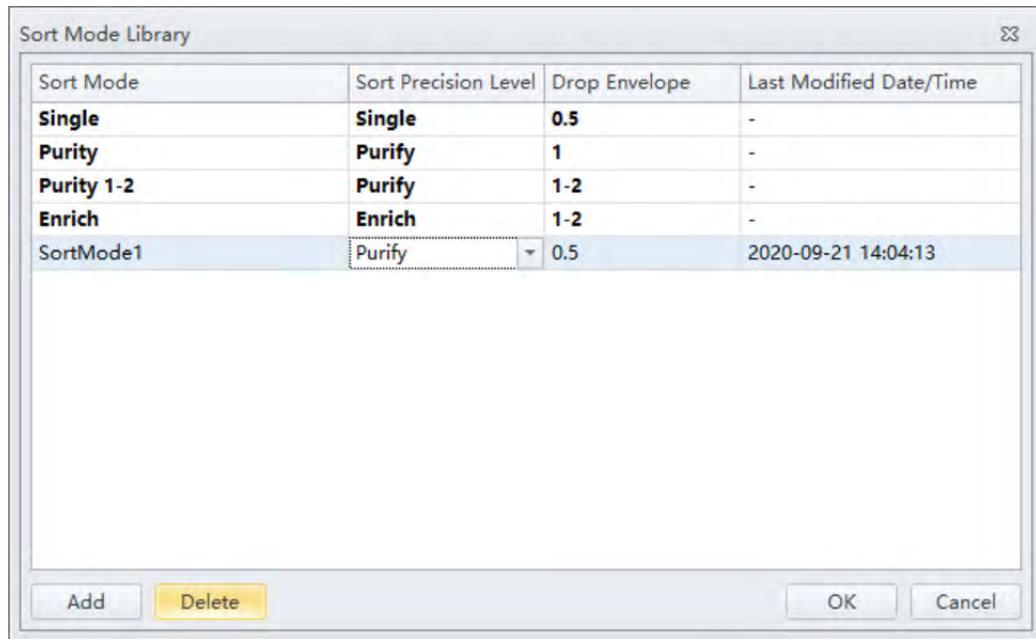
Deleting a Sort Mode

IMPORTANT The system default sort modes that are in bold cannot be deleted.

- 1 Select **Sort Mode Library** from the Advanced menu. The Sort Mode Library window appears.



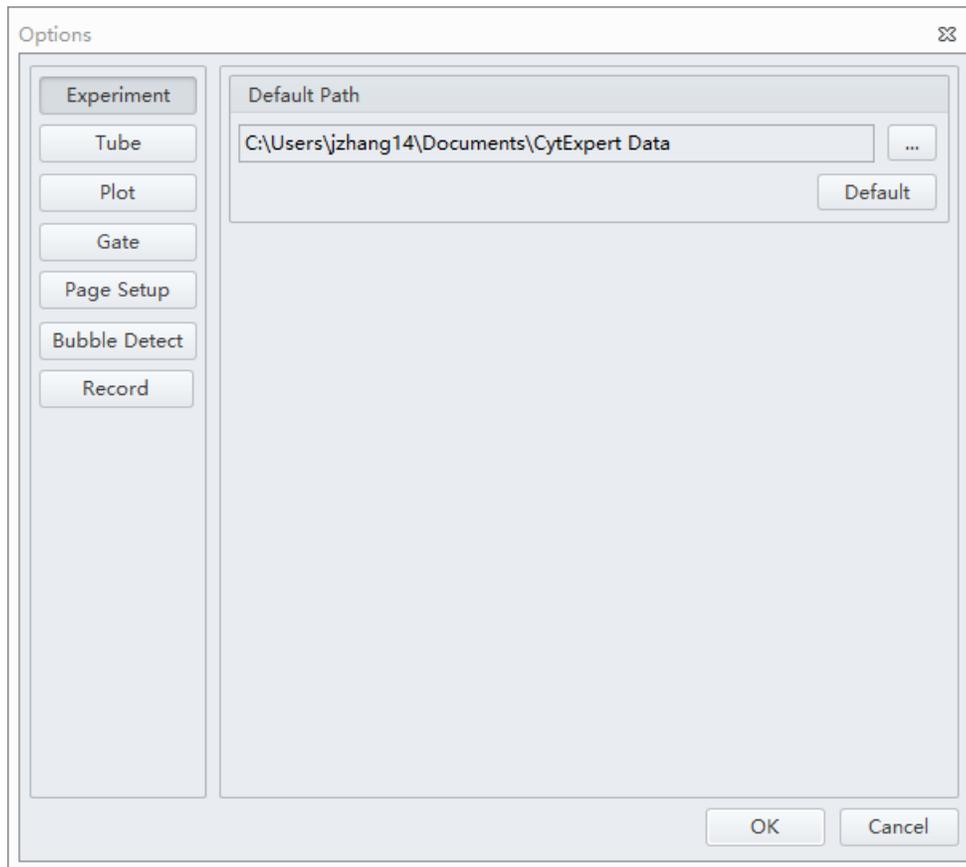
- 2 Select a sort mode to be deleted, and select **Delete**.



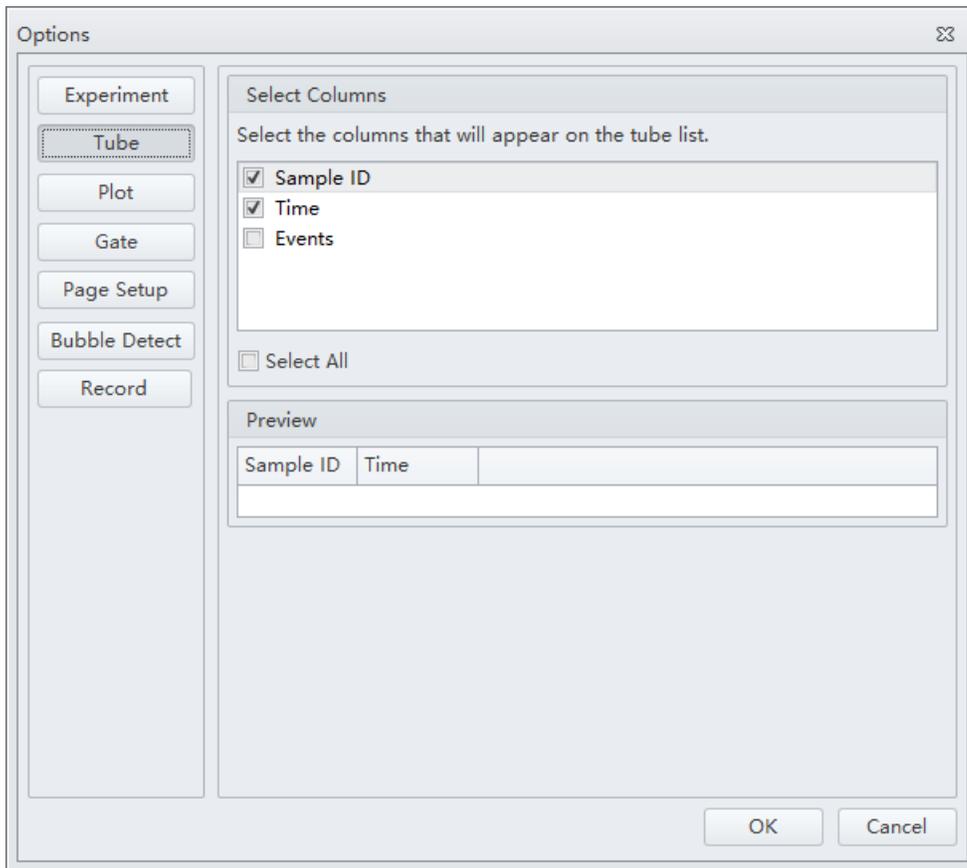
Software Settings

Select **Options** in the Settings menu to configure the software settings.

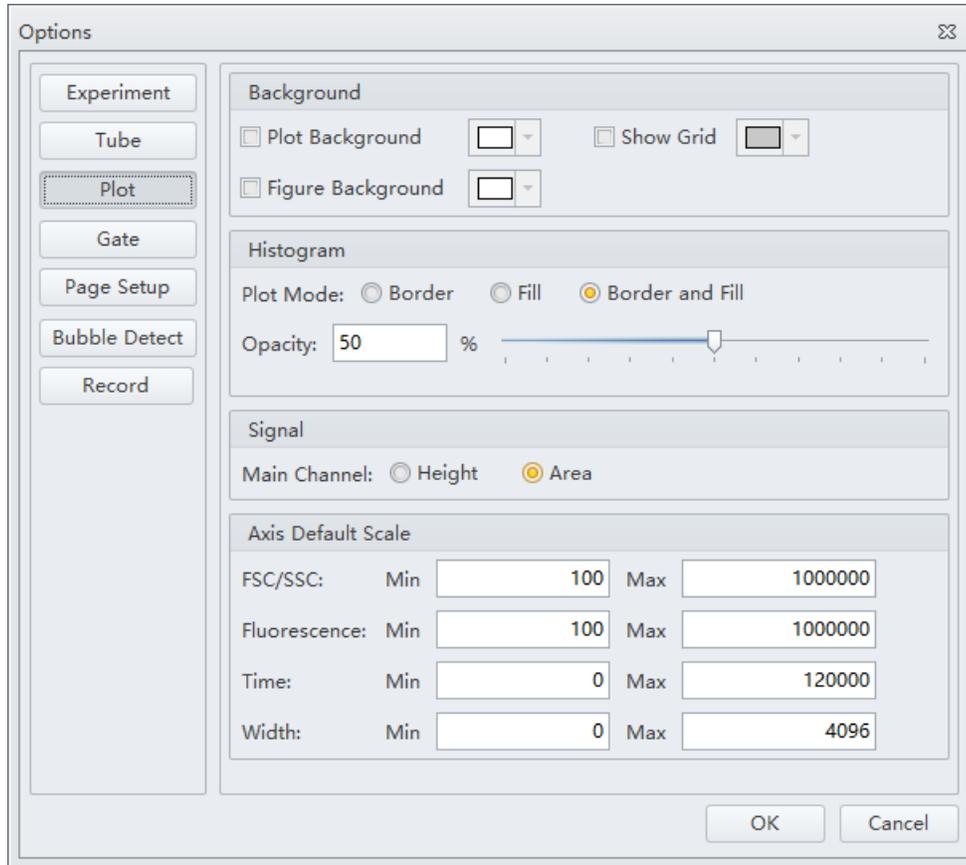
In the experiment settings, you can set the experiment's default save path.



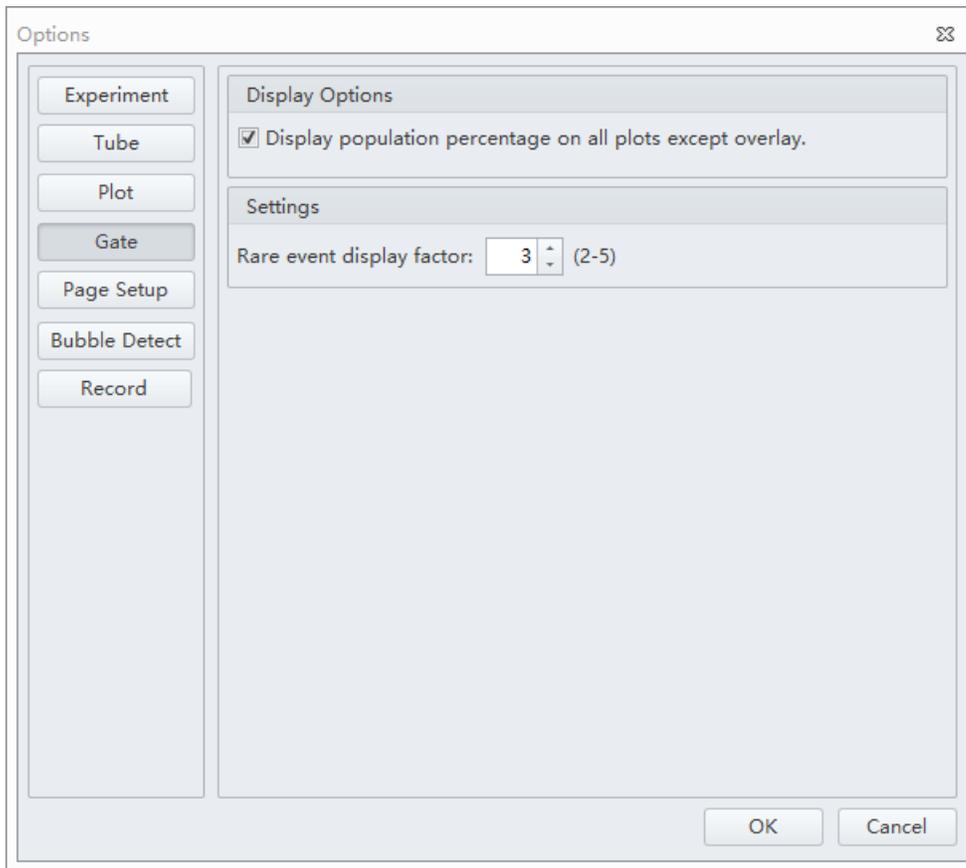
In the tube settings, you can select the columns that display in the tube section of the screen.



In the plot settings, you can define the background of the graphics display area, configure the histograms, and set the default signal parameters to either the channel's area or the channel's height. The default is area. You can also set the default axis display range.

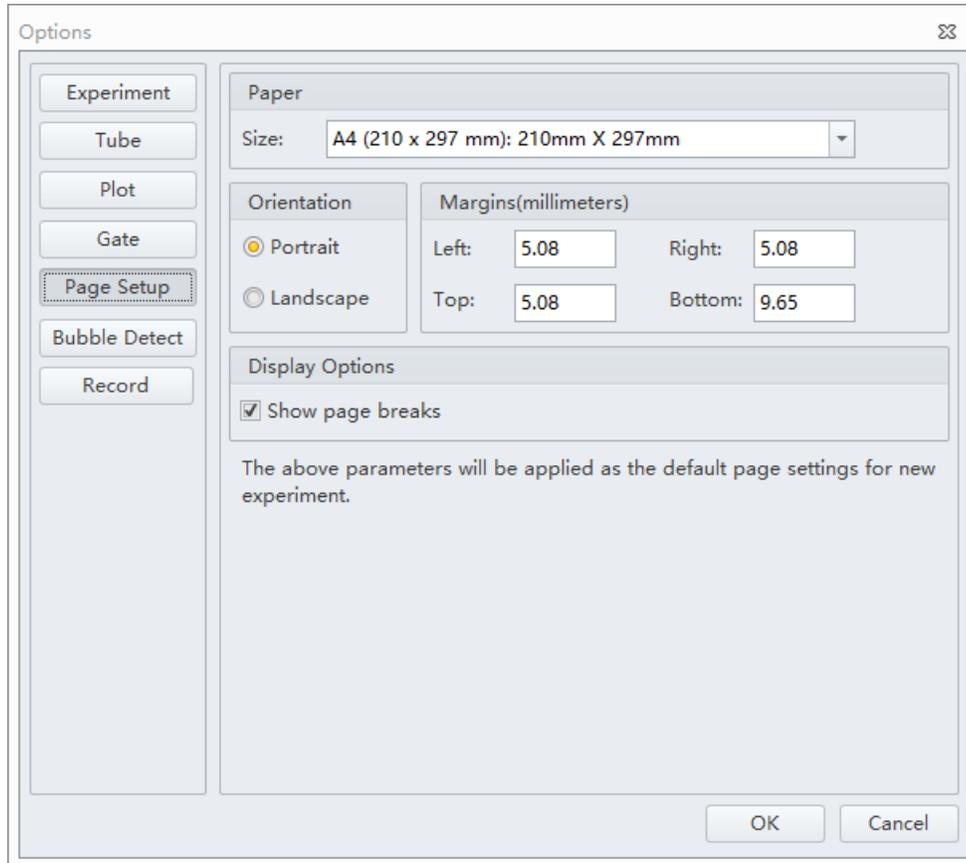


In the Gate settings, you can choose to display population percentage on all plots except overlay.

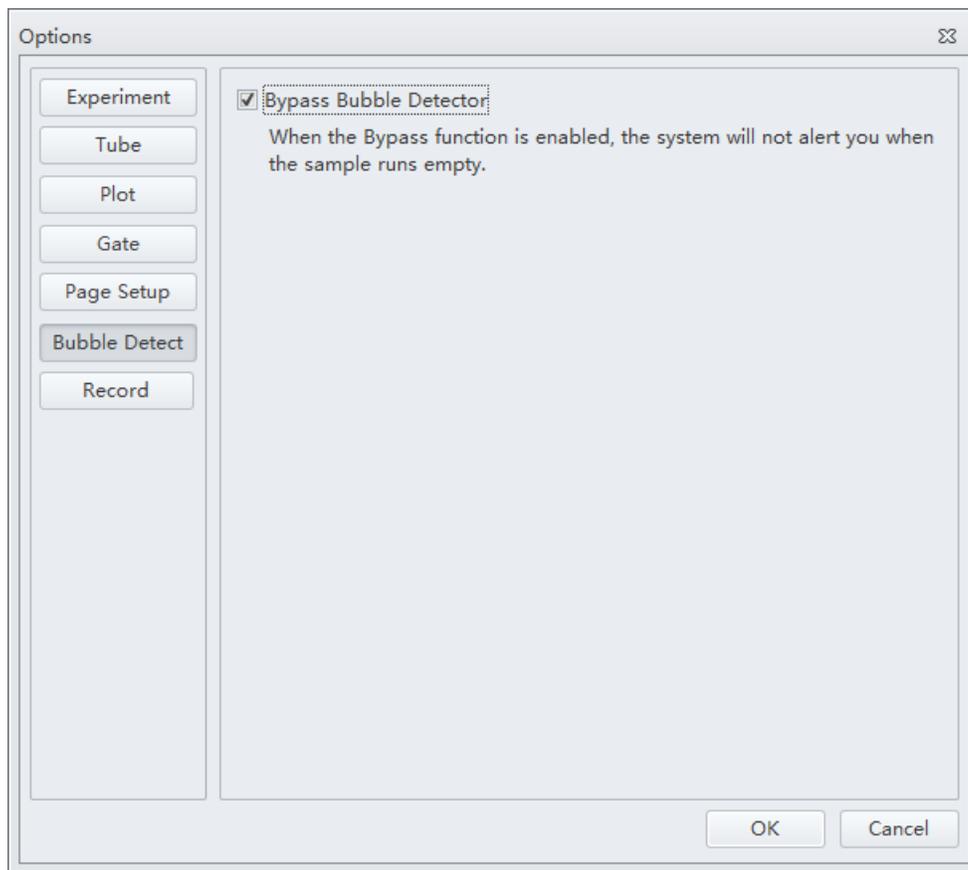


NOTE **Show as rare events** is to increase the visibility of the gated population anywhere they appear on the plots. **Rare event display factor** is to set the degree to which the rare event size is increased on the plots.

In the Page Setup settings, you can change the page size, orientation, margin size, and display options. Select **Show page breaks** to display page boundaries within the Acquisition or Analysis views for simplifying plot arrangement for printing.



In the Bubble Detect settings, you can enable/disable the Bubble Detector function.

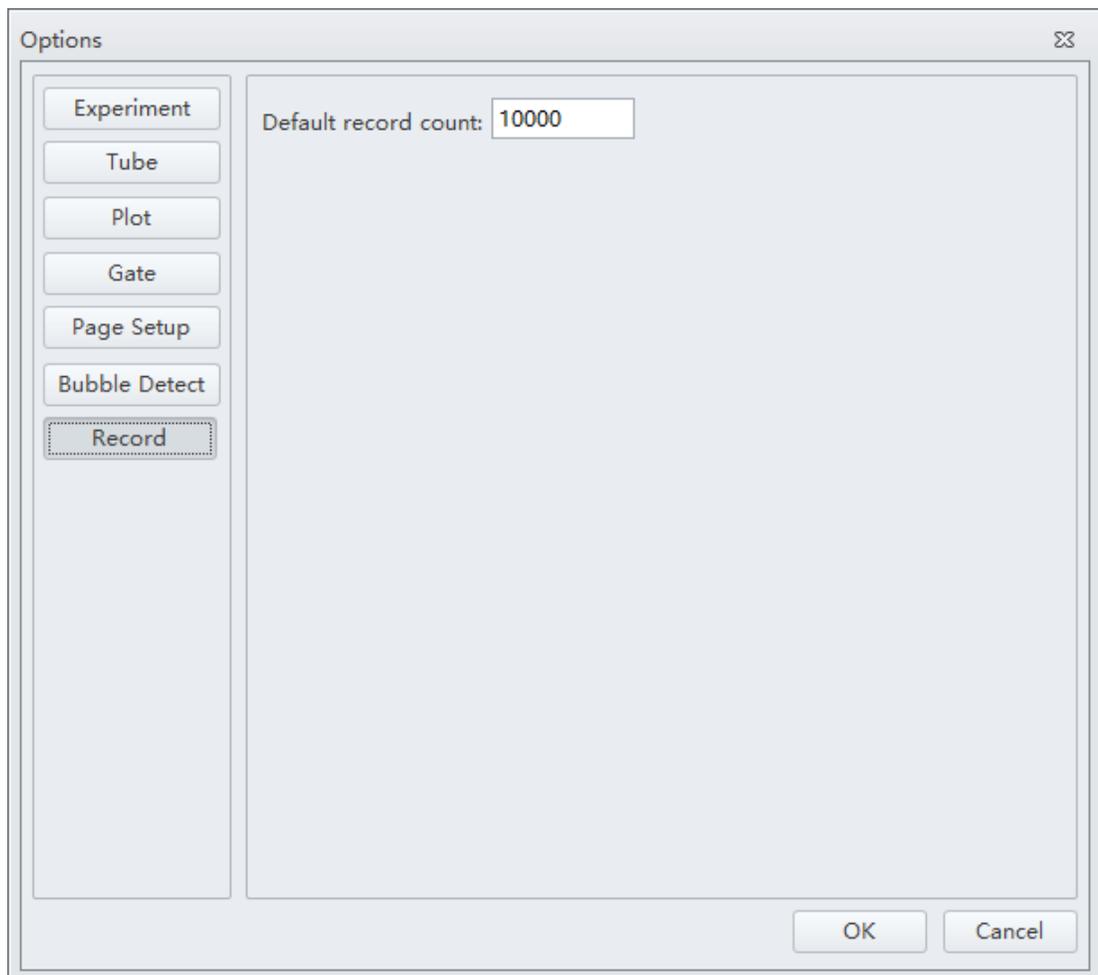


IMPORTANT Only use the Bypass Bubble Detector for the colored or turbid samples.

The bubble detector is sensitive to light scattering and attenuation. When running colored or turbid samples, or sample with high concentration, the error 090025 is likely to occur. If this occurs, select **Bypass Bubble Detector**.

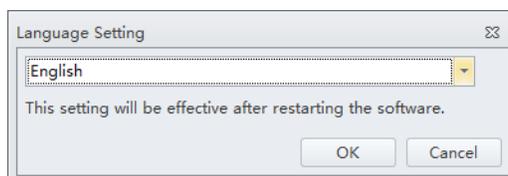
NOTE If the Bypass function is enabled, the system will not alert you when the sample runs empty.

In the Record settings, you can set the default record count.



Language Settings

Select **Settings > Language Settings** to open the Language Settings window. In the Language Settings window, you can select which language to use for the software menus and graphical statistics. The two options currently offered are English and Simplified Chinese.

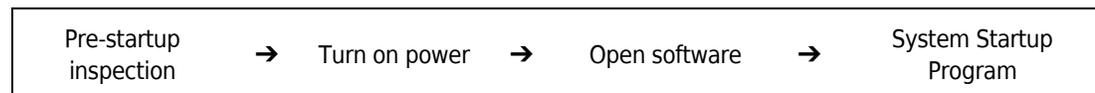


Overview

IMPORTANT Verify that the correct USB configuration key is securely connected to a computer USB port. If the USB configuration key is not connected, the following error message appears: *CytExpert cannot find the license. Please check whether the correct USB configuration key has been plugged in.*

This chapter describes the instrument startup procedure.

Workflow:



This chapter contains information on:

- [Pre-Startup Inspection](#)
- [Turning On the Instrument](#)
- [Logging Into the Software](#)
- [System Startup Program](#)
- [Selecting Experiments from the Start Page](#)

Pre-Startup Inspection

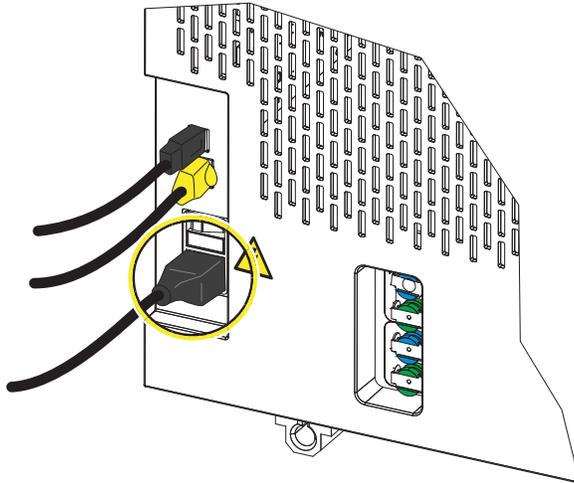
Before using the CytoFLEX SRT instrument, perform the following system checks.

System Connections Inspection

- 1 Verify that the power cable of the computer is securely connected to the power source.
- 2 Check that the monitor, mouse, keyboard, and the Sorter USB cable are properly connected to the computer. Refer to [Figure 1.34](#).

- 3 Verify that all sheath fluid tubing, waste fluid tubing, shutdown fluid tubing, and the sensor cable from the fluidics cart are properly connected to the Sorter. Refer to [Figure 1.11](#).
- 4 Verify that the power cable located below the power switch on the lower left side of the Sorter, and verify it is securely connected to both the Sorter and the power source. Refer to [Figure 3.1](#).

Figure 3.1 Power Cable



- 5 Check the Ethernet cable located above the power switch on the lower left side of the Sorter, and verify it is securely connected to the workstation. Refer to [Figure 1.34](#).

Checking Fluid Levels



- 1 Examine the sheath tank, waste container, and the shutdown fluid container. Refer to [Figure 1.4](#). Verify that there is sufficient fluid in the sheath tank and shutdown fluid container, and the waste container is empty.

NOTE You can view the fluidics status information from the fluidic status bar on the CytExpert SRT software if the software has been started.

CAUTION

Risk of instrument damage. Fluid spills in the fluidics cart can damage the instrument electronics. Remove the sheath tank from the fluidics cart before filling the sheath tank to avoid damage to instrument electronics.

- 2 If necessary, fill the sheath fluid tank while not exceeding the upper position ring. Refer to [Filling the Sheath Tank](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).
- 3 If necessary, fill the shutdown fluid container with CytoFLEX SRT Shutdown fluid while not exceeding the bottom of the neck. Refer to [Filling the Shutdown Fluid Container](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

WARNING

Risk of chemical injury from bleach. To avoid contact with the bleach, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

- 4 If necessary, empty all waste liquid from the waste container. If biohazardous samples are used for data collection, add 400 mL high-quality, fragrance-free, gel-free bleach (5 to 6% solution of sodium hypochlorite - available chlorine) to the waste container. Refer to [Emptying the Waste Container](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).
- 5 Verify that all sheath fluid tubing, waste fluid tubing, shutdown fluid tubing, and sensor cables are properly connected on the fluidics cart. Refer to [Figure 1.6](#).

Turning On the Instrument

IMPORTANT For users who have the optional Biosafety Cabinet, the Biosafety Cabinet must be turned on first (manually) or left on from the previous day for the instrument to be ready to run right away. Otherwise, allow at least 30 minutes for the system to reach thermal equilibrium.

- 1 **Optional:** Turn on the Biosafety Cabinet (if part of the system).
- 2 Turn on the main power switch located on the lower left side of the Sorter.

NOTE Or select **Turn on** from the Cytometer menu to remote power on the Sorter if the software has been open.

- 3 Wait for the Sorter to finish powering on, then turn on the Workstation.

Logging Into the Software

- 1 Log into the Windows operating system and double-click the CytExpert SRT desktop icon



to open the software.

If you are running the CytExpert Default software installation, login is not required. Proceed to Step 4.

If you are running the CytExpert User Management software installation, the login window appears.

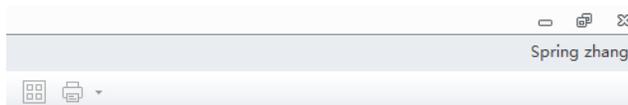


NOTE The default software shortcut appears on the desktop. If you do not see the icon, the default installation path is under C:/Program Files/CytExpert SRT. Double-click CytExpert SRT.exe to run the software.

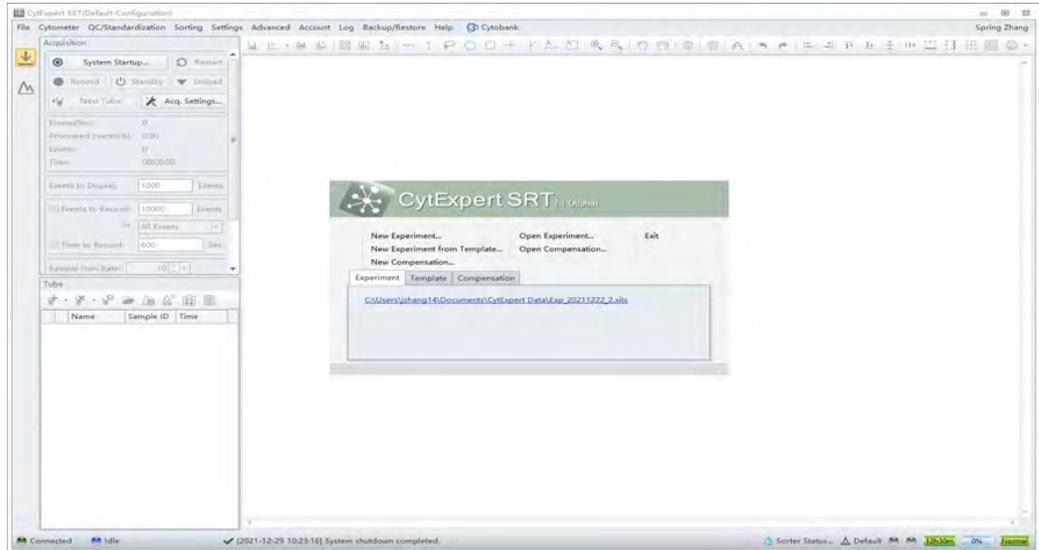
- 2 Enter your username and password.

- 3 Select .

NOTE The full name of the user that is currently logged in displays in the top right corner of the software screen.



- 4 Confirm that the software and the Sorter are properly connected.
 - a. Open the software. The Startup screen appears.



- b. Verify that the connection indicator light in the lower left corner of the software screen is green, and *Connected* is displayed. The left side shows the connection status, the middle shows the instrument status, and the right side shows the status details.

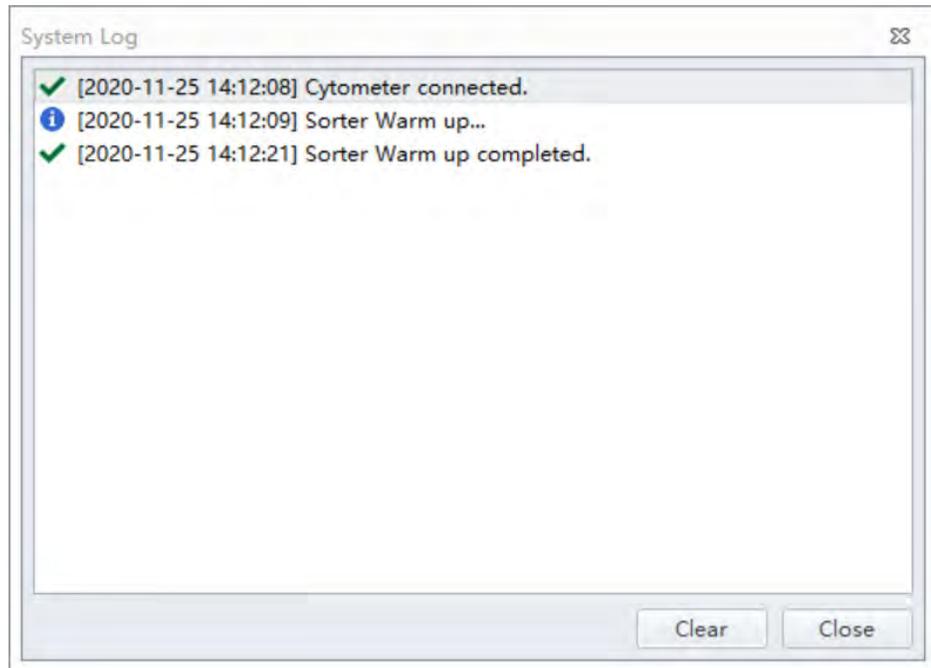


NOTE

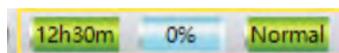
- A red connection indicator light indicates that there is a faulty connection. Ensure that the instrument is properly turned on and connected. If necessary, restart both the Sorter and the Workstation.



- Select the status information in the lower left to open the system log. Send a copy of the system log to your Beckman Coulter Representative for support if a service call is requested.



- c. Verify that the *Sheath*, *Waste* and *Shutdown* flow indicators in the lower right corner of the software screen are green indicating that the fluidics system is normal.



NOTE When the flow indicator is yellow or red, the fluid containers need your attention.

Table 3.1 Sheath Status

	Normal
	Insufficient
 (with audible warning)	Empty
 (with audible warning)	Overfilling

The value in the symbol indicates the remaining time that the sheath can sustain.

NOTE When the sheath flow indicator displays “empty”, refill the sheath tank as soon. Otherwise, the system stops the fluid and goes into standby automatically. Refer to [Filling the Sheath Tank](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

NOTE When the sheath flow indicator displays “full”, pour the excessive sheath and ensure that the sheath level is lower than the position ring (refer to step 6) inside the sheath tank.

Table 3.2 Waste Status

	Waste volume < 25%
	25% ≤ Waste volume ≤ 50%
	50% ≤ Waste volume ≤ 75%
 (with audible warning)	Waste volume ≥ 75%
 (with audible warning)	Approaching full

NOTE When the waste flow indicator is yellow, empty the waste container as soon. Otherwise, system stops the fluid and goes into standby immediately when the waste flow indicator turns red. Refer to [Emptying the Waste Container](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

Table 3.3 Shutdown Fluid Status

	Normal
	Empty

NOTE When the shutdown flow indicator is red, refill the shutdown fluid container. Refer to [Filling the Shutdown Fluid Container](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

IMPORTANT Ensure that the sheath tank you used matches with the sheath tank type you selected. Otherwise, you could encounter System Startup failure, Aseptic Cleaning failure, Long-term Shutdown failure, or sheath flow indication error.

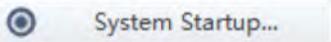
5 Verify that the sheath tank you used matches with the sheath tank type you selected. Select Sheath Tank Scale Reset from the Advanced menu to display the current sheath tank type.

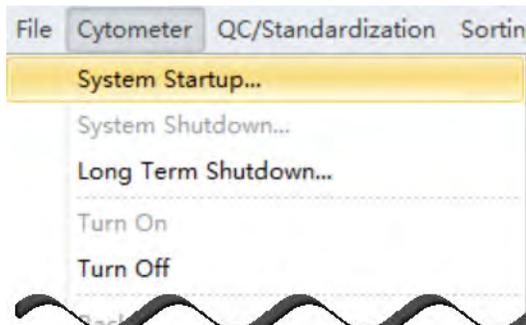
System Startup Program

The System Startup Program is required every time when you turn on the instrument. The System Startup Program allows the system to pressurize the sheath tank, check fluidic system, debubble, stabilize the pressure, and clean sample probe. The program takes 10–15 minutes depending on the sheath tank type you used.

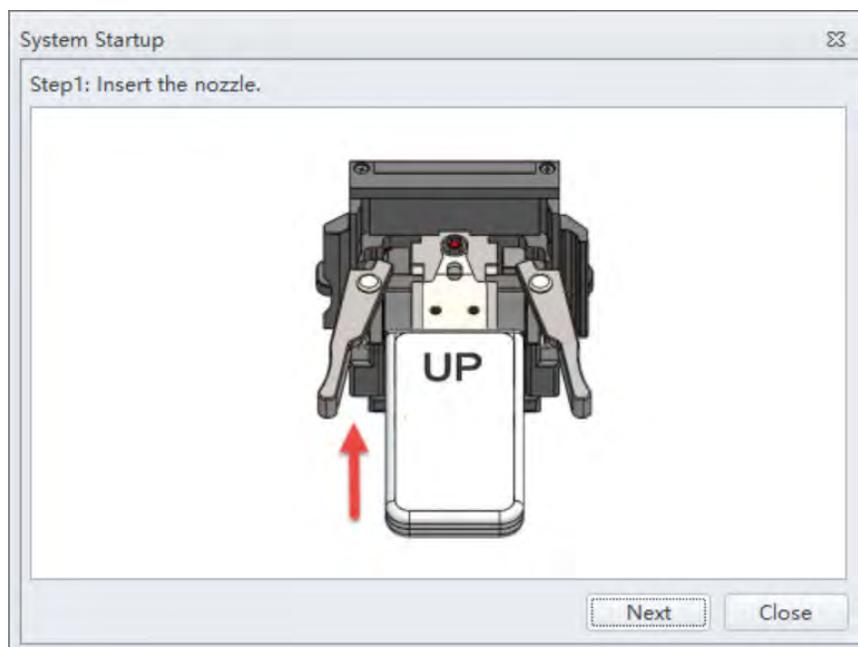
IMPORTANT Ensure that the sheath tank you used matches with the sheath tank type you selected. Otherwise, you could encounter startup failure, aseptic cleaning failure, long-term shutdown failure, or sheath flow indication error.

Restarting after the Daily Shutdown (CytoFLEX SRT Shutdown Fluid in the Instrument)

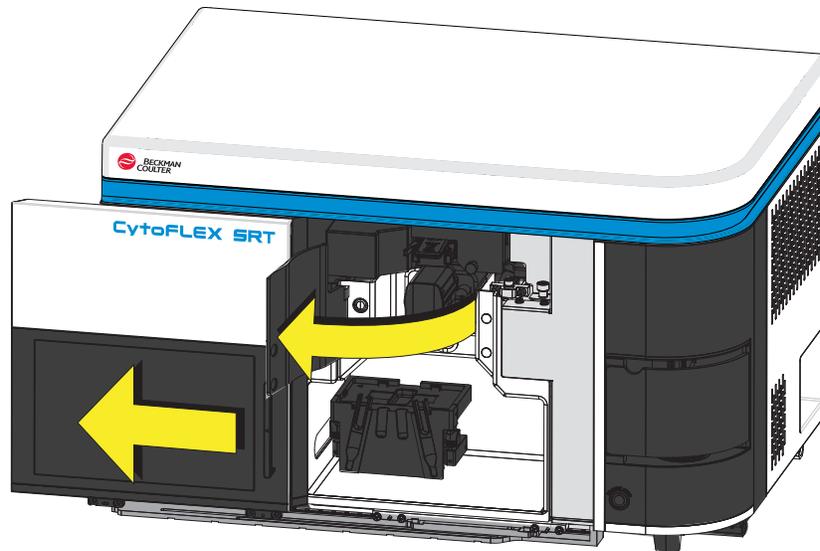
- 1 Select  or **System Startup** in the Cytometer menu.



The System Startup window appears.



- 2 Open the sort chamber sliding door and the sort protection door.

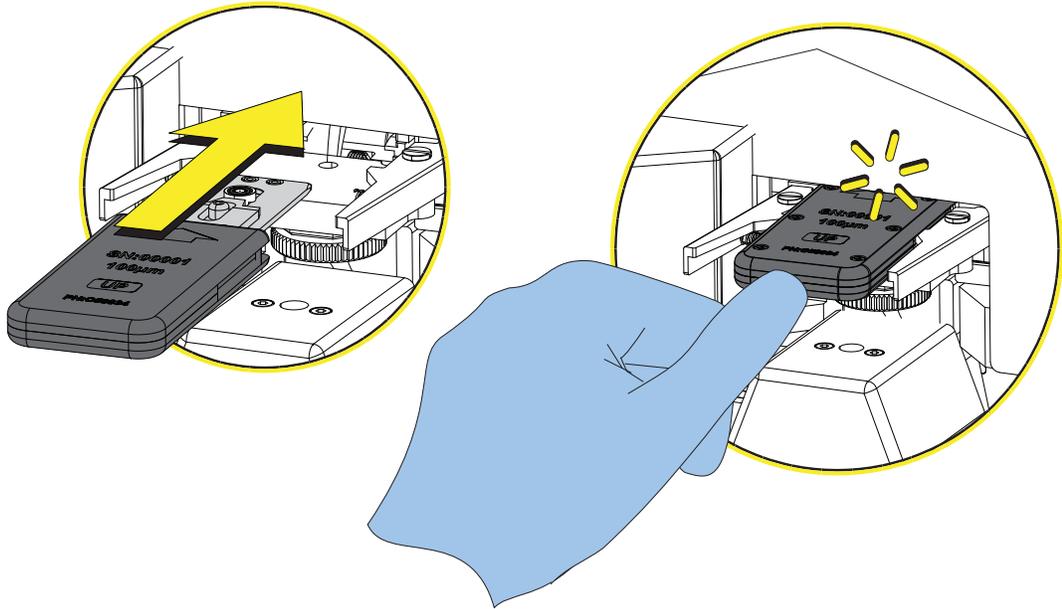


- 3 Clean the bottom of the flow cell, the sliding rails for the nozzle lift, and the V-plate surface. For instructions, refer to [Daily Decontamination During Shutdown](#) in [CHAPTER 10, Cleaning Procedures](#).

IMPORTANT A wet or clogged nozzle might cause the fluid stream off center or even no stream.

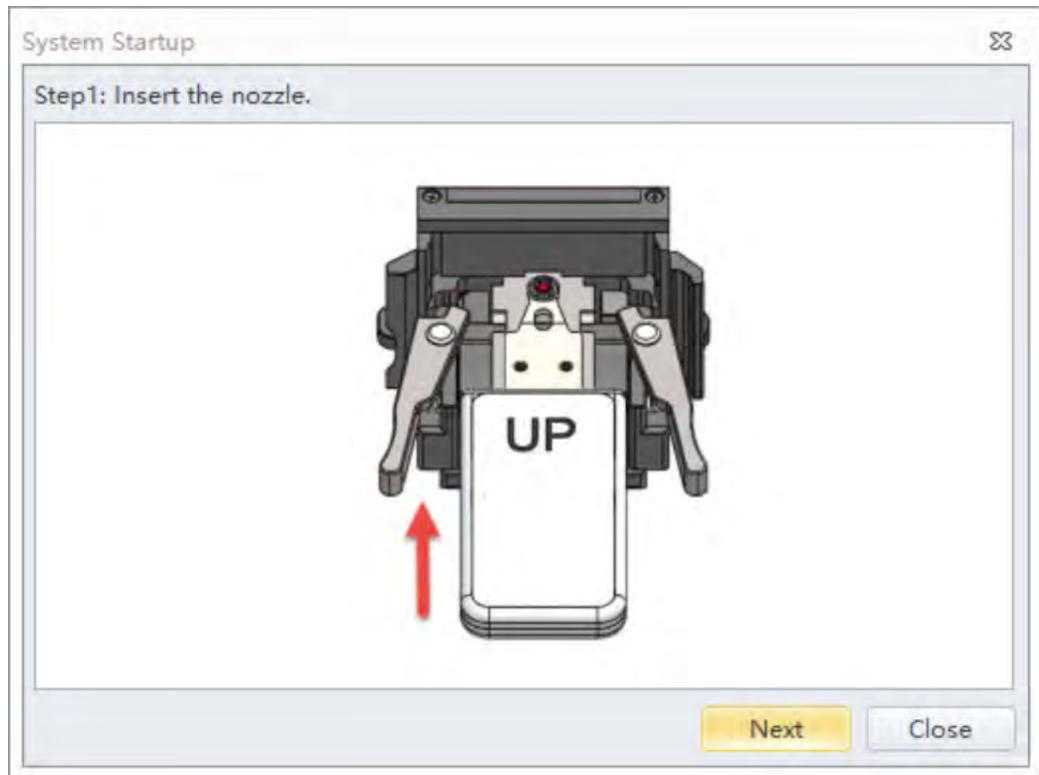
- 4 Verify that the nozzle is clean and dry. If necessary, clean the nozzle. For instructions, refer to [Cleaning the Nozzle](#) in [CHAPTER 10, Cleaning Procedures](#).

- 5 Insert the nozzle module carefully into Sorter with the UP symbol facing up. The nozzle module is locked into its position when you hear a click.

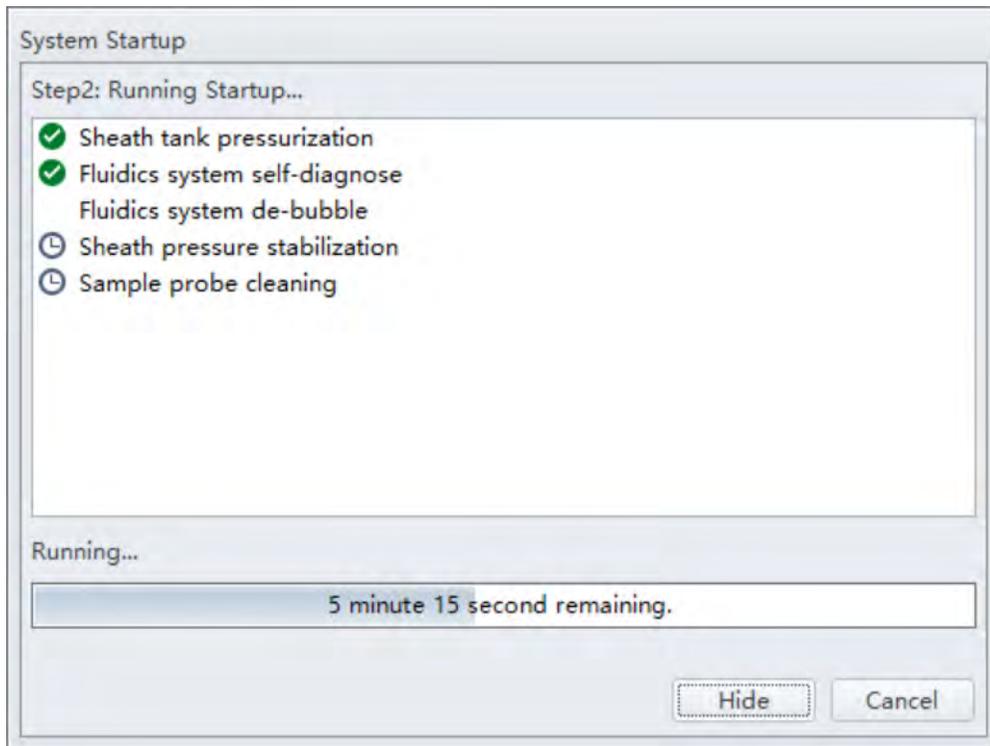


- 6 Close the sort protection door, the sort chamber sliding door, and the sample station door.

7 Select **Next**.

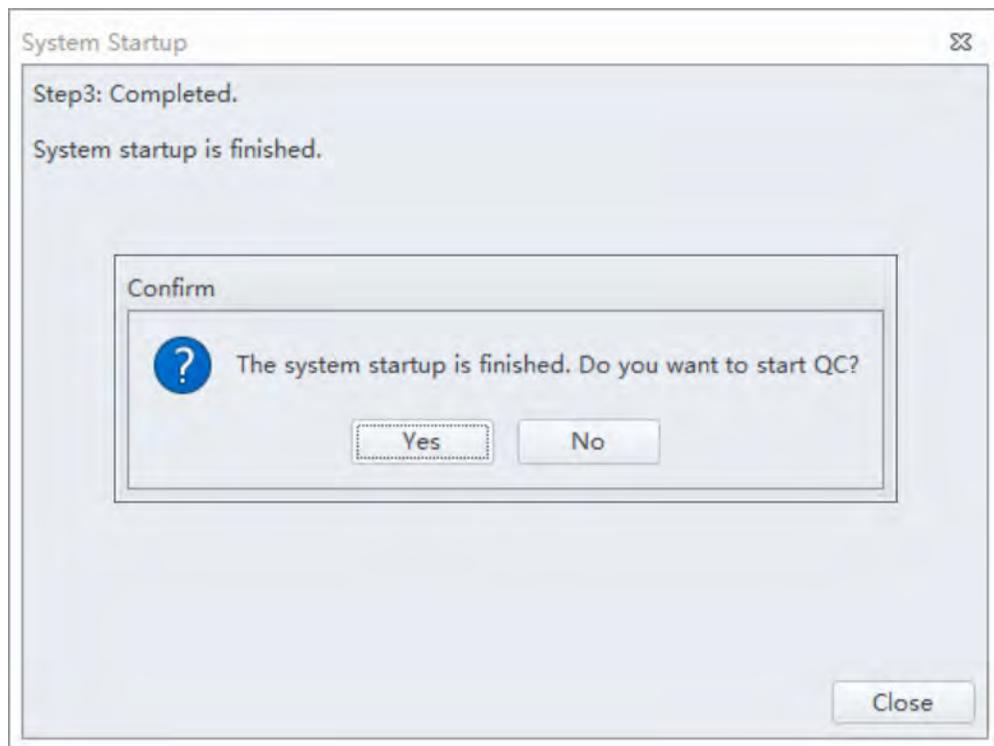


The system starts running Startup Program.



NOTE Select **Hide** to hide the System Startup window.

The Confirm window displays when the System Startup Program is finished,



NOTE Select **Yes** to start QC now. For instructions on running QC, refer to [CHAPTER 4, Instrument Quality Control and Standardization](#).

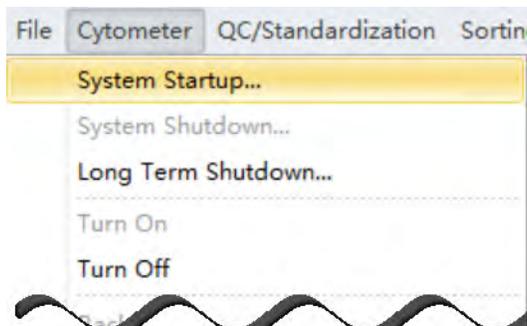
Or select **No** to defer the QC.

8 Select **Close** to quit the System Startup program. The system is now ready for use.

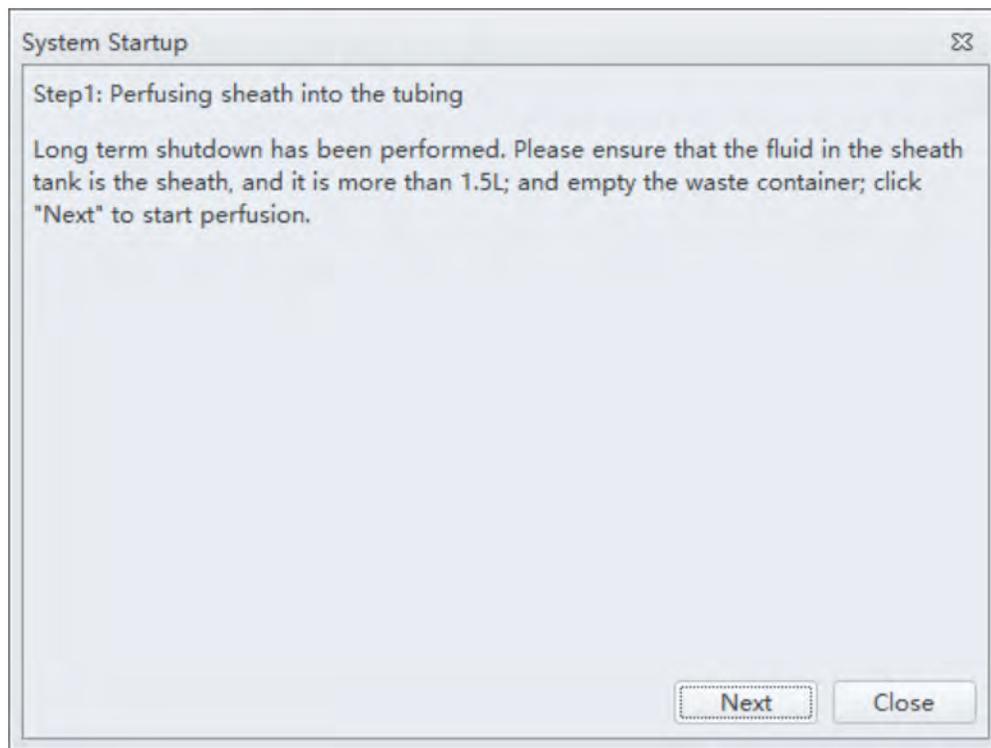


Restarting after the Long-Term Shutdown (70% Ethanol in the Instrument)

- 1 Select  or **System Startup** in the Cytometer menu.



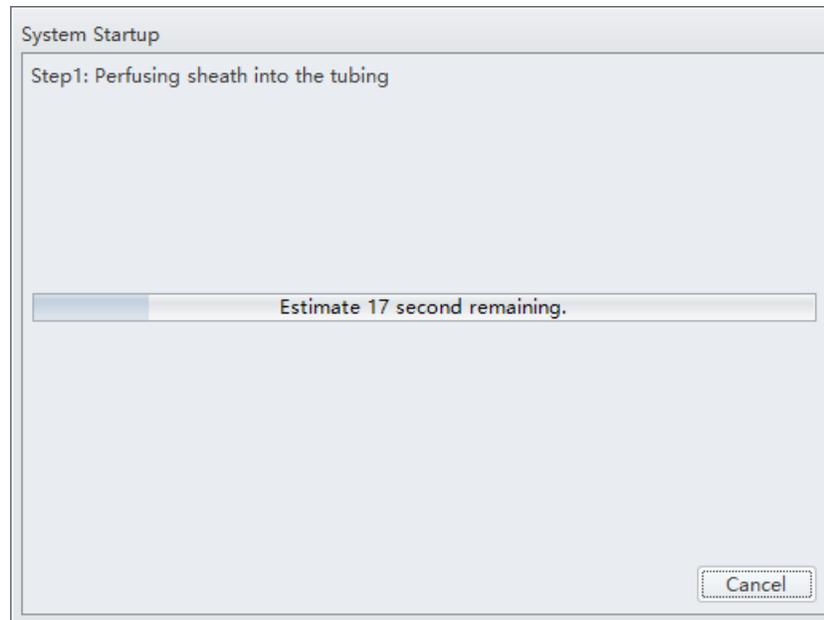
The following message appears.



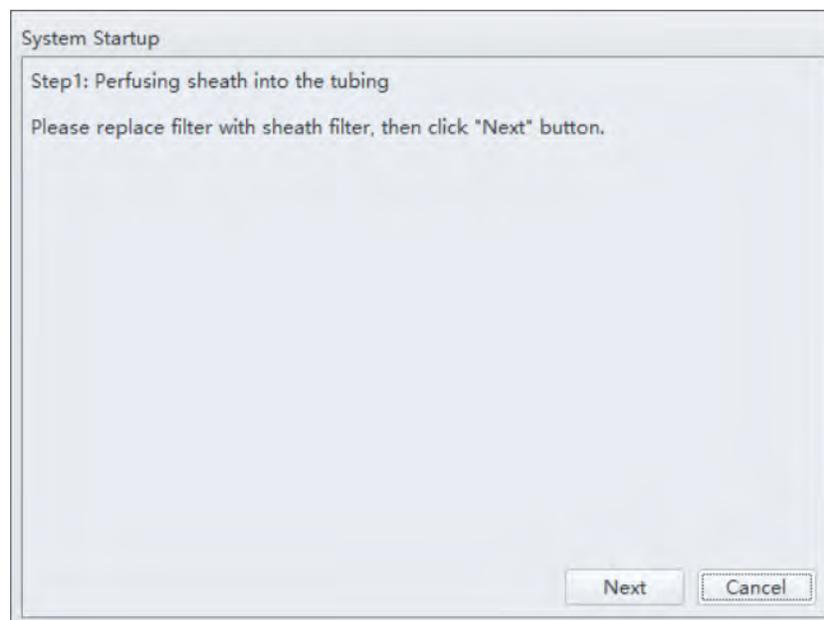
IMPORTANT Ensure the sheath is sterile.

- 2 Empty the sheath tank and refill the sheath tank with at least 1.5 L sheath. Refer to [Filling the Sheath Tank](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).
- 3 Empty the waste container. Refer to [Emptying the Waste Container](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

- 4 Select **Next**. The system starts to rinse the sheath line with sheath.

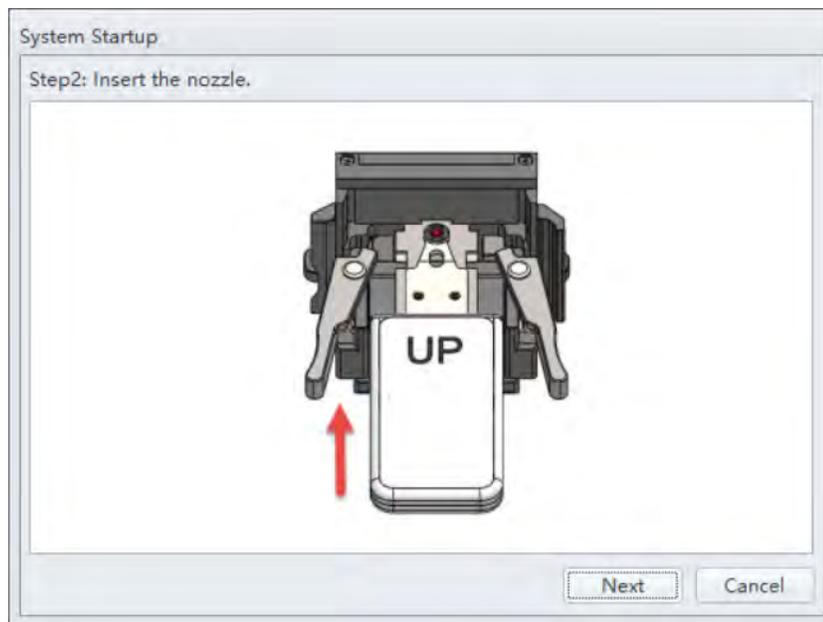


The following message appears when the system finishes the perfusion.

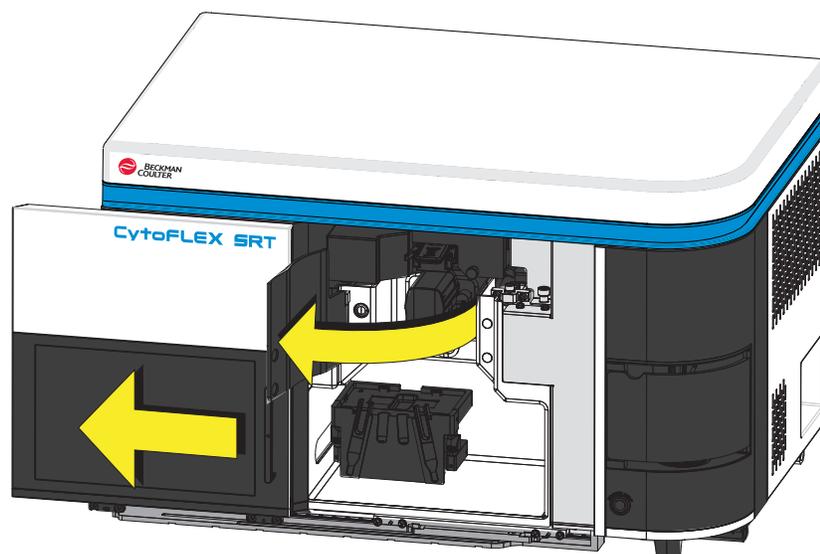


- 5 Switch the aseptic cleaning filter with the sheath fluid filter. Refer to [Replacing the Aseptic Cleaning Solution Filter](#) in CHAPTER 11, Replacement/Adjustment Procedures.

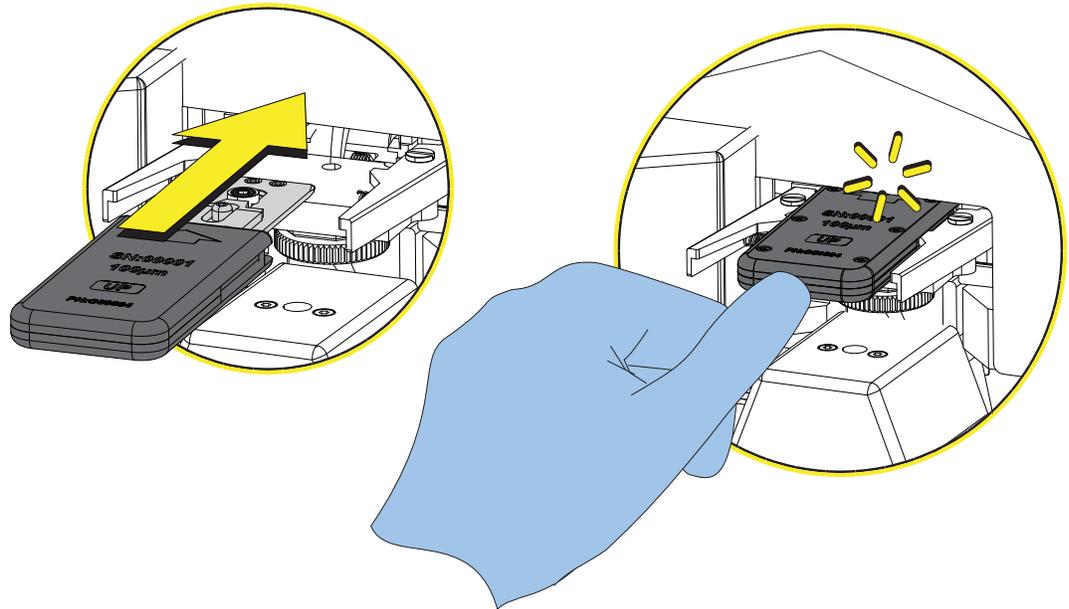
6 Select **Next**.



7 Open the sort chamber sliding door and the sort protection door.

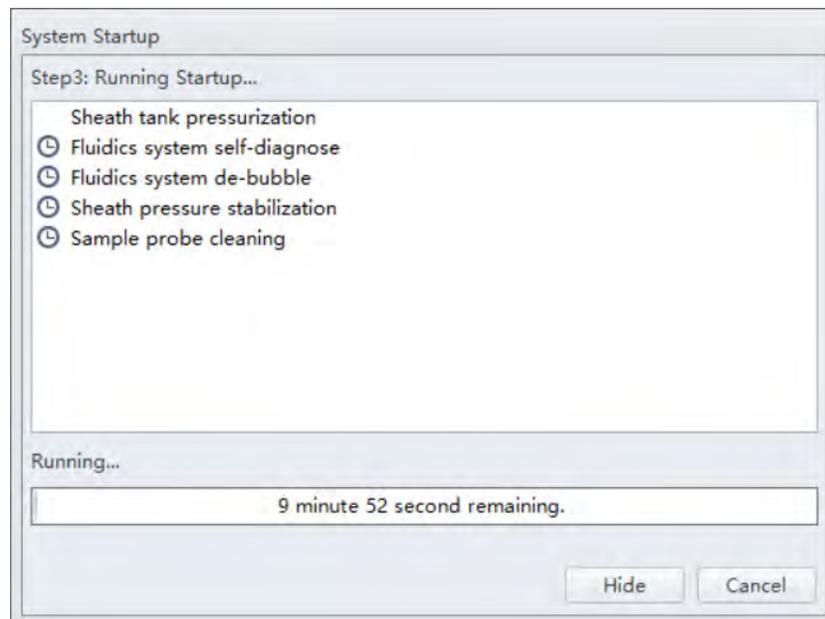


- 8 Insert the nozzle module carefully into Sorter with the UP symbol facing up. The nozzle module is locked into its position when you hear a click.



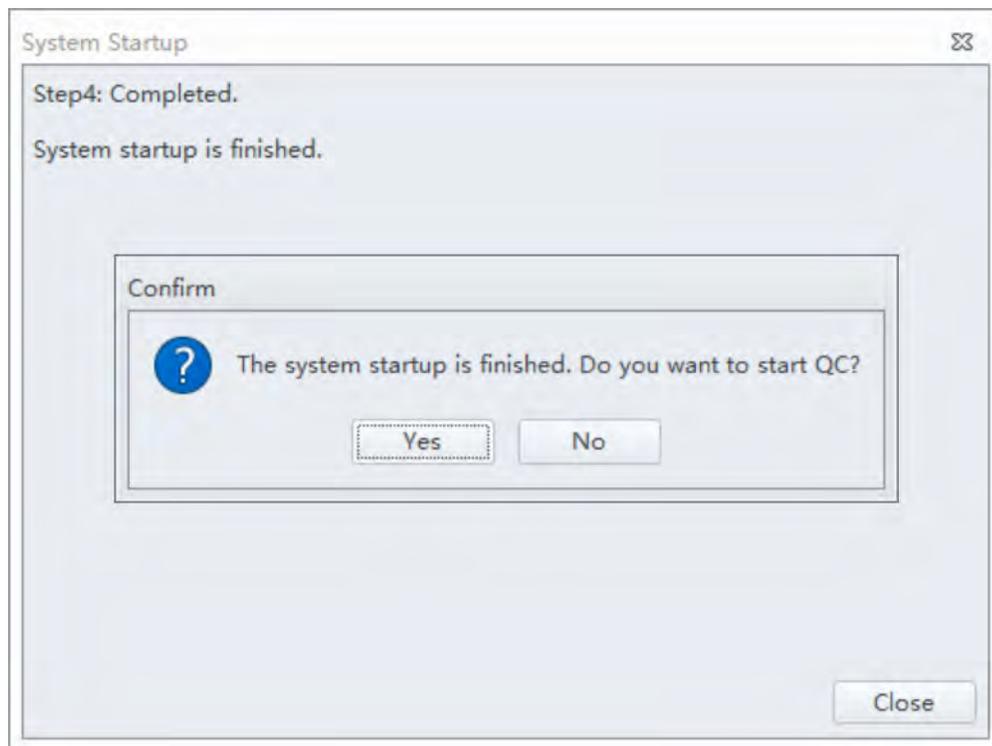
- 9 Close the sort protection door, the sort chamber sliding door, and the sample station door.

- 10 Select **Next**. The system starts running Startup Program.



NOTE Select **Hide** to hide the System Startup window.

The Confirm window displays when the System Startup Program is finished,



NOTE Select **Yes** to start QC now. For instructions on running QC, refer to [CHAPTER 4, Instrument Quality Control and Standardization](#).

Or select **No** to defer the QC.

11 Select **Close** to quit the System Startup program. The system is now ready for use.

12 Optional: Sample DI water at 10 $\mu\text{L}/\text{min}$ for 1 hour. This is to remove the tiny air bubbles which might be introduced in the Long-term Shutdown program.

NOTE For more instructions on removing air bubbles, refer to [Removing Trapped Air Bubbles in CHAPTER 11, Replacement/Adjustment Procedures](#).

Selecting Experiments from the Start Page

Refer to [Start Page](#) in [CHAPTER 2, Using the CytExpert SRT Software](#).

CHAPTER 4

Instrument Quality Control and Standardization

Overview

This chapter provides information on performing daily Quality Control (QC) on the CytoFLEX SRT Sorter and how to confirm that the instrument is working properly within the specified parameters. Quality Control allows you to determine whether your instrument can provide adequate signal strength and precision.

This chapter also provides information on performing standardization. CytoFLEX Daily QC Fluorospheres, CytoFLEX Ready to Use Daily QC Fluorospheres, or any other reference material that is relevant for your application may be used as the standardization sample. The system can only recognize a single peak.

Standardization can be used to monitor the Median Fluorescent Intensities (MFI), or target values for scatter and fluorescent parameters that have been defined and optimized for a specific application. All channels in the current configuration, those with/without an assigned QC target, can be tracked as necessary via Standardization since this portion of the CytExpert SRT Software is used to assess application specific settings. Standardization, however, does NOT replace QC as the Sorter's optical alignment (rCV statistical analysis), Laser Power and Laser Delay outputs are not measured during the run.

NOTE Beckman Coulter recommends performing QC prior to performing Sort Calibration on a daily basis or after using a new nozzle.

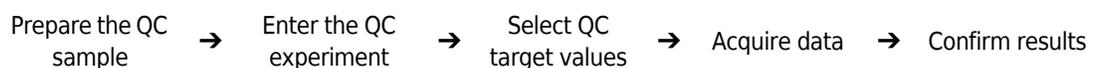
NOTE QC target values apply exclusively to standard channels. A channel is a laser-filter combination. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in [CHAPTER 5, Sorting](#) to verify that the default factory detector configuration is selected before running QC.

NOTE CytExpert SRT QC includes an automated QC routine with Levey-Jennings (LJ) charts tracking and logging.

NOTE CytExpert SRT Standardization allows for application-specific settings to be established and applied to future experiments.

NOTE Perform QC on the same day prior to performing the CytExpert SRT standardization.

QC Workflow:



Standardization Workflow:



This chapter contains information on:

- [Quality Control](#)
 - [Preparing the QC Sample](#)
 - [Importing Lot-Specific Target Values](#)
 - [Collecting QC Data](#)
 - [Confirming Results](#)
- [Standardization](#)
 - [Preparing the Standardization Sample](#)
 - [Generating Target Median Values](#)
 - [Adding a New Standardization Item](#)
 - [Performing the Standardization](#)
 - [Applying the Standardized Acquisition Settings](#)
 - [Standardization Target Library](#)

Quality Control

The QC process verifies important system functions. The system:

1. Verifies that the unit hardware configuration matches the default configuration specified in the software. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Sorting](#).
2. Measures the laser power of each individual laser and ensures that each laser meets the system specifications.
3. Loads the QC sample and begins to acquire data.
4. Verifies that the actual laser delays match those set in the software and will adjust the delay accordingly.
5. If laser delay is $\leq 2 \mu\text{s}$ from the previous setting, the software automatically changes the laser delay setting.

OR

Notifies you if laser delay is within $2 \mu\text{s}$ - $5 \mu\text{s}$ from the previous setting. The software prompts you to confirm the laser delay setting.

OR

Notifies you if laser delay is $> 5 \mu\text{s}$ from the previous setting. Manual laser delay adjustments are required. Refer to [Setting Laser Delay](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

6. Verifies and calibrates the gain settings. If any of these parameters are outside of the operating limits, the system automatically adjusts these parameters. If the system is unable to adjust these parameters to fall within the operating limits, the system notifies you.
7. Optimize the fluid stream and verify whether the jitter of T1 signal is acceptable. Then determine the DD1 value.

Preparing the QC Sample

Required Materials

The following materials are required to complete the QC process:

- CytoFLEX Daily QC Fluorospheres or CytoFLEX Ready to Use Daily QC Fluorospheres
- IsoFlow Sheath Fluid or another saline sheath fluid
- Sample tubes (12 x 75 mm)
- Vortexer

CytoFLEX Daily QC Fluorospheres Preparation Process

IMPORTANT For preparation instructions for the CytoFLEX Ready to Use Daily QC Fluorospheres, refer to [CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process](#).

-
- 1 Take one sample tube and label it as the QC sample tube.

 - 2 Add approximately 1 mL of deionized water to the sample tube.

 - 3 Use the Vortexer or shake vigorously to thoroughly mix the bottle of CytoFLEX Daily QC Fluorospheres.

 - 4 Add three drops of CytoFLEX Daily QC Fluorospheres to the sample tube.

 - 5 Vortex the sample tube to uniformly suspend the fluorospheres.

-
- 6 Place the sample tube in a dark location at 2-8 °C until ready to load the tube into the instrument for QC.

NOTE Tubes containing diluted CytoFLEX Daily QC Fluorospheres should be sealed and stored in a dark location at 2-8 °C for up to 5 days.

CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process

IMPORTANT For preparation instructions for the CytoFLEX Daily QC Fluorospheres, refer to [CytoFLEX Daily QC Fluorospheres Preparation Process](#).

- 1 Take one sample tube and label it as the QC sample tube.
- 2 Use the Vortexer or shake vigorously to thoroughly mix the bottle of CytoFLEX Ready to Use Daily QC Fluorospheres.
- 3 Add 10 drops of CytoFLEX Ready to Use Daily QC Fluorospheres to the sample tube.
- 4 Vortex the sample tube to uniformly suspend the fluorospheres.
- 5 Place the sample tube in a dark location at 2-8 °C until ready to load the tube into the instrument for QC.

NOTE Tubes containing CytoFLEX Ready to Use Daily QC Fluorospheres should be sealed and stored in a dark location at 2-8 °C for up to 5 days.

Importing Lot-Specific Target Values

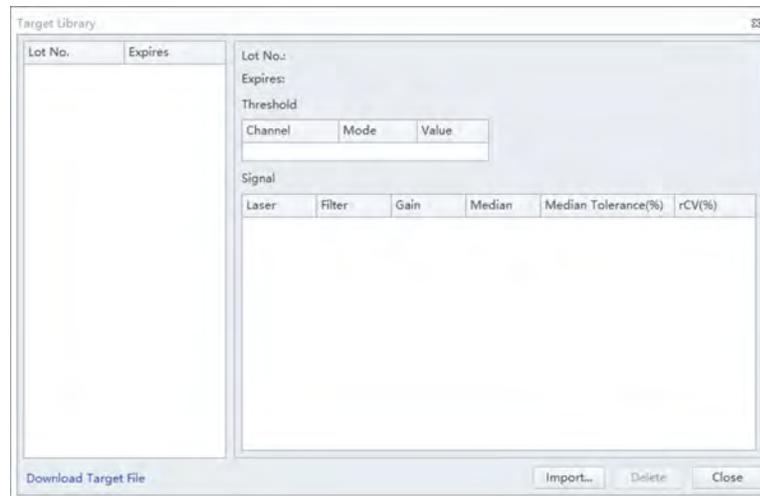
Import lot-specific target values for each new lot of CytoFLEX Daily QC Fluorospheres, CytoFLEX Ready to Use Daily QC Fluorospheres.



Risk of erroneous QC results. Different target value information corresponds to different lot numbers. Selecting the wrong lot number will lead to erroneous QC results.

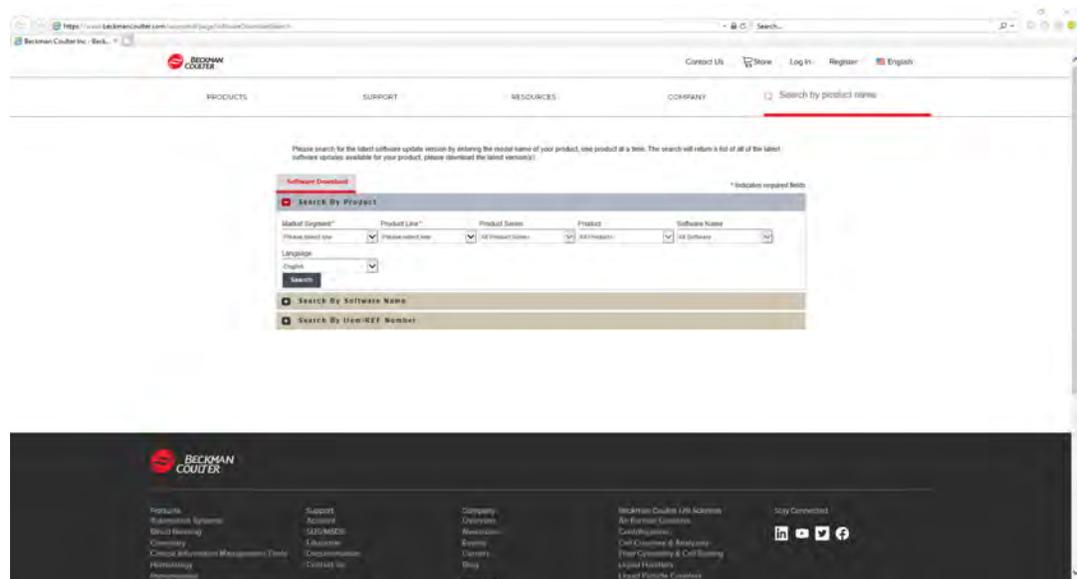
- 1 Open the CytExpert QC screen.

- 2 Select **Target Library** from the Settings menu. The Target Library window appears.



IMPORTANT The Beckman Coulter website may prompt you to select your Region and Country prior to the Beckman Coulter Technical Documents and Software page.

- 3 Select **Download Target File**. The Beckman Coulter Software Downloads page appears.



NOTE If your CytoFLEX SRT Workstation does not have access to the internet, navigate to <https://www.beckmancoulter.com/wsrportal/page/softwareDownloadSearch> using a computer with access to the internet and save the file to a USB drive. If the website is not accessible, [contact us](#).

- 4 If necessary, register and log in to the Beckman Coulter website.

- 5 In the Search By Product section of the screen, select the following:
 - a. Select **Research & Discovery** from the Market Segment drop-down menu.
 - b. Select **Flow Cytometry** from the Product Line drop-down menu.
 - c. Select **Instruments** from the Product Platform drop-down menu.
 - d. Select **CytoFLEX** from the Product drop-down menu.
 - e. Select **CytoFLEX QC Fluorospheres Target Values** or **CytoFLEX Ready to Use QC Fluorospheres Target Values** from the Software Name drop-down menu.
 - f. Select **All** from the Lot Number drop-down menu.
 - g. Select **English** from the Language drop-down menu.

[CytoFLEX QC Fluorospheres Target]

Technical Documents | My Technical Documents | Safety Data Sheets (SDS/MSDS) | Software Download * Indicates required fields

Search By Product

Market Segment* | Product Line* | Product Series | Product | Software Name
Research & Discovery | Flow Cytometry | Instruments | CytoFLEX | CytoFLEX QC Fluorospheres T

Lot Number | Language
All | English

Search

+ Search By Software Name
+ Search By Item/REF Number
+ Search By Lot Number

[CytoFLEX Ready to Use Daily QC Fluorospheres Target]

Technical Documents | My Technical Documents | Safety Data Sheets (SDS/MSDS) | Software Download * Indicates required fields

Search By Product

Market Segment* | Product Line* | Product Series | Product | Software Name
Research & Discovery | Flow Cytometry | Instruments | CytoFLEX | CytoFLEX Ready to Use Daily QC Fluor

Lot Number | Language
All | English

Search

+ Search By Software Name
+ Search By Item/REF Number
+ Search By Lot Number

- 6 Select **Search**.

- 7 The search results appear below the Search By Lot Number tab.

[CytoFLEX QC Fluorospheres Target]

Technical Documents | My Technical Documents | Safety Data Sheets (SDS/MSDS) | Software Download * Indicates required fields

Search By Product

Market Segment*: Research & Discovery | Product Line*: Flow Cytometry | Product Series: Instruments | Product: CytoFLEX | Software Name: CytoFLEX QC Fluorospheres Target Values

Lot Number: All | Language: English

Search

Search By Software Name

Search By Item/REF Number

Search By Lot Number

Software Download Search Results

First Prev 1 Next Last View 25 per page

Software Name	Product	Lot No.	Version	Item/REF NO.	Release Date	Language
CytoFLEX QC Fluorospheres Target Values	CytoFLEX	12345 ...		B53230		English

First Prev 1 Next Last Displaying 1 to 1 of 1 software found.

[CytoFLEX Ready to Use Daily QC Fluorospheres Target]

Search By Product

Market Segment*: Research & Discovery | Product Line*: Flow Cytometry | Product Series: Instruments | Product: CytoFLEX | Software Name: [CytoFLEX Ready to Use Daily QC Fluor.](#)

Lot Number: All | Language: English

Search

Search By Software Name

Search By Item/REF Number

Search By Lot Number

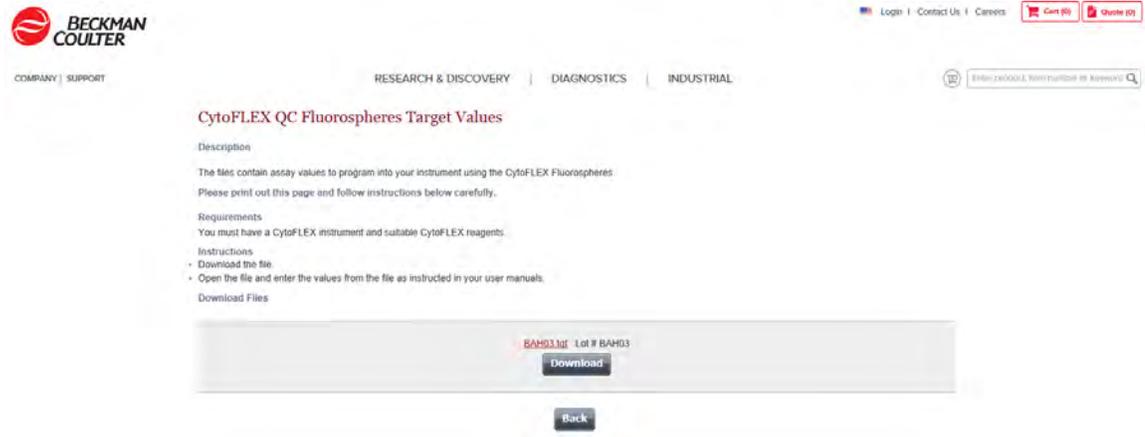
Software Download Search Results

First Prev 1 Next Last View 25 per page

Software Name	Product	Lot No.	Version	Item/REF NO.	Release Date	Language
CytoFLEX Ready to Use Daily QC Fluorospheres Target Values	CytoFLEX ...	AM03		C65719	05/16/2021	English

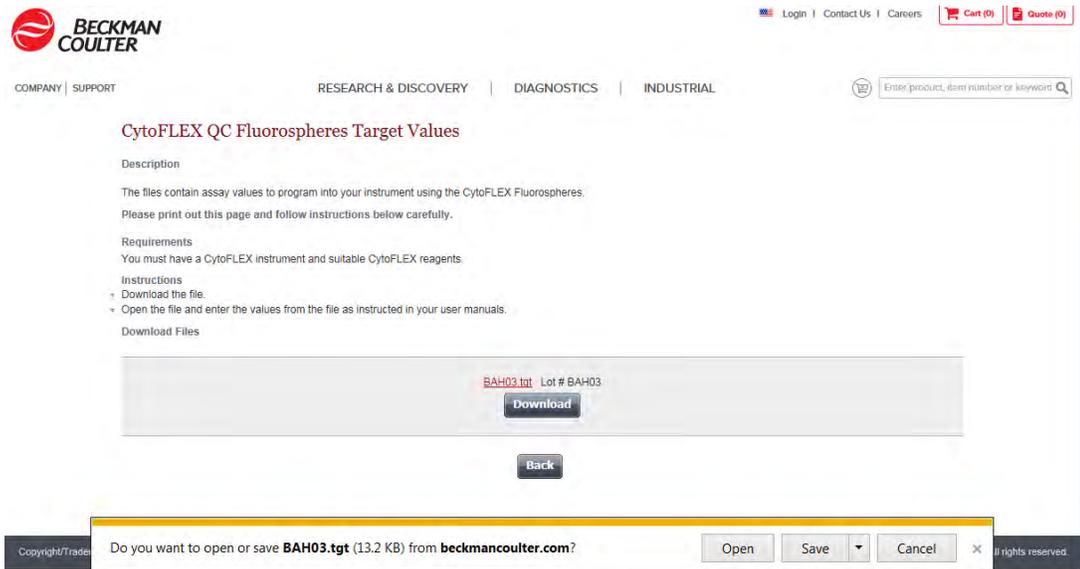
First Prev 1 Next Last Displaying 1 to 1 of 1 software found.

- 8 Select **CytoFLEX QC Fluorospheres Target Values** under the Software Name column. The CytoFLEX QC Fluorospheres Target Values page appears.

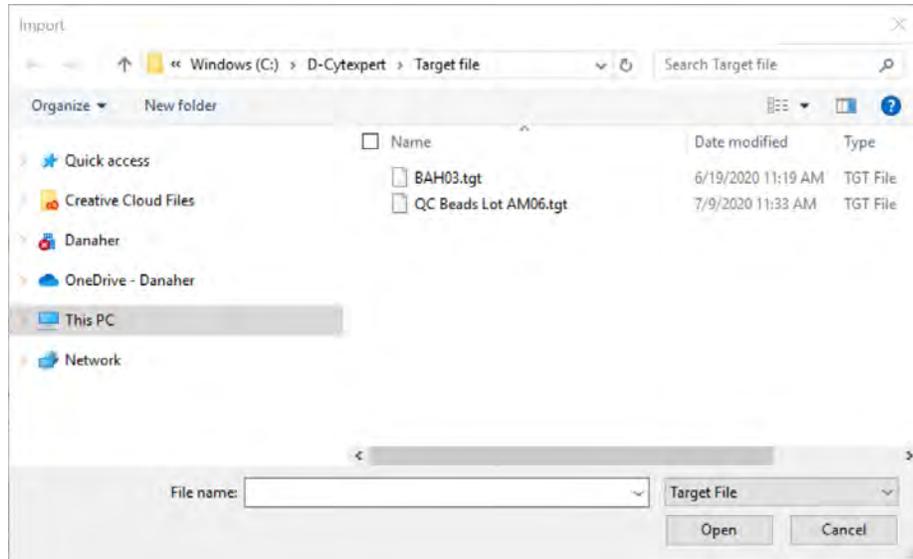


- 9 Select **Download** under the correct lot number from the CytoFLEX QC Fluorospheres Target Values page.

- 10 If the File Download pop up window appears, select **Save** and browse to the desired file path.

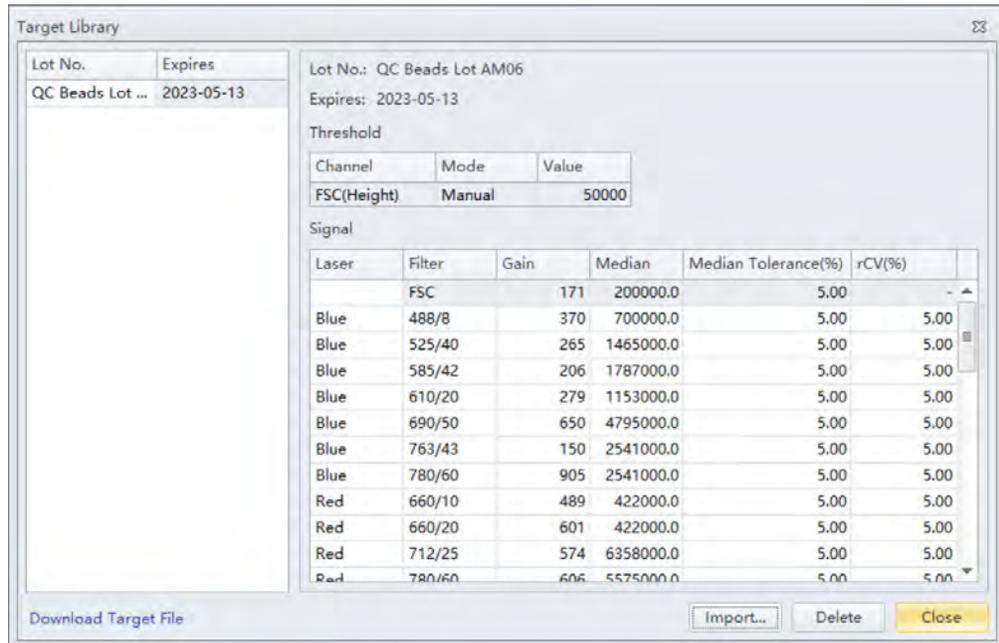


11 Select **Import** from the Target Library window in the CytExpert SRT software.



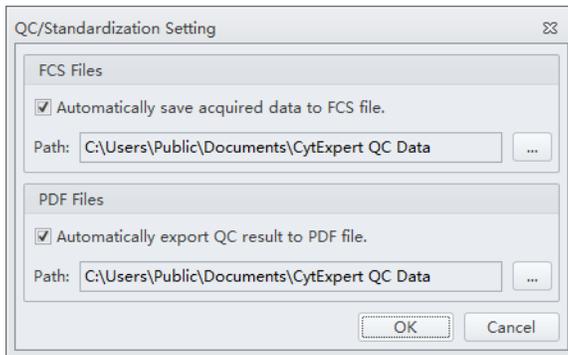
12 Navigate to the file saved in step 10 and select **Open**.

13 Select **Close** to exit the Target Library window.



Collecting QC Data

QC data and reports are saved by default. Select **QC/Standardization Setting** in the Settings menu to change the default save settings or modify the file path these files are saved to.



- 1 Double-click  to start the CytExpert SRT software.
 - a. Ensure that the **Connected** icon on the Status Bar near the bottom-left side of the display is green.



- b. If the icon is not green, ensure that the Sorter USB is securely connected to the Workstation and restart the Workstation.
- 2 Verify the detector configuration. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in [CHAPTER 5, Sorting](#).

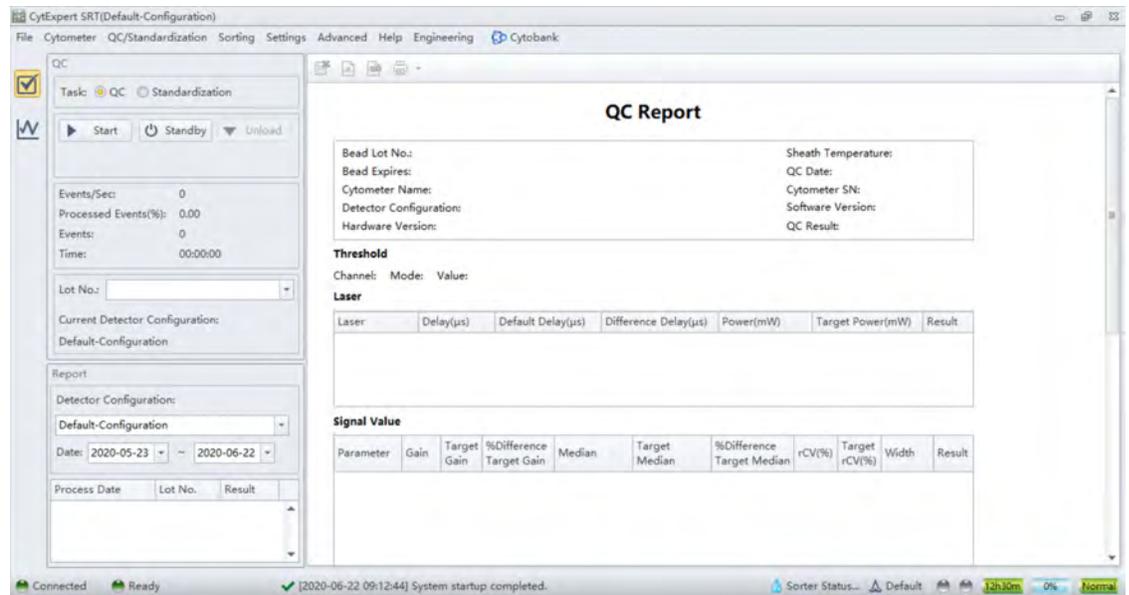
NOTE Ensure that the instrument configuration is properly configured for the QC experiment. The QC experiment may not be completed or may end in erroneous results if incorrect settings are chosen. Beckman Coulter recommends using the factory configuration and ensuring that the proper optical filters are in place.

- 3 Verify the laser settings. Refer to [Laser Settings](#) in [CHAPTER 5, Sorting](#).

- 4 Select **Initialize** to put the instrument in the initialized state.

NOTE Skip this step if the instrument has already been in the initialized state.

- 5 Select **Start QC/Standardization** in the QC/Standardization menu to access the QC experiment.

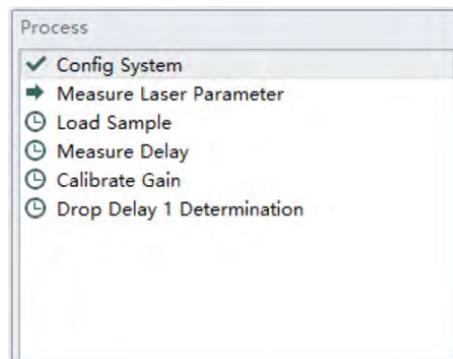


Ensure that the QC bead lot number is selectable in the Lot No. drop-down menu. If the lot number is not selectable, refer to [Importing Lot-Specific Target Values](#), then select the proper lot number.

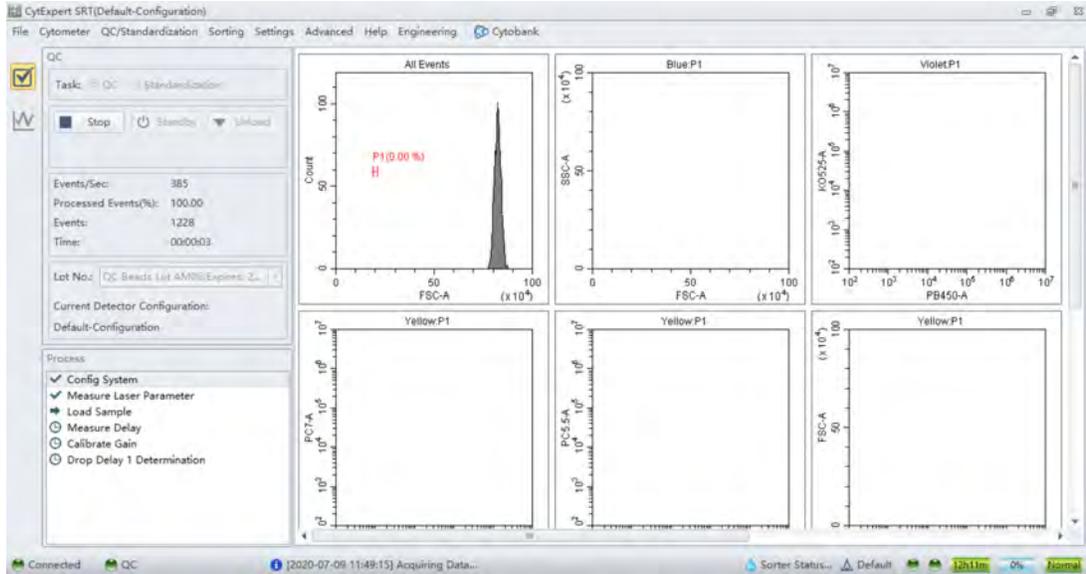
- 6 Insert the prepared QC sample tube (refer to [CytoFLEX Daily QC Fluorospheres Preparation Process](#) or [CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process](#)) into the tube holder.

- 7 Select **Start** to load the sample and begin to run the QC procedure.

Completed processes appear on the left. Plots appear on the right. The QC experiment sequentially detects the system configuration, laser power, laser delay, signal strength, coefficient of variation, and Drop Delay 1.



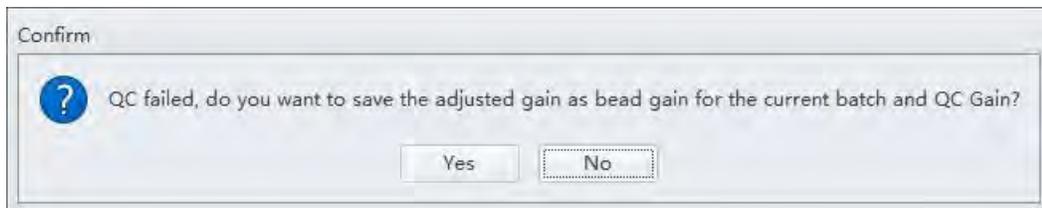
During QC, the software automatically seeks the CytoFLEX QC Fluorospheres and computes the results. The software returns to the QC Report screen after the QC run is complete.



- 8 If the sampling rate is too low, the Sorter stops the QC run and displays a prompt alerting you that the QC run failed to reach the required event flow rate. This is not considered a QC failure. If this situation occurs, increase the sample concentration by adding one drop of CytoFLEX Daily QC Fluorospheres to the sample tube or prepare a new tube of CytoFLEX Ready to Use Daily QC Fluorospheres, and then perform the QC.

NOTE Mix the fluorospheres dropper bottle thoroughly before dispensing it into a sample tube.

- 9 If the lot number of CytoFLEX QC Fluorospheres is new and QC fails, the following software message appears. Select **Yes**.



NOTE Target gain values must be established for each new lot number of CytoFLEX QC Fluorospheres. QC could fail up to 3 times upon running each new lot number for the first time until target gain values are established.

If the lot number of CytoFLEX QC Fluorospheres is NOT new and QC fails, refer to Step 3 of [Confirming Results](#), or [CHAPTER 9, Troubleshooting](#).

If QC passes, proceed to Step 10.

10 Select **Yes** to start Sort Calibration now. Refer to [Sort Calibration \(Auto Drop Delay\)](#) in [CHAPTER 5, Sorting](#).

Or

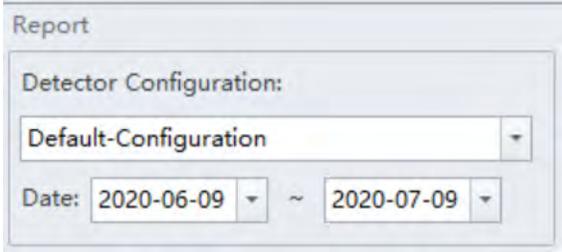
Select **No** to defer the Sort Calibration.

NOTE Sort calibration is required prior to the sorting. For analysis experiments, skip the Sort Calibration.

Confirming Results

Select **Start QC/Standardization** in the QC/Standardization menu to return to the QC Setting screen at any time to review completed experiment results.

1 Select the desired default configuration and date range from the drop-down menus located on the left side of the QC screen to sort by the configuration used during the specified date range.



The screenshot shows a 'Report' window with two main sections. The first section is 'Detector Configuration:' with a dropdown menu currently set to 'Default-Configuration'. The second section is 'Date:' with two date pickers separated by a tilde (~). The first date picker is set to '2020-06-09' and the second is set to '2020-07-09'.

NOTE At least one date range must be specified.

2 Select a QC run from the QC Process list on the left and a QC report appears on the right.

Process Date	Lot No.	Result	
2020-04-13 13:44	QC Beads...	✓	▲
2020-04-14 08:42	QC Beads...	✓	
2020-04-14 09:31	QC Beads...	✓	
2020-04-15 11:19	QC Beads...	✓	
2020-04-15 11:51	QC Beads...	✗	
2020-04-15 12:15	QC Beads...	✗	

NOTE The results column indicates a passing QC result with a ✓ and a failed QC result with ✗.

QC results must meet the following criteria to pass:

- DD1 must be within the range of 720 - 880 μ s.
- DD laser must be within the range of 90 - 110 mW.
- The delay differences must be ≤ 5 μ s.
- The gain differences must be $\leq 20\%$ from the target gain.
- The median fluorescence intensity (MFI) differences must be $\leq 5\%$ from the target MFI.
- The rCV must be within the range.

The report area on the right displays detailed experiment results, including laser power, delay, testing conditions, and signal results. The same  and  symbols are used to indicate each result. For items that fail, values falling outside the prescribed range are displayed in red font. In the Comment area, an explanation appears for each failed item.

QC Report										
Bead Lot No.:	QC Beads Lot AM05					Sheath Temperature:	24.46			
Bead Expires:	2023-04-16					QC Date:	2020-11-04 14:18			
Cytometer Name:	CytoFLEX SRT					Cytometer SN:	MP07			
Detector Configuration:	Default-Configuration					Software Version:	1.0.0.10181			
Firmware Version:	1.0.0.213					QC Result:				
Threshold										
Channel: FSC(Height) Mode: Manual Value: 50000										
Laser										
Laser	Delay(μs)	Default Delay(μs)	Difference Delay(μs)	Power(mW)	Target Power(mW)	Result				
Violet	-57.52	-57.52	0.00	92	70-120					
Blue	0.00	0.00	0.00	50	40-60					
Yellow	31.12	31.20	-0.08	30	20-40					
Red	-30.08	-30.08	0.00	101	80-120					
Signal Value										
Parameter	Gain	Target Gain	%Difference Target Gain	Median	Target Median	%Difference Target Median	rCV(%)	Target rCV(%)	Width	Result
FSC	89	83	7.23	197959.3	200000.0	-1.02	-	-	1107.4	
SSC	279	258	8.14	693418.7	699000.0	-0.80	-	-	1204.7	
FITC	153	153	0.00	1412793.0	1440000.0	-1.89	7.98	5.00	1198.4	
PerCP	434	434	0.00	4563440.0	4665000.0	-2.18	7.89	5.00	1203.9	
APC	404	404	0.00	416309.0	420000.0	-0.88	2.24	5.00	1374.2	
APC-A700	288	288	0.00	6250893.0	6294000.0	-0.68	1.89	5.00	1380.1	
APC-A750	304	304	0.00	5457445.0	5523000.0	-1.19	2.02	5.00	1382.3	
PB450	95	99	-4.04	772407.2	769000.0	0.44	6.87	5.00	836.3	
KO525	65	69	-5.80	196136.8	193000.0	1.63	6.02	5.00	836.1	
Violet610	337	353	-4.53	298425.1	296000.0	0.82	5.09	5.00	832.6	
Violet660	348	367	-5.18	84968.3	84000.0	1.15	5.05	5.00	828.2	
V780	416	416	0.00	449354.3	433000.0	3.78	3.50	5.00	840.1	
PE	382	359	6.41	469427.0	469000.0	0.09	5.65	5.00	1189.0	
ECD	257	241	6.64	347661.6	347000.0	0.19	5.77	5.00	1201.8	
PC5	370	370	0.00	398091.4	415000.0	-4.07	5.44	5.00	1201.0	
PC5.5	179	179	0.00	1011244.0	1029000.0	-1.73	5.41	5.00	1203.3	
PC7	670	670	0.00	1775460.0	1813000.0	-2.07	5.63	5.00	1207.6	
Drop Delay 1 Determination										
Item	Value	Target	Result							
DD1	836	720-880								
DD Laser	101	80-120								

3 If QC fails, follow the procedure below:

- Verify whether the beads used were within their shelf life and stored in accordance with the appropriate instruction manual.
- Verify whether the allocated sample tube was prepared as required and correctly positioned.
- Verify whether the detector configuration selected for QC matches with the current detector configuration. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in [CHAPTER 5, Sorting](#).
- Run [Performing the Flow Cell De-bubble](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#), and retest.

- e. Run [Performing the Sheath Filter De-bubble](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#), and retest.
- f. Run Backflush. Select **Cytometer > Backflush**, and retest.
- g. Verify whether the Nozzle is clean and installed properly. Refer to [Cleaning the Nozzle](#) in [CHAPTER 10, Cleaning Procedures](#), and retest.
- h. Run [Removing Trapped Air Bubbles](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#), and retest.
- i. Restart the system, and retest.

NOTE If QC fails after repeating Steps a-i, [contact us](#).

-
- 4 If necessary, you can select  (for CSV format) or  (for PDF format) in the top left corner of the report area to export the QC results.

-
- 5 Select **Close QC/Standardization** in the QC/Standardization menu to exit the QC screen.
-

Creating Levey-Jennings Charts

- 1 Select **Start QC/Standardization** in the QC/Standardization menu to open the QC screen.
-
- 2 Select LJ chart  on the left side of the screen.

- 4 Select the **Laser** tab, and select the power and/or delay checkboxes for each laser as needed.

LJ Charts Setting

Detector Configuration: Default-Configuration

Lot No.	Laser	Channel	Alarm Boundary and Scale Range
<input type="checkbox"/>	Violet	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	Blue	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	Yellow	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	Red	<input type="checkbox"/>	<input type="checkbox"/>

Select All Clear All

OK Cancel Apply

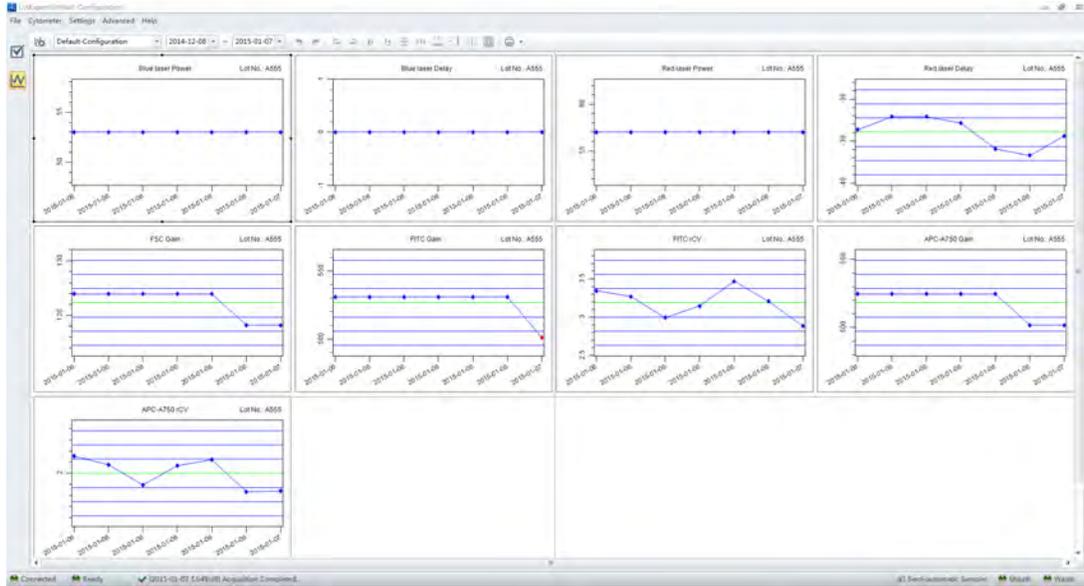
- 5 Select the **Channel** tab, and select each channel checkbox as needed.

Lot No.	Channel	Gain	rCV
<input type="checkbox"/>	FSC	<input type="checkbox"/>	-
<input type="checkbox"/>	SSC	<input type="checkbox"/>	-
<input type="checkbox"/>	B525	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	B690	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	R660	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	R712	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	R780	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	V450	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	V525	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	V610	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	V660	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	V780	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	Y585	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	Y610	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	Y675	<input type="checkbox"/>	<input type="checkbox"/>

- 6 Select **Apply**.

- 7 Select **OK**.

- 8 Select the Levey-Jennings plot and select the start and end date from the drop-down boxes at the top of the LJ Chart screen to specify the desired date range.



NOTE Select the desired configuration and date range from the drop-down menus located at the top of the LJ Chart screen to sort by the configuration used during the specified date range.

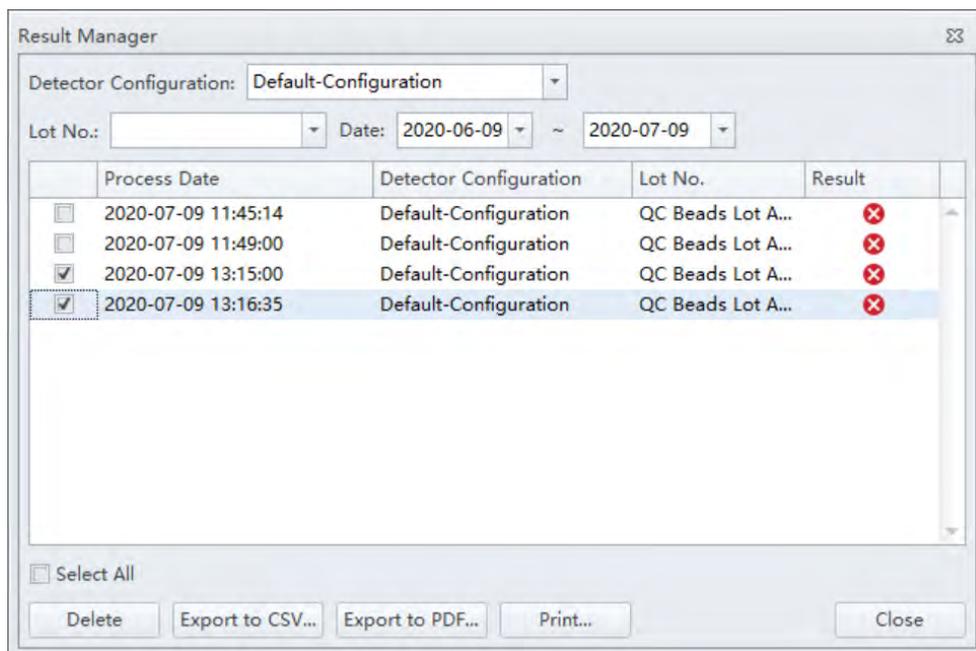
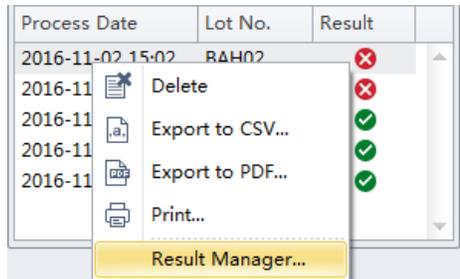


- 9 Select **Close QC/Standardization** in the QC/Standardization menu to exit the QC screen.

QC Result Manager

The QC Result Management window can be used to search, delete, print, and export QC results.

To access the QC Result Manager, right-click the desired QC result and select **Result Manager** in the QC screen. The QC Result Manager window appears.



Standardization

Ensure that the standardization sample has been run at optimized experiment settings to determine the standardization sample threshold setting as well as median values for all relevant channels.

Preparing the Standardization Sample

Use Beckman Coulter CytoFLEX Daily QC fluorospheres or CytoFLEX Ready to Use Daily QC Fluorospheres or any other reference material that is relevant for your application.

Required Materials

The following materials are required to complete the QC process:

- CytoFLEX Daily QC Fluorospheres or CytoFLEX Ready to Use Daily QC Fluorospheres, or other material applicable for your application
- IsoFlow or ISOTON II sheath
- Sample tubes (12 x 75 mm).
- Vortexer

Preparation Process

For procedures, refer to [CytoFLEX Daily QC Fluorospheres Preparation Process](#) or [CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process](#).

Generating Target Median Values

- 1 Double-click  to start the CytExpert SRT software.
 - a. Ensure that the **Connected** icon on the Status Bar near the bottom-left side of the display is green.



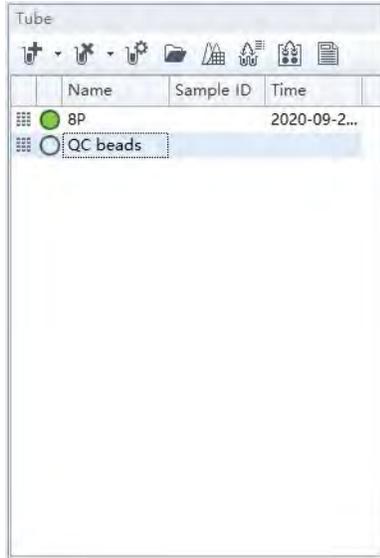
- b. If the icon is not green, ensure that the Sorter USB is securely connected to the Workstation and restart the Workstation.
-

- 2 Select **Open Experiment** to open an experiment with the desired acquisition settings to standardize.

NOTE Ensure the Gain and Threshold settings are optimal. Refer to [Adjusting the Gain](#) and [Adjusting the Threshold](#) in [CHAPTER 5, Sorting](#).

- 3 Right-click the tube and select **Duplicate without Data** to create a tube with the same acquisition settings.

- 4 Change the tube name. Refer to [Changing the Tube Name](#) in [CHAPTER 5, Sorting](#).



- 5 Select **Save As** from the File menu to save the experiment.

- 6 Select  to delete all the remaining tubes.



7 Select . The Compensation Matrix window appears.

Compensation Matrix - QC beads

Use Show Autofluorescence Sync Area and Height Area ▾

Autofl.	Channel	-FITC%	-PC5%	-APC%	-APC-A700%	-APC-A750%	-PB45 0%	-KO52 5%	-V610%	-V660%	-V780%	-PE%	-ECD%	-PC5-Y...	-PC5.5%	-PC7%
0.00	FITC		0.00	0.00	0.12	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.06
0.00	PC5	1.27		0.00	1.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	226.36	0.21
0.00	APC	0.02	0.00		0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.06	0.00
0.00	APC-A700	0.01	0.00	0.00		0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	83.67	0.05
0.00	APC-A750	0.42	0.00	0.00	76.42		0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	65.69	4.76
0.00	PB450	0.03	0.00	0.00	0.09	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
0.00	KO525	1.58	0.00	0.00	0.06	0.00	7.25		0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
0.00	V610	0.24	0.00	0.00	0.01	0.00	0.32	0.00		0.00	0.00	0.00	0.00	0.00	0.02	0.00
0.00	V660	0.14	0.00	0.00	0.04	0.00	0.14	0.00	0.00		0.00	0.00	0.00	0.00	12.05	0.00
0.00	V780	0.22	0.00	0.00	7.87	0.00	0.09	0.00	0.00	0.00		0.00	0.00	0.00	88.85	3.32
0.00	PE	0.03	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.04	0.29
0.00	ECD	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.03	0.06
0.00	PC5-Y	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		33.02	0.05
0.00	PC5.5	0.04	0.00	0.00	14.52	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
1.09	PC7	5.24	0.00	0.00	32.48	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	112.73	

Import from Library... Import... Export... Clear Apply to... Close

8 Select **Clear** to clear the compensation matrix. The message *Are you sure you want to clear the compensation matrix?* appears. Select **Yes**.

Compensation Matrix - QC beads

Use Show Autofluorescence Sync Area and Height Area ▾

Autofl.	Channel	-FITC%	-PC5%	-APC%	-APC-A700%	-APC-A750%	-PB45 0%	-KO52 5%	-V610%	-V660%	-V780%	-PE%	-ECD%	-PC5-Y...	-PC5.5%	-PC7%
0.00	FITC		0.00	0.00	0.12	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.06
0.00	PC5	1.27		0.00	1.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	226.36	0.21
0.00	APC	0.02	0.00		0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.06	0.00
0.00	APC-A700	0.01	0.00	0.00		0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	83.67	0.05
0.00	APC-A750	0.42	0.00	0.00	76.42		0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	65.69	4.76
0.00	PB450	0.03	0.00	0.00	0.09	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
0.00	KO525	1.58	0.00	0.00	0.06	0.00	7.25		0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
0.00	V610	0.24	0.00	0.00	0.01	0.00	0.32	0.00		0.00	0.00	0.00	0.00	0.00	0.02	0.00
0.00	V660	0.14	0.00	0.00	0.04	0.00	0.14	0.00	0.00		0.00	0.00	0.00	0.00	12.05	0.00
0.00	V780	0.22	0.00	0.00	7.87	0.00	0.09	0.00	0.00	0.00		0.00	0.00	0.00	88.85	3.32
0.00	PE	0.03	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.04	0.29
0.00	ECD	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.03	0.06
0.00	PC5-Y	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		33.02	0.05
0.00	PC5.5	0.04	0.00	0.00	14.52	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
1.09	PC7	5.24	0.00	0.00	32.48	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	112.73	

Confirm
? Are you sure you want to clear the compensation matrix?
Yes No

Import from Library... Import... Export... **Clear** Apply to... Close

9 Load the sample tube.

NOTE The sample tube holder only accommodates 12 x 75 mm sample tubes.

10 Select **Run**.

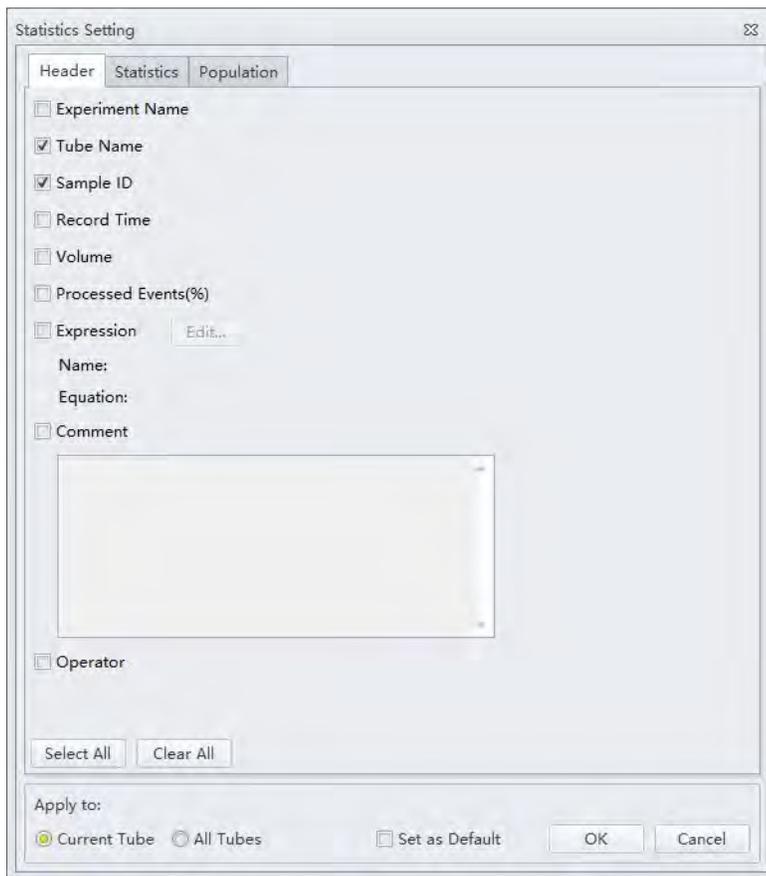
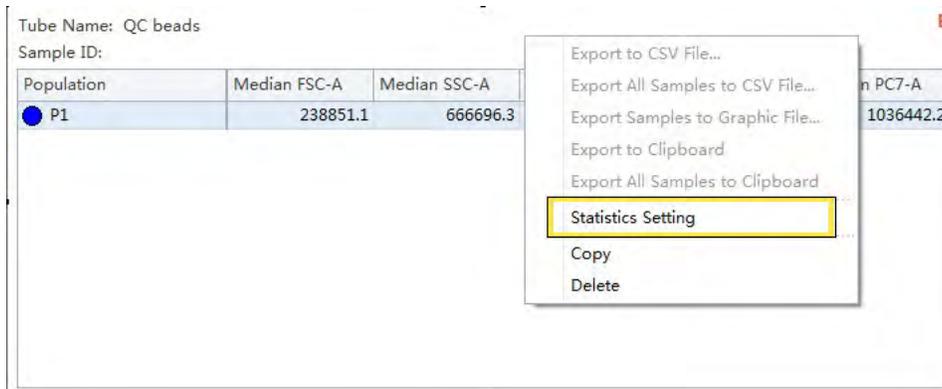
11 View the plots and establish the gates. Refer to [Creating Plots and Gates](#) in [CHAPTER 5, Sorting](#).

NOTE Use the FSC channel as the trigger channel and select **Automatic** threshold.

NOTE The threshold may need to be adjusted to visualize the QC beads populations. If so, record this value for future reference.

12 Select the **Statistics** tab.

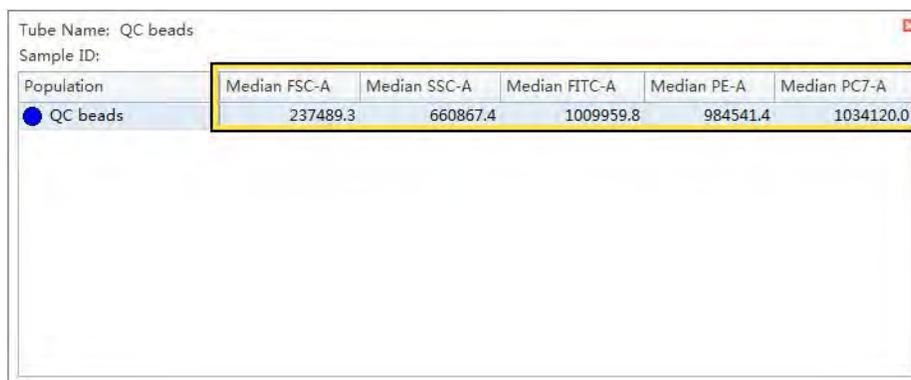
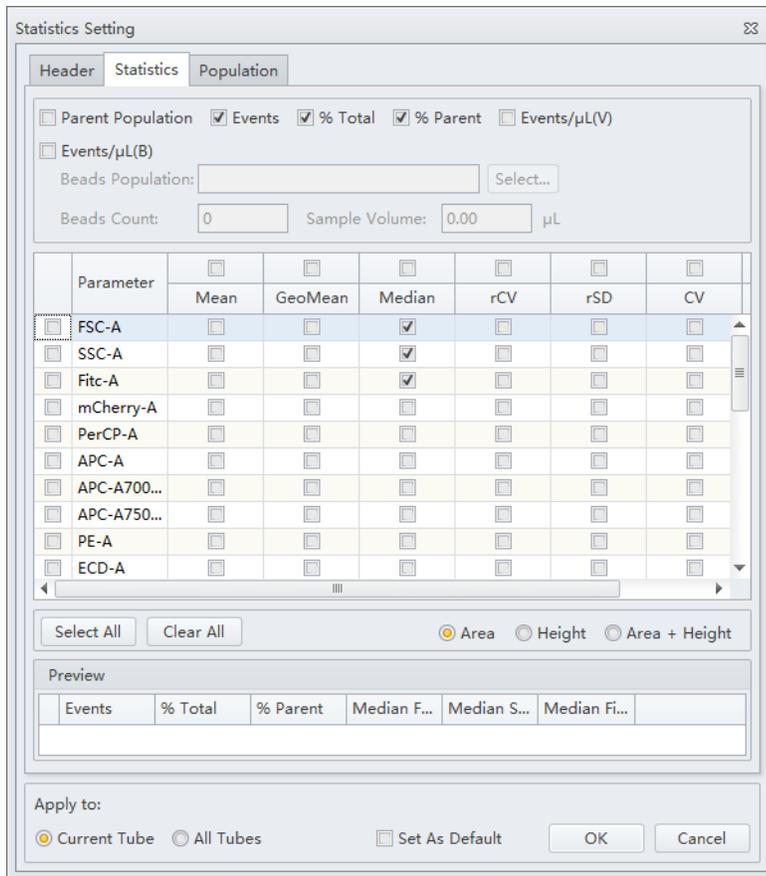
13 Right-click the table and select **Statistics Settings**. The Statistics Setting window appears.



- 14 Select the Population tab and select the relevant population for the tube.



15 Select the **Statistics** tab then select the Median Fluorescence value for all parameters used.



NOTE The median values are the target settings that will be used for standardization.

16 Select **Stop**.

17 Right-click the statistics table and select **Export to CSV file**.

If Excel is not available, manually record all the median values or take a screen shot.

18 Save the experiment.

NOTE Rerun the experiment if:

- You change the standardization fluorosphere used.
- The Lot number for the standardization fluorosphere is changed.

Adding a New Standardization Item

- 1 Select **Start QC/Standardization** in the QC/Standardization menu to access the Standardization screen.

The screenshot displays the 'Standardization Report' window in the CytExpert SRTI software. The report contains the following information:

Task Information:

- Task Item: 1
- Bead Lot No.: AM04
- Cytometer Name: Default-Configuration
- Firmware Version: 1.0.0.M213
- Date: 2020-09-17 17:00
- Bead Expires: 2020-09-17
- Cytometer S/N: 1.0.0.10191
- Software Version: 1.0.0.10191
- Standardization Result: ✔

Threshold: Channel: FSC(Height) Mode: Manual Value: 20000

Signal Value Table:

Parameter	Gain	Median	Target Median	%Difference Target Median	Result
FSC	57	199050.8	200000.0	-0.47	✔
SSC	113	201927.3	200000.0	0.96	✔
FITC	201	988319.1	1000000.0	-1.17	✔
APC	1288	496054.6	500000.0	-0.79	✔
PB450	202	1006547.0	1000000.0	0.63	✔
PE	198	499915.1	500000.0	-0.02	✔

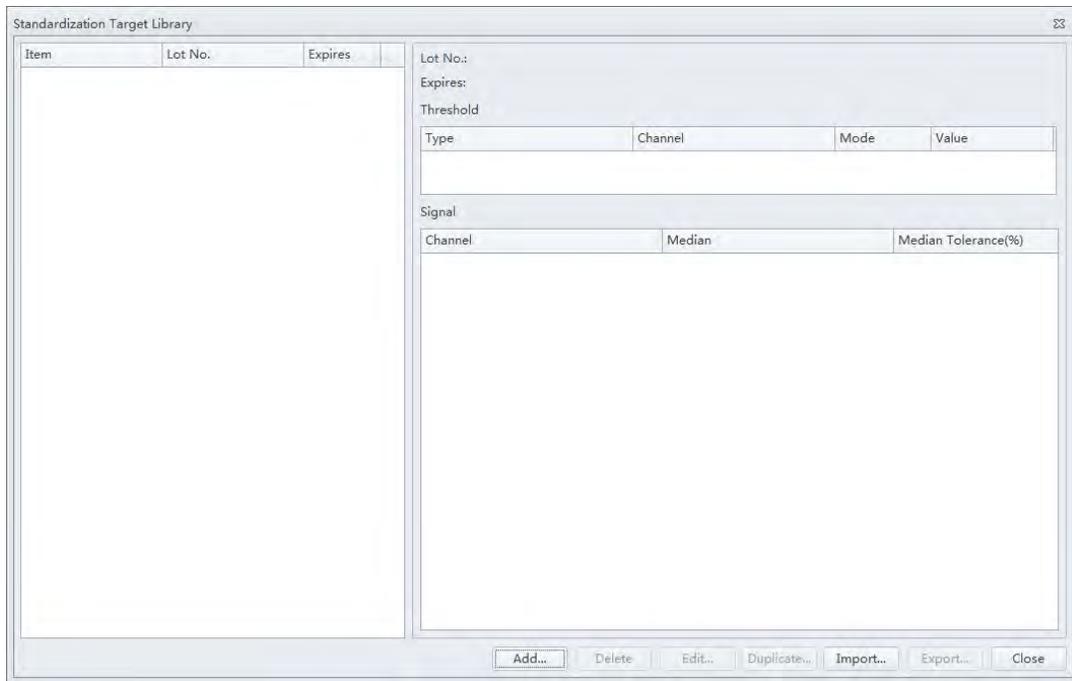
Specifications: Medians -5.00% ≤ %Difference Target Median ≤ 5.00%.

Result: Standardization Passed.

The interface also shows a 'Process Date' table with the following entries:

Process Date	Lot No.	Task Item	R
2020-09-17 16:52	AM04	1	
2020-09-17 16:54	AM04	1	
2020-09-17 16:56	AM04	1	
2020-09-17 16:57	AM04	1	

2 Select **Standardization Target Library** from the Settings menu.



- 3 Select **Add...**. The Add Standardization Target Value window appears.

	Channel	Median	Median Tolerance(%)
<input checked="" type="checkbox"/>	FSC	0.00	5.00
<input type="checkbox"/>	SSC	0.00	5.00
<input type="checkbox"/>	FITC	0.00	5.00
<input type="checkbox"/>	PC5	0.00	5.00
<input type="checkbox"/>	APC	0.00	5.00
<input type="checkbox"/>	APC-A700	0.00	5.00
<input type="checkbox"/>	APC-A750	0.00	5.00
<input type="checkbox"/>	PB450	0.00	5.00
<input type="checkbox"/>	KO525	0.00	5.00
<input type="checkbox"/>	V610	0.00	5.00
<input type="checkbox"/>	V660	0.00	5.00
<input type="checkbox"/>	V780	0.00	5.00
<input type="checkbox"/>	PE	0.00	5.00
<input type="checkbox"/>	ECD	0.00	5.00
<input type="checkbox"/>	PC5-Y	0.00	5.00
<input type="checkbox"/>	PC5.5	0.00	5.00
<input type="checkbox"/>	PC7	0.00	5.00

- 4 Enter the Item, Lot No., and Expire date from the dropdowns located at the top of the Add Standardization Target Value window.

NOTE A single Lot No. can include several Items, but you cannot add duplicate Items under the same Lot No..

NOTE If the Lot No. selected already exists, the Expire date cannot be edited.

- 5 Select either Manual or Automatic threshold from the Standardization Test Setting or Acquisition Setting section of the window.

NOTE If you select Manual threshold, enter a value greater than 0, but less than 8,388,600.

NOTE Keep the threshold setting the same as previous Step 11 in [Generating Target Median Values](#).

NOTE Unchecking the *Use the same threshold setting for acquisition setting* checkbox allows you to specify custom threshold settings.

6 Set the channels to be standardized.

NOTE The contents of the channel, laser, and filter column come from the current detector configuration setting. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in [CHAPTER 5, Sorting](#).

NOTE Do not set the median tolerance range any lower than 5%.

NOTE FSC is a required channel.

7 Enter the target median values saved in [Step 15](#) into the corresponding channels.

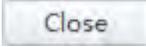
Channel	Median	Median Tolerance(%)
<input checked="" type="checkbox"/> FSC	708979.00	5.00
<input checked="" type="checkbox"/> SSC	3864471.00	5.00
<input checked="" type="checkbox"/> FITC	2160286.00	5.00
<input type="checkbox"/> PC5	0.00	5.00
<input type="checkbox"/> APC	0.00	5.00
<input type="checkbox"/> APC-A700	0.00	5.00
<input type="checkbox"/> APC-A750	0.00	5.00
<input type="checkbox"/> PB450	0.00	5.00
<input type="checkbox"/> KO525	0.00	5.00
<input type="checkbox"/> V610	0.00	5.00
<input type="checkbox"/> V660	0.00	5.00
<input type="checkbox"/> V780	0.00	5.00
<input checked="" type="checkbox"/> PE	554880.00	5.00
<input type="checkbox"/> ECD	0.00	5.00
<input type="checkbox"/> PC5-Y	0.00	5.00
<input type="checkbox"/> PC5.5	0.00	5.00
<input checked="" type="checkbox"/> PC7	6065859.00	5.00

Or copy the median values from the previously exported CSV file and paste into the corresponding median column.

NOTE Verify that the target values are entered correctly.

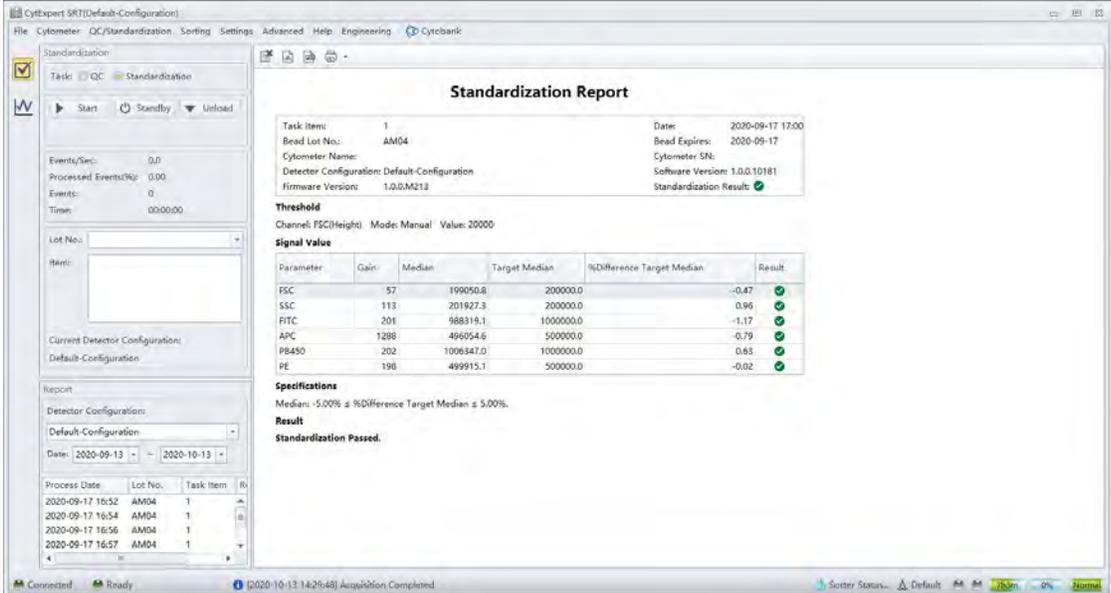
- 8 Select **OK** to save the target value.

The saved results display in the Standardization Target Library window. This item is ready to be run through the Standardization experiment. Refer to [Performing the Standardization](#) in CHAPTER 4, [Instrument Quality Control and Standardization](#).

- 9 Select  to exit the Standardization Target Library window.

Performing the Standardization

- 1 Select **Start QC/Standardization** in the QC/Standardization menu to access the Standardization screen.



Standardization Report

Task Item: 1
Bead Lot No.: AM04
Cytometer Name: Default-Configuration
Detector Configuration: Default-Configuration
Firmware Version: 1.0.0.M213

Date: 2020-09-17 17:00
Bead Expires: 2020-09-17
Cytometer SN: 1.0.0.10191
Software Version: 1.0.0.10191
Standardization Result: 

Threshold
Channel: FSC(Height) Mode: Manual Value: 20000

Signal Value

Parameter	Gain	Median	Target Median	%Difference Target Median	Result
FSC	57	199050.8	200000.0	-0.47	
SSC	113	201627.3	200000.0	0.86	
FITC	201	988319.1	1000000.0	-1.17	
APC	1288	496054.6	500000.0	-0.79	
PB450	202	1006347.0	1000000.0	0.63	
PE	196	499915.1	500000.0	-0.02	

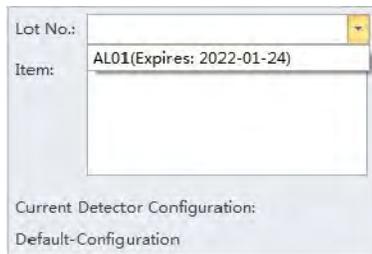
Specifications
Medians: -5.00% ≤ %Difference Target Median ≤ 5.00%.

Result
Standardization Passed.

- 2 Select the **Standardization** radio button.

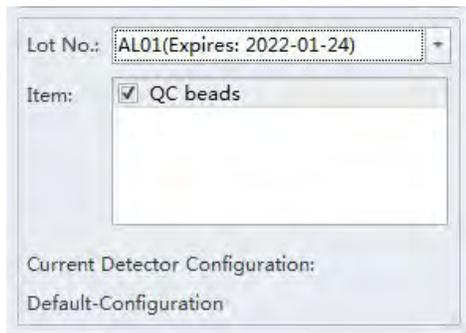


- 3 Select the correct Lot No. from the Lot No. dropdown.

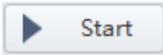


NOTE Ensure the Lot No. corresponds to the standardization sample that generated the target median values.

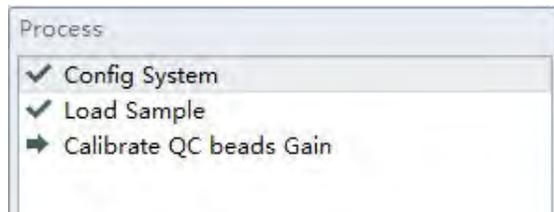
- 4 Select the Items to be standardized.



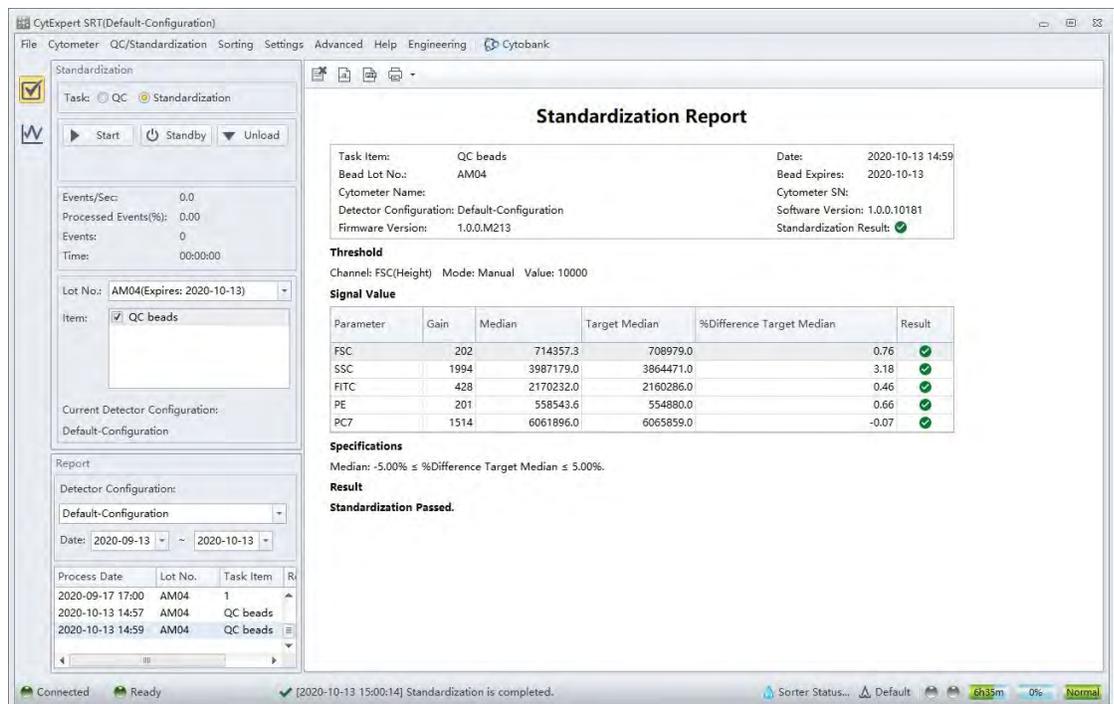
- 5 Select the proper detector configuration. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in CHAPTER 5, [Sorting](#).

- 6 Select .

The Process section of the screen displays the process details.



Once the process is complete, the Standardization Report displays.



Standardization Report

Task Item: QC beads Date: 2020-10-13 14:59
 Bead Lot No.: AM04 Bead Expires: 2020-10-13
 Cytometer Name: Cytometer SN:
 Detector Configuration: Default-Configuration Software Version: 1.0.0.10181
 Firmware Version: 1.0.0.M213 Standardization Result: 

Threshold
 Channel: FSC(Height) Mode: Manual Value: 10000

Parameter	Gain	Median	Target Median	%Difference Target Median	Result
FSC	202	714357.3	708979.0	0.76	
SSC	1994	3987179.0	3864471.0	3.18	
FITC	428	2170232.0	2160286.0	0.46	
PE	201	558543.6	554880.0	0.66	
PC7	1514	6061896.0	6065859.0	-0.07	

Specifications
 Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.

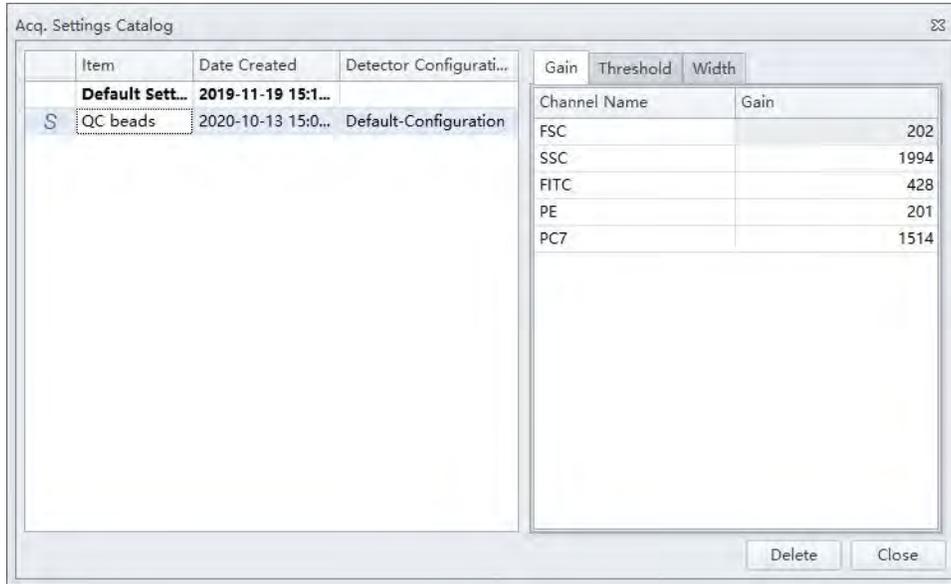
Result
Standardization Passed.

Process Date Lot No. Task Item Ri
 2020-09-17 17:00 AM04 1
 2020-10-13 14:57 AM04 QC beads
 2020-10-13 14:59 AM04 QC beads

Connected Ready [2020-10-13 15:00:14] Standardization is completed. Sorter Status... Default 6h35m 0% Normal

NOTE The updated Standardization item is added to the Acquisition Catalog automatically and overwrites the previously existing standardized settings for this item.

- 7 Select **Acq. Setting Catalog** from the Cytometer menu to verify the gain settings. The Acq. Setting Catalog window appears.



NOTE **S** designates test items from Standardization.

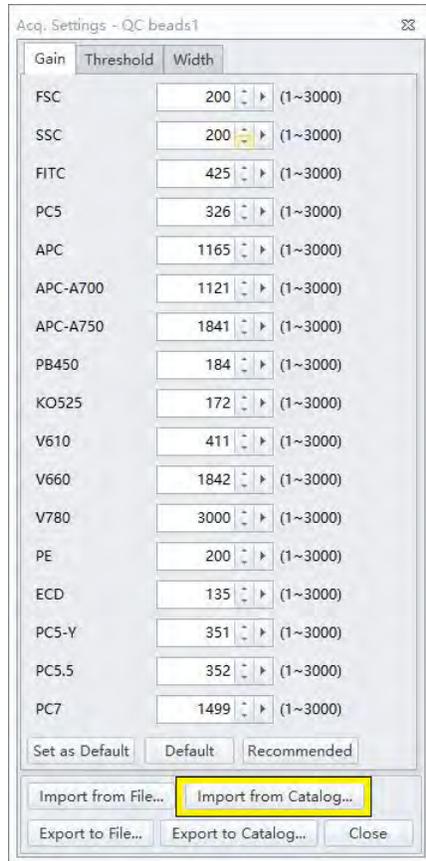
- 8 Run Daily Clean. Refer to [Daily Clean Program](#) in [CHAPTER 10, Cleaning Procedures](#).
- 9 Select **Close QC/Standardization**.

Applying the Standardized Acquisition Settings

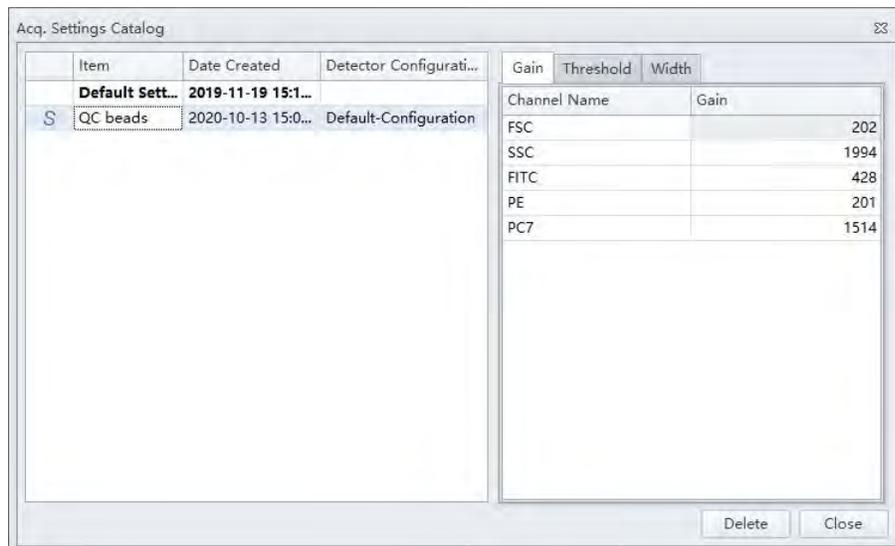
- 1 Open an experiment.

NOTE The corresponding compensation matrix should have been determined as the optimal settings. Refer to [CHAPTER 6, Compensation](#) for detailed instructions on setting compensation.

2 Select **Acq.Setting** from the Cytometer menu. The Acq. Setting window appears.

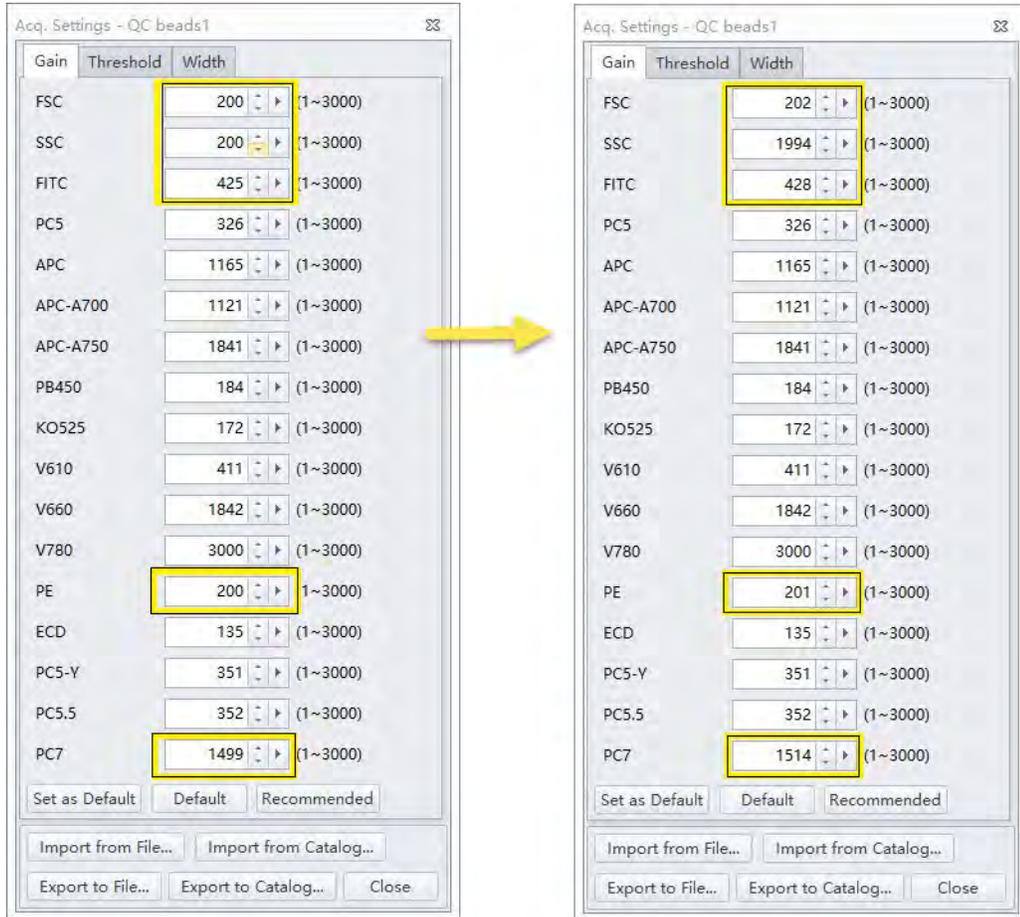


3 Select **Import from Catalog**. The Acq. Setting Catalog window appears.

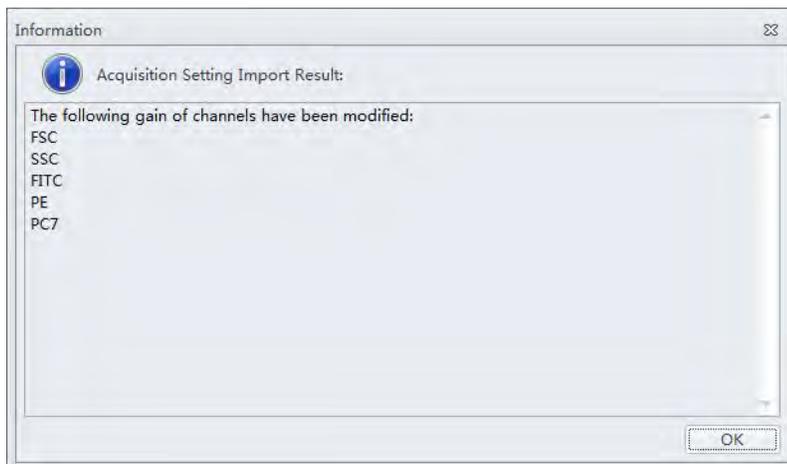


4 Browse for the item to import and select **Import**.

The standardized settings are applied to the sample tube.



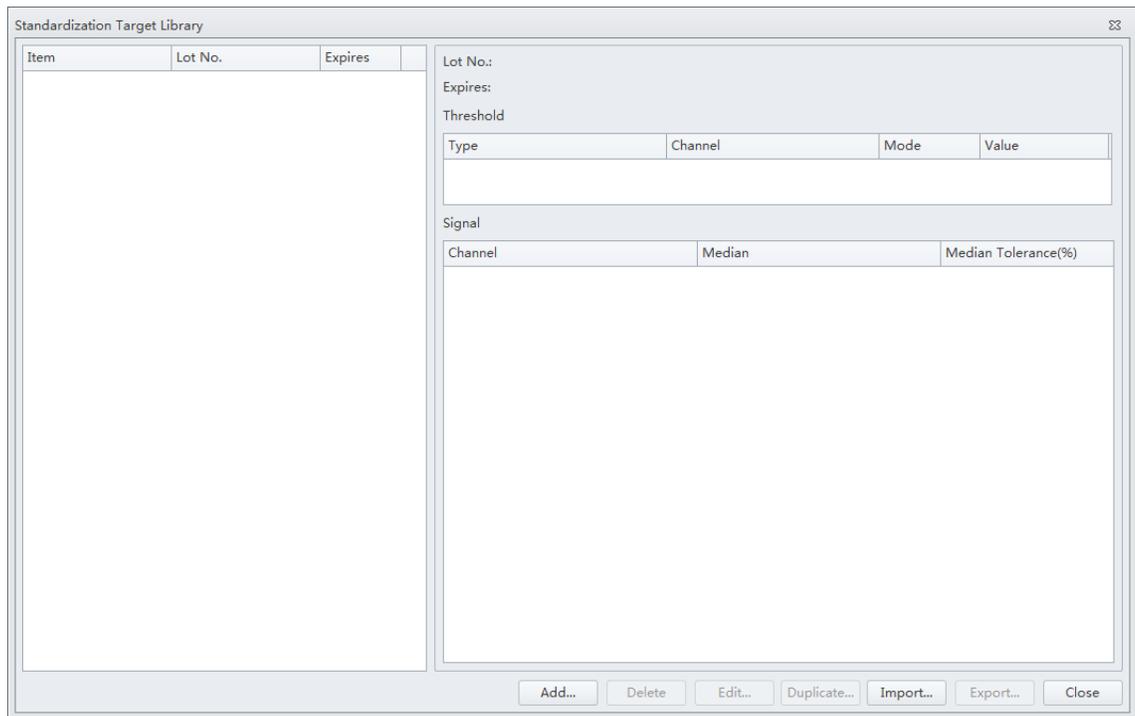
The Information window appears to notify of the corresponding channels with the changed gain as a result of the Standardization.



- 5 Select **OK**.

Standardization Target Library

Select **Standardization Target Library...** from the Settings menu. The standardization Target Library window appears.



NOTE The Item name displays in the Acquisition Setting Catalog window as the saved acquisition setting name.

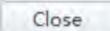
Importing a Standardization Item

- 1 Select **Import...** on the Standardization Target Library window.

- 2 Browse for the Item to import and select **Open**.

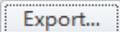
The imported item displays at the top of the list in the Standardization Target Library window.

NOTE If the standardization item is existing in the target library, the importing will overwrite the existing item. The system will prompt to ask you to confirm.

-
- 3 Select  to exit the Standardization Target Library window.
-

Exporting a Standardization Item

- 1 Browse for the Item to export.
The available items display on the left panel of the Standardization Target Library window.
-

- 2 Select  on the Standardization Target Library window.
-

- 3 Navigate to the desired file path and select **Save**.

NOTE The standardization items save as .stgt file. This file can be used to standardize the settings between different instruments with the same laser and optical filter configuration.

- 4 Select  to exit the Standardization Target Library window.
-

Editing Standardization Item Parameters

- 1 Select an item from the Item column on the Standardization Target Library window and select .
-

- 2 Edit the parameters for that item and select **OK**.

NOTE Task Item, Lot No., and Expire date cannot be edited.

- 3 Ensure the item parameters are correct then select  and save the file.
-

- 4 Select  to exit the Standardization Target Library window.
-

Duplicating Standardization Items

- 1 Select an existing item from the Item column on the Standardization Target Library window and select .

-
- 2 Edit Item, Lot No., Expire date and the parameters for that item and select **OK**.

NOTE Perform a new standardization if the Lot No. of standardization sample is changed.

- 3 Ensure the item parameters are correct then select  and save the file.
-

- 4 Select  to exit the Standardization Target Library window.
-

Deleting Standardization Items

- 1 Select an item from the Item column on the Standardization Target Library window.
-

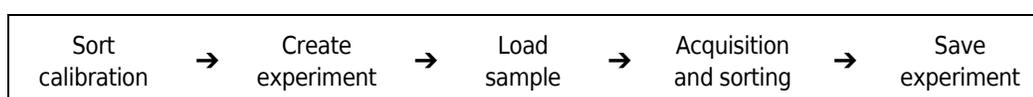
- 2 Select . The confirm message *The target values will be permanently deleted. Are you sure?* displays. Select **Yes** to confirm.
-

- 3 Select  to exit the Standardization Target Library window.
-

Overview

This chapter contains information on how to use your CytoFLEX SRT instrument to perform an acquisition or sorting experiment.

Workflow:



This chapter contains information on:

- [Sort Calibration \(Auto Drop Delay\)](#)
- [Setting Drop Delay Manually \(Optional\)](#)
- [Creating an Experiment](#)
- [Configuring Acquisition Settings](#)
- [Load Sample and Record Data](#)
- [Sorting](#)
 - [Setting Up Tube Sorting](#)
 - [Setting Up Plate/Slide Sorting](#)
 - [Stop Criteria for Sorting](#)
 - [Calibrating the Sort Collection Device](#)
 - [Manual Side Stream Calibration \(Optional\)](#)
 - [Adjusting Tube Position \(Optional\)](#)
 - [Starting and Monitoring a Sort](#)
 - [Pause Sorting and Resume Sorting](#)
 - [Index Sorting](#)
 - [Sort Report](#)
 - [Additional Information for Aseptic Sorting](#)
- [Exporting Data](#)
- [Saving the Experiment](#)

Sort Calibration (Auto Drop Delay)

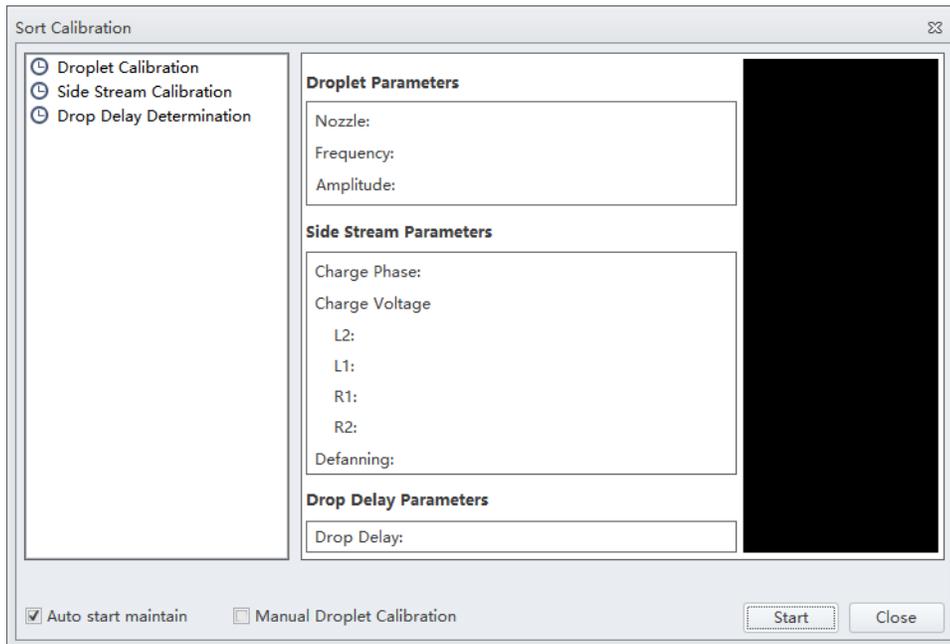
IMPORTANT Beckman Coulter recommends performing the sort calibration immediately after passing QC for better sorting performance. For analysis experiments, skip the Sort Calibration.

IMPORTANT Sort calibration is mandatory after reinstalling a nozzle or replacing a new nozzle. Beckman Coulter recommends re-running the sort calibration to ensure the auto drop delay is accurate if the system enters standby state during a sorting.

Sort Calibration allows the system to achieve an optimal droplet break-off point, optimal side stream settings, and generate the drop delay value automatically (auto drop delay). Effective sorting is highly dependent on the appropriate drop delay. For detailed explanations about drop delay, refer to [Operation Principles in CHAPTER 1, System Overview](#).

The whole Sort Calibration process takes about 10 minutes.

- 1 Select **Initialize** to turn on the sheath flow.
- 2 Select **Sort Calibration** in the Sorting menu. The Sort Calibration window appears.

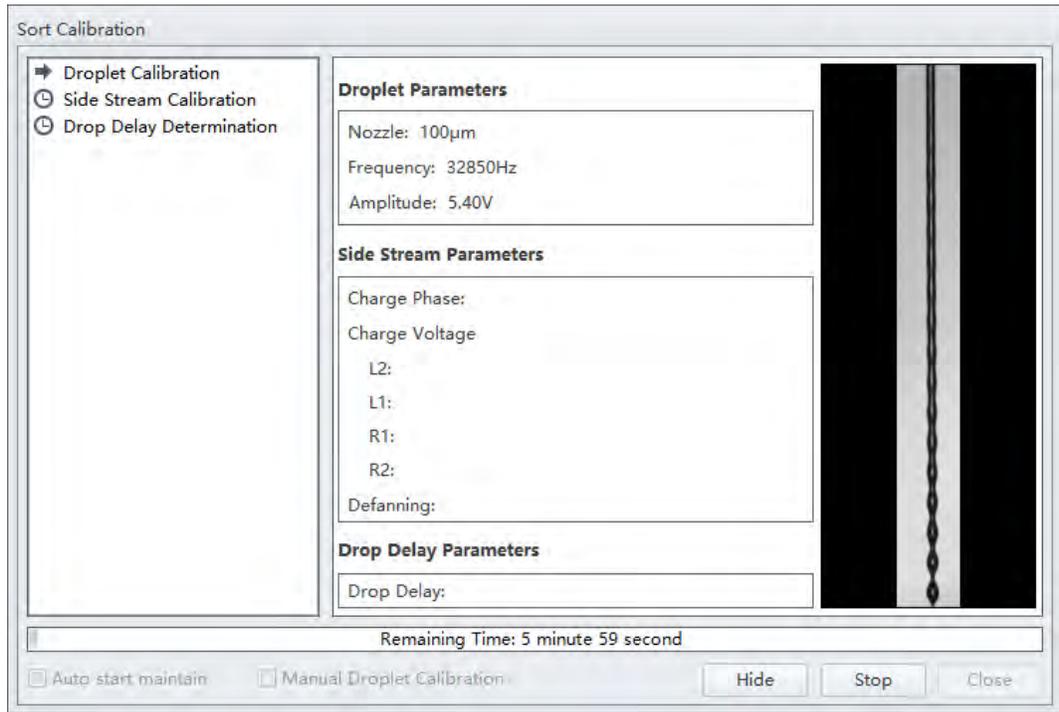


NOTE **Auto start maintain** is selected by default. To view the status of Auto maintain during a sort, select **Sorter Status** from the CytExpert status bar.

NOTE **Manual Droplet Calibration** is used to scan the Frequency and Amplitude parameters manually during the Droplet Calibration. Refer to Step 4.

IMPORTANT The system is sensitive to airflow interference. Ensure that the sort protection door and the sort chamber sliding door are always closed during the sort calibration.

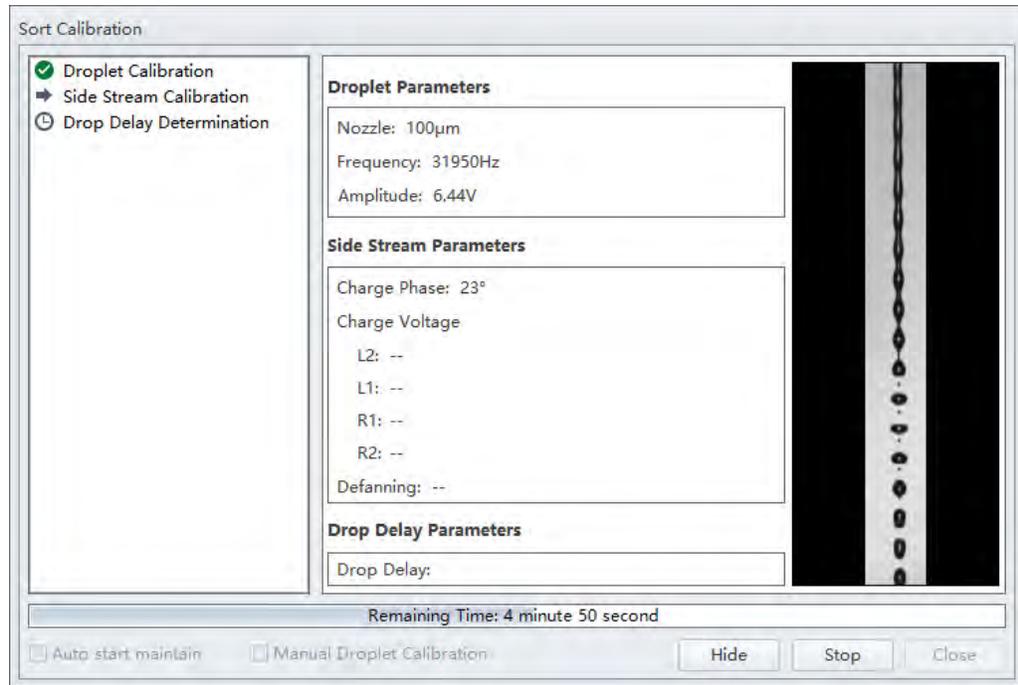
3 Select **Start** to begin the Sort Calibration procedure.



Sorting

Sort Calibration (Auto Drop Delay)

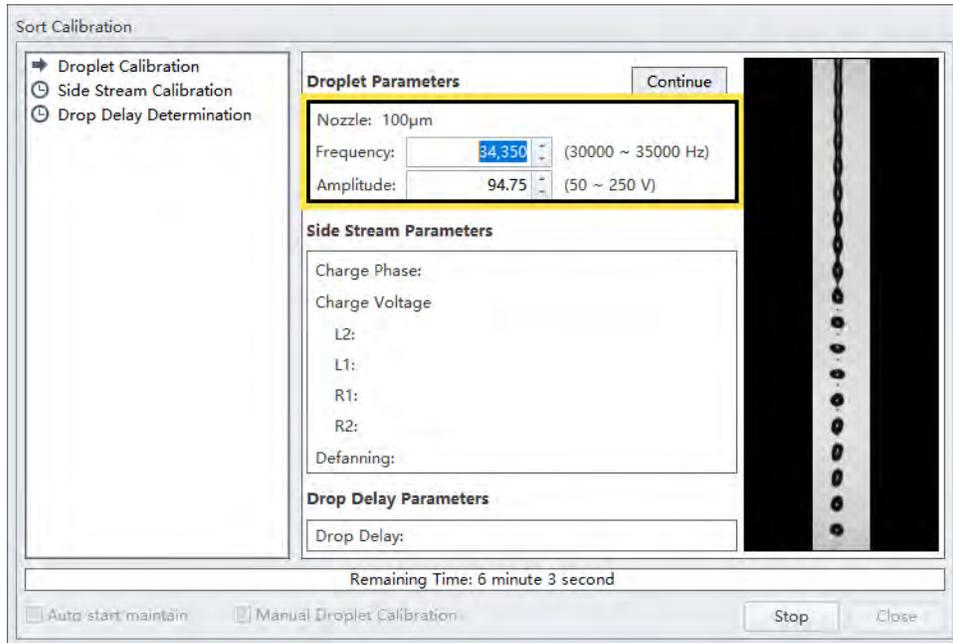
- a. The system automatically scans the frequency and amplitude to form an optimal droplet. The dynamic stream appears on the right. Completed processes appear on the left.



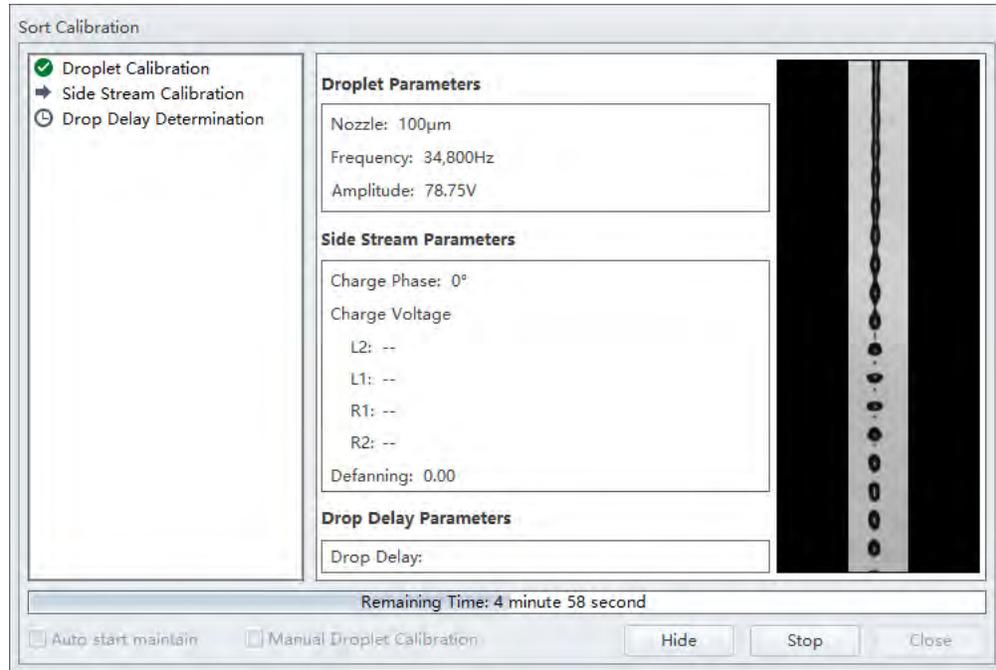
Or perform Manual Droplet Calibration.

IMPORTANT Manual Droplet Calibration should be activated before the Droplet Calibration starts. Refer to Step 2.

Enter the frequency and amplitude values manually and select **Continue** to begin the manual droplet calibration. The dynamic stream appears on the right.

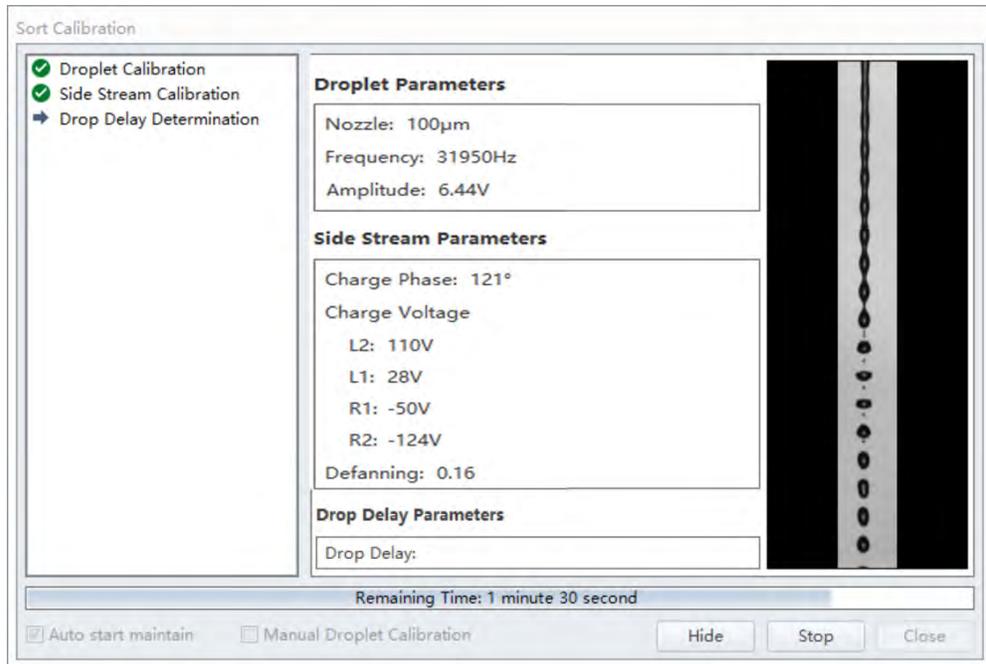


- b. After completing the droplet calibration, the system sequentially calibrates charge phase, charge voltage, and defanning automatically. These calibrated parameters appear in the middle.

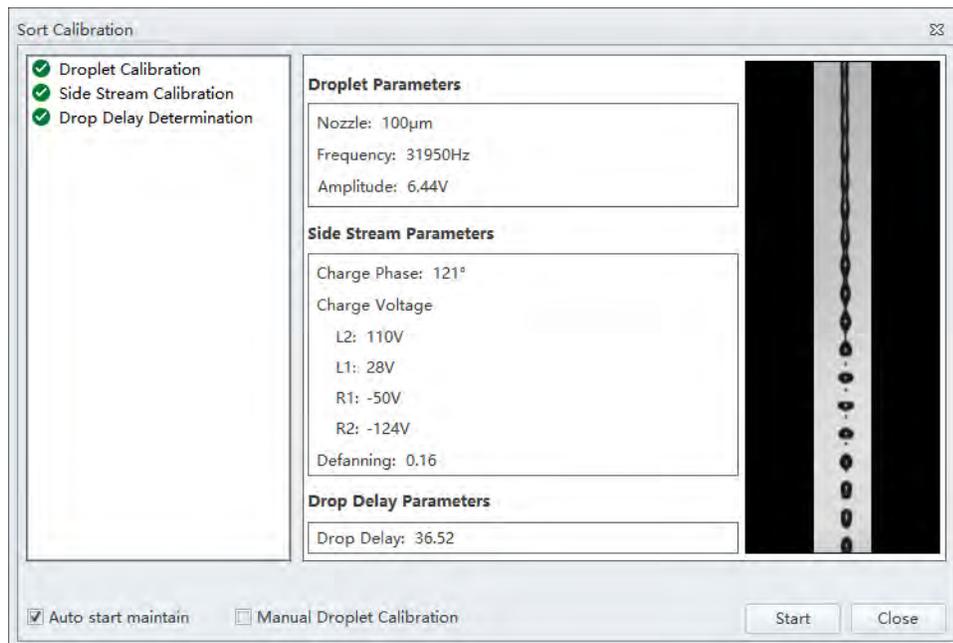


- **Frequency:** The rate at which the crystal in the nozzle vibrates.
- **Amplitude:** The force with which the crystal in the nozzle vibrates.
- **Charge Phase:** Used to determine where the inconsistent charging occurs and then optimize for single side stream.
- **Charge Voltage:** Used to adjust the amount of charge applied to the droplet.
- **Defanning:** Used to place a little residual charge on the droplet following a charged droplet to minimize the potential impact of charged droplet.
- **Drop Delay:** Defined as the amount of time it takes for a particle to travel from the interrogation point of the primary laser to the last attached drop in the stream.

- c. After completing the droplet calibration, the system determines the drop delay automatically.



- 4 When the Sort Calibration procedures finish and pass, select **Close**.



Default Amplitude Setting (Optional)

This function allows you to set the default amplitude as the datum point to scan the amplitude during the automatic Sort Calibration. Use this function only when the Droplet Calibration fails and prompts the error code 070015 or error code 070030. Increase the Amplitude when the break-off point is too low, and decrease the Amplitude when the break-off point is too high. For the criteria of break-off point, refer to [Figure 5.1](#). Then, rerun the Sort Calibration until it passes.

Select **Advanced > Default Amplitude Setting** to access the Default Amplitude Setting window.

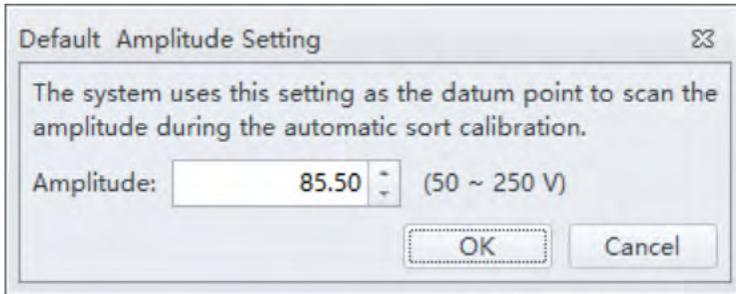
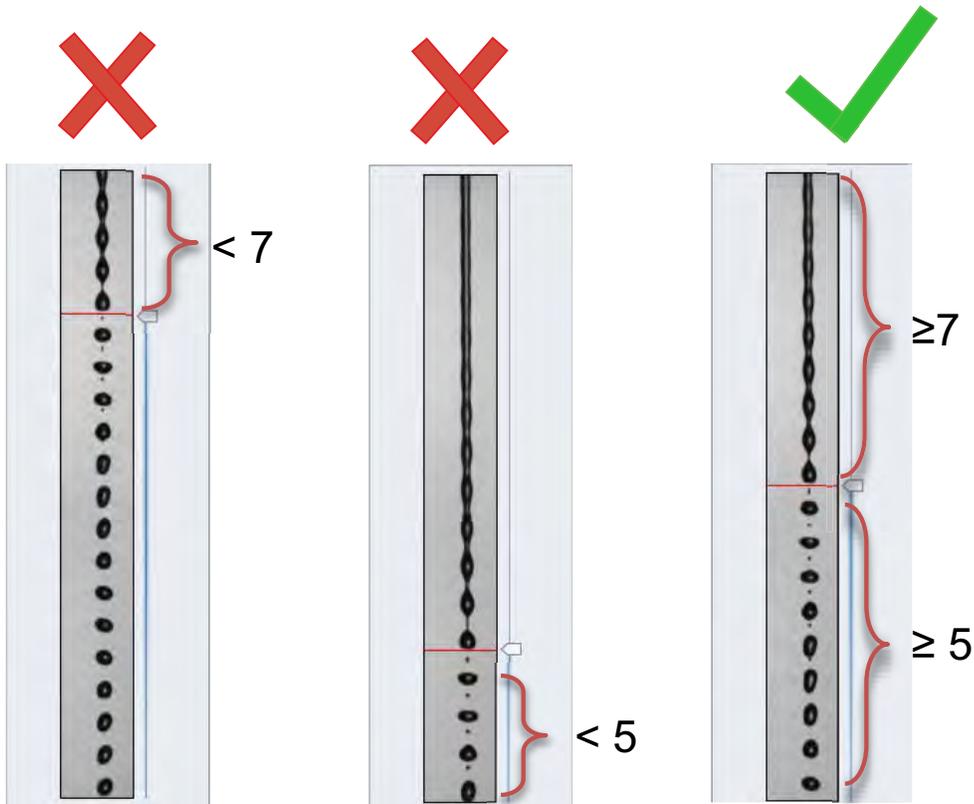


Figure 5.1 Break-off Point Position Reference

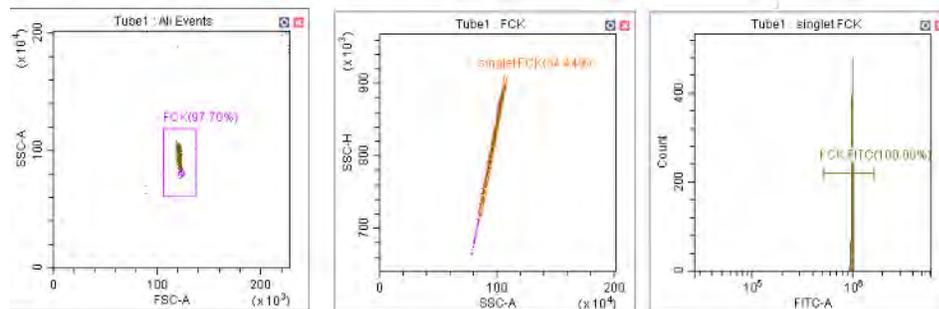


Setting Drop Delay Manually (Optional)

IMPORTANT The Auto Drop Delay generated from the Sort Calibration may differ from Manual Drop Delay due to different measurement criteria. Manual Drop Delay is the most accurate, while the Auto Drop Delay is accurate to $\pm 10\%$.

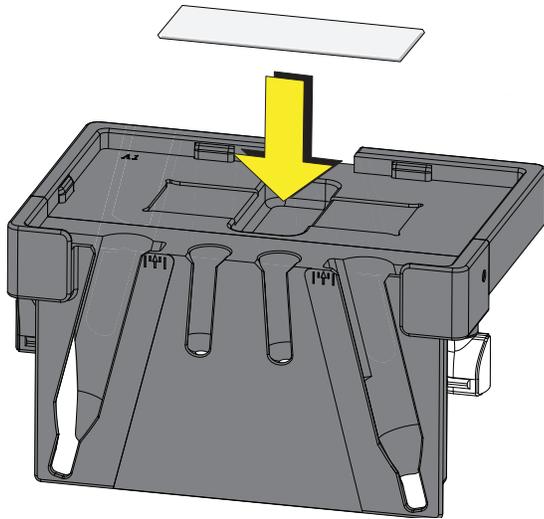
The following procedures can be used to determine the drop delay manually or verify the accuracy of automatic drop delay.

- 1 Run the System Startup procedure. Refer to [CHAPTER 3, Daily Startup](#).
- 2 Run the QC procedure. Refer to [CHAPTER 4, Instrument Quality Control and Standardization](#).
- 3 Run [Sort Calibration \(Auto Drop Delay\)](#).
- 4 Create an experiment. Refer to [Creating an Experiment](#).
- 5 Run Flow-Check Fluorospheres (approximately 0.5 mL undiluted) at low sample flow rate.
- 6 Create a plot of two fluorescent parameters and gate this plot.

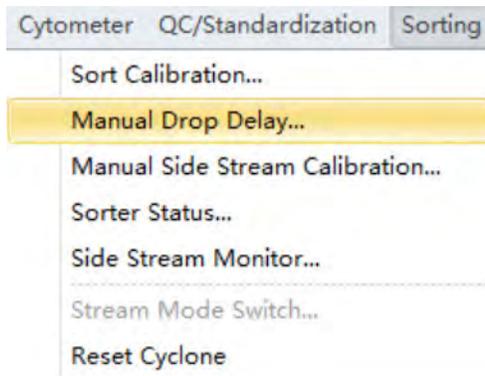


NOTE Ensure the beads are positive on the two channels.

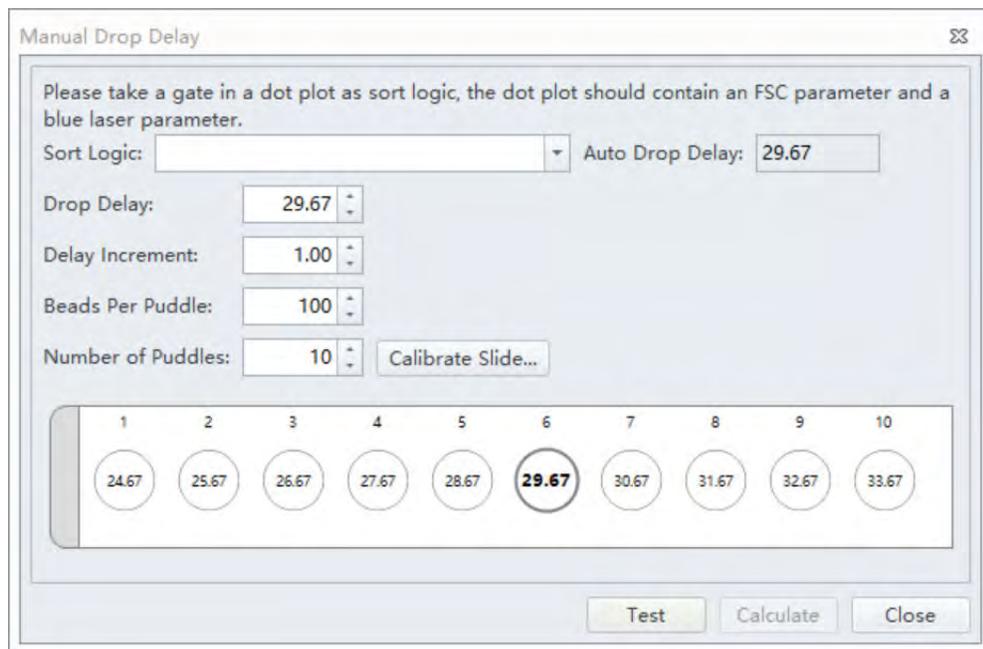
-
- 7 Place a clean slide on the sort output holder.



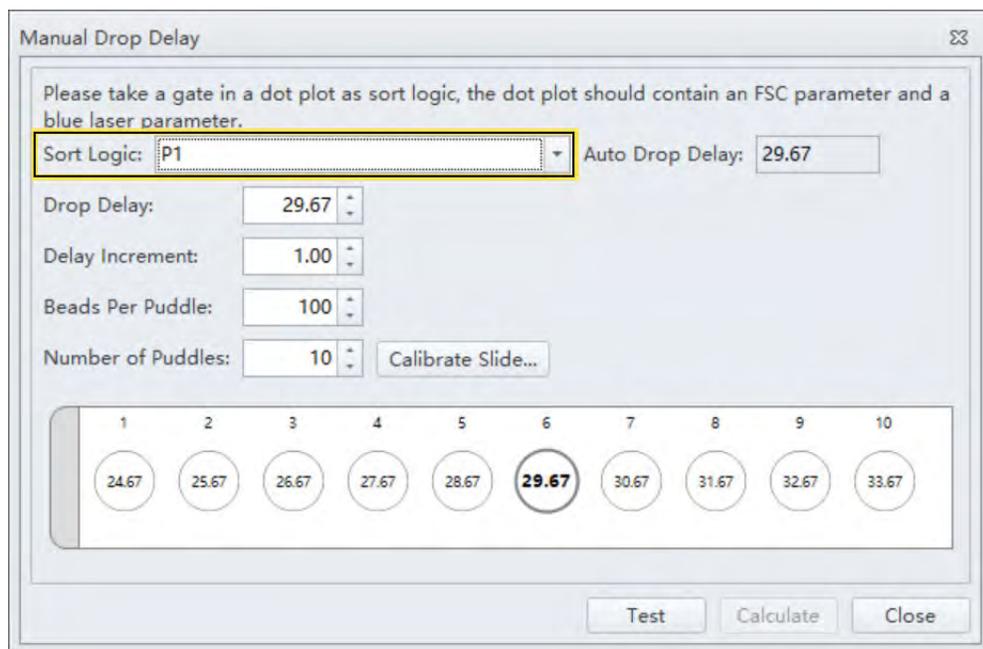
-
- 8 Select **Manual Drop Delay** from the Sorting menu.



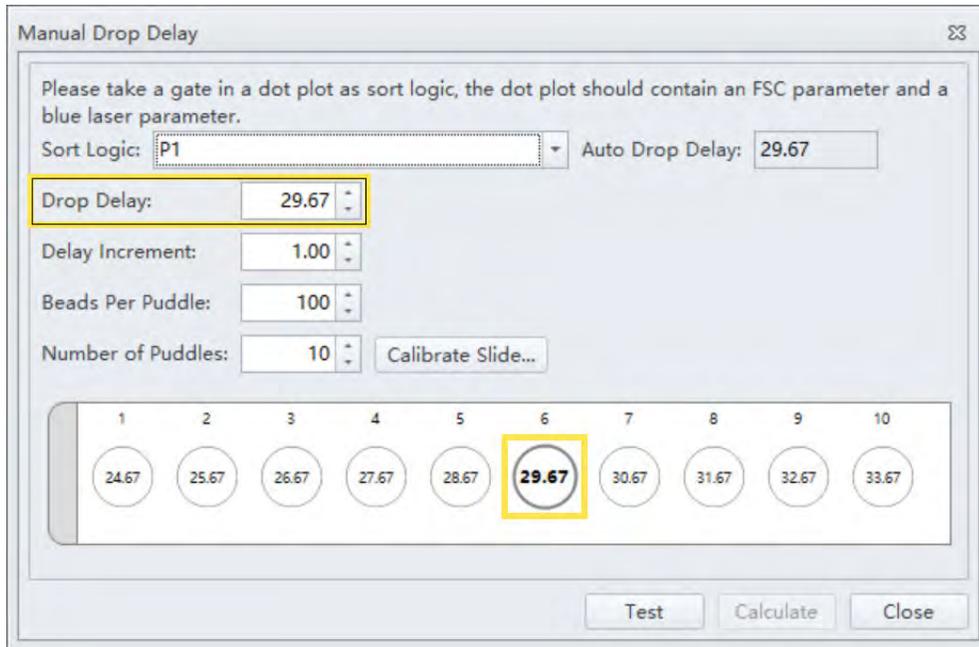
The Manual Drop Delay window displays.



9 Select the desired gate for the Sort Logic.



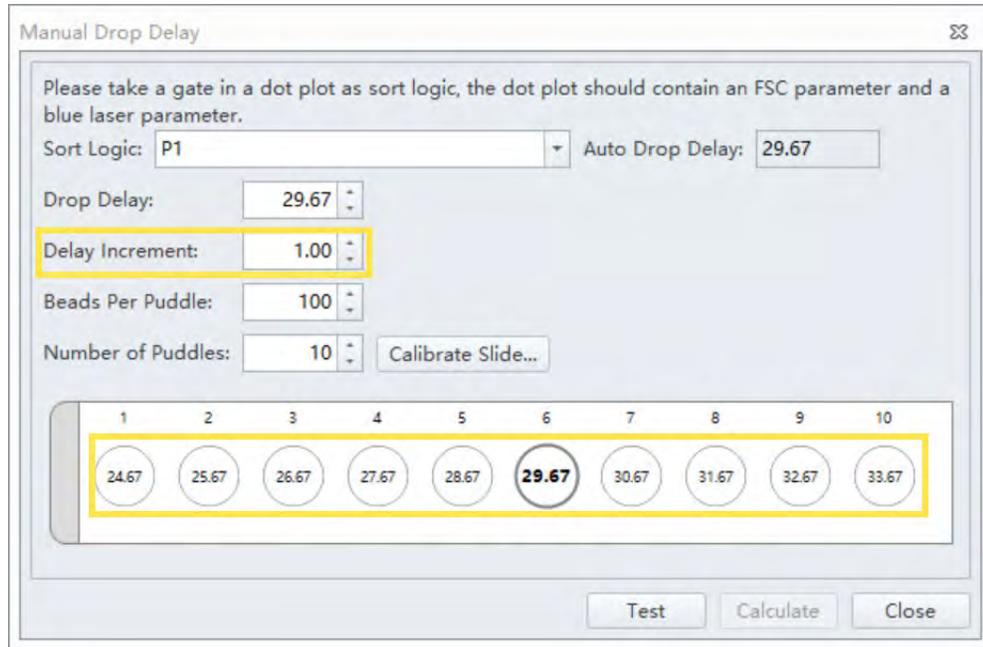
- Enter a desired Drop Delay for the puddle in the middle. The Drop Delay setting appears in the puddle.



NOTE If you did not run the Manual Drop Delay test, the system starts with an Auto Drop Delay as the initial Drop Delay setting. If you have run the Drop Delay test, the initial Drop Delay will be the same as the last time you used the instrument.

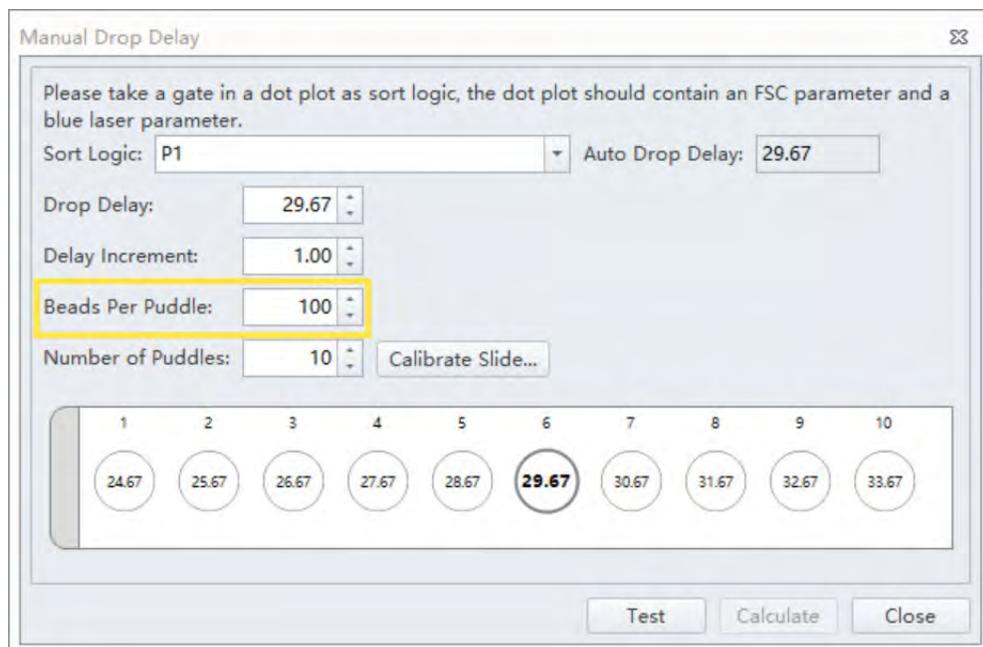
NOTE The range of Drop Delay is 0.01-100.

- 11 Set a Delay Increment to specify a set of drop delays for the adjacent puddles.



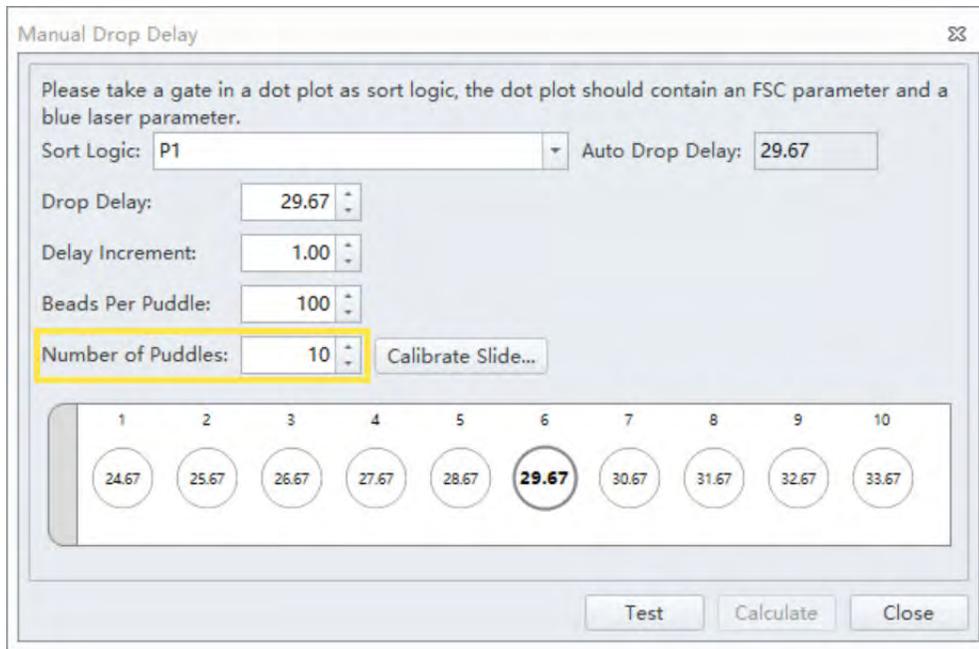
NOTE The range of Delay increment is 0.01-100.

- 12 Set Beads Per Puddle to specify the target count of beads for each puddle.



NOTE The range of Beads Per Puddle is 1-1,000,000.

13 Set the Number of Puddles.

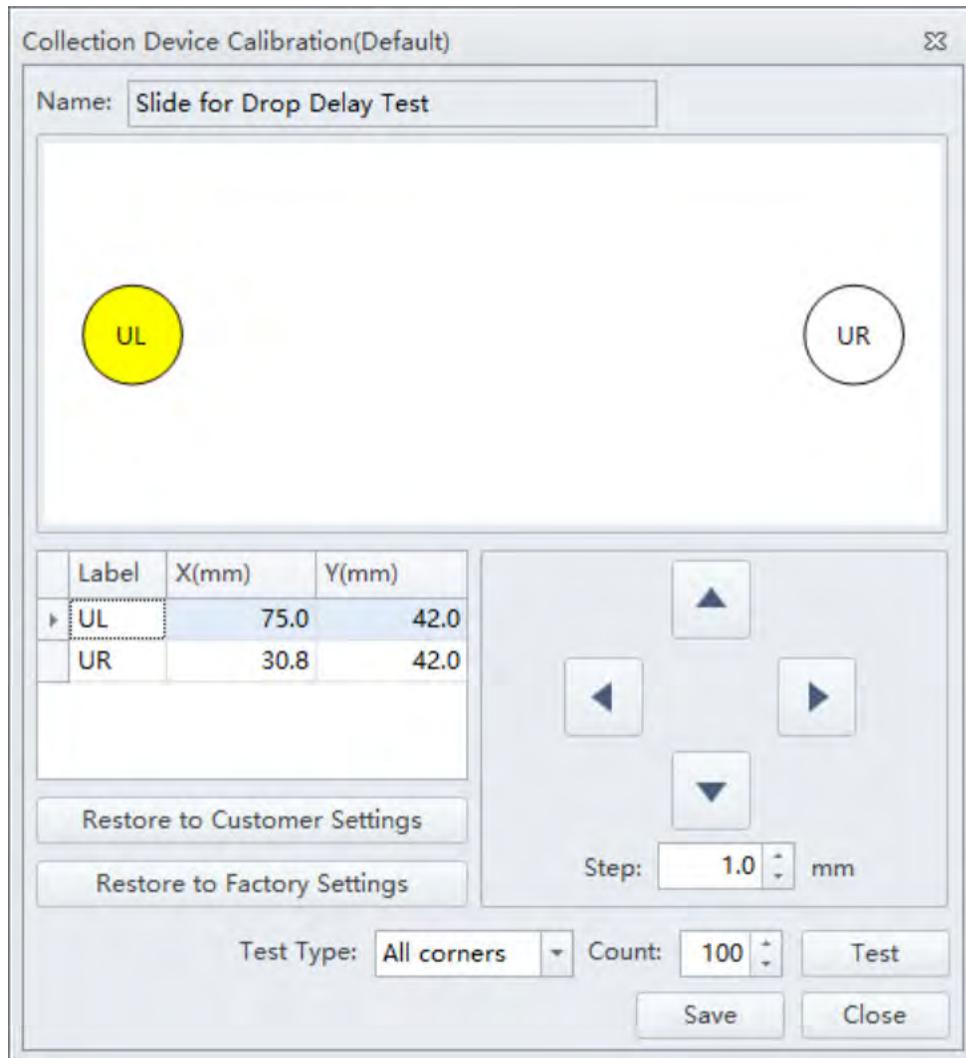


NOTE The range for Number of Puddles is 3-20. The default setting is 10 puddles.

14 Select **stop** to stop loading.

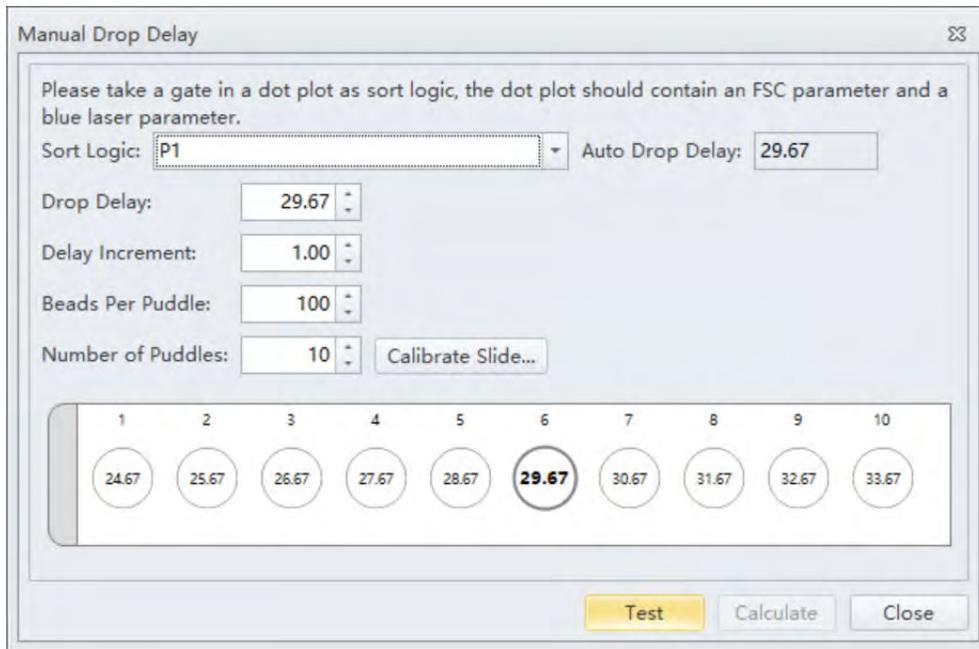
IMPORTANT The slide calibration archived in the Collection Device Library cannot be used for the Manual Drop Test. Slide calibration is mandatory for the initial use of a slide during the Manual Drop Delay Test. Skip the slide calibration if you have calibrated a slide for a Manual Drop Delay Test.

15 Select **Calibrate Slide** to calibrate the slide position if needed.

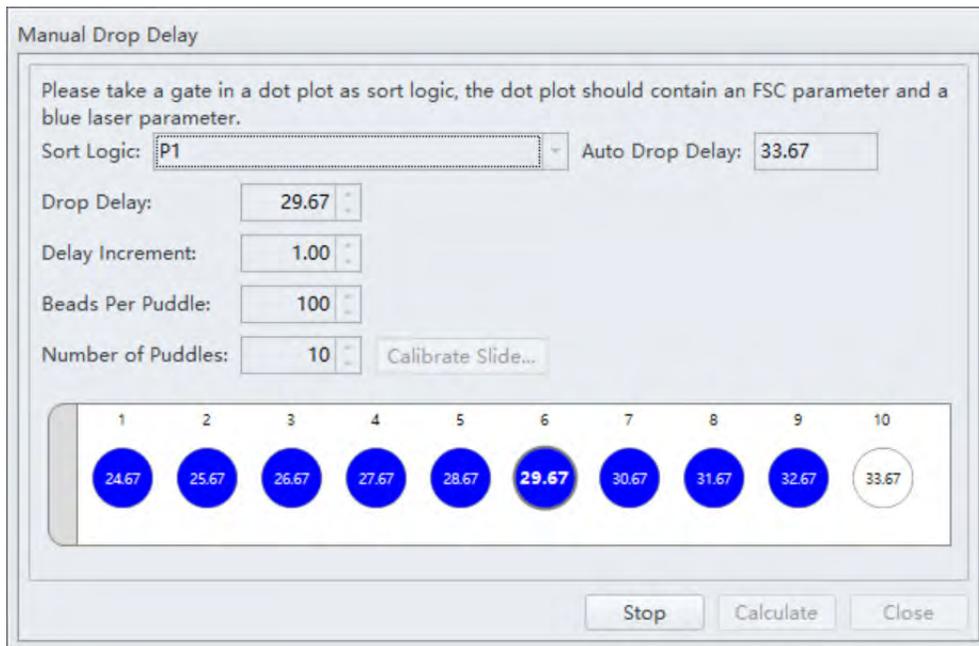


NOTE For instructions on calibrating a slide, refer to [Calibrating the Sort Collection Device](#).

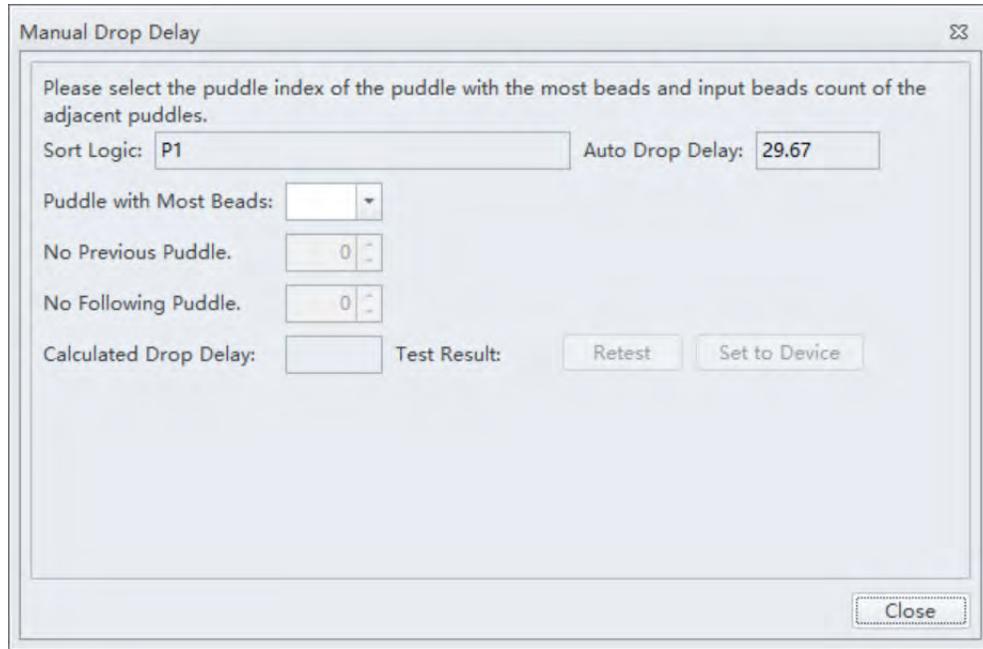
16 Select Test.



You will see the circles turn blue as the puddles are created.



17 Select **Calculate** when all puddles have been created. The following window displays.



18 Remove the slide and inspect the puddles under a fluorescent microscope.

19 Determine the puddle that contains the most beads. Puddle number one is located on the edge of the slide closest to the CyClone arm when the test was run.

20 Count the beads in the puddles adjacent to the puddle that contains the most beads.

21 Enter the values in the Drop Delay Test window.

Manual Drop Delay

Please select the puddle index of the puddle with the most beads and input beads count of the adjacent puddles.

Sort Logic: P1 Auto Drop Delay: 29.67

Puddle with Most Beads: 6

Beads in Puddle 5: 4

Beads in Puddle 7: 4

Calculated Drop Delay: 29.71 Test Result: ❌ Retest Set to Device

Close

The system automatically calculates the Drop Delay.

Manual Drop Delay

Please select the puddle index of the puddle with the most beads and input beads count of the adjacent puddles.

Sort Logic: P1 Auto Drop Delay: 29.67

Puddle with Most Beads: 6

Beads in Puddle 5: 4

Beads in Puddle 7: 4

Calculated Drop Delay: 29.71 Test Result: ❌ Retest Set to Device

Close

NOTE The Test Result indicates a passing result with a  and a failed result with .

NOTE The passing criteria: the difference between the number of beads in the puddles adjacent to the target puddle is less than 3%.

22 If the test result passes, select **Set to Device** to save the calibrated manual drop delay.

The screenshot shows the 'Manual Drop Delay' dialog box. At the top, it says 'Please select the puddle index of the puddle with the most beads and input beads count of the adjacent puddles.' Below this, there are several input fields: 'Sort Logic' is set to 'P1', 'Auto Drop Delay' is 29.67, 'Puddle with Most Beads' is a dropdown menu set to '6', 'Beads in Puddle 5' is a spinner set to '1', and 'Beads in Puddle 7' is a spinner set to '0'. The 'Calculated Drop Delay' is 29.66. The 'Test Result' is a green checkmark, indicating success. There are three buttons: 'Retest' (disabled), 'Set to Device' (highlighted in yellow), and 'Close' (bottom right).

Or

If the test result fails, select **Retest** and repeat Steps 18-21 until the test result passes.

The screenshot shows the 'Manual Drop Delay' dialog box with a failed test result. The 'Sort Logic' is 'P1', 'Auto Drop Delay' is 29.67, 'Puddle with Most Beads' is '6', 'Beads in Puddle 5' is '4', and 'Beads in Puddle 7' is '8'. The 'Calculated Drop Delay' is 29.71. The 'Test Result' is a red 'X', indicating failure. The 'Retest' button is highlighted in yellow, and the 'Set to Device' button is disabled. The 'Close' button is at the bottom right.

23 Select **Close**.

24 Optional: Save this experiment as a template if needed.

Creating an Experiment

Creating an Experiment



Risk of file corruption. When modifying experiment (*.xit) file names in Windows Explorer, ensure you modify the corresponding experiment folder name to match the new file name.

- 1 Open the CytExpert SRT software and confirm that the instrument is connected. Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#).
- 2 Verify the detector configuration. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#).
- 3 Verify the laser settings. Refer to [Laser Settings](#) in [CHAPTER 5, Sorting](#).
- 4 Create or open an experiment using one of the following methods:
 - Create a new experiment:
 - Select **New Experiment** on the Start page, specify the file path, and save the experiment.
 - Or
 - Select **New Experiment** in the File menu, specify the file path, and save the experiment.
 - Create a new experiment from a template:
 - Select **New Experiment from Template** on the Start page. Select **Browse** next to New Experiment and specify the file path for the new experiment, then select **Browse** next to Template and specify the file path to the existing template.
 - Or
 - Select **New Experiment from Template** in the File menu, specify the file path and save the experiment.
 - Or
 - Select the Template tab on the Start page and select the template from the list of recently used templates. Specify the file path and save the experiment.
 - Open an existing experiment:
 - Select **Open Experiment** on the Start page, specify the file path and save the experiment.

- Or
- Select **Open Experiment** in the File menu, specify the path and save the experiment.
- Or
- Select the Experiment tab on the Start page and select the experiment from the list of recently opened experiments. Specify the file path and save the experiment.

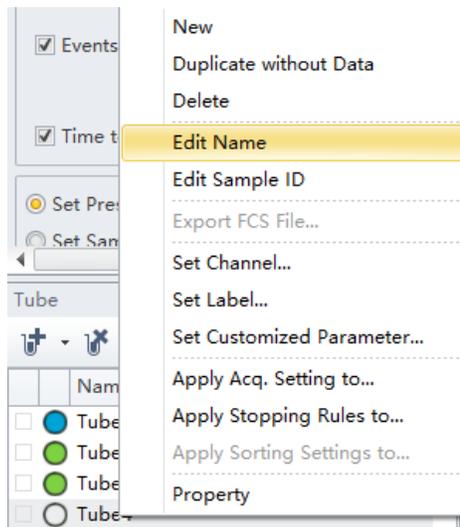
NOTE Experiments are saved as an .xits file. Template are saved as an .xitm file.

NOTE The CytoFLEX SRT software can read the experiments with the suffix xits. Selecting **Convert CytExpert Experiment** from the File menu to convert the .xit file to the .xits file if you want to use the CytoFLEX SRT software to analyze the experiments with the suffix xit.

NOTE If desired, import saved settings/standardization settings from the catalog.

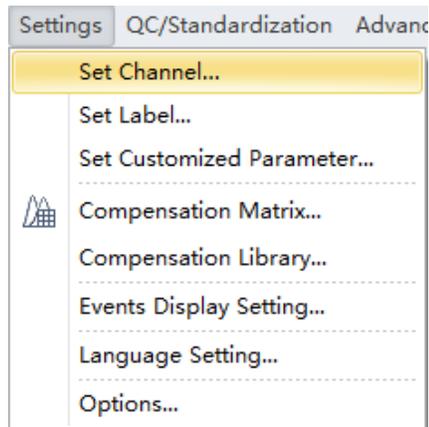
Changing the Tube Name

To change the name of a new sample tube or the sample ID, right-click the tube name or the sample ID name in the Tube section of the screen and select **Edit Name** or simply double-click the sample tube or sample ID name.

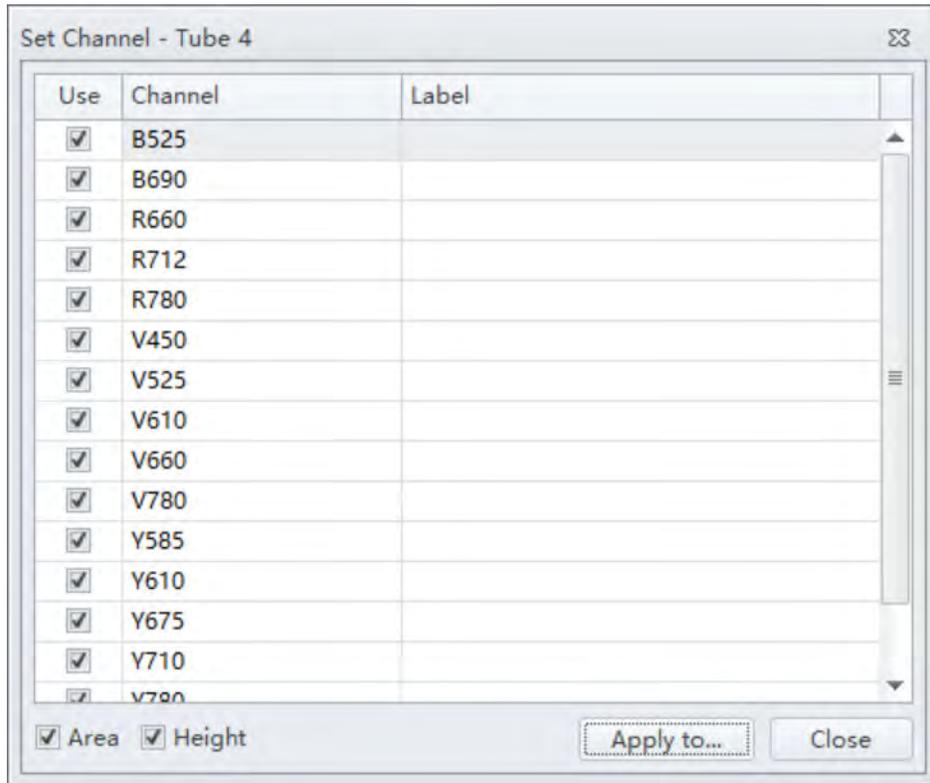


Setting the Channel and Label

- 1 Select **Set Channel** in the Settings menu. The Set Channel window appears.

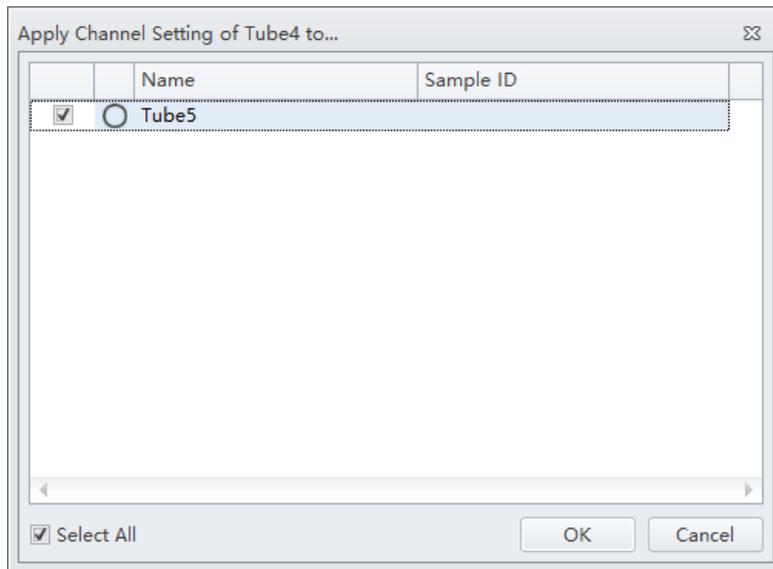


- 2 In the Set Channel window, modify which channels are used and how they are displayed.
 - a. Select the channel signal check box, then you can add the reagent name in the Label column. The information you add appears in the corresponding axis of the relevant plot in the plot area. Unselected channel signals are not stored in the data file.

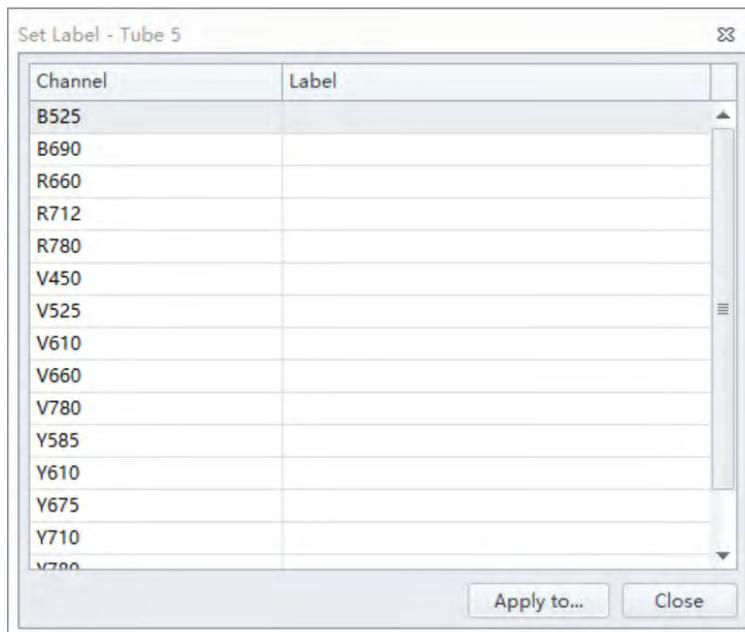


NOTE You can select which signal type to use Height or Area.

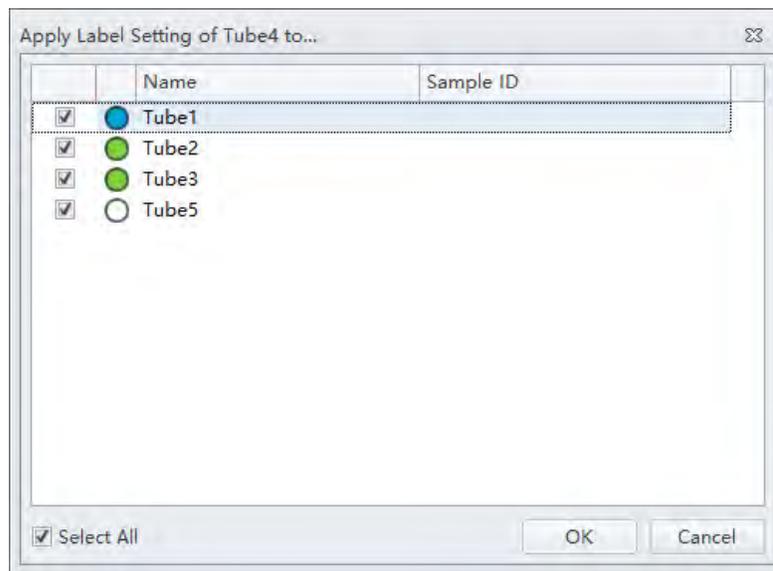
- b. Select **Apply to**. The Apply Channel Setting window appears.



- c. Select the tubes to apply the channel settings to and select **OK**.
- d. If you only need to modify the label name, select **Set Label** in the Settings menu to make the required changes. The Set Label screen appears. The Set Label screen does not allow you to select which channels to use, but it does allow you to apply the modified label to all the sample tubes.



- e. Select **Apply to**. The Apply Label Setting window appears.



- f. Select the tubes to apply the label settings to and select **OK**.

Creating Plots and Gates

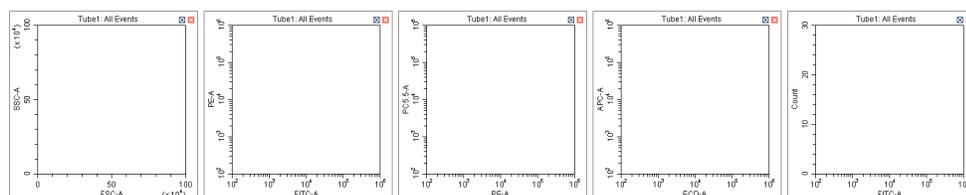
IMPORTANT The maximum number of elements allowed in an experiment is 200. Elements include plots, statistics tables, and gate hierarchy tables.

IMPORTANT The maximum number of gates allowed in an experiment is 200.

- 1 Use the plotting controls (refer to [Figure 2.1](#)) in the plot area to create plots and gates and to generate graphs as shown.

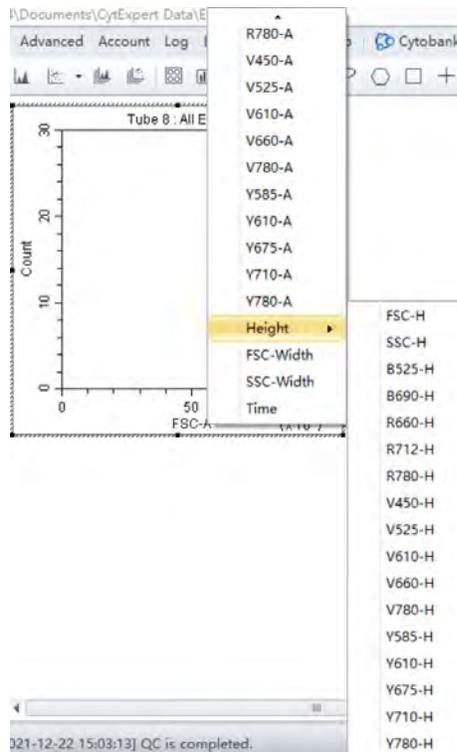
Use the  icons to generate histograms, dot plots, density plots, pseudo color plots, and contour plot.

The experiment uses scatter plots, histograms, polygon gating, four-quadrant gating, and line-segment gating.



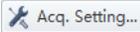
- a. After selecting a plot, click and drag the mouse to adjust the position and select and drag the sizing handles at the edge of the graph to adjust the size of the graph.

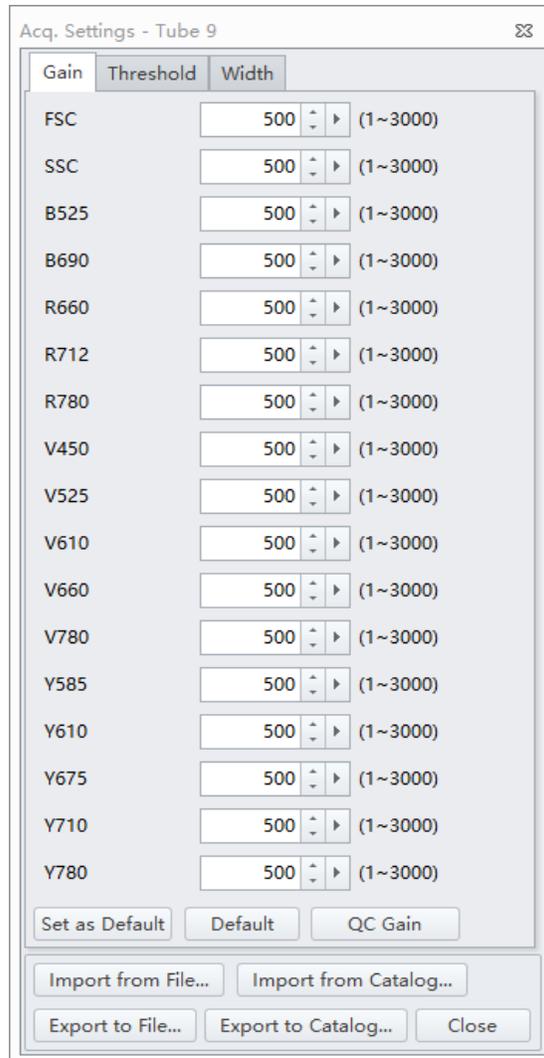
- b. Select an axis name to change which channel is displayed. An “A” after the channel name indicates signal pulse area, while an “H” indicates height. The default setting is "A".



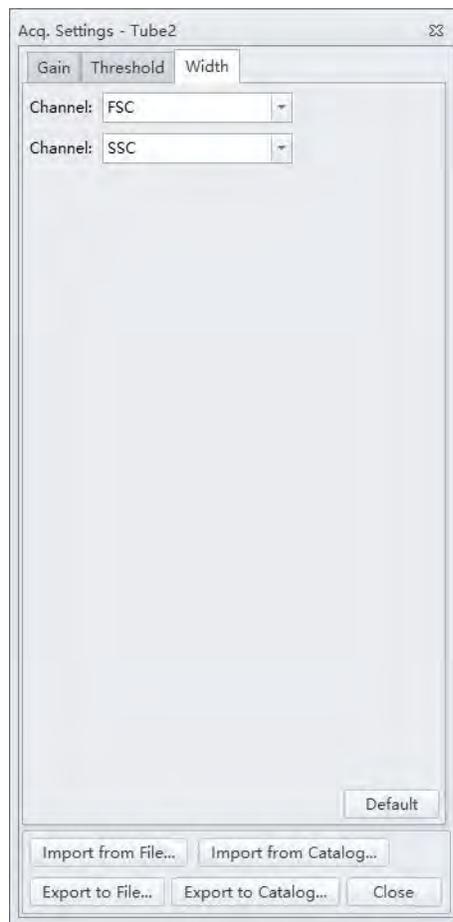
NOTE To modify the default settings, select **Options** in the Settings menu. The Options window appears. Select **Plot** on the left side of the Options window. Under the Signal section of the window, change the Main Channel default by selecting the **Height** or **Area**.

NOTE When using both Height and Area signals, ensure the gain setting is set to where the Height signal does not reach its upper range.

- c. Signal width can be used as a tool for doublet discrimination and to differentiate somatic cell adhesion. If necessary, select  to open the Acq. Setting window.

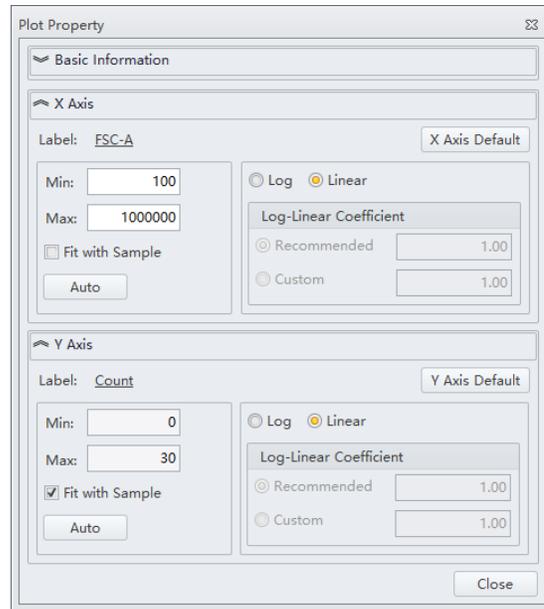


- d. Select the **Width** tab, and select a channel with the required signal width.



- e. Plot properties can be configured to display axes in Log, Log-Linear, or Linear format.

- 1) Double-click the plot or right-click the plot and select **Property** from the drop-down menu. The Plot Property screen appears.



- 2) Select whether to display the axes in logarithmic or linear format for both the X-axis and Y-axis. Enter a value for log-linear coefficient if the log-linear view is desired.
- 3) Select **Close**.

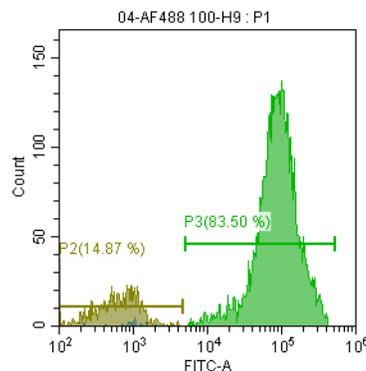
Or

Select the logarithmic axis on the plot. The slider appears. Drag the slider along the axis to change the log-linear coefficient and view events that are not shown, including events with negative values.

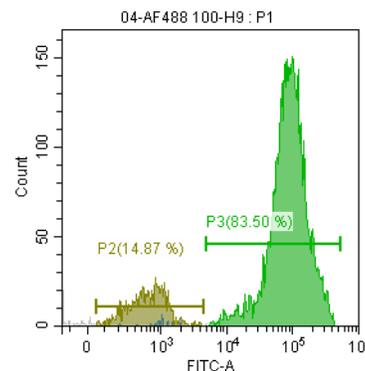
NOTE The log-linear slider is also available during data acquisition.

NOTE To reset the axis back to logarithmic, right-click on the axis and select **Property**. Select **X axis Default** or **Y axis Default** to reset the axis.

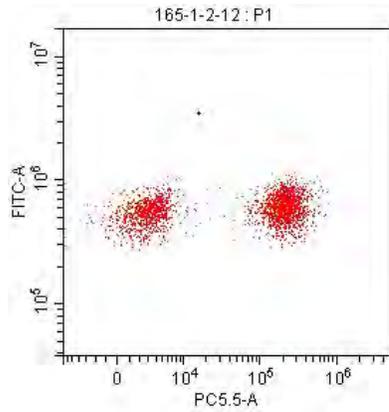
Histogram with logarithmic X-axis



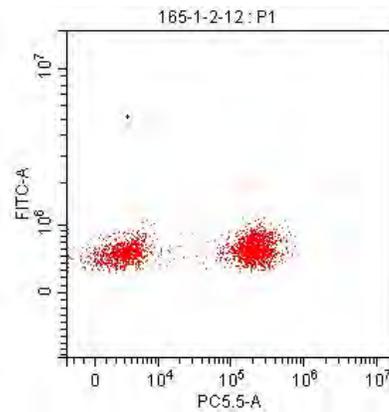
Histogram with log-linear X-axis



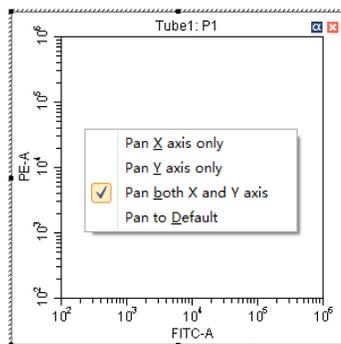
Dot plot with logarithmic X-axis



Dot plot with log-linear X-axis



- f. You can adjust axis ranges using the pan axis display controls located at the top of the screen.
- Select , to zoom-in and define which area of a plot to enlarge. The selected area can be magnified to fill the entire graph. By selecting the zoom-out function, you can click on the graph and restore the plot to its original appearance before magnification.
 - Select  to shift the axes. The mouse pointer appears as a hand. It allows you to drag the graph to reveal the axis segment you need.
 -  Pan: Modifies the axis display range dimensions when panning both axes. When the pan control is selected, you can right-click the graph and select which axis you need to adjust when dragging. You can also pan directly to the default axis range.



-  Single side pan: Modifies the axis display range dimensions when panning one axis.

NOTE Only the low end of the axis can be adjusted by the single side pan tool.

- Double-click the border area of the plot to open the Plot Property window, or right-click the plot, then select **Property** to open the same Plot Property window.

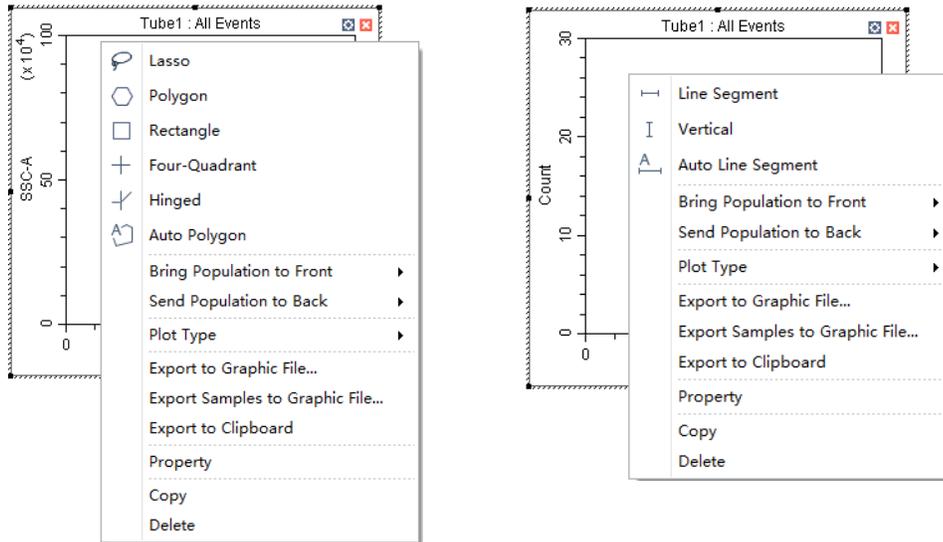
- In the Plot Property window, manually enter the minimum and maximum display values for the X- and Y-axes. You can also select **Fit With Sample** to let the software automatically adjust the lower limit according to the signal and perform the corresponding log-linear transformation. The X- and Y-axes **Default** settings are the default parameters. The default parameters are 100-1,000,000.

NOTE Select **Fit With Sample** to identify the signal's lower limit, adjusting automatically as warranted. Selecting this item is recommended whenever the signal appears to be relatively low.

NOTE Select **Auto** to automatically set the upper and lower display limits of the axes based on the data already collected.

NOTE Select **Options** in the Settings menu, then select **Plot** to modify the default setting of the axis range under the Axis Default Setting section of the window.

- 2 To create gates, use the  control buttons or right-click the plot and select the gate type required. Gates can be set according to different requirements to differentiate cell populations.



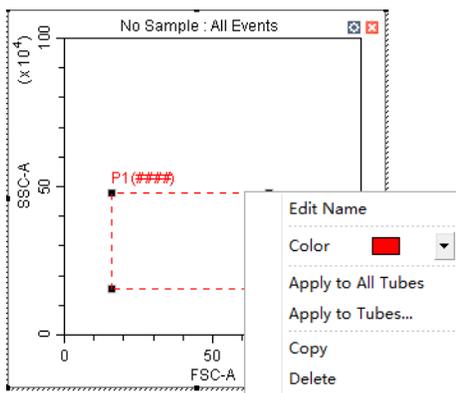
NOTE To add a vertex to a polygon gate:

1. Select the gate.
2. Hover your cursor over the perimeter of the gate until the cursor changes to the hand icon.
3. Select the desired location for the new gate vertex.

NOTE A newly created gate becomes a subset of the plot where it appears. The relationship between parent and progeny/daughter gates can be changed when a displayed gate is subsequently modified.

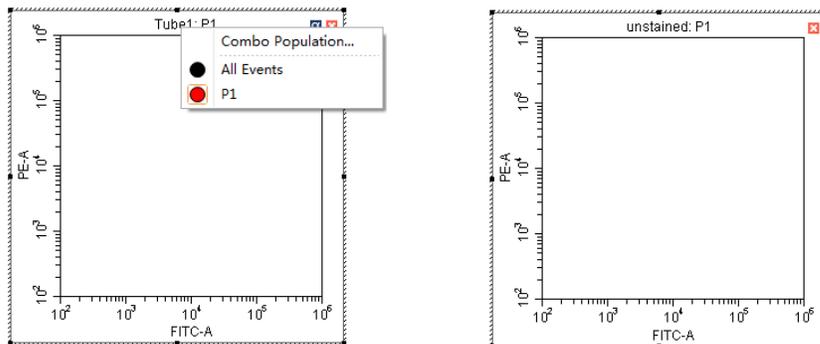
The position of the same gate in different sample tubes may vary. To change the position of a gate and apply the change to all sample tubes accordingly, you can right-click the gate and select **Apply to All Tubes**.

You can also apply the change to select tubes by selecting **Apply to Tubes**.



3 Select the gates to display.

- a. Select the heading area of the plot, select the parent population/gate to display in the plot from the drop-down menu. The selected parent gate appears in the tab area of the plot.



NOTE The auto gates cannot be used as the sort logic.

NOTE The CytExpert SRT software will not list gates which would create circular gating logic.

Figure 5.2 shows all gates defined in the example experiment below. Note that the only gate option in plot 1 of Figure 5.3 is P2 for the following reasons:

- Plot 1 cannot be gated on P1 because P1 is on that plot.
- Plot 1 cannot be gated on P2 because P2 is gated on P1.
- Plot 1 cannot be gated on the P2 OR P1 combo population because the gate logic contains P1.

Figure 5.2 All Gates - Example Experiment

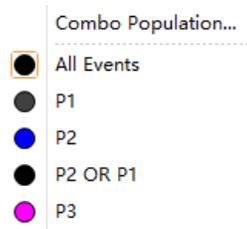
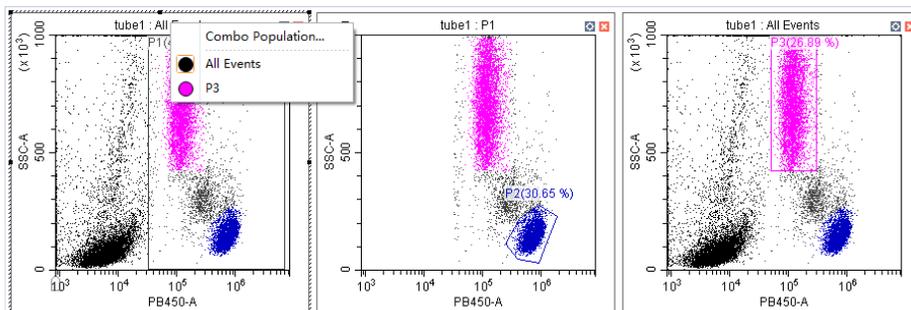
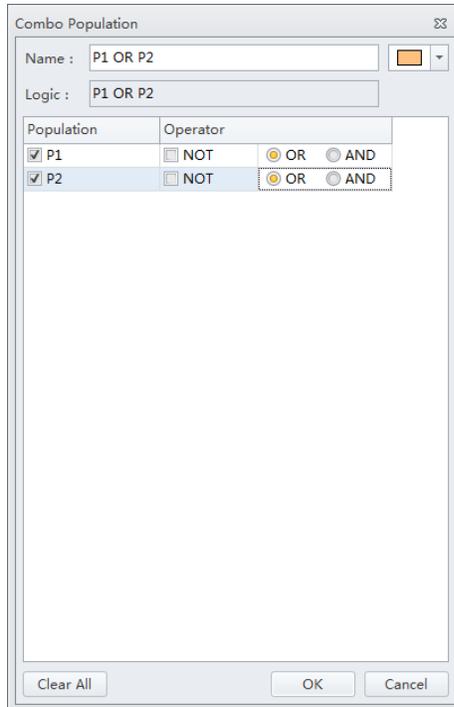


Figure 5.3 Circular Gating Logic - Example Experiment



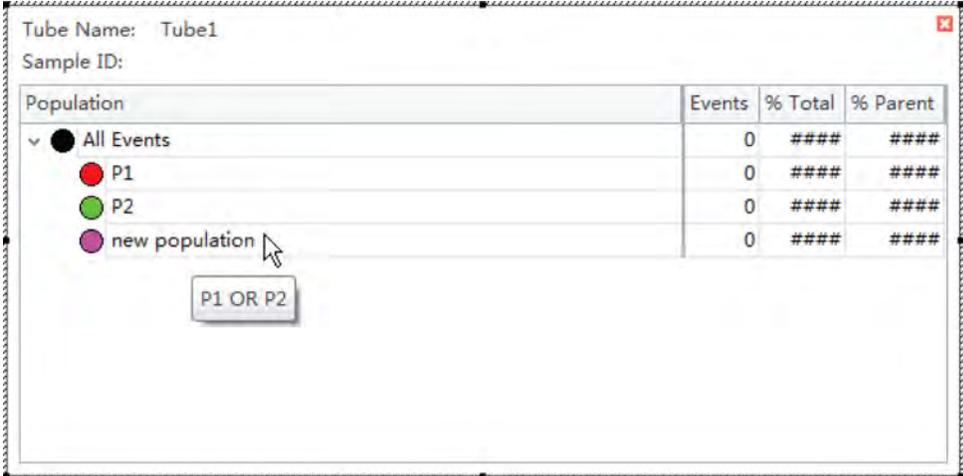
- b. If necessary, you can select the **Combo Population** option from the drop-down menu to create a combination gate, using the Boolean relationships “and”, “or”, and “not” to produce a new gate. You can also select the population color or change the gate name.



- “And” indicates that all selections must be satisfied. For example, “P1 and P2” means that the data for the newly added gate represent the intersection of P1 and P2.
- “Or” indicates that only one of the selections needs to be satisfied. For example, “P1 or P2” means that the data for the newly added gate represent the union of P1 and P2.
- “Not” indicates exclusion from the selection. For example, “Not P1” means that the data for the newly added gate represent the events that are not part of P1.

- 4 Select  to display the population hierarchy.

The Population Hierarchy function allows you to view how gates rank in relation to one another. To change the display color, double-click the default color and select the desired color from the drop-down color palette. To change the name of each gate, double-click the name of the desired gate. By hovering your mouse pointer over a combo population whose display name has just been changed, you can view its corresponding Boolean logical operation.

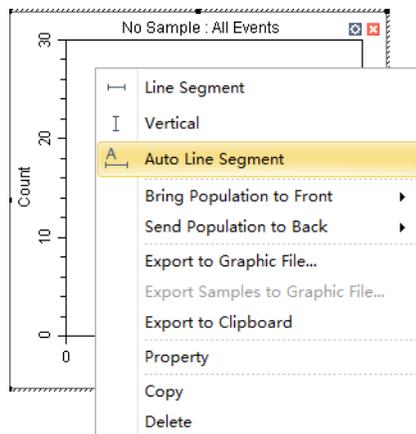


Population	Events	% Total	% Parent
● All Events	0	####	####
● P1	0	####	####
● P2	0	####	####
● new population	0	####	####

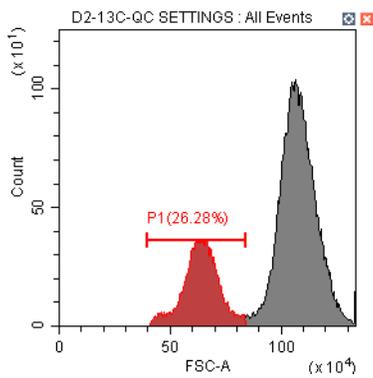
Creating and Adjusting Auto Gates

Two types of auto gates are available in the CytExpert SRT software: auto line segment and auto polygon.

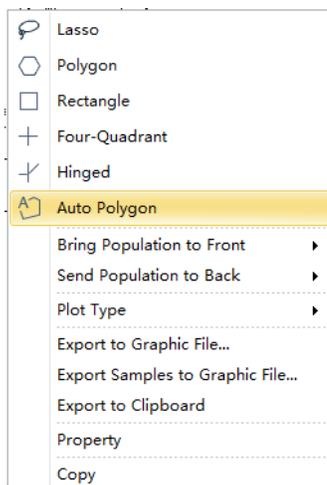
To create an auto line segment gate, select  from the tool bar or right-click on the histogram and select **Auto Line Segment** from the drop-down menu.



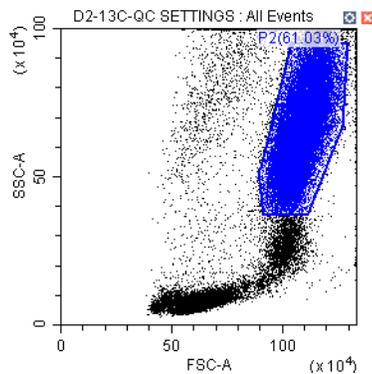
Select the population you want to gate in the histogram to automatically gate that population.



To create an auto polygon gate, select  from the tool bar or right-click on the 2D plot and select **Auto Polygon** from the drop-down menu.



Select the population you want to gate in the 2-D plot. The gate will automatically be drawn to fit the population.



NOTE To add a vertex to an auto polygon gate:

1. Select the gate.
2. Hover your cursor over the perimeter of the gate until the cursor changes to the hand icon.
3. Select the desired location for the new gate vertex.

Turning Auto Recalculate On/Off

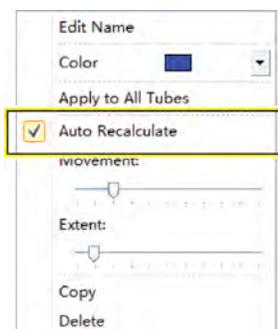
When auto recalculate is turned on, all auto gates will recalculate when:

- The current tube is changed
- Compensation is changed
- Gating is changed
- Collection stops
- An FCS file is imported to the tube or well

Auto recalculate turns off after a gate is moved or the size of a gate is altered. You must select **Auto Recalculate** from the auto gate menu again to turn auto recalculate back on.

NOTE Auto recalculate turns on after adjusting movement or extent.

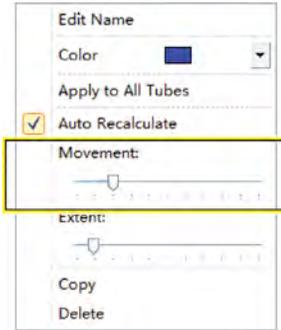
Right-click an auto gate and select **Auto Recalculate** from the auto gate menu to toggle auto recalculate on and off.



Adjusting Autogate Movement and Extent

Movement — The distance an autogate can move to find the target population.

To adjust movement, right-click an autogate and drag the Movement handle in the auto gate menu left and right.



NOTE The default value setting for movement is 20 units. The minimum value setting for movement is 0 units and the maximum value setting for movement is 100 units.

If a target population is consistently in the same location, movement is not needed. However, if a target population is periodically missing from some samples, or events are rare, movement can be used to move the gate within a certain percentage of its axis to capture the correct population. Refer to [Figure 5.4](#) for an example of the default movement setting. Refer to [Figure 5.5](#) for an example of the maximum movement setting.

Figure 5.4 Movement - Default Setting

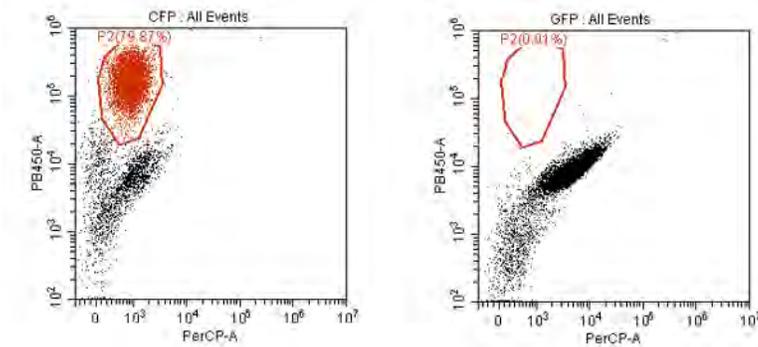
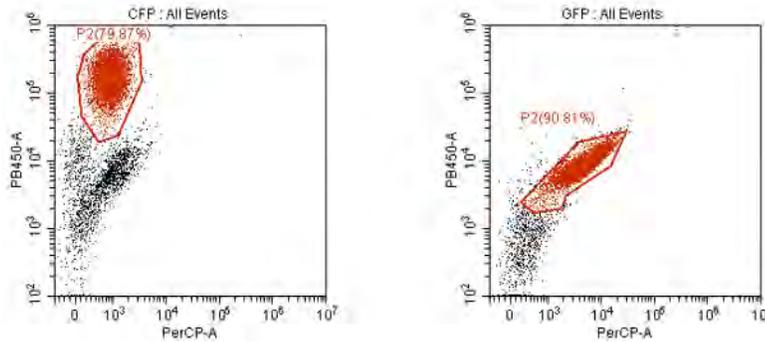
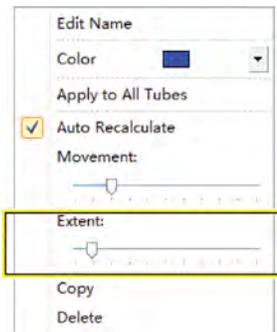


Figure 5.5 Movement - Max Setting



Extent — Shrinks or expands the gate around the population.

To adjust extent, right-click an autogate and drag the Extent handle in the auto gate menu left and right.



NOTE The default value setting for extent is 20 units. The minimum value setting for extent is 0 units and the maximum value setting for extent is 100 units.

Refer to [Figure 5.6](#) for an example of the default extent setting. Refer to [Figure 5.7](#) for an example of the maximum extent setting.

Figure 5.6 Extent - Default Setting

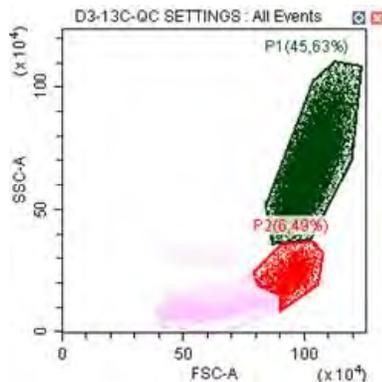
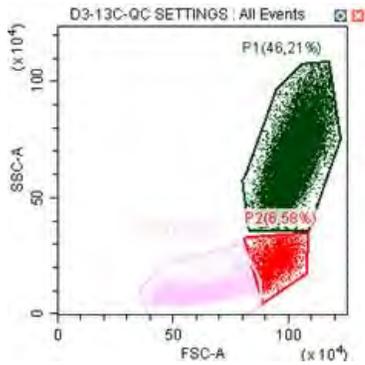


Figure 5.7 Extent - Maximum Setting



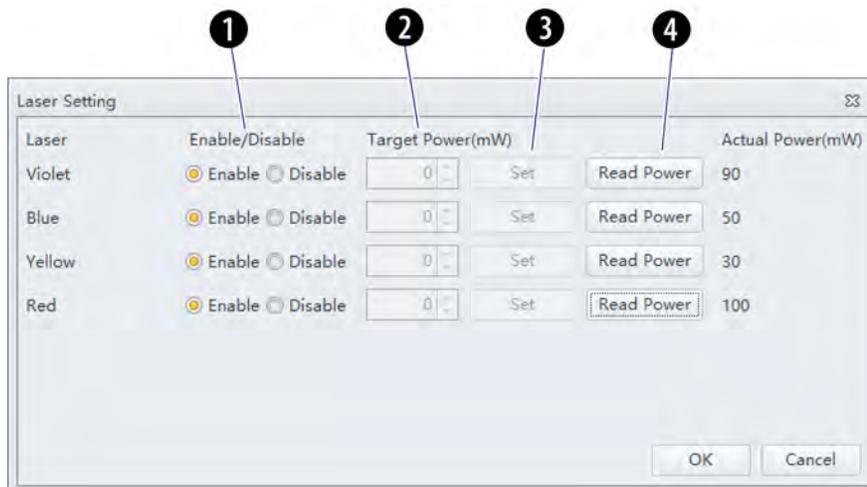
Configuring Acquisition Settings

Laser Settings

To access the Laser Setting window, select **Advanced > Laser Setting**. The Laser Setting window appears. Refer to [Figure 5.8](#).

NOTE The instrument must be in Standby mode to access the Laser Setting window.

Figure 5.8 Laser Setting Window



1. **Enable/Disable:** Enables or disables the laser.

IMPORTANT The laser target power setting is not available to users.

2. **Target Power (mW):** Used to change the laser target power.

NOTE The power detector has ± 1 mW tolerance. Refer to [Table 5.1](#) or [Figure 2.2](#) (the target Power column in the QC report area) for range limits of each laser.

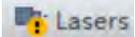
3. **Set**: Sets the laser target power setting.

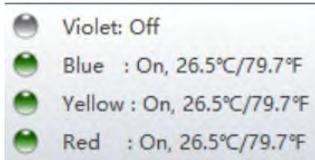
IMPORTANT The Actual Power readings are +/- 1 mW.

4. **Read Power**: Reads the current laser power before the flow cell assembly and displays the current laser power in the Actual Power (mW) column of the Laser Setting window.

Table 5.1 Target Power Ranges in the Laser Setting Screen

Laser	Power Range (mW)	Max Power (mW)
405	90	120
488	50	60
561	30	40
638	100	120

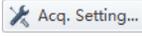
Select the **Enable** or **Disable** radio button next to each laser on the Laser Setting window to enable or disable lasers. When a laser is disabled or abnormal, the laser status icon  appears in the software status bar. Hover your mouse over  to display details for each laser.

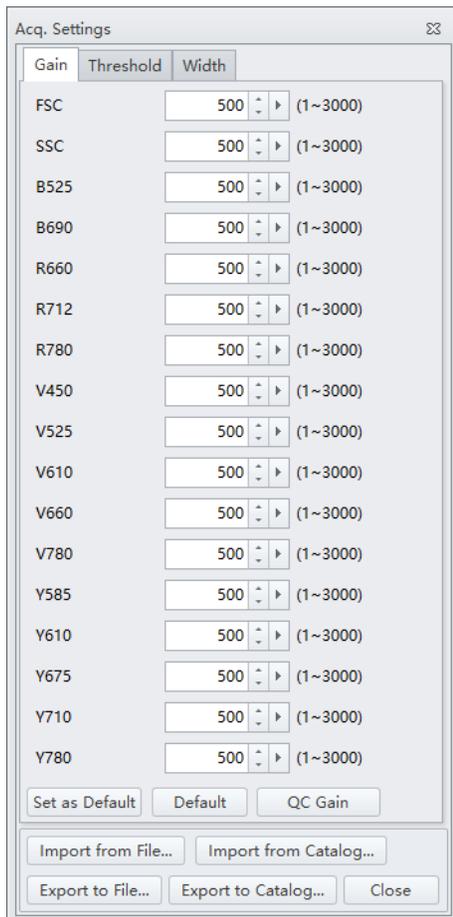


NOTE Lasers can only be enabled or disabled when the system is in standby state.

Adjusting the Gain

While the instrument is in use, the signal value can be increased or decreased by adjusting the instrument's gain configuration.

- 1 Select  in the Cytometer menu. The Acq. Setting window appears.



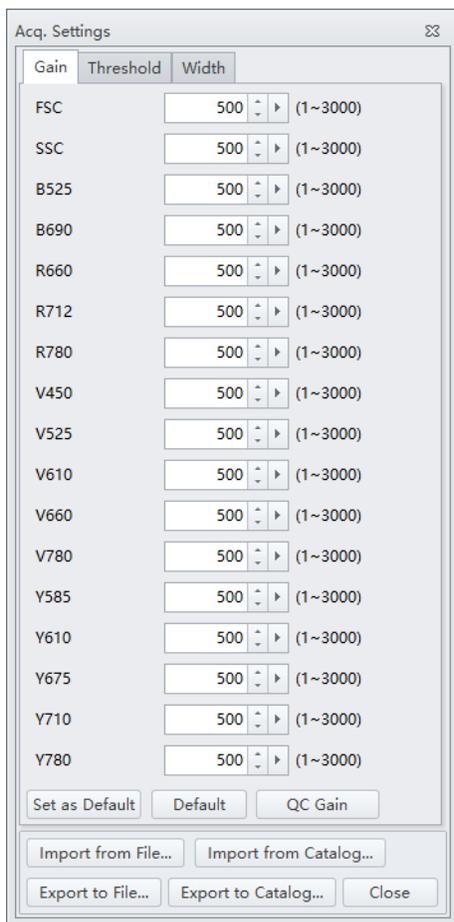
2 Select the **Gain** tab in the Acq. Setting window.

Select or edit the instrument's default gain settings using one of the following methods:

- Edit the gain settings and select **Set as Default** to create a new default setting.
- Select **Default** to return to your saved default settings.
- Select **QC Gain** to use the instrument's QC settings.

NOTE In cases where you do not specify your own default parameters, the recommended settings and default settings are identical.

3 Adjust the gain setting of each channel under the Gain tab in the Acq. Setting window. Raising the gain increases the signal. Lowering the gain reduces the signal.



NOTE Optimize the gain settings according to your own experimental goals. The recommended values are only for reference.

Another option is to use the **Gain Control** button  on the tool bar in the graphic control area to adjust the gain values for cell population data to their desired levels, directly on the plots where the data appears during data collection.



NOTE Gain adjustments have a predefined range between 1 and 3,000.

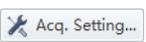
-
- 4 If necessary, change the coordinate display range and the plot type.
-

Adjusting the Threshold

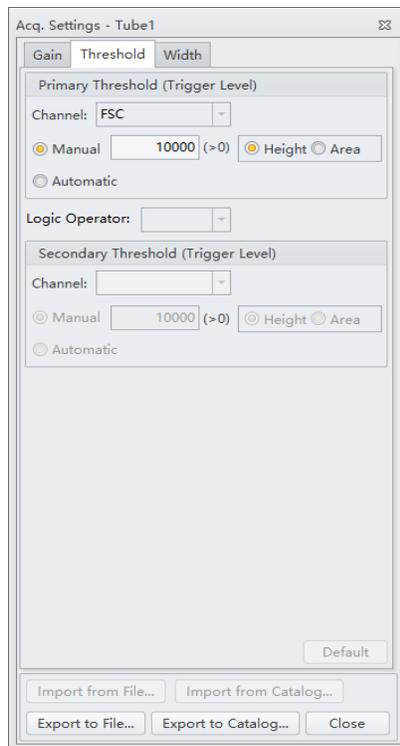
By adjusting the threshold, the user can remove unnecessary signal noise to ensure that most of the data collected consists of desired signal data. After the threshold settings have been configured for a given channel, the acquisition of data from this channel will only be triggered by signals that exceed the established threshold. Threshold settings have considerable bearing on whether the appropriate events can be acquired.

-
- 1 Create a plot to view the channels where the threshold will occur. Generally, a bivariate plot showing FSC and SSC is used.

NOTE Threshold can be defined for any of the fluorescence channels.

-
- 2 Select  on the left side of the screen.

- 3 Select the **Threshold** tab in the Acq. Setting window.



- 4 Set the desired threshold using one of the following methods:

- Choose the channel that is used for setting the threshold. Manually enter the threshold value in the Threshold tab.

NOTE For dual-parameter plots, you can right-click the plot and select both parameters if desired. Then, select the desired threshold boundary for the second parameter.

- Select **Automatic** in the Primary Threshold Trigger Level section of the Acq. Setting screen to seek the target signal based on the background signal. It can quickly help find the target population if the signal-to-noise ratio (SNR) of the channel is comparatively good. The threshold can be set to either “H” (signal height) or “A” (signal area).

NOTE The automatic threshold value is based on the relative signal difference. When adjusting gain, you do not need to update the threshold settings. For channels with a low SNR or an excessively impure signal, manually setting the threshold parameters is recommended.

Moreover, “and” as well as “or” can be applied to as many as two channels, so as to allow these Boolean logical operators to be used in setting the threshold value.

- “and”: Data is displayed and collected only when two threshold conditions are met simultaneously.
- “or”: Data is displayed and collected when at least one of two threshold conditions are met.

- Select  from the plot control area. Move your mouse pointer to the desired threshold position in the desired plot and select once.

5 Select **Close**.

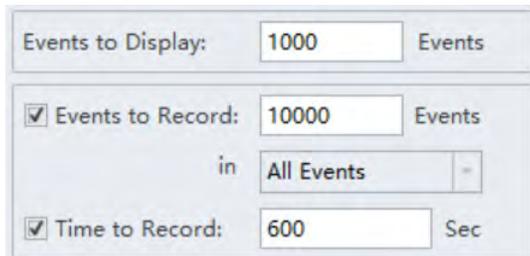
Setting Collection Conditions

- 1 Check mark the conditions required to set the necessary stop count events on the left side of the Acquisition screen.

Two stop conditions are available for sample recording:

- **Events to Record.** Used to set the number of events to record in the specified population.
- **Time to Record.** Used to set the collection time duration in seconds.

For example, if the event to record is set to record 1,000 P1 events, the software automatically stops recording when P1 events reach 1,000 events. However, the software saves all data acquired, including events outside of P1, when 1,000 P1 events is reached. You can also specify the time to store if necessary. When multiple acquisition conditions are established, any one of these conditions stops the collection process.



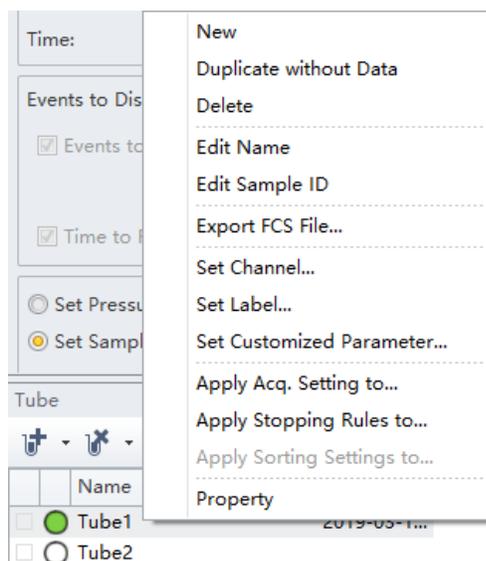
NOTE The sorting stop criteria takes precedence over the collection stop condition in a sort. Refer to [Stop Criteria for Sorting](#).

NOTE Navigate to Settings > Options > Record to customize the default value for Events to Record. Refer to [Software Settings](#) in [CHAPTER 2, Using the CytExpert SRT Software](#).

- 2 Select **Record** and wait for the software to complete collecting the data, at which time the sample chamber and sample probe are in loading position.

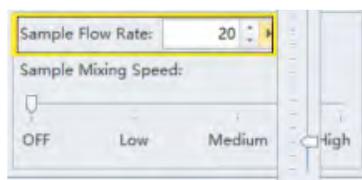


- 3 If you made changes to the data acquisition conditions and need to apply these changes to an established sample tube, right-click the sample tube and select **Apply Acq. Settings To**, to apply the conditions accordingly.



Setting Sample Flow Rate

Set the sample flow rate by specifying the sample pressure located in the lower left corner of the software screen. The allowable range is 10-100. The value can be adjusted in 1 increment.

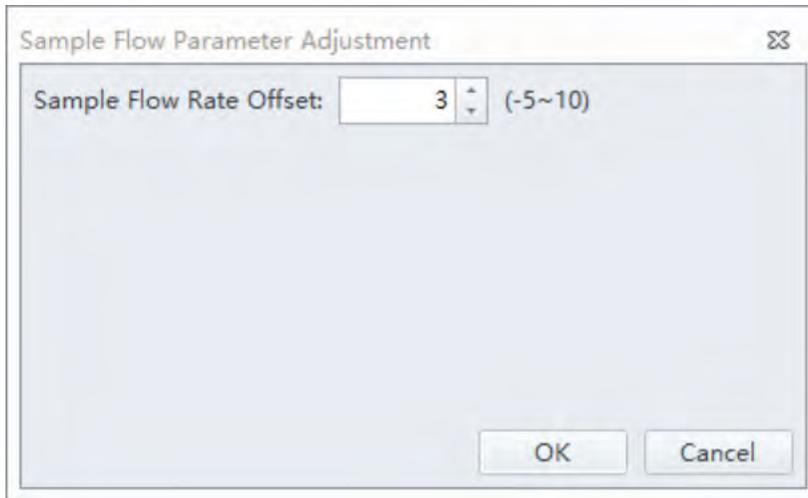


- 10: approximately 10 $\mu\text{L}/\text{min}$
- 30: approximately 30 $\mu\text{L}/\text{min}$
- 60: approximately 60 $\mu\text{L}/\text{min}$

NOTE Customize the pressure differential according to the nature of the sample. Big pressure differential may increase the measurement CVs.

The sample flow rate setting might not be accurate. If the deviation between the setting and the actual (calculated flow rate based on known sample concentration) is $\leq 30\%$, and leads to QC failure,

select **Sample Flow Parameter Adjustment** from the Cytometer menu to offset the sample flow rate. The allowable range is -5 to 10.



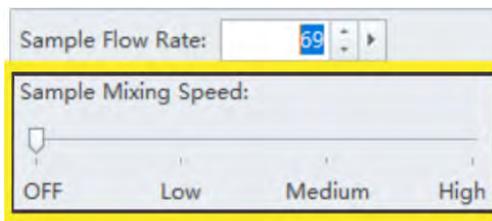
10: increase by approximately 10 $\mu\text{L}/\text{min}$

-5: decrease by approximately 5 $\mu\text{L}/\text{min}$

NOTE If the sample flow rate deviation is > 30%, or the QC problem persists after adjusting the sample flow rate offset, [contact us](#) to perform the sample flow rate calibration.

Setting Mixing Speed

Set the mixing speed located in the lower left corner of the software screen.



Low: approximately 100 r/min

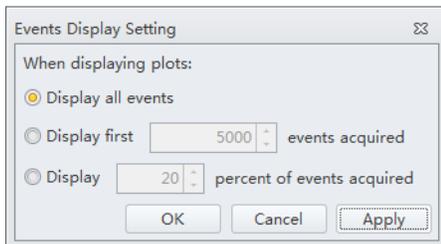
Medium: approximately 300 r/min

High: approximately 500 r/min

NOTE You can customize the sample mixing speed for each tube. The sample mixing automatically stops when the acquisition ends.

Setting Plot Display Conditions

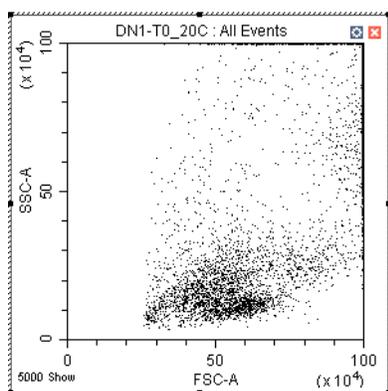
Select **Events Display Setting** in the Settings menu. The Events Display Setting window appears.



Three display options are available:

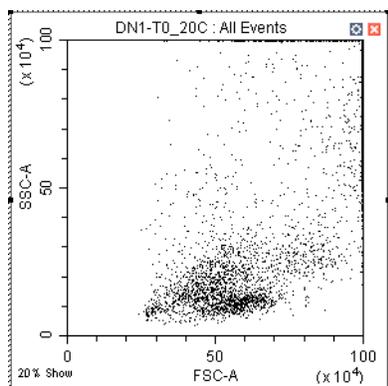
- **Display all events.** Used to view all events on the plot.
- **Display first XXXX events.** Used to set the set number of events to display.

NOTE The selected number of events displays in the bottom, left corner of the plot. For example, if you choose to show 5000 events, the bottom, left corner of the plot displays *5000 Show*.



- **Display XX percent of events acquired.** Used to set the percentage of events to display.

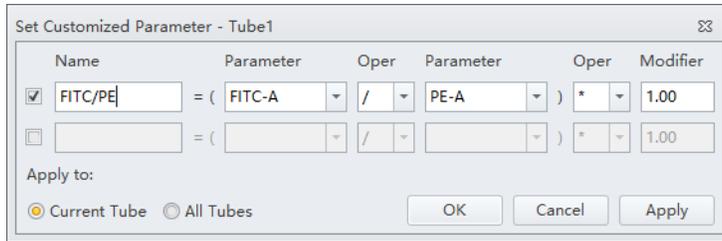
NOTE The selected percentage of events displays in the bottom, left corner of the plot. For example, if you choose to show 20 percent of events acquired, the bottom, left corner of the plot displays *20% Show*.



Setting Customized Parameters

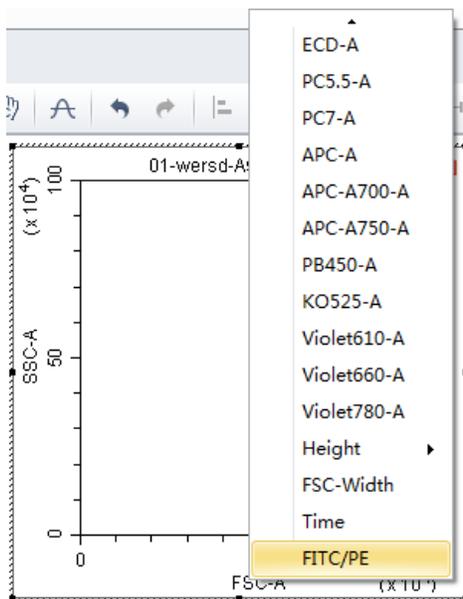
Set custom parameter to create fluorescence calculations.

- 1 Select **Set Customized Parameter** from the Settings menu. Or, right-click a test tube from the test tube menu and select **Set Customized Parameter**. The Set Customized Parameter window appears.



- 2 Enter a name for the parameter in the Name section.
- 3 Select the parameters for calculation in the Parameter dropdowns.
- 4 Select **OK**.
- 5 Select the equation operations from the **Oper** dropdown menu.

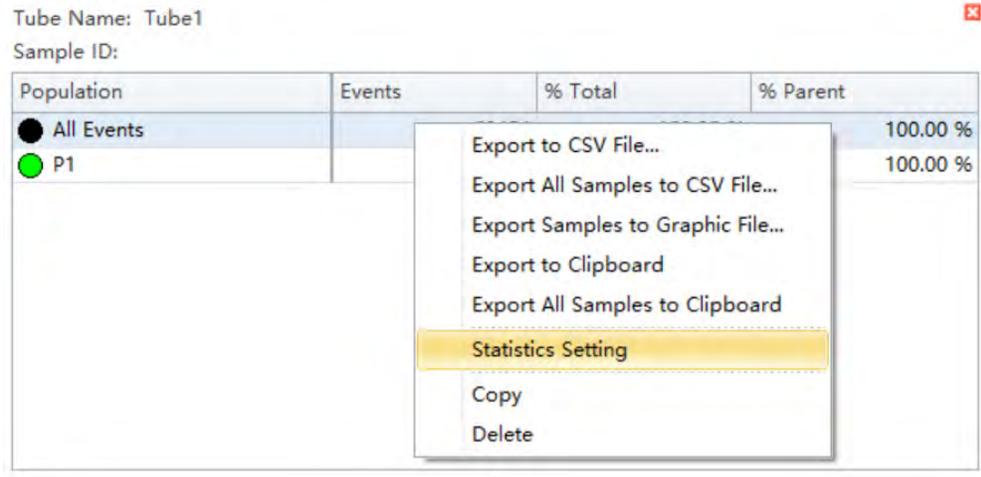
The new parameter name is displayed in the list of parameters and statistic items.

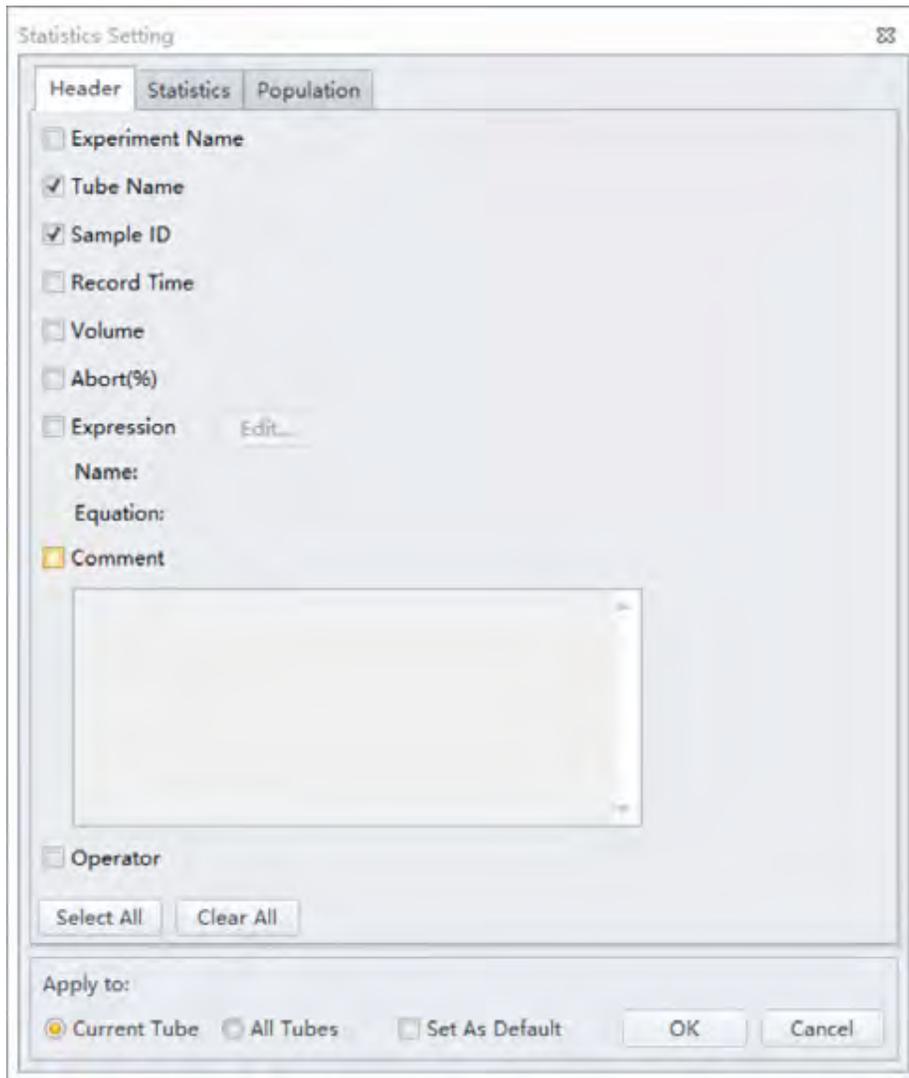


Setting Custom Statistics

Set custom statistics to create calculations based on populations of interest.

- 1 Right-click the statistics table and select **Statistics Setting**. The Statistics Setting window appears.

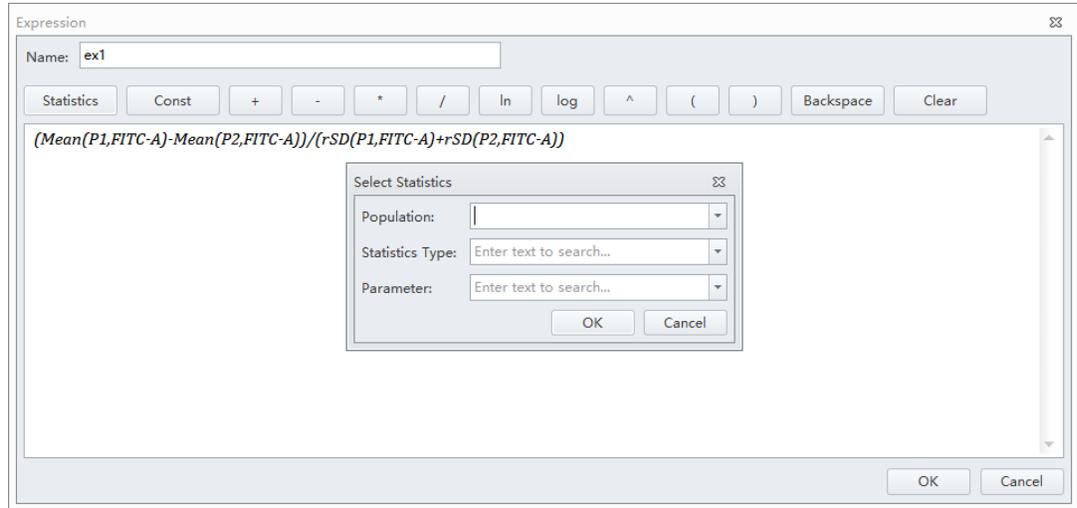




2 Select **Expression**.

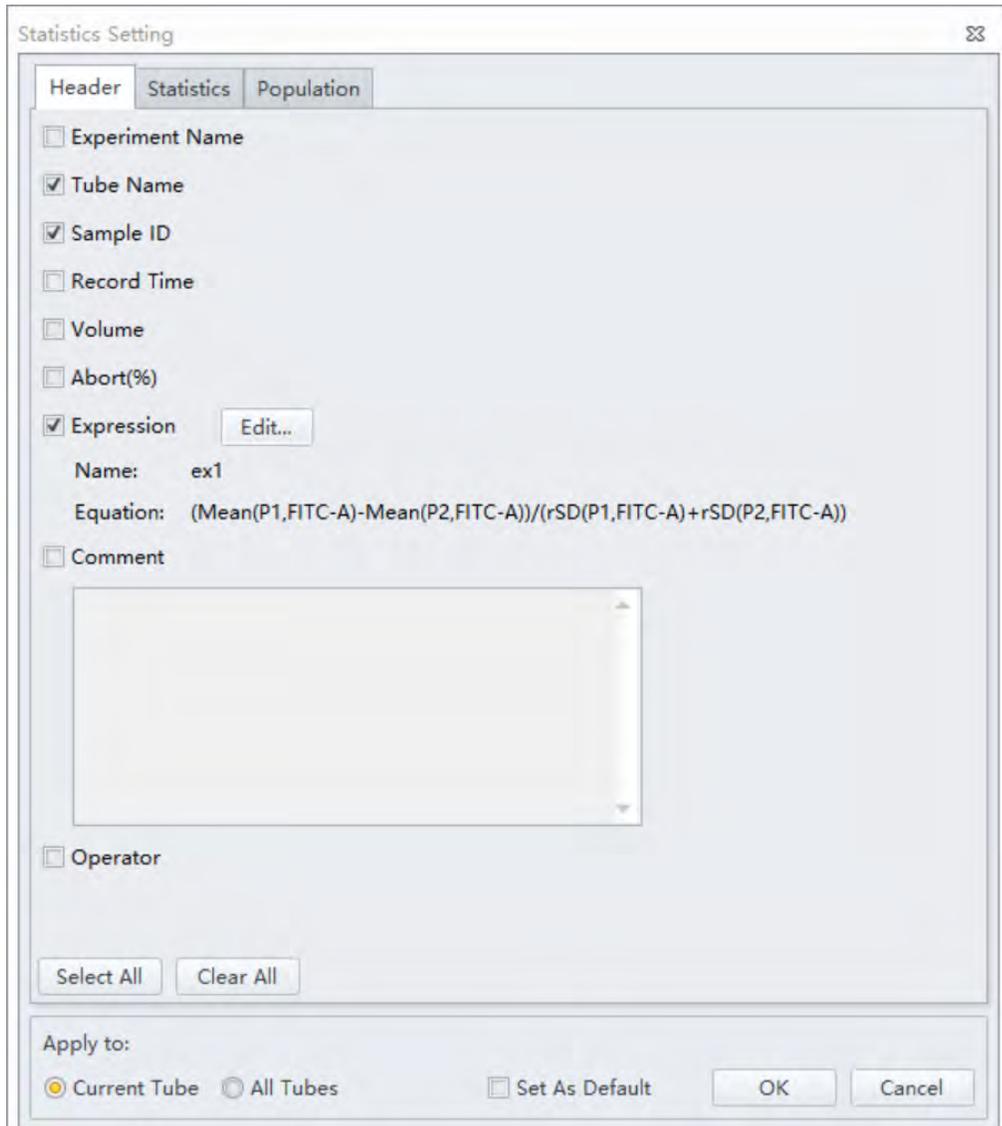
3 Select **Edit**. The Expression window appears.

- 4 Enter the expression name in the Name section and enter the expression using the equation buttons.



5 Select **OK**.

NOTE The equation populates in the Statistics Setting window under the Expression selection.



Tube Name: Tube1
Sample ID:
ex1: 0.00

Population	Events	% Total	% Parent
● All Events	60454	100.00 %	100.00 %
● P1	60454	100.00 %	100.00 %
● P2	54905	90.82 %	90.82 %

Load Sample and Record Data

Before Running Samples

 **CAUTION**

Risk of erroneous results if the Sorter has been idle for an extended period of time. Perform a prime if the system has been idle for an extended period of time. Refer to [Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures](#).

- 1 Run the [System Startup Program](#) procedure.
- 2 Run the [Instrument Quality Control and Standardization](#) procedure.
- 3 Create an experiment. Refer to [Creating an Experiment](#).
- 4 Run Sort Calibration. Refer to [Sort Calibration \(Auto Drop Delay\)](#).
- 5 Ensure that there is sufficient space on your hard drive for sample processing and data acquisition.
- 6 Verify the detector configuration. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#).

- 7 Verify the laser settings. Refer to [Laser Settings](#) in [CHAPTER 5, Sorting](#).

Setting Up Violet Side Scatter (VSSC) Channel

For micro-particles, a VSSC option can be added to better separate side scatter signals from noise. Beckman Coulter recommends using this channel to detect particles smaller than 500 nm.

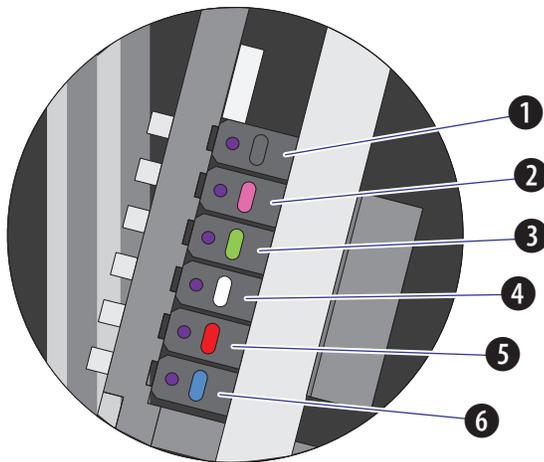
NOTE Since the total available channel numbers remain the same when the VSSC channel is used, the number of fluorescent channels in the violet WDM is reduced by 1 channel.

CAUTION

Risk of erroneous results. Beckman Coulter recommends using the VSSC channel to detect side scatter signals for particles smaller than 0.5 μm . VSSC could be too sensitive when large particles are being acquired. Switch back to the original detector configuration for particles larger than 5 μm . For particles larger than 5 μm , set the gain of the VSSC to 1 to increase the threshold and decrease the collection of sample noise.

- 1 Open the Violet WDM lid (refer to [Replacing the Optical Filter](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)) and remove the 405-nm filter, the 450-nm filter, and a third filter not required for the test, for example, the 780-nm filter.

NOTE Refer to [Table 5.1](#) to identify the WDM filter mount color codes.

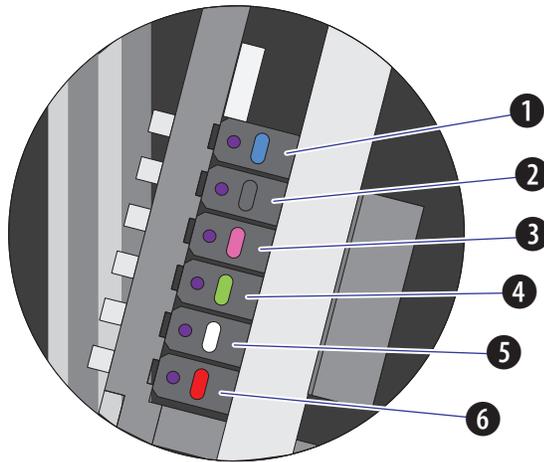


- | | |
|---------------|---------------|
| 1. Position 1 | 4. Position 4 |
| 2. Position 2 | 5. Position 5 |
| 3. Position 3 | 6. Position 6 |

NOTE The orientation for position 1 through 6 starts with position 1 located closest to the fiber coming into the WDM, and position 6 located on the side furthest from the fiber coming into the WDM.

- Place the sixth filter in position 1, the 405-nm filter in position 2, and the 450-nm filter in position 3.

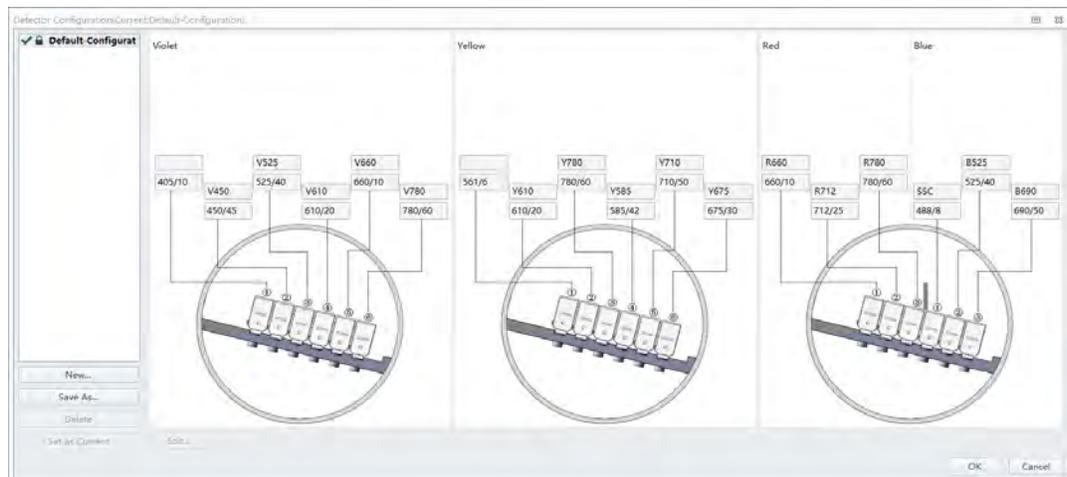
NOTE For the Violet WDM, Beckman Coulter recommends placing the filters in sequential order from the shortest wavelength to the longest wavelength in positions 2 to 6. Position 1 will always contain the unused filter.



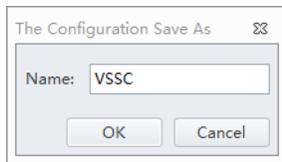
- | | |
|---------------|---------------|
| 1. Position 1 | 4. Position 4 |
| 2. Position 2 | 5. Position 5 |
| 3. Position 3 | 6. Position 6 |

- Start the CytExpert SRT software. Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#).

- Select **Detector Configuration** from the Cytometer menu. The Detector Configuration window appears.

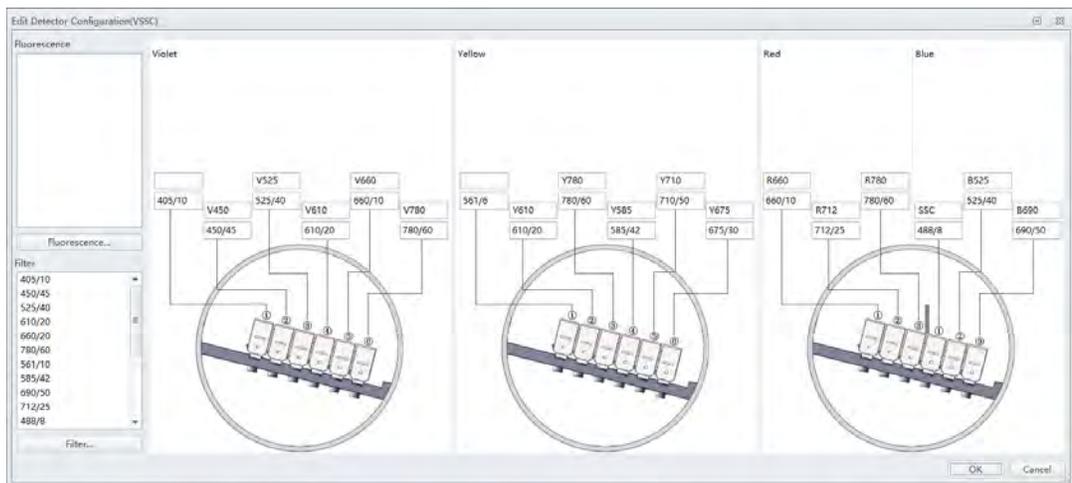


- 5 Select the Default Configuration and select **Save As**. The Configuration Save As window appears.



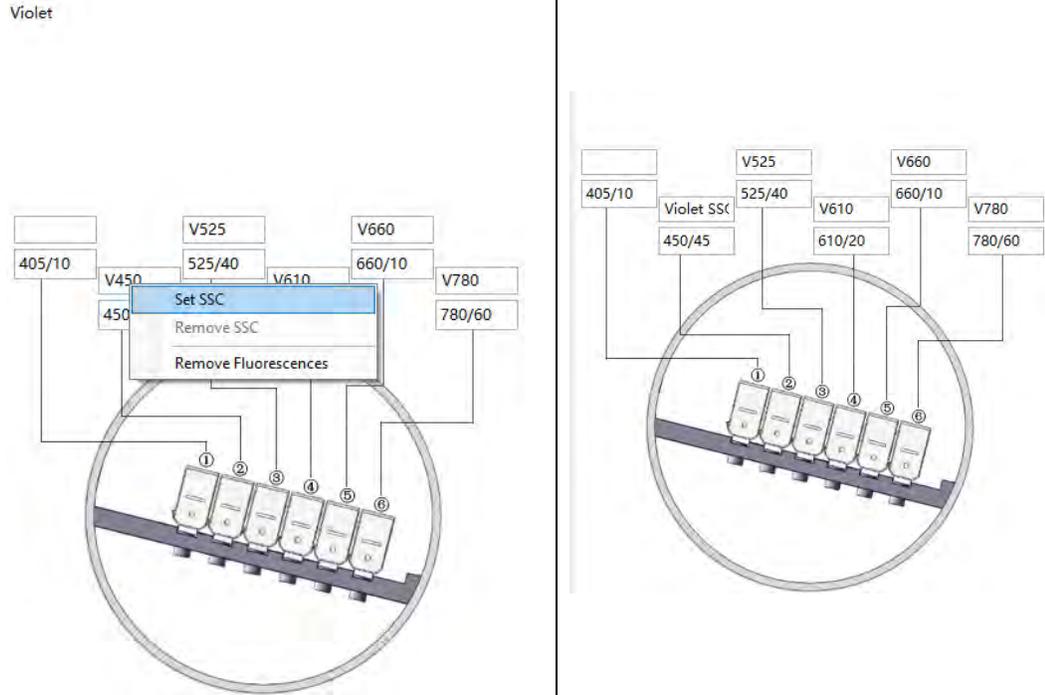
- 6 Name the new configuration VSSC and select **OK**.

- 7 Select the VSSC configuration and select **Edit**. The Edit Detector Configuration window appears.



- 8 Change the filters and channel names according to the filter order in the violet WDM.

9 Right-click the VSSC channel, and select **Set SSC** to set it as the Violet SSC channel.



10 Select **OK** to save the changes and close the Edit Detector Configuration window.

11 Select **Set as Current**.

12 Select **OK** to save the changes and close the Detector Configuration window.

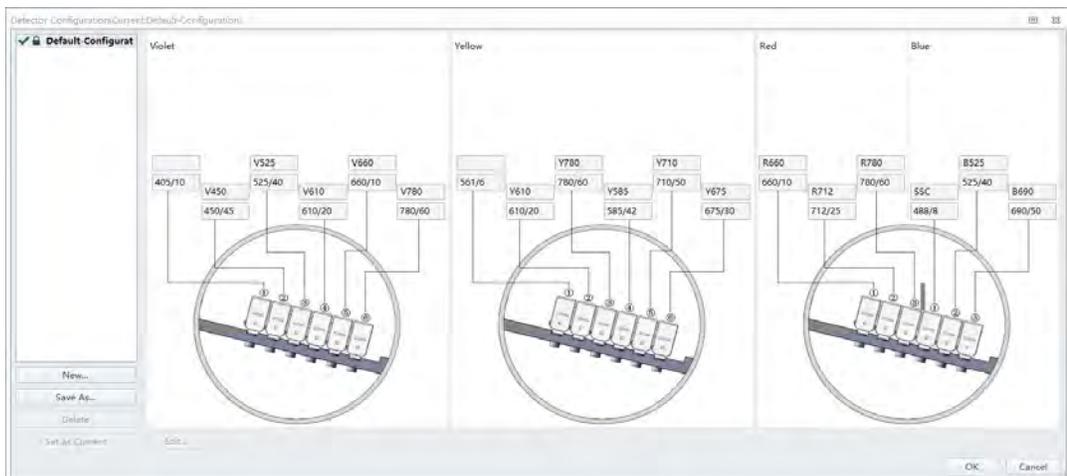
13 Create a new experiment using the VSSC configuration. Refer to [Creating an Experiment](#).

Verifying, Selecting, Editing, and Creating Detector Configuration



Risk of erroneous results. The system will read the selected Detector Configuration even if the optical filters do not match the selected Detector Configuration. You must verify the installed optical filters match the selected Detector Configuration.

- 1 Select **Detector Configuration** in the Cytometer menu to verify the correct detector configuration is selected. To change the configuration:
 - a. Select the desired configuration.
 - b. Select **Set as Current**.
A green check mark appears in front of the selected configuration.



NOTE A configuration is locked when  appears to the left of a configuration. A configuration locks for two reasons:

- QC was run using the configuration.
- The compensation library contains data for the configuration.

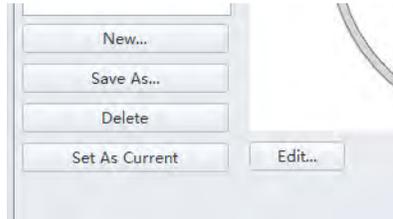
Locked configurations can be deleted, but not edited.

- 2 Select **OK** to close the Detector Configuration screen.
- 3 Proceed to Step 4 if you need to edit the Detector Configuration settings, or skip to Step 5 if you need to create a new Detector Configuration, or skip to Step 12 if you need to delete a Detector configuration.

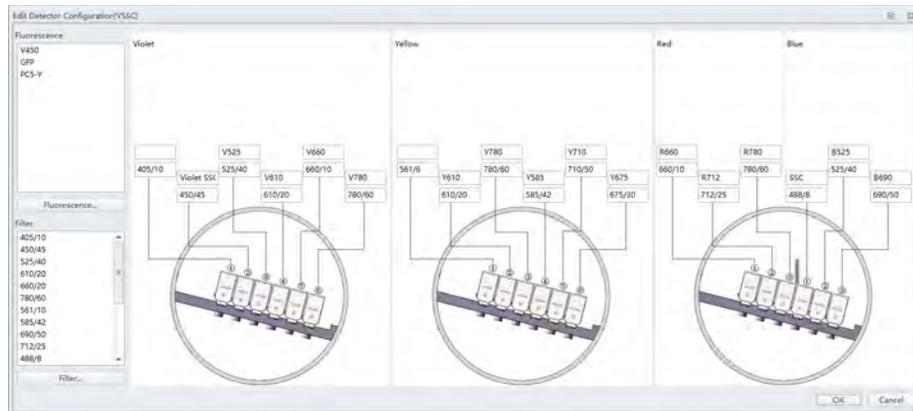
4 If a saved configuration requires changes, edit that configuration.

NOTE The factory configuration is in bold and cannot be edited.

- a. Select the configuration, then select **Edit** to access the Edit Detector Configuration screen.



- b. Channels with a white background can be edited. Drag the names of the appropriate fluorescence channels and optical filters on the left to the correct channels.

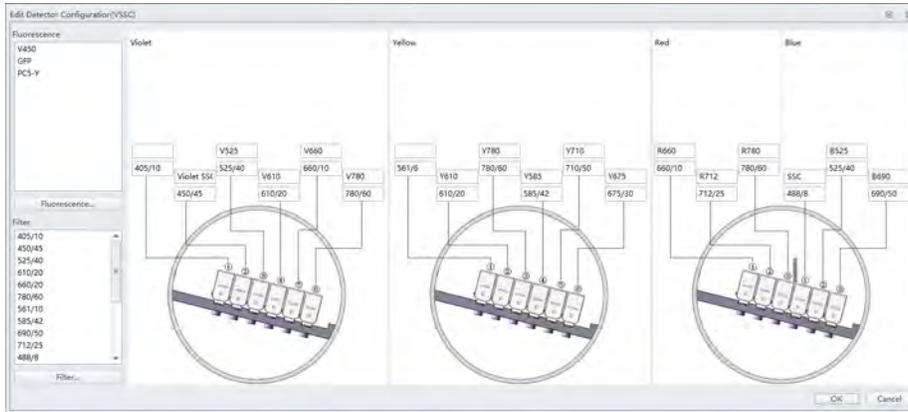


- c. Continue to Step 6.

5 If an appropriate configuration is not saved, create a new configuration.

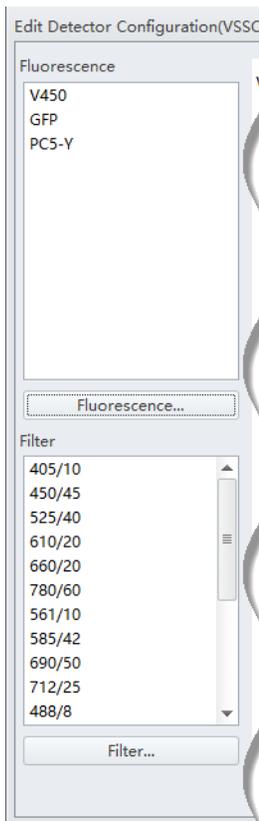
- a. Select **Detector Configuration...** in the Cytometer menu.
- b. Select **New...** and name your configuration.
You can also select a previously saved configuration and select **Save As** to create a copy.
- c. Select **OK**.

- d. Ensure the new configuration is highlighted, then select **Edit**. The Edit Detector Configuration window appears.

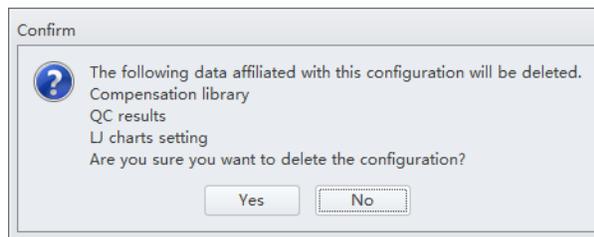


- e. Customize the new configuration. Channels with a white background can be edited. Drag the names of the appropriate fluorescence channels and optical filters on the left to the correct channels.
- f. Continue to Step 6.

- 6 If a required channel name or filter is not listed on the left, select **Fluorescence** or **Filter** to add or modify the channel name or the filter.



-
- 7** When finished, select **OK**.
-
- 8** Select the appropriate configuration.
-
- 9** Verify that the correct optical filters are installed in the Sorter and match the newly created configuration.
-
- 10** Select **Set As Current**.
-
- 11** Select **OK**.
-
- 12** To delete a configuration created in error, select **Delete**. The following confirmation message appears. Select **OK**.



Sampling and Collecting Data



Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

CAUTION

Risk of clogging the sample line. Filter the biological sample using a 70- μ m mesh aperture filter before sampling.

NOTE Settings can be imported from the Acquisition Settings Catalog. Refer to [Importing and Exporting Instrument Settings](#).

If compensation settings are desired, import the compensation from the Compensation Library or import the compensation file. Refer to [Importing and Exporting Compensation](#) in [CHAPTER 6, Compensation](#).

NOTE If the instrument has been idle for more than two hours, select **Backflush** from the Cytometer menu before sampling.

-
- 1 Select  from the Test Tube screen to create the new sample tube.

NOTE The first sample tube is already created by default.

-
- 2 Change the tube name if necessary. Refer to [Changing the Tube Name](#).

WARNING

Risk of biohazardous contamination. When using 12x75 mm tube for sample loading, do not exceed 4 mL sample volume. Running sample with volumes exceeding 4 mL can result in sample splashing. Clean up spills immediately. Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

WARNING

Risk of instrument damage. When using 12x75 mm tube, always cut the cap off. Running samples with a cap attached to the sample tube can result in a bent sample probe.

CAUTION

Risk of clogging the sample line if the sample contains aggregates or clumps. Filter the sample using an appropriately sized mesh aperture filter before loading sample. Enable the sample mixing.

- 3 Place the sample tube in the sample tube holder.

- 4 Select the desired acquisition parameters (Events/Time to Record, Sample Flow Rate, and Sample Mixing) on the left side of the screen.

Events to Display: 1000 Events

Events to Record: 10000 Events
in All Events

Time to Record: 600 Sec

Sample Flow Rate: 10

Sample Mixing Speed:
OFF Low Medium High

- 5 Select **Run** to load the sample.

NOTE When you select a tube that only contains acquired data, as indicated by the blue tube  in the test tube section of the screen, the following message appears:

Confirm

The current tube already contains data. Would you like to:

Create a new tube

Overwrite the data

OK Cancel

- **Create new tube.** Saves the current tube and creates an additional tube.
- **Overwrite the data.** Overwrites the current tube data with new data.

- 6 View the plots and establish the gates. Refer to [Creating Plots and Gates](#). Adjust the gate and instrument settings as necessary. Refer to [Configuring Acquisition Settings](#).

- 7 Adjust the gain settings. Refer to [Adjusting the Gain](#).

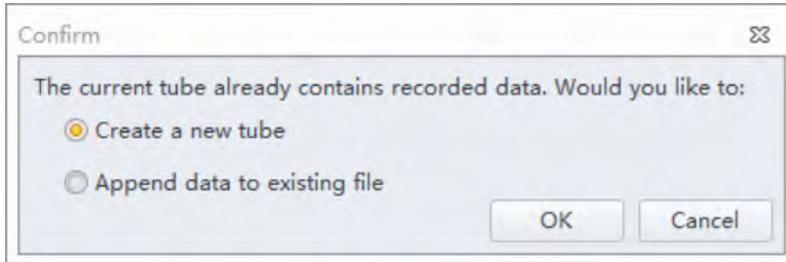
- 8 Adjust the threshold settings. Refer to [Adjusting the Threshold](#).

- 9 Adjust the Acquisition conditions. Refer to [Setting Collection Conditions](#).

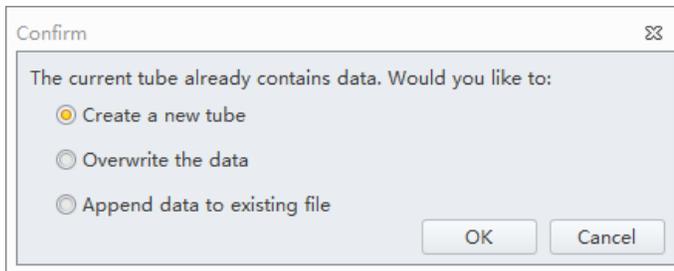
10 Select **Record** to save the data.

Wait for the saving process to finish.

NOTE When you select a tube that contains recorded data, as indicated by the green tube ● in the test tube section of the screen, the following message appears:



NOTE When you select a tube that only contains acquired data, as indicated by the blue tube ● in the test tube section of the screen, the following message appears:



- **Create new tube.** Creates a new tube in the test tube section of the screen for the data.
- **Overwrite existing data.** Overwrites the current tube data with new data.
- **Append data to existing file.** Adds new data to the existing data.

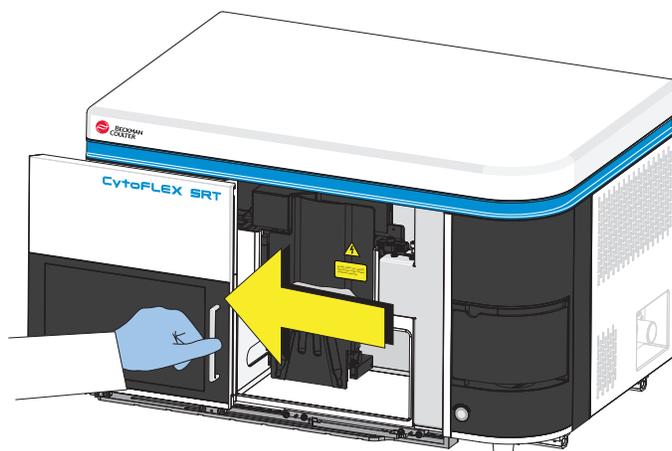
11 Repeat steps 1-10 until all sample tube data required for testing has been collected.

NOTE If the rate suddenly appears to drop, check to see if the sample has run dry or the sample probe is clogged. Any time the sample probe becomes clogged, immediately select **Stop** to unload the sample. Then select **Backflush** to clean the sample probe. Refer to [Daily Clean Program](#) in [CHAPTER 10, Cleaning Procedures](#) to flush out the sample probe. If you are still unable to clear the sample probe, [contact us](#).

Sorting

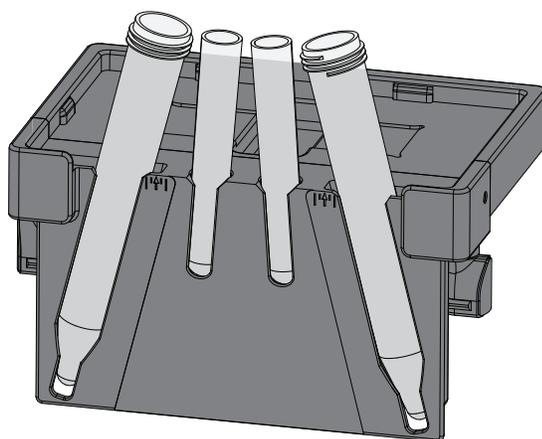
Setting Up Tube Sorting

- 1 Open the sort chamber sliding door.



- 2 Place the appropriate sorting tubes into the output holder and ensure they are secure.

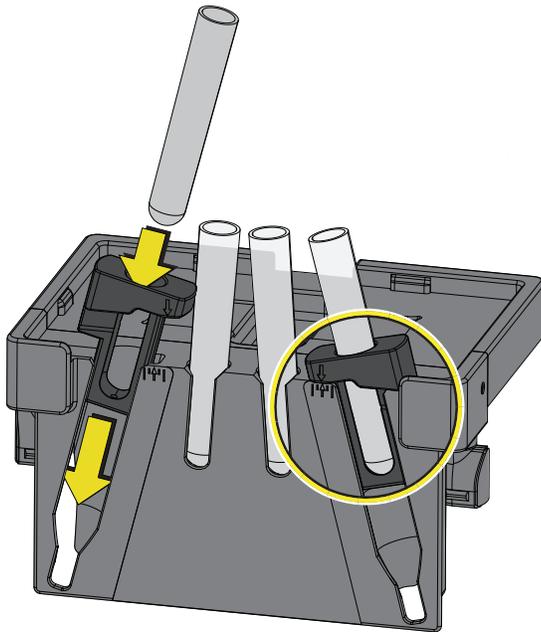
[Two 5 ml tubes and two 15 ml tubes]



CAUTION

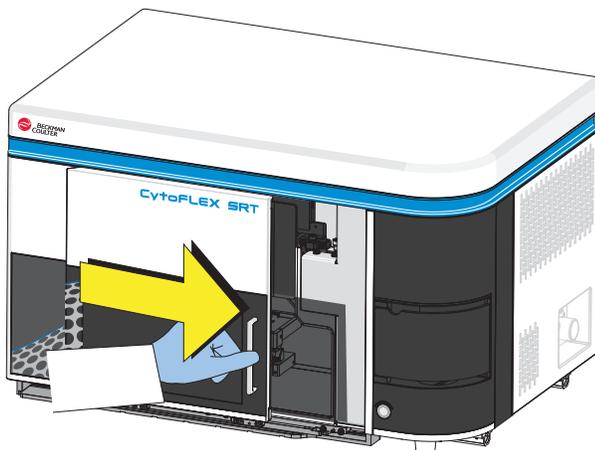
Risk of sample loss and biohazardous contamination. Ensure that the tube adapters are installed in the output holder when using the 12x75 mm tubes to collect the L2 and/or R2 side stream during the sorting. Otherwise, the sorted sample can spray outside the collection tube and contaminate the sort output holder.

[Four 5 ml tubes]

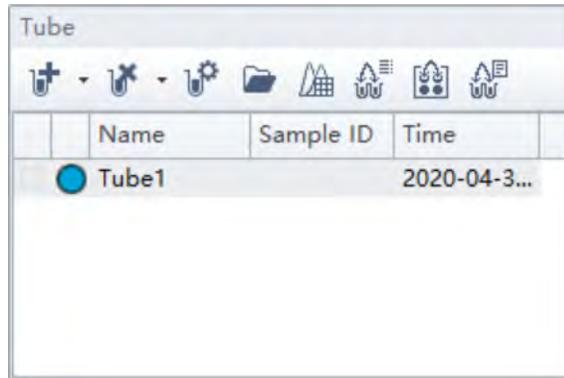


IMPORTANT Ensure that the sort protection door is closed.

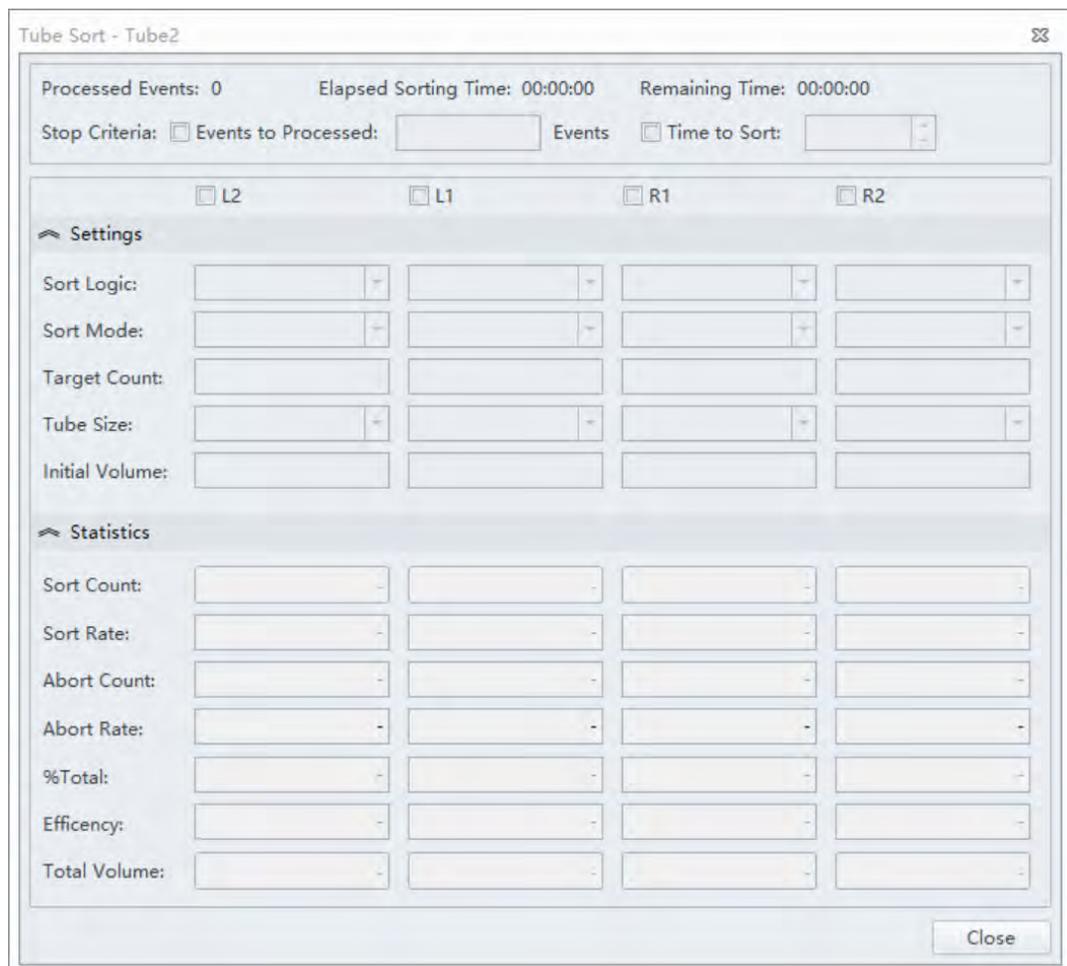
3 Close the sort chamber sliding door.



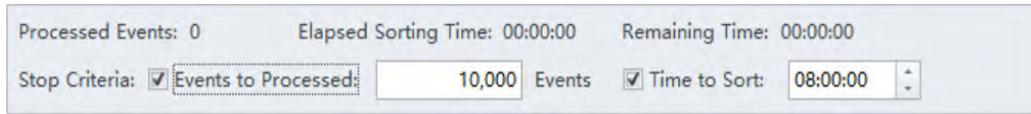
- 4 Add a sample tube from the Tube Management screen.



- 5 Select  in the Tube Management Control screen to set the sorting settings. The Tube Sort window appears.



6 Set the stop conditions for sorting.



The screenshot shows a control panel for sorting. At the top, it displays 'Processed Events: 0', 'Elapsed Sorting Time: 00:00:00', and 'Remaining Time: 00:00:00'. Below this, the 'Stop Criteria' section is active, with two checked options: 'Events to Processed' and 'Time to Sort'. The 'Events to Processed' option has a text input field containing '10,000' and the unit 'Events'. The 'Time to Sort' option has a time input field set to '08:00:00' with up and down arrow buttons.

Explanation of restrictions:

Events to Process: 1 ~ 864,000,000

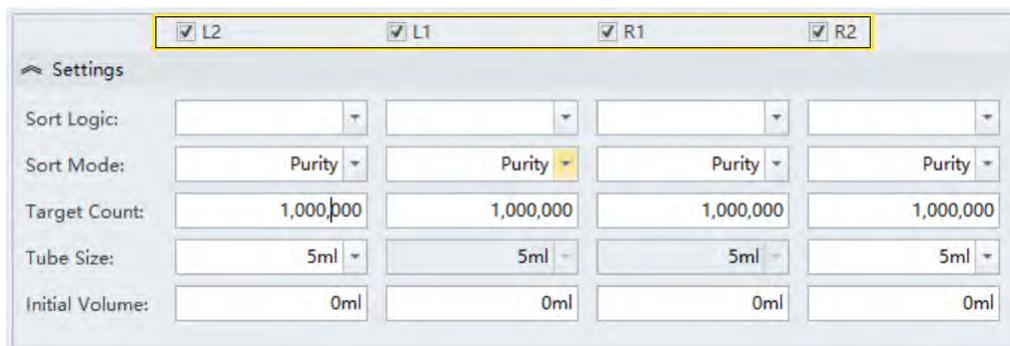
Time to Sort: 1 second ~ 8 hour

NOTE When multiple conditions are established, any one of these conditions stops the sorting process.
Refer to [Stop Criteria for Sorting](#).

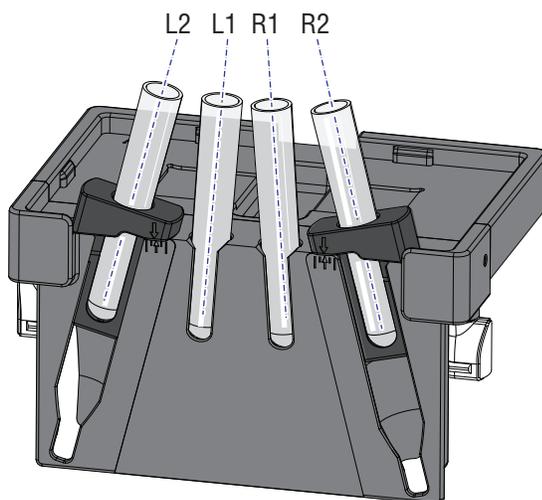
NOTE The Elapsed Sorting Time records the sorting duration, exclusive of the sorting pause.

IMPORTANT Place the most precious and/or rare events in the outer streams (L2/R2) for maximum purity. However, it is better to use the inner streams (L1/R1) for sorting macro-particles ($\geq 15 \mu\text{m}$).

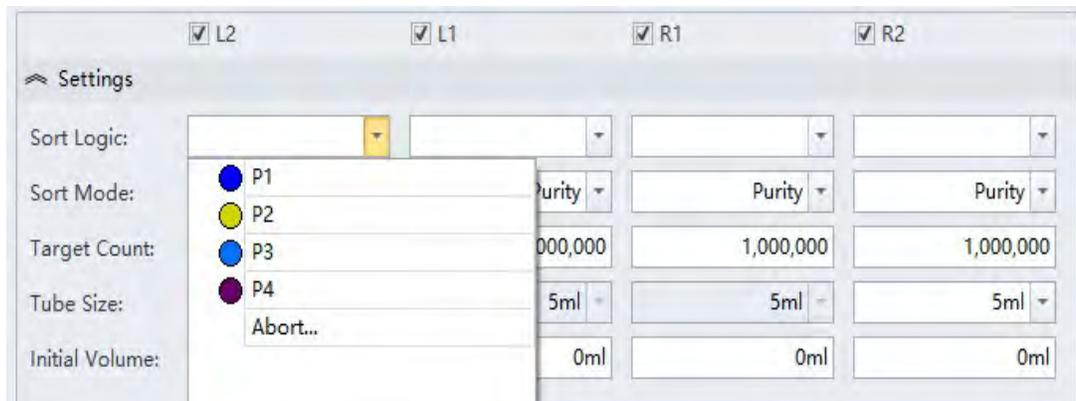
7 Set the side streams to be sorted.



NOTE L1 refers to the left side stream closer to the core stream and L2 refers to the outer left side stream. R1 refers to the right-side stream closer to the core stream and R2 refers to the outer right side stream.



8 Set the Sort Logic.

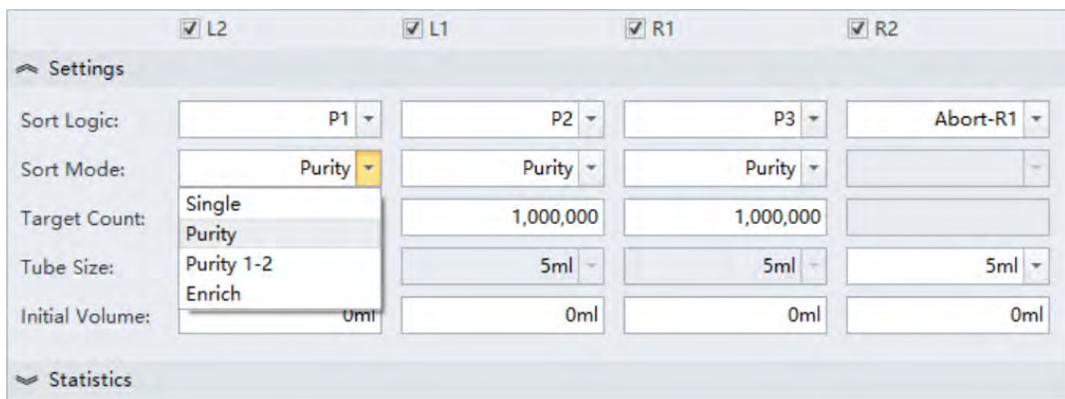


NOTE Sort Logic is the combinational Boolean logic of regions and gates to determine if an event is positive (desired for sorting). Events that fall outside the sort logic are considered negative events.

NOTE The available logic displays in the dropdown list depends on the gates established in the plots. Refer to [Creating Plots and Gates](#).

NOTE The auto gates cannot be used as the sort logic.

9 Verify that the desired Sort Mode is selected.



NOTE For explanations about the Sort Mode or setting the default Sort Mode, refer to [Sort Mode](#) in [CHAPTER 2, Using the CytExpert SRT Software](#).

10 Set the target count of cells to be sorted for each stream.

NOTE The allowable range is 0~ 864,000,000. Target count "0" means unlimited.

NOTE If the target volume setting exceeds the tube volume in theory, the system will display  in the corresponding sort stream.

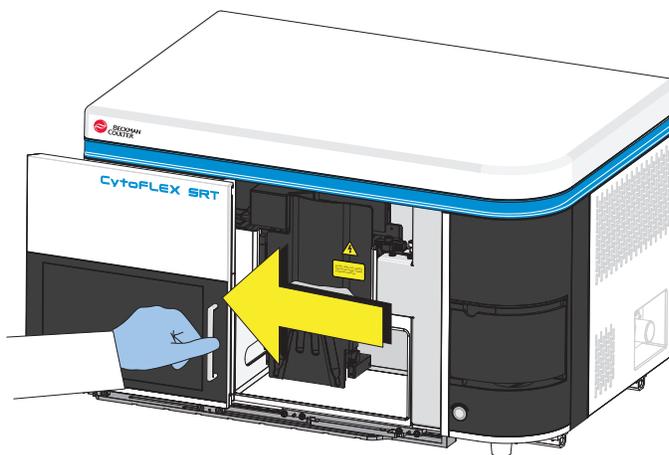
11 Set the tube size.

12 Enter the initial buffer volume.

NOTE If the target volume setting exceeds the tube volume in theory, the system will display  in the corresponding sort stream.

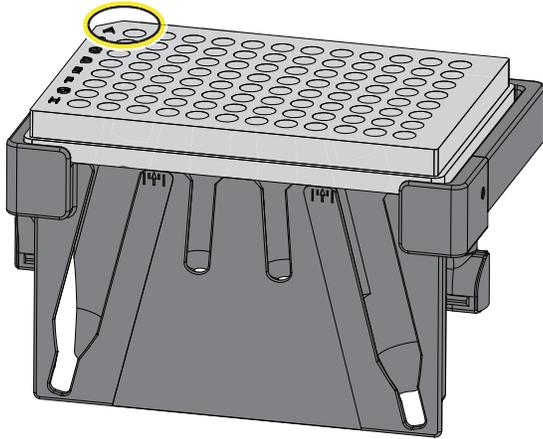
Setting Up Plate/Slide Sorting

1 Open the sort chamber sliding door.

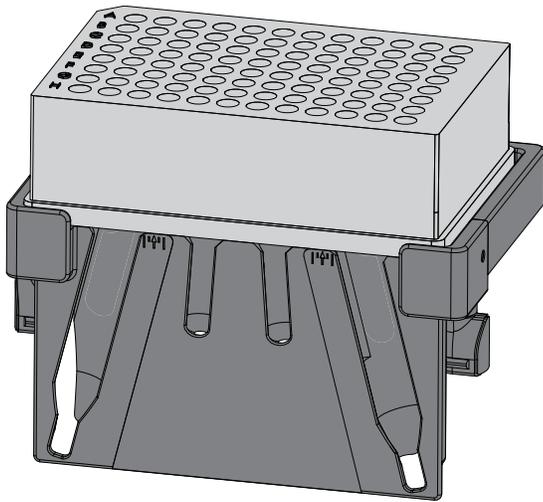


- 2 Place the appropriate sorting plate/slide on the output holder and ensure it is secure.

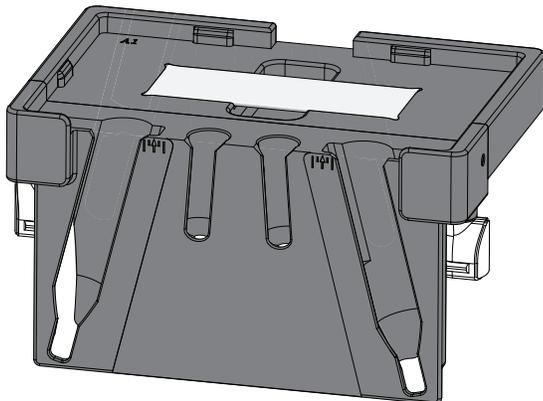
[Standard 96-well plate]



[96-well deep well plate]



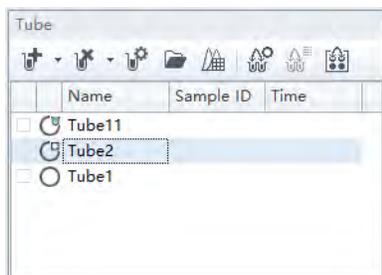
[Slide]



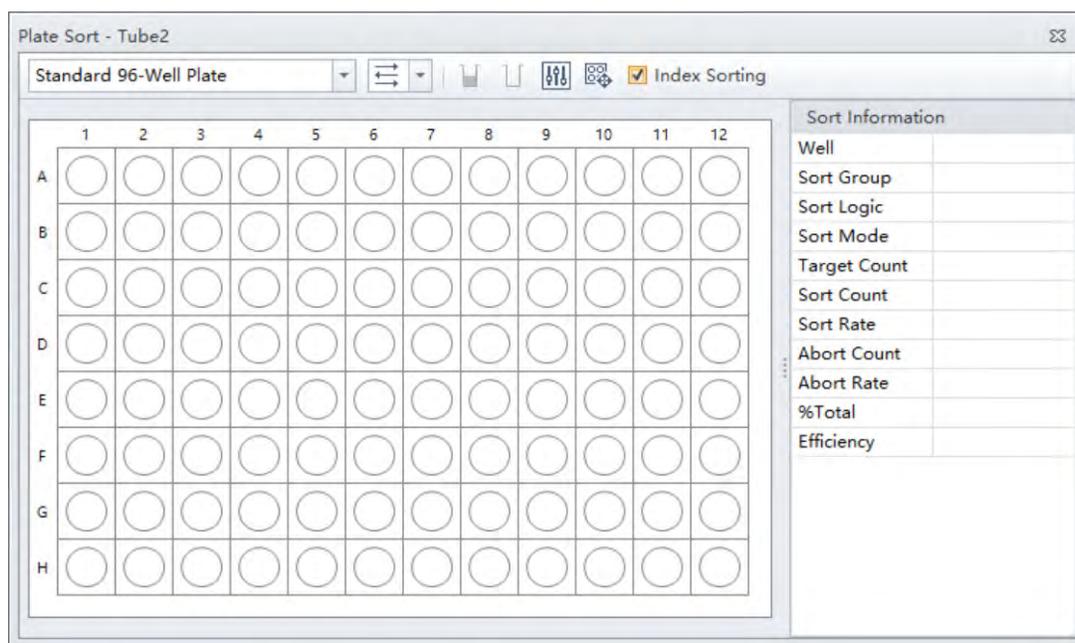
IMPORTANT Ensure that the sort protection door is closed.

3 Close the sort chamber sliding door.

4 Select a sample tube from the Tube Management screen.

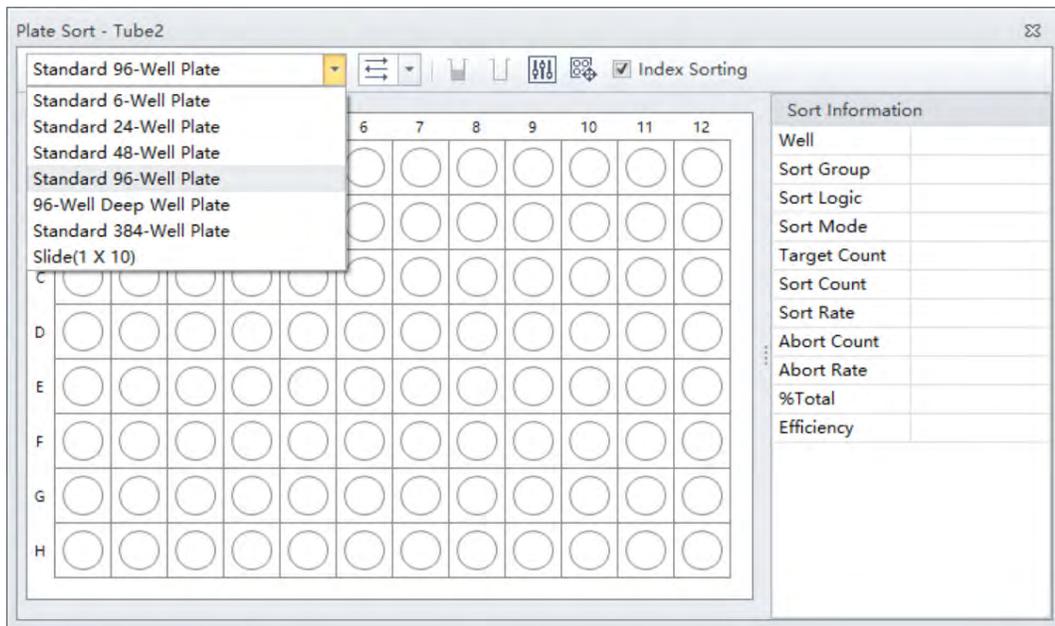


5 Select  in the Tube Management Control screen. The Plate Sort window appears.

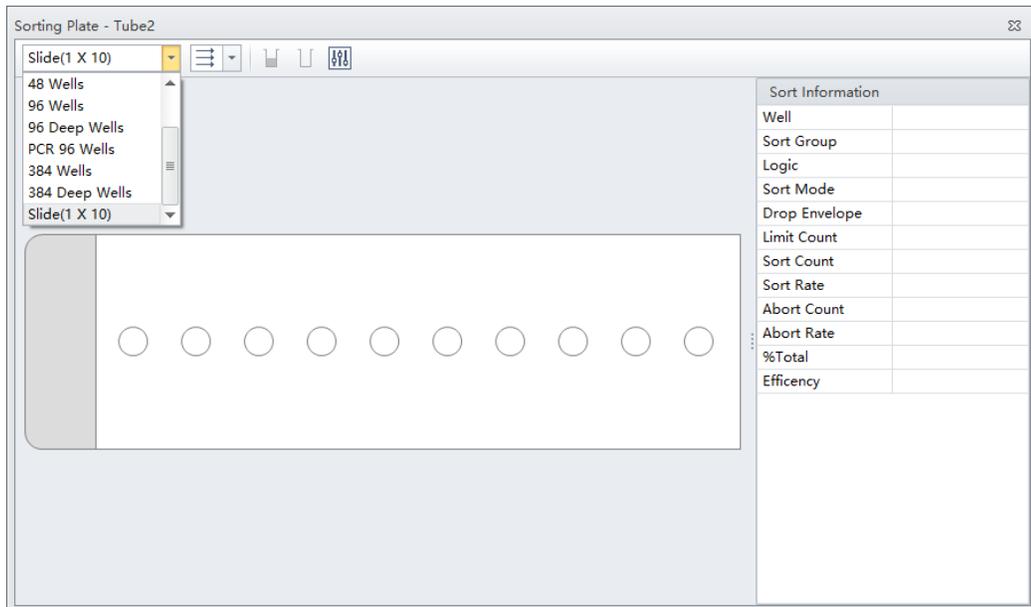


- 6 Select the desired plate type from the Plate Type drop-down menu.

[Standard 96-well plate]

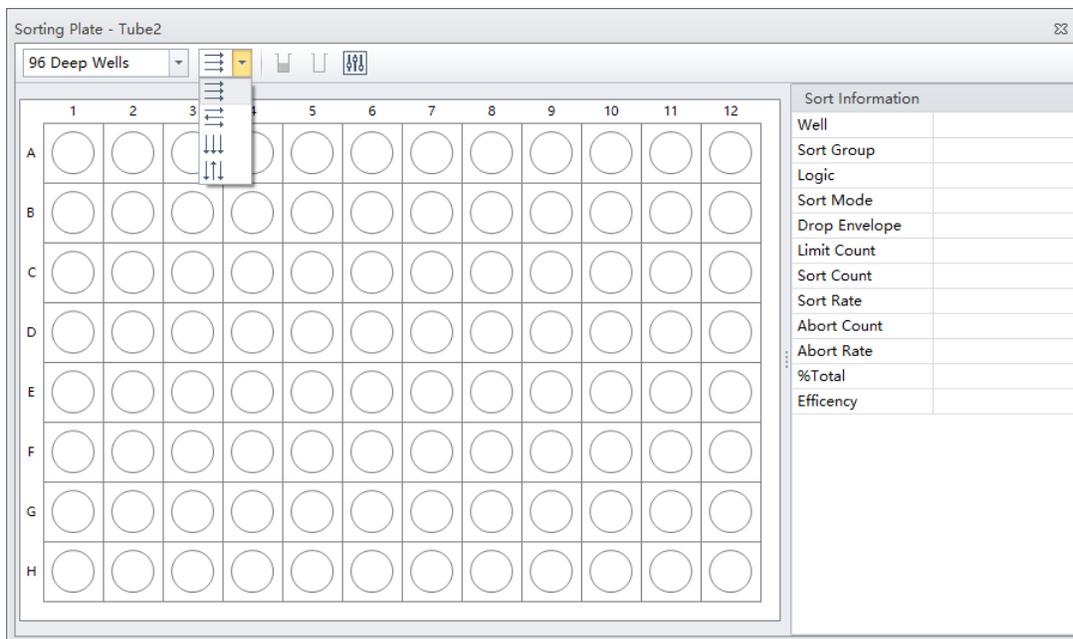


[Slide]

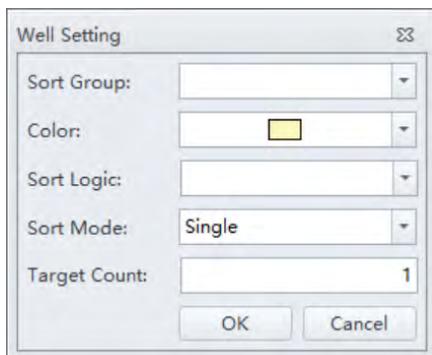


NOTE The screen varies according to the type of collection devices.

7 Set the sorting sequence.

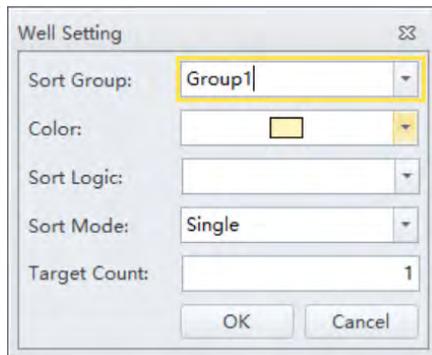


8 Select the desired wells, and select  to set the well settings for the desired wells. The Well Setting window appears.

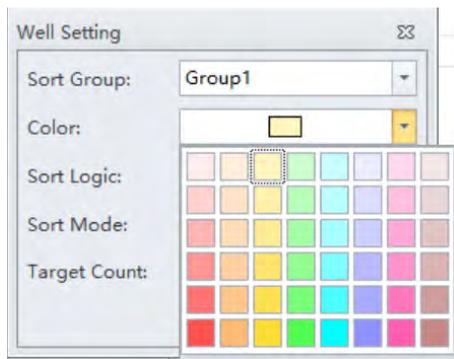


NOTE Ensure that the gates are created prior to this well settings.

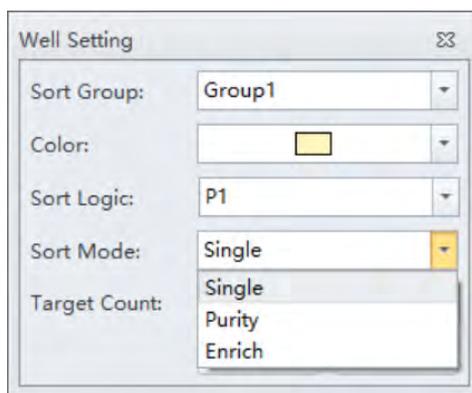
- a. Enter a name for the group of desired wells.



- b. Select a color for the group of desired wells.

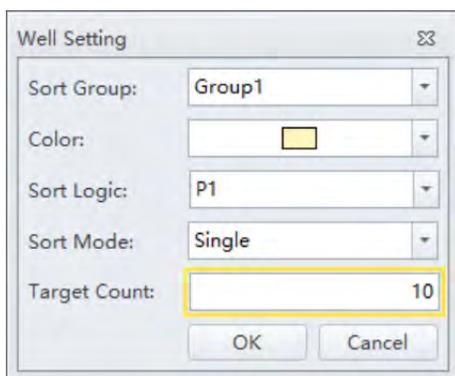


- c. Set the Sort logic.
- d. Set the Sort Mode for this group.



NOTE For explanations about Sort Mode, refer to [Sort Mode](#) in [CHAPTER 2, Using the CytExpert SRT Software](#).

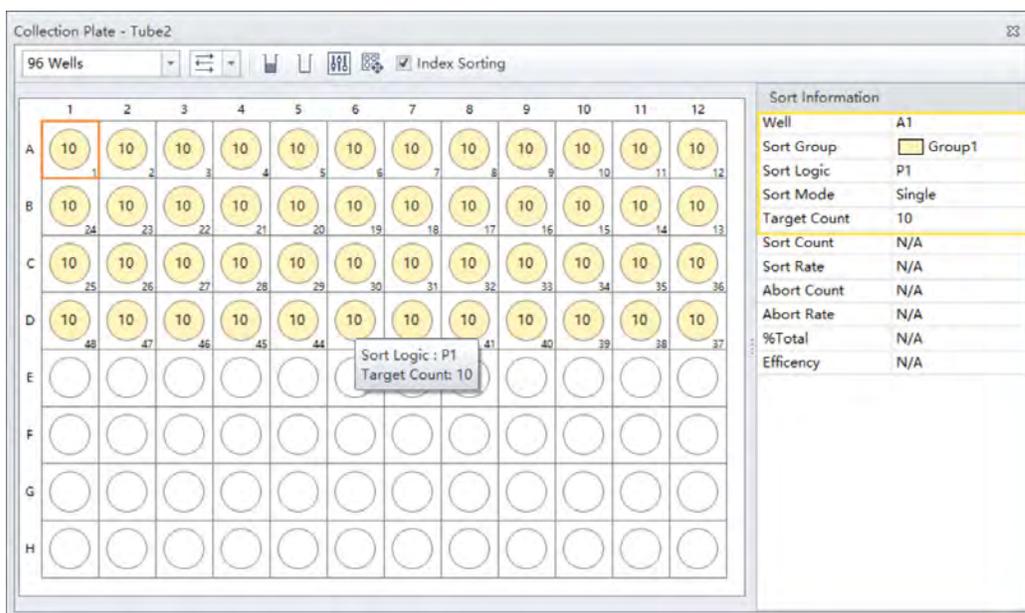
- e. Set the Target Count for each well.



NOTE The range of target count is 1- 1,000,000.

The CyClone Movement system automatically moves to next well when the sorted events in a well reach the target. The sorting stops until all the desired wells reach the target.

- f. Select **OK**. The settings display in the Sort Information area.

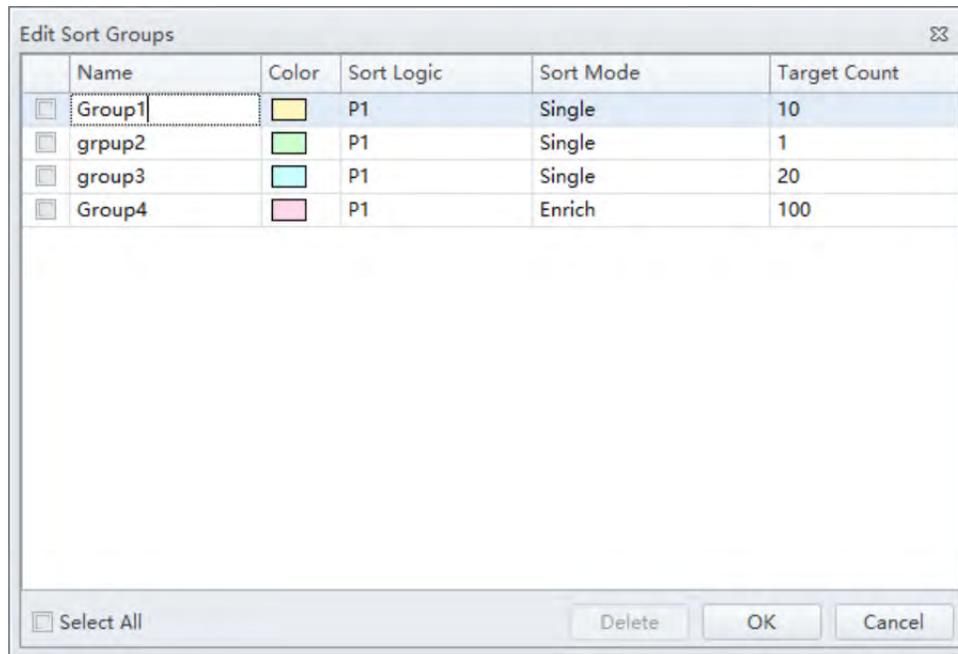


NOTE **Index Sorting** is selected by default. For explanations, refer to [Index Sorting](#).

NOTE To cancel the well settings, select the desired wells and select .

OR

Select  to set the well settings for several groups of desired wells.



9 Exit the Collection Plate window.

Stop Criteria for Sorting

Three stop sorting conditions are available for tube sorting. When multiple stop conditions are established, any one of these conditions stops the sorting process.

- **Events to Process.** Used to set the number of total events acquired.
- **Time to Sort.** Used to set the sorting duration.
- **Target Count.** Used to set the target number of cells to be sorted for each side stream L2/L1/R1/R2.

Explanation of restrictions:

Events to process: 1- 864,000,000

Time to sort: 1 - 28,800 seconds

Target count: 0- 864,000,000

NOTE Target count "0" means unlimited. Use this feature for the continuous sorting.

NOTE In plate sorting mode, only Target Count is available. Select  to access the Target Count tab.

NOTE The sorting stop criteria takes precedence over the collection stop condition in a sort. Refer to [Setting Collection Conditions](#).

Calibrating the Sort Collection Device

Calibrate the plate or slide position for the following cases:

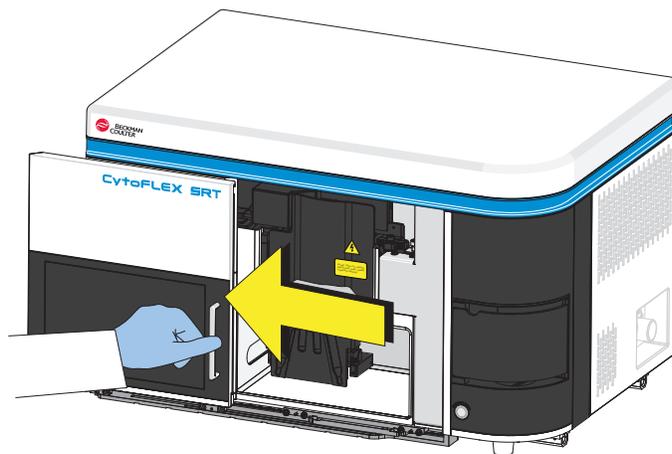
- Every time after running the sort calibration
- When switching the sort collection device from tube to plate, or vice versa.
- After using a new plate type
- After changing the manufacturers of a plate type
- After replacing the nozzle module

The software supports two ways to access the Collection Device Calibration window:

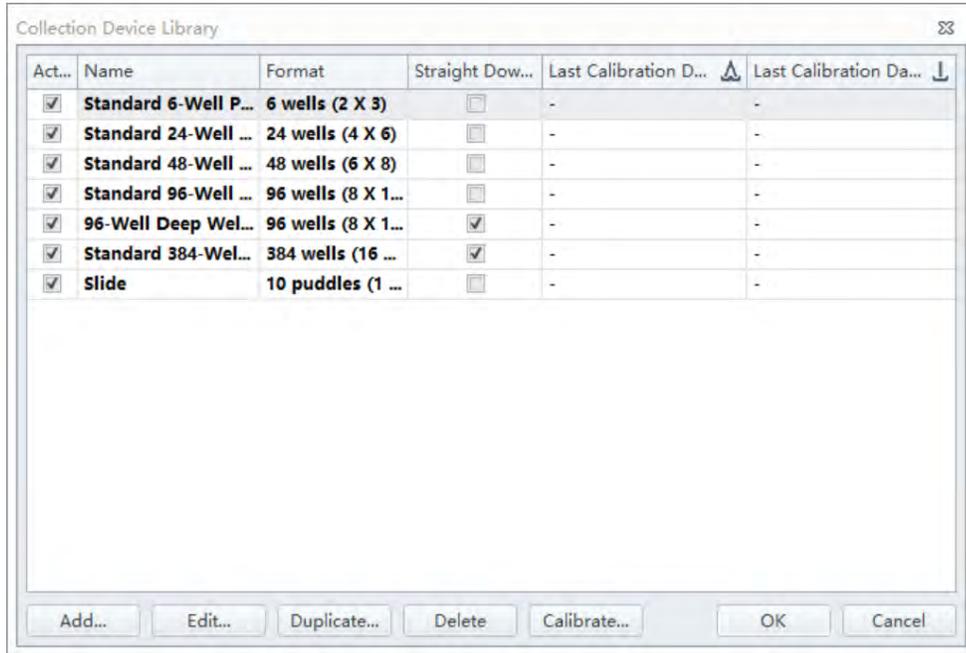
- Select **Advanced** > **Collection Device Library**. Then select **Calibrate** from the Collection Device Library window.
- Select  from the Tube management area to open the Collection Plate window. Then select  on the Collection Plate window.

NOTE The slide calibration archived in the Collection Device Library cannot be used for the Manual Drop Delay Test. Calibrate the slide again for the Manual Drop Delay Test at the initial use. Refer to [Setting Drop Delay Manually \(Optional\)](#).

- 1 Open the sort chamber sliding door.

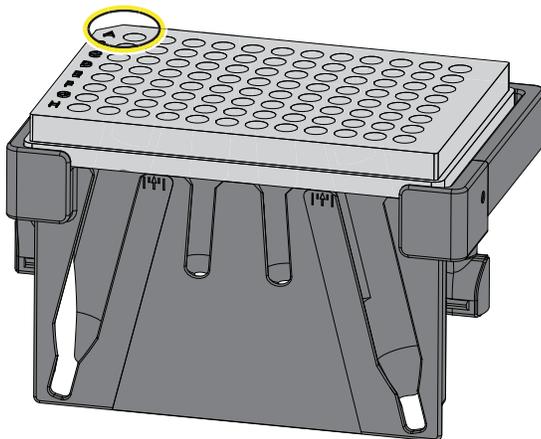


- 2 Select **Collection Device Library** in the Advanced menu. The Collection Device Library window appears.



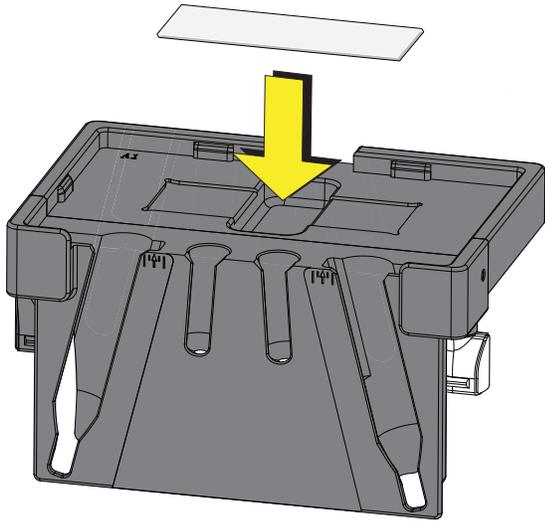
- 3 Select the collection device type from the Collection Device Library window and place a plate or slide on the output holder.

[Standard 96-Well Plate]



NOTE Ensure plate well A1 aligns with position A1.

[Slide]



- 4 Select **Calibrate** on the Collection Device Library window. The Collection Device Calibration window appears.

[Standard 96-Well Plate]

Collection Device Calibration(Default) ⌵

Name:



UL



UR



LL



LR

Label	X(mm)	Y(mm)
▶ UL	111.0	72.0
UR	12.2	72.0
LL	111.0	9.2
LR	12.2	9.2

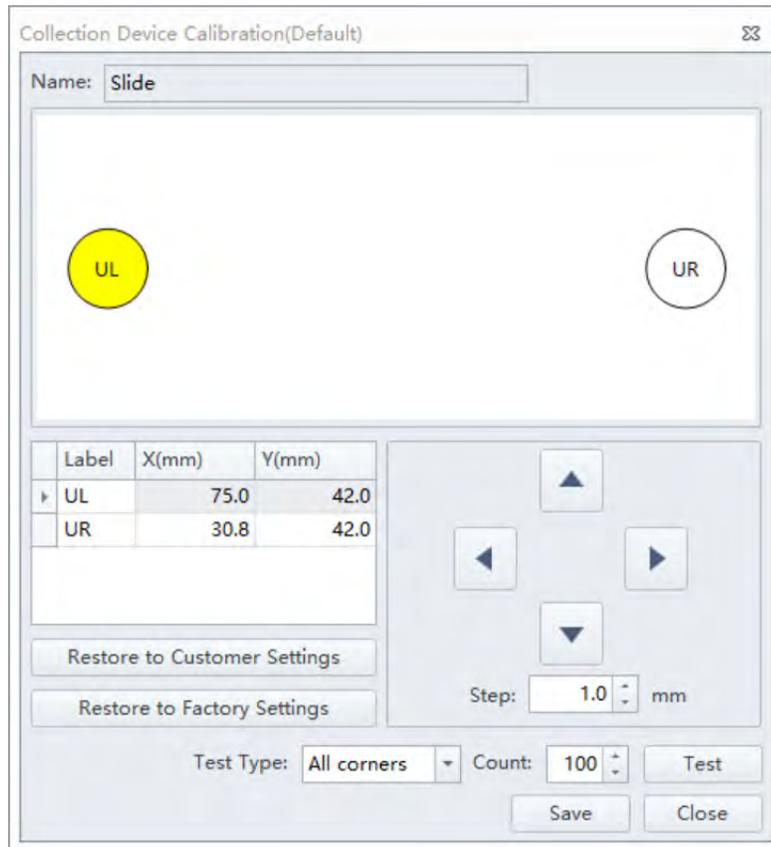


Step: mm

Test Type:

Count:

[Slide]



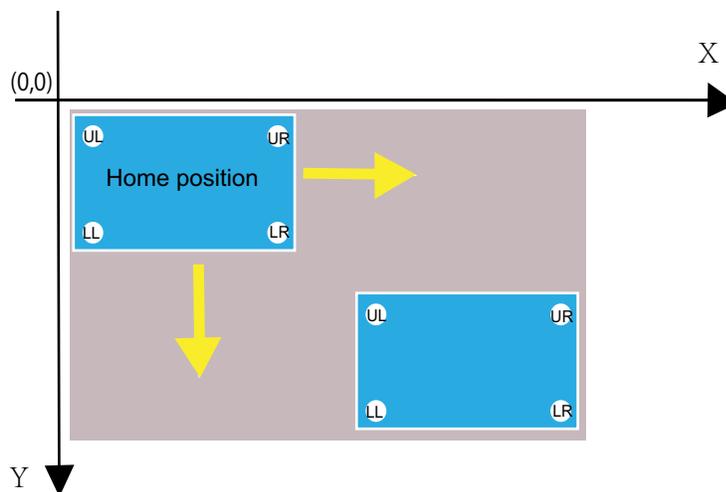
5 Select the well to be calibrated.

NOTE The selected well is highlighted in yellow.

- UL: upper-left well A1.
- UR: upper-right well A12.
- LL: lower-left well H1.
- LR: lower-right well H12.

- 6 Select  and  or  and  to adjust the well positions in the X-, and Y-axes. You can type a number as well.

The X-axis arrows moves the well position left and right. The Y-axis arrows moves the well position forward and back.



NOTE Use **Step** to set the increment for each step.

- 7 Set the Test Type to specify how many wells to deposit each time. **All corners** is set by default.

[Standard 96-Well Plate]

Collection Device Calibration(Default) ☰

Name:



UL



UR



LL



LR

Label	X(mm)	Y(mm)
UL	110.0	72.0
UR	12.2	72.0
LL	111.0	9.2
LR	12.2	9.2

▲

◀ ▶

▼

Step: mm

Test Type: All corners ▼

- Current well
- All corners

Count:

8 Set the count of droplets for each well.

[Standard 96-Well Plate]

Collection Device Calibration(Default) ⌵

Name:



UL



UR



LL



LR

Label	X(mm)	Y(mm)
UL	110.0	72.0
UR	12.2	72.0
LL	111.0	9.2
LR	12.2	9.2

Restore to Customer Settings

Restore to Factory Settings

Test Type: All corners

Step: mm

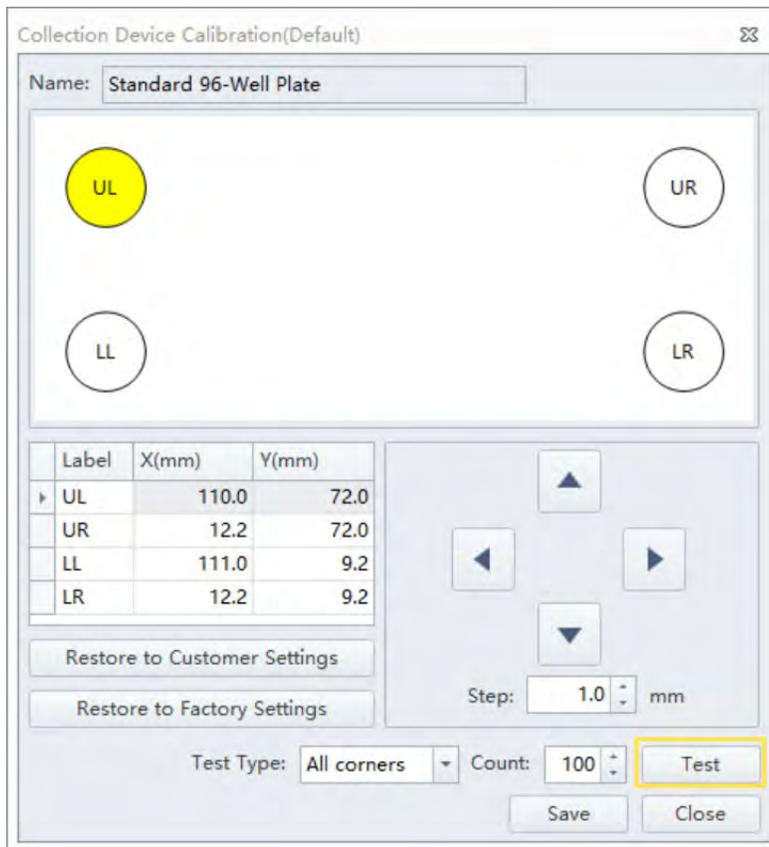
Count:

Test

Save Close

- 9 Select **Test** to deposit a small puddle over the wells.

[Standard 96-Well Plate]

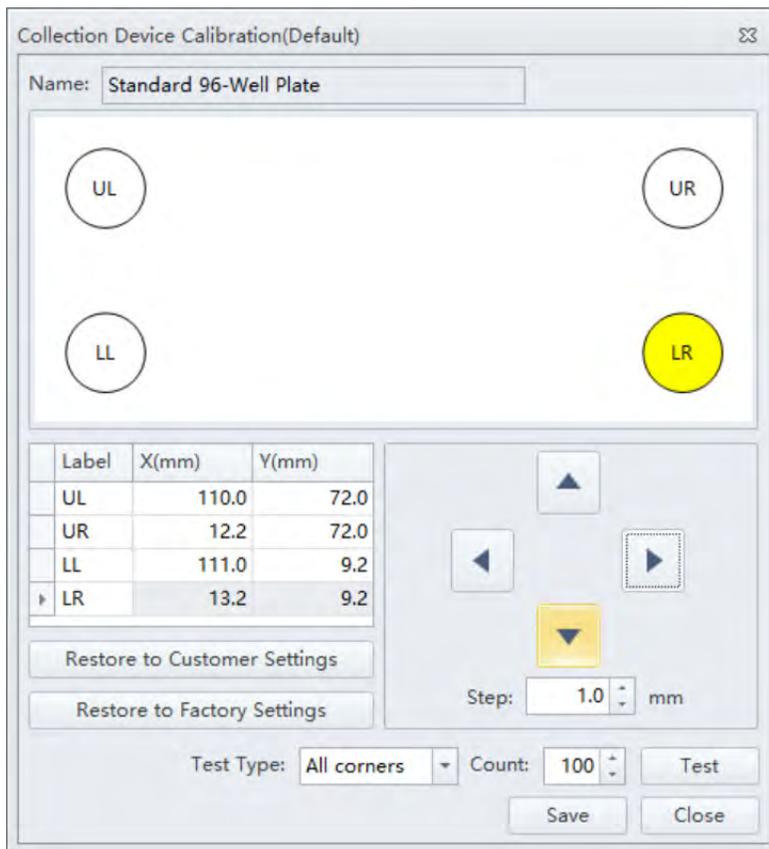


- 10 Remove the plate lid or slide and verify that the drops are located in the center of each well at the corners.

NOTE If the drops are not aligned with the center of the well, repeat steps 6-9 until the stream R1 hits the center of each well.

- 11 Select **Save** to save the calibrated settings.

[Standard 96-Well Plate]



NOTE Select **Restore to Customer Settings** to discard the current calibration and use the previous calibration setting.

Select **Restore to Factory Settings** to set back to the factory default.

12 Select **Close** to exit.

13 Close the sort chamber sliding door.

Manual Side Stream Calibration (Optional)

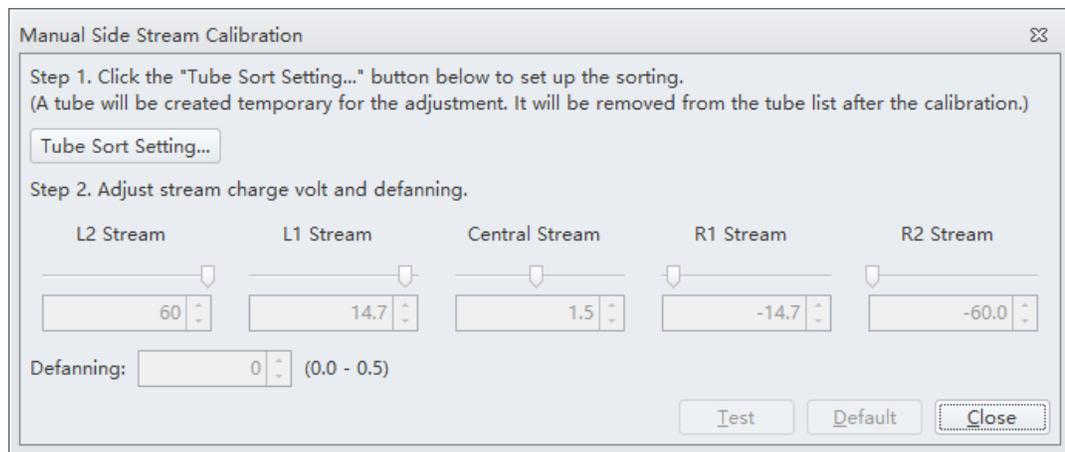
This function is for advanced users to fine tune the charge voltage settings to ensure that the drops are deposited into the center of a tube.

IMPORTANT The charge voltage and defanning setting can cause the side stream detection to be inaccurate. The system will deactivate the Side Stream Monitor function when the manual charge voltage or defanning setting takes effect. To restart the Side Stream Monitor, you need to perform the Sort Calibration again. Refer to [Sort Calibration \(Auto Drop Delay\)](#).

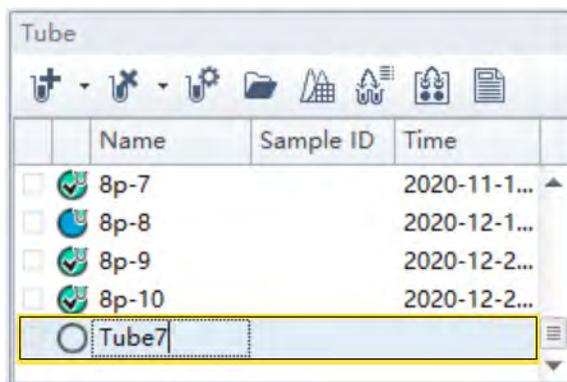
1 Select **Initialize** to put the instrument in the Ready state.

NOTE Skip this step if the instrument has already been initialized.

2 Select **Manual Side Stream Calibration** from the Sorting menu. The Manual Side Stream Calibration window displays.



3 Select **Tube Sort Setting**. A new tube is created automatically in the Tube Management screen.



The Tube Sort window displays.

Tube Sort - Tube7

Processed Events: 0 Elapsed Sorting Time: 00:00:00 Remaining Time: 00:00:00

Stop Criteria: Events to Processed: Events Time to Sort: -

L2 L1 R1 R2

⌵ Settings

Sort Logic:	<input type="text" value="P1"/>	<input type="text" value="P2"/>	<input type="text" value="P3"/>	<input type="text" value="P4"/>
Sort Mode:	<input type="text" value="Purity"/>	<input type="text" value="Purity"/>	<input type="text" value="Purity"/>	<input type="text" value="Purity"/>
Target Count:	<input type="text" value="1,000,000"/>	<input type="text" value="1,000,000"/>	<input type="text" value="1,000,000"/>	<input type="text" value="1,000,000"/>
Tube Size:	<input type="text" value="5ml"/>	<input type="text" value="5ml"/>	<input type="text" value="5ml"/>	<input type="text" value="5ml"/>
Initial Volume:	<input type="text" value="0ml"/>	<input type="text" value="0ml"/>	<input type="text" value="0ml"/>	<input type="text" value="0ml"/>

⌵ Statistics

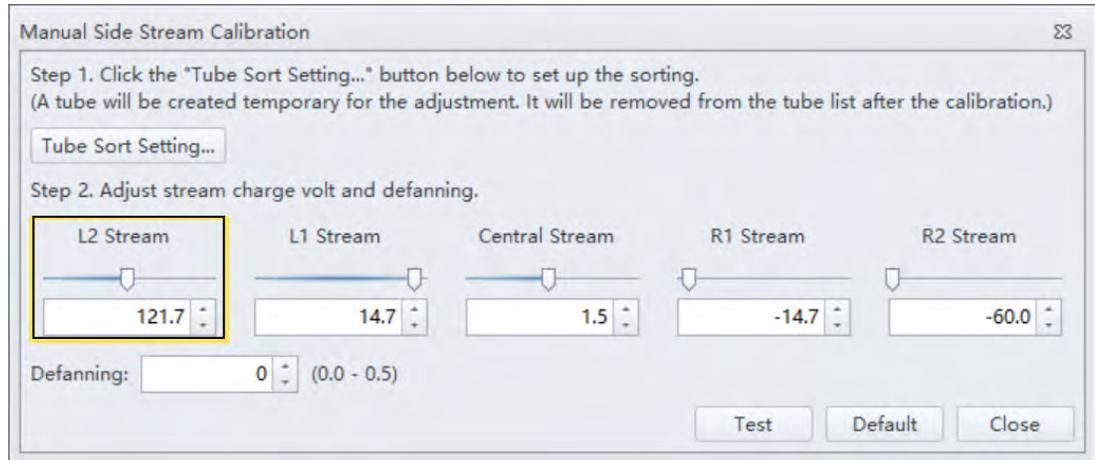
Sort Count:	<input type="text" value="0/1,000,000"/>	<input type="text" value="0/1,000,000"/>	<input type="text" value="0/1,000,000"/>	<input type="text" value="0/1,000,000"/>
Sort Rate:	<input type="text" value="0.0 evt/s"/>			
Abort Count:	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
Abort Rate:	<input type="text" value="0.0 evt/s"/>			
%Total:	<input type="text" value="0.00%"/>	<input type="text" value="0.00%"/>	<input type="text" value="0.00%"/>	<input type="text" value="0.00%"/>
Efficiency:	<input type="text" value="0.00%"/>	<input type="text" value="0.00%"/>	<input type="text" value="0.00%"/>	<input type="text" value="0.00%"/>
Total Volume:	<input type="text" value="0%"/>	<input type="text" value="0%"/>	<input type="text" value="0%"/>	<input type="text" value="0%"/>

Close

- 4 Set up the tube sort settings. Refer to [Setting Up Tube Sorting](#).
- 5 Open the sort chamber sliding door and place a clean slide over the top of tubes.

NOTE The slide is for viewing the position of drops into a tube.
- 6 Select **Close** to exit the Tube Sort window.

- Adjust the charge voltage to set the deflection angle for L2 stream.



Explanation of restrictions:

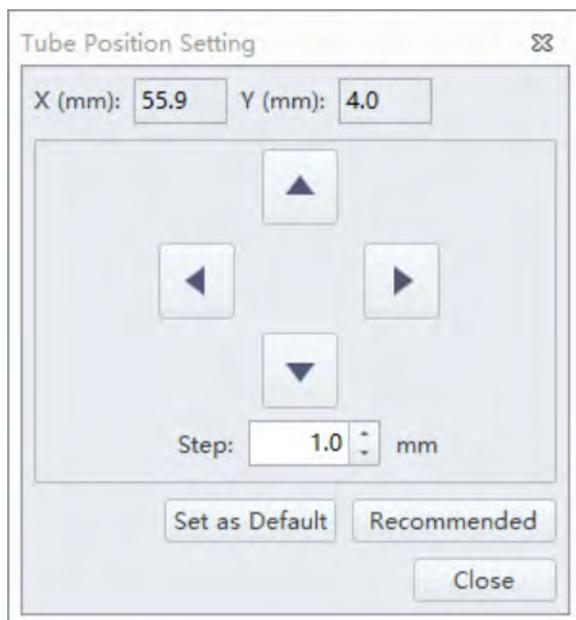
- L2 Stream: 60 ~180 V
- L1 Stream: 10 ~ 100 V
- Core Stream: -40 ~ 40 V
- R1 Stream: -10 ~ 100 V
- R2 Stream: -60 ~ -180 V

- Adjust the defanning.
- Select **Test** to deposit drops on the slide.
- Select **Stop** to stop the sorting.
- View the drops that were deposited on the slides. If the drops are not in the center of the tubes, repeat Steps 7-10 until the drops are correctly positioned.
- Repeat Steps 7- 11 to adjust the charge voltage for other side streams.
- Select **Close** to exit the Manual Side Stream Calibration. The calibrated charge voltages are applied automatically.

NOTE Select **Default** to discard the calibrated charge voltage setting and use the charge voltage setting which was calibrated by the last sort calibration.

Adjusting Tube Position (Optional)

The Tube Position Setting allows you to slightly adjust the output holder position in the X-axis and Y-axis to align the sort tubes with the side streams when a sorting is in process. This function is helpful when all the side streams are slightly tilted to one side. Select **Sorting > Tube Position Setting** to access the Tube Position Setting window.

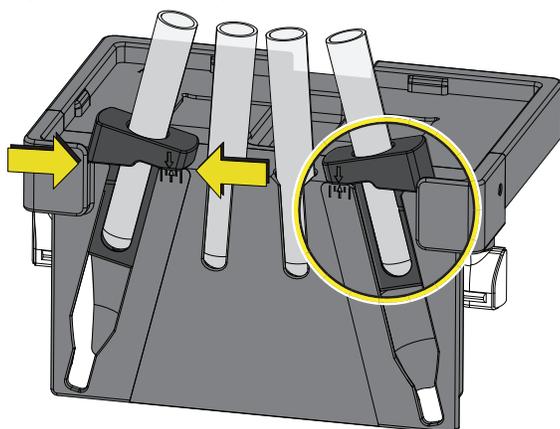


NOTE The Tube Position Setting is only available when tube sorting is in process.

NOTE Select **Set as Default** to set the current position as the default setting. Select **Recommended** to return to the factory setting.

NOTE You can manually fine tune the L2/R2 tube positions in the X-axis if your sort output holder has already been upgraded to the new design (refer to [Figure 5.9](#)). This feature is helpful when the L1/R1 streams are aligned with the L1/R1 tubes, but L2/R2 stream cannot be deposited into the L2/R2 tube center.

Figure 5.9 New Output Holder



Starting and Monitoring a Sort

During a Sort



Risk of contaminating the sorted sample. Do NOT open the sort protection door (refer to [Figure 1.22](#)) when a sorting is in process. Otherwise, the system disables the voltage to the deflection plates immediately, and a small amount of the waste stream (L1 stream) in the Straight Down mode will drop on a plate or slide due to the response delay of the waste catcher.

NOTE If you open the sort protection door during a sort, you will find that:

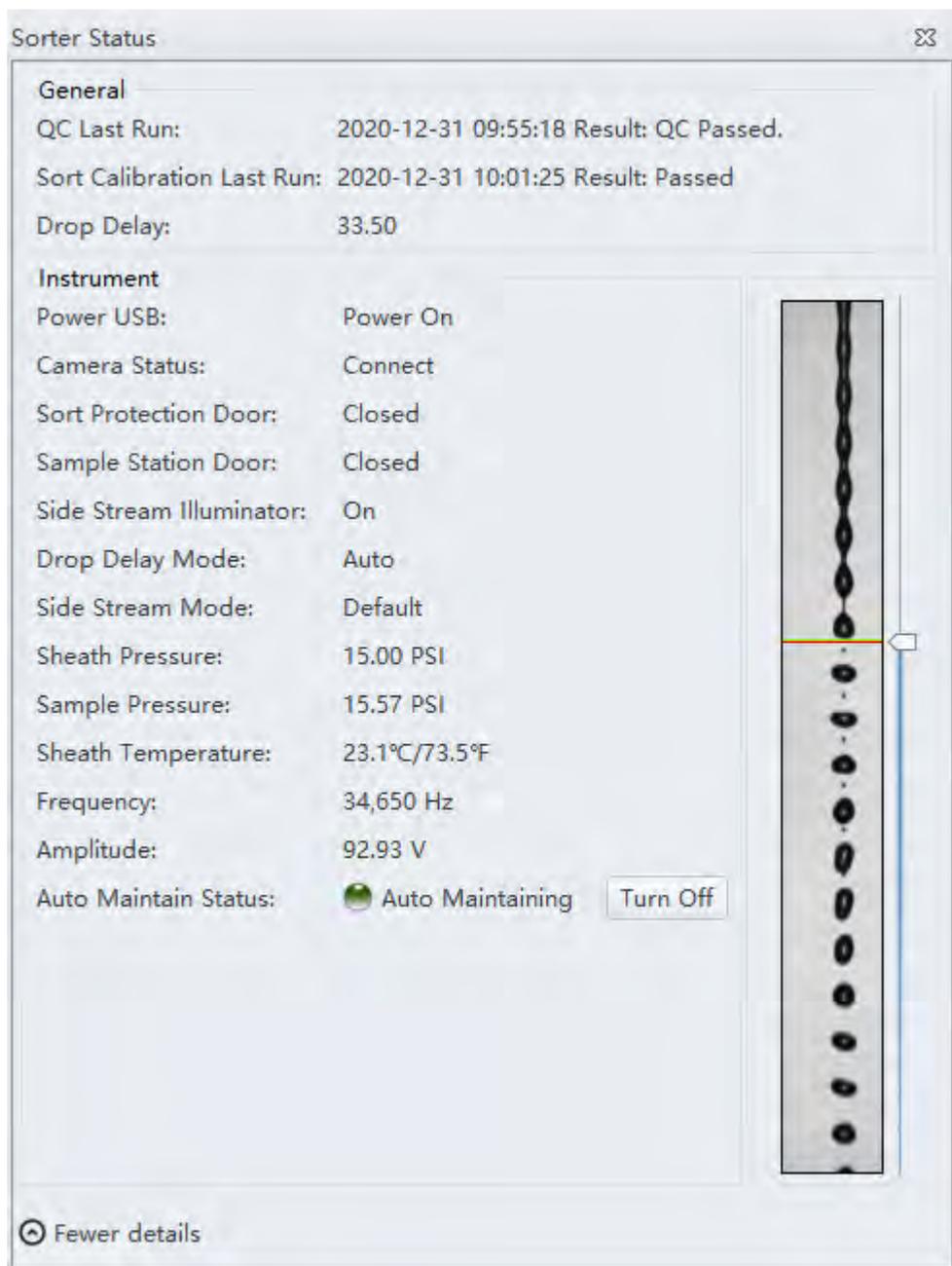
- the system stops the sorting abruptly
- the waste catcher moves into place
- sample flow halts
- the CyClone movement stops.

During a sort the following events occur:

- An event is detected at the interrogation point.
- A decision is made on the event to determine if it is a particle or cell that satisfies the sort and/or abort logic.
- If it is determined that a cell is to be sorted, the system waits until the cell reaches the Last Attached Drop.
- The electronics sends a charge through the sheath and sample stream. The charge can be various positive, negative, or neutral amplitudes depending upon the direction the drop is to be sorted.
- The last attached drop breaks off carrying the charge that was applied to the stream.

Viewing Sorter Status

Select  **Sorter Status...** in the lower right corner of the software screen, or select **Sorter Status** from the Sorting menu to access the Sorter Status window.



NOTE The green line indicates the break-off position at the moment when the Sort Calibration finishes. The green line is created automatically, and can be used as a baseline.

To mark the real-time break-off position, you can create a red line by dragging the slider where the droplet breaks. These two lines help you to monitor the stability of fluid.

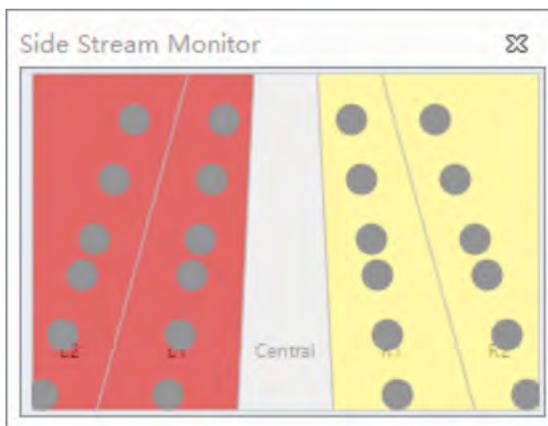
Side Stream Monitor

IMPORTANT The Side Stream Monitor is not available if the default voltage or defanning has ever been changed manually. You can re-start the Side Stream Monitor only after performing the Sort Calibration again. For instructions on Sort Calibration, refer to [Sort Calibration \(Auto Drop Delay\)](#).

IMPORTANT Open the Side Stream Monitor window and continue to monitor the side stream status during a sort. A neglected error might impact the sorting result.

Eight photo-diodes (PDs) behind the side stream detection windows, the side stream illumination source (refer to [Figure 1.22](#)) and the software make it possible to monitor whether the desired droplets are directed to the targeted sort output device and whether the waste stream is deposited into the waste catcher.

Select **Side Stream Monitor** from the Cytometer menu to access the Side Stream Monitor screen.



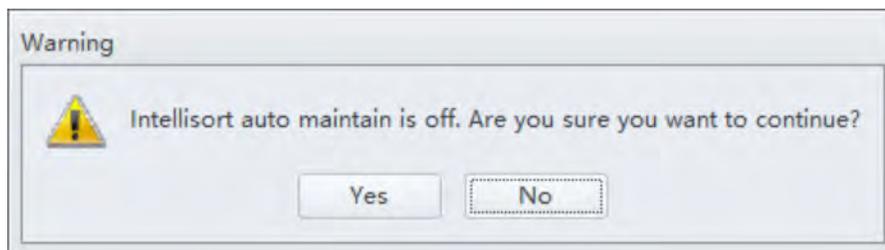
NOTE The droplet animation, which updates proportionally with the real-time sorting, is only for your reference.

Explanation of Background Colors:

- : Indicates a normal state that the percent of the detected droplets of a side stream L1/R1 relative to the charged droplets is $\geq 60\%$, or the percent of the detected droplets of a side stream L2/R2 relative to the charged droplets is $\geq 50\%$.
- : Indicates an abnormal state which requires your attention, for example, the sample contains macro-particles, or clumps, or the droplets are too quick for the software to respond.
- : Indicates that the detected droplets of a side stream are less than 30% of the charged droplets. You need pause the sorting and troubleshoot the problem.

Auto Maintain

IMPORTANT Ensure that the Auto Maintain is enabled prior to starting a sort. Otherwise, the following warning message appears.

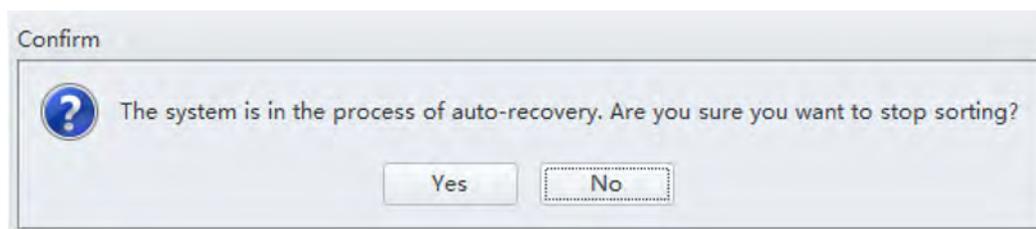


When a sort is in process, the software with the IntelliSort technology monitors the droplet stream and maintains the Drop Delay automatically, which is called Auto Maintain. If the software detects an instability, Auto Maintain modifies the amplitude parameter (without affecting the determined DD) to ensure that the sort continues uninterrupted with no operator intervention.

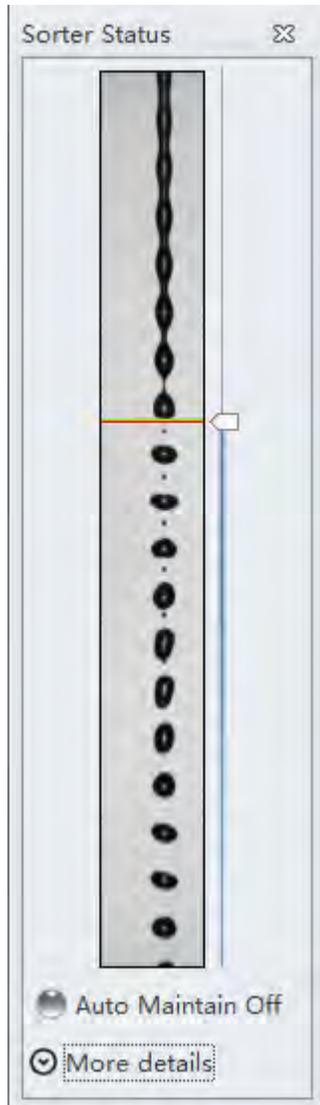
If the software detects a dramatic droplet-formation failure or stream instability which cannot be restored by Auto Maintain within 1 minute, Auto Maintain turns off and the system starts Auto Recovery automatically.

In Auto Recovery, the system sequentially stops the sample flow, extends the waste catcher, and starts Flow Cell De-bubble. If Auto Recovery succeeds, the system re-enters the Auto Maintain state and restarts sorting automatically. If Auto Recovery fails, the sorting stops and user intervention is required, for example, cleaning the Nozzle, removing the bubbles, and performing the sort calibration. For instructions, refer to [Cleaning the Nozzle](#) in [CHAPTER 10, Cleaning Procedures](#), or [Removing Trapped Air Bubbles](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

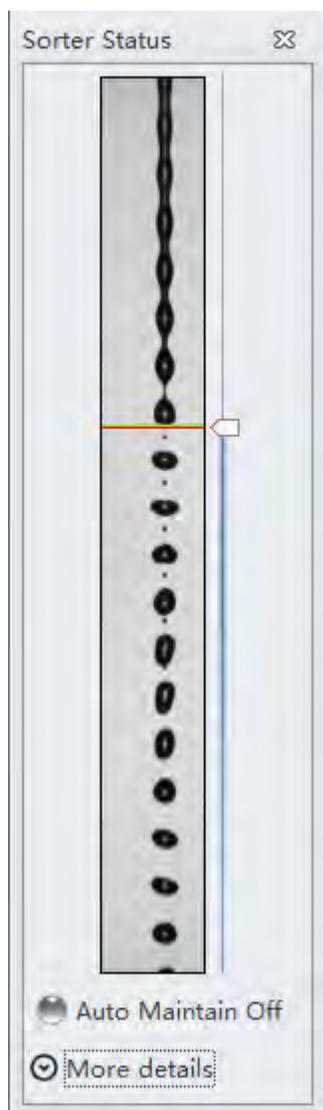
NOTE Auto Recovery can be stopped at any time by selecting **Stop** from the Sorting control screen. If you select **Stop**, the following message appears.



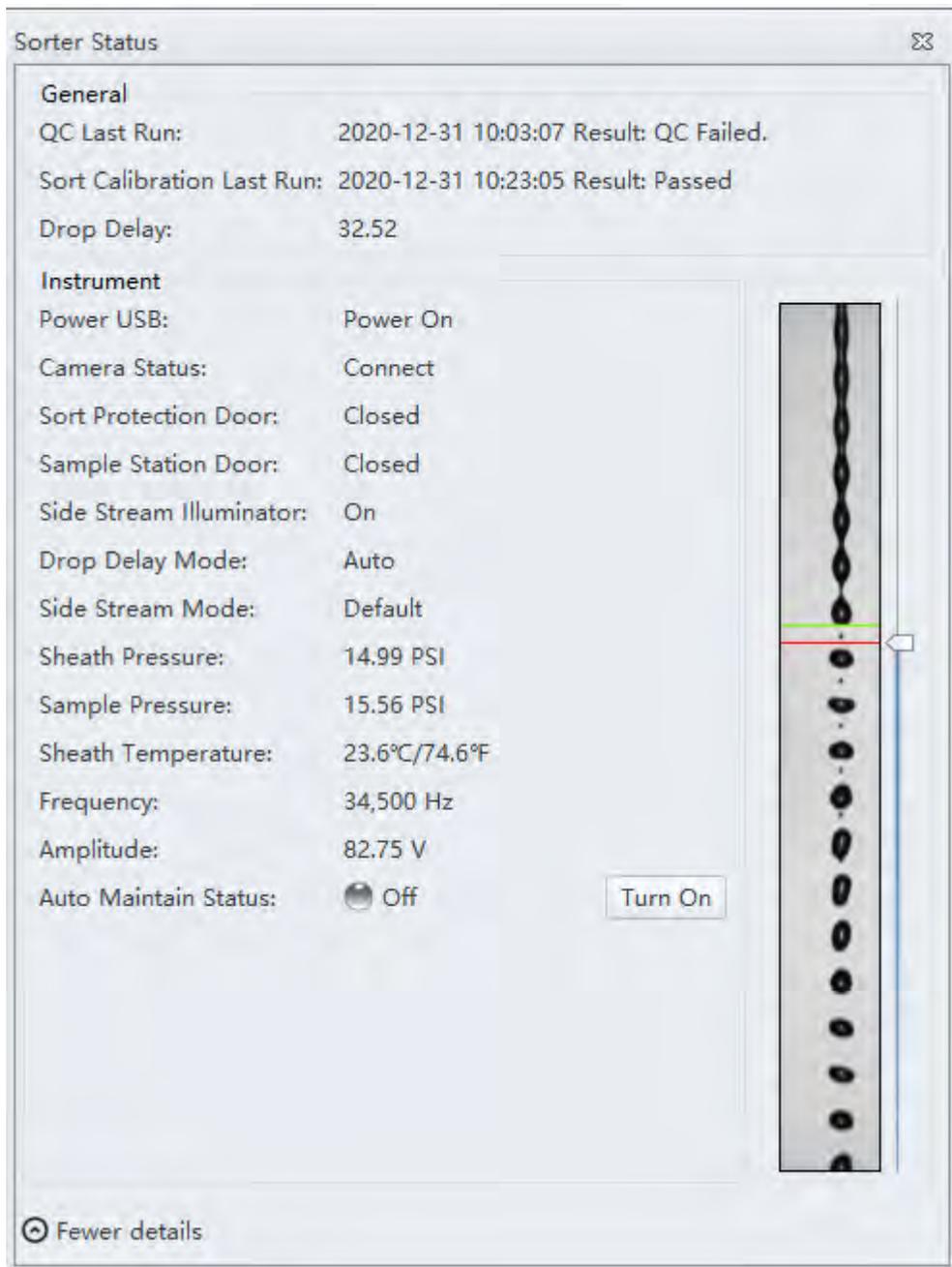
The status of Auto Maintain can be viewed by the icon  below the droplet view on the Sorter Status window. Following the instructions below to enable the Auto Maintain.



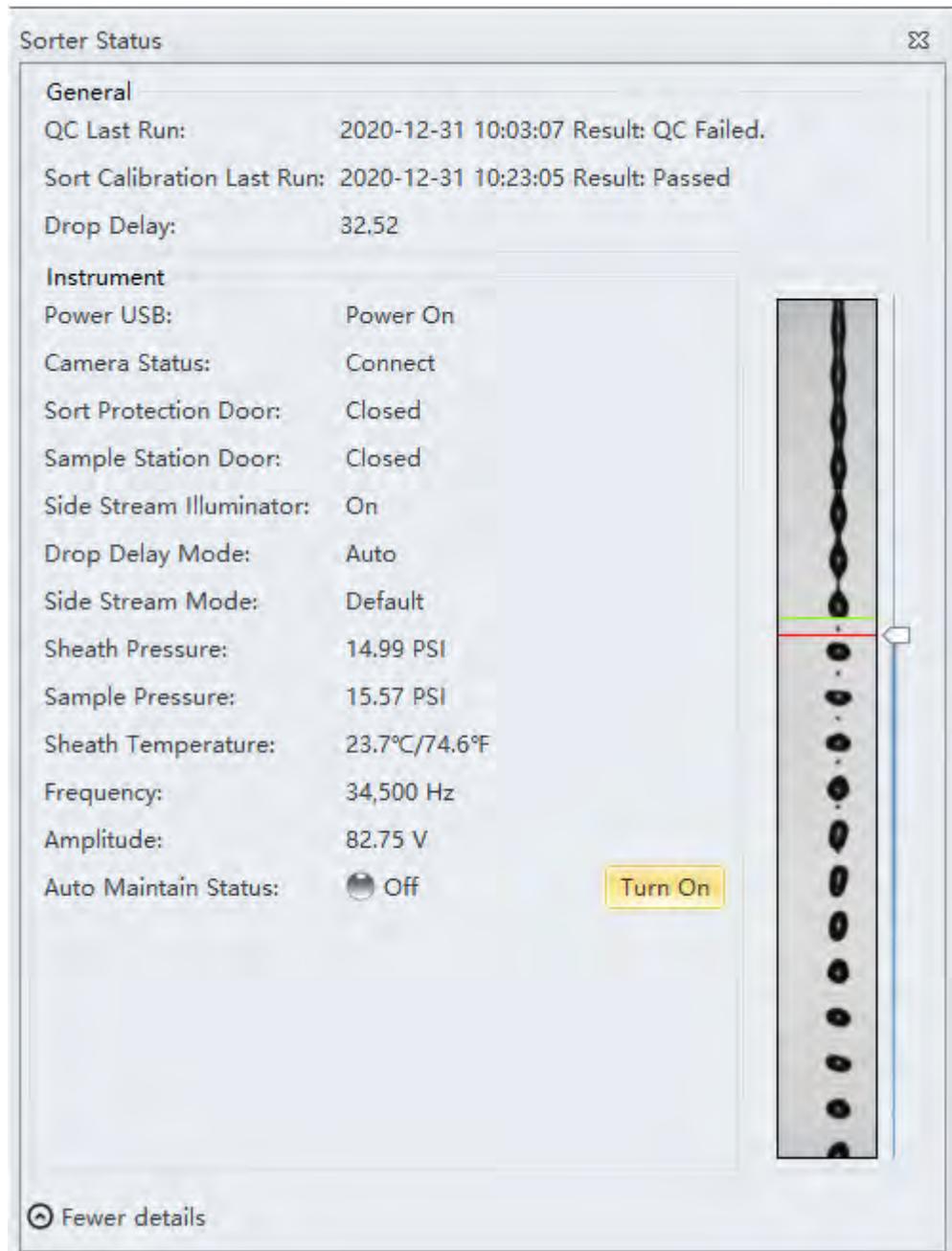
- 1 Select  **Sorter Status...** in the lower right corner of the software screen. The Sorter Status window appears.



- 2 Select **More details** to unfold the Sorter Status window.



3 Select **Turn On** to turn on the Auto Maintain.



The Auto Maintain icon turns green.



Viewing Sorting Statistics



Risk of sample loss and biohazardous contamination. Remove the sample collection tube when the tube is nearly full. The Tube Volume value on the software might not be accurate due to the various sample conditions. Use suitable laboratory attire when removing the sample.

Table 5.2 Sort Statistics

Screen Element	Function
Sort Count	Total positive events that have been sorted for the stream.
Sort Rate	Total sorted events per second for the stream.
Abort Count	Total positive events that have been aborted for the stream.
Abort Rate	Total aborted events per second for the stream.
% Total	The percent of positive sorted events relative to the Total Events for the acquisition.
Efficiency	The number of positive events sorted, divided by the total events that could have been sorted for the stream. $\text{sorted}/(\text{sorted} + \text{aborted})$
Total Volume	<p>The percent of the collection volume relative to the tube size. $(\text{number of charged drops} \times \text{droplet volume} + \text{initial volume})/\text{tube volume}$</p> <ul style="list-style-type: none"> • Grey: indicates the initial volume • Green: indicates that the collection volume is normal • Red: indicates that the collection volume reaches the limit. You need pause sorting and replace a new tube. <p>NOTE This Total Volume is based on theoretical estimation and may be inaccurate. Check the status of sample collection tube on a regular basis and replace the tube if it is nearly full.</p>

[Tube Sorting]

Tube Sort - Tube8

Processed Events: 447,922 Elapsed Sorting Time: 00:14:58 Remaining Time: 00:00:00

Stop Criteria: Events to Processed: Events Time to Sort:

L2 L1 R1 R2

Settings

Sort Logic: L2 L1 R1 R2

Sort Mode: Purity Purity Purity Purity

Target Count: 50,000 50,000 50,000 50,000

Tube Size: 5ml 5ml 5ml 5ml

Initial Volume: 0.5ml 0.5ml 0.5ml 0.5ml

Statistics

Sort Count:	50,000/50,000	50,000/50,000	50,000/50,000	50,000/50,000
Sort Rate:	500 evt/s	103 evt/s	158 evt/s	56 evt/s
Abort Count:	0	0	0	0
Abort Rate:	0.0 evt/s	0.0 evt/s	0.0 evt/s	0.0 evt/s
%Total:	11.16%	11.16%	11.16%	11.16%
Efficiency:	100.00%	100.00%	100.00%	100.00%
Total Volume:	61.31%	61.31%	61.31%	61.31%

Close

[Plate Sorting]

Plate Sort - Tube12

Standard 96-Well Plate

Index Sorting

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1

Sort Information

Well	E12
Sort Group	1
Sort Logic	P1
Sort Mode	Single
Target Count	1
Sort Count	1
Sort Rate	5 evt/s
Abort Count	0
Abort Rate	0.0 evt/s
%Total	100.00%
Efficiency	100.00%

Table 5.3 Additional Information for Plate Sorting

	<p>The green dot at the upper right corner indicates that the sorting of this well has completed. The target count of this well is 100. The sorting sequence is the 75th.</p>
	<p>The red square indicates that the sorting of this well is in process. 8 events have been collected.</p>
	<p>“0” indicates that this well is to be sorted. The sorting sequence of this well is the 77th.</p>

Pause Sorting and Resume Sorting

In certain situations, you may want to pause data acquisition but continue the sampling so you can view events, and then resume data acquisition later. If a sort collection device has become full, but the sort is not complete, pause the sort to replace the sort collection device.

NOTE If the sample tube is empty, you must stop the sorting and then select **Unload**.

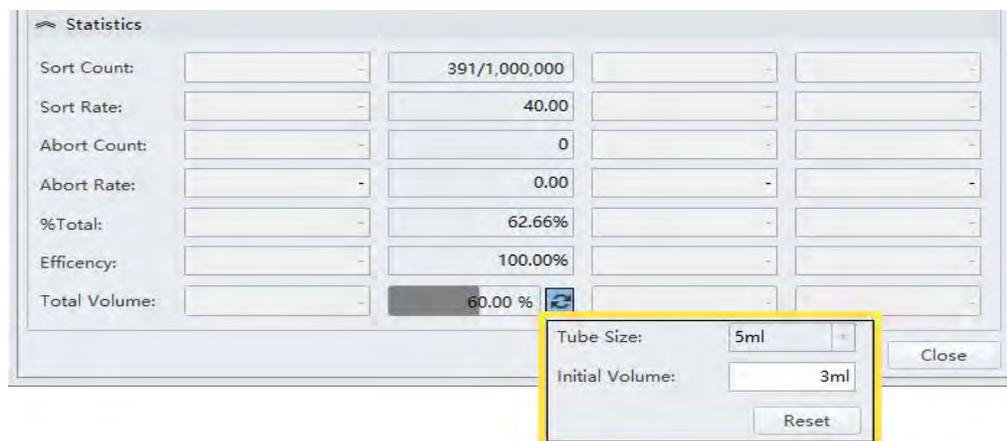
CAUTION

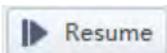
Risk of sample loss and biohazardous contamination if the sample collection tube overflows. Check the status of sample collection tube on a regular basis. Remove the sample collection tube when the tube is nearly full. Use suitable laboratory attire when removing the tube.

Replacing the Collection Devices

- 1 Select  .
- 2 Open the sort chamber door.
- 3 Replace the full collection device with a new one.

- 4 Select  to reset the initial buffer volume.



- 5 Select  to resume sorting and acquiring data.

Index Sorting

You can use the index sorting to sort single cells onto a plate or slide, and index the well or slide location to the collected parameters for that cell. The index sorting helps to ensure that a sorted cell with a specific phenotype has been sorted. Index sorting is useful in characterizing subpopulations of phenotypically similar events using post-sort genetic, chemical, and/or metabolic applications.

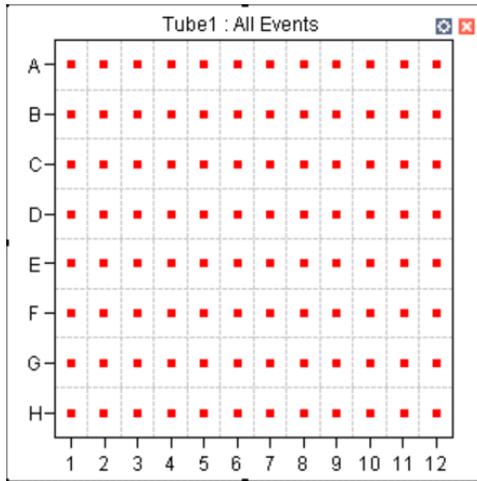
- 1 Create an experiment. Refer to [Creating an Experiment](#).
- 2 Load sample. Refer to [Sampling and Collecting Data](#).
- 3 Create the plots and gates needed to define the populations of interest.
- 4 Set up for sorting. Refer to [Setting Up Plate/Slide Sorting](#).

IMPORTANT If **Index Sorting** selected, the entire index sort data (within the acquisition recording limit) is automatically recorded during a sorting. You can export the data for post-sort analysis.

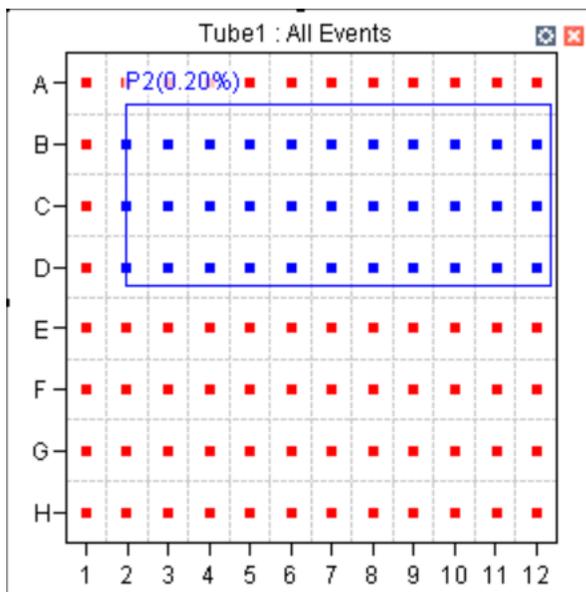
- 5 Verify that **Index Sorting** is selected.

6 Select **Sort** to start sorting.

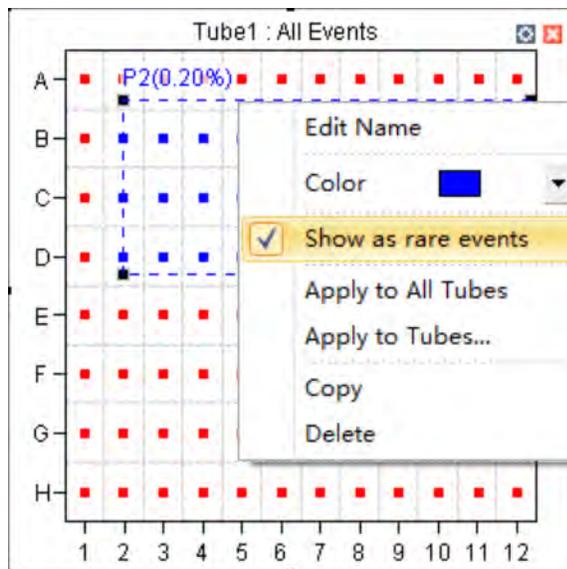
7 Select  from the drawing control tool bar to view the index sorting result.



8 Select the wells you want to index on the index sorting plot.

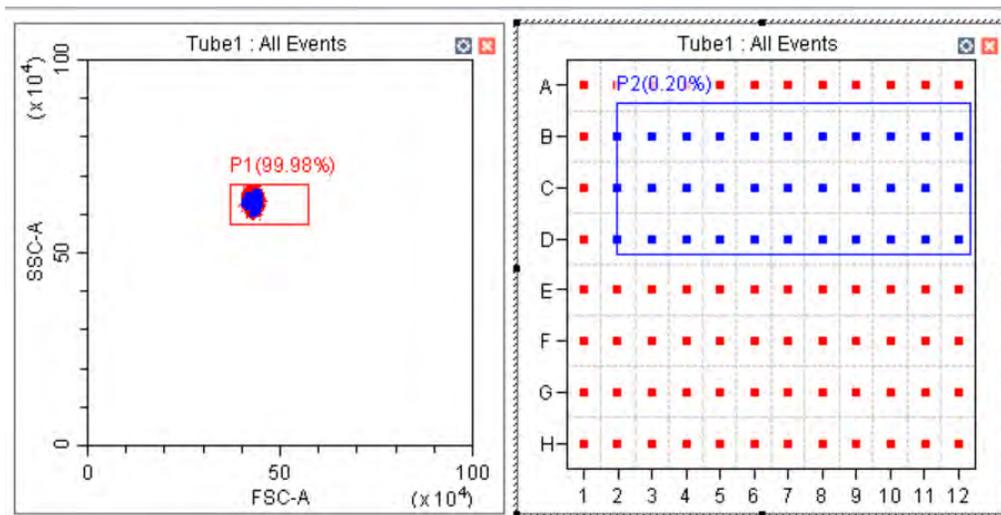


9 Right-click on the gate and select **Show as rare events**.



NOTE Show as rare events is to increase the visibility of the gated population anywhere they appear on the plots.

You can see that the location of the populations is highlighted on the plot.



Sort Report

Creating a Sort Report

To create a sort report, select  from the tube management area. A new sort report page appears.

Sort Report
☰ ☰

📄 🖨️ 🔧

Sort Report

Cytometer Name: Exp_20201105_T0032-Tube Sorting

Last QC Result: Failed on 2020-12-28 09:34:03

Stop Status: Manual

Processed Events(%): 100.00

Frequency: 34,800Hz

Drop Delay: 32.79

Auto Maintain State: On

Side Stream Mode: Auto

Software Name: CytExpert SRT

Software Version: 1.0.0.10193

Cytometer SN: 8p-10

Sort Device: Tube

Total Events: 3,851,636

Sorting Stream Mode: Default

Sheath Pressure: 15.00

Guardband: 15%

Drop Delay Mode: Auto

Start Time: 2020-12-28 09:40:20

End Time: 2020-12-28 11:49:13

Operator: JZHANG14

Threshold

Channel: FSC Height Mode: Manual Threshold Value: 101271

TriggerLogic: AND

Channel: SSC Height Mode: Manual Threshold Value: 251245

Gain

Channel	FSC	SSC	FITC	PerC P	APC	APC- A700	APC- A750	PB45 0	KO52 5	Viole t610	Viole t660	V780	PE	ECD	PC5	PC5.5	PC7
Gain	58	228	235	381	213	193	186	137	79	431	422	500	204	139	249	102	353

Compensation-Area

Autofl.	Channel	-FITC%	-Per CP%	-APC%	-AP C-A 700%	-AP C-A 750%	-PB4 50%	-KO 525%	-Vio let6 10%	-Vio let6 60%	-V7 80%	-PE%	-ECD%	-PC5%	-PC 5.5%	-PC7%
0.00	FITC	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PerCP	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC-A700	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC-A750	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PB450	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	KO525	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	Violet610	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	Violet660	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	V780	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
0.00	PE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
0.00	ECD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
0.00	PC5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
0.00	PC5.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00
0.00	PC7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Population Hierarchy

Designing a Report

Select  on the sort report tool bar then select a property element to add to a report.

NOTE The Sort Report Setting is different for tube experiment and plate experiment. Refer to [Figure 5.10](#) and [Figure 5.11](#).

Figure 5.10 Sort Report Setting-Tube Experiment

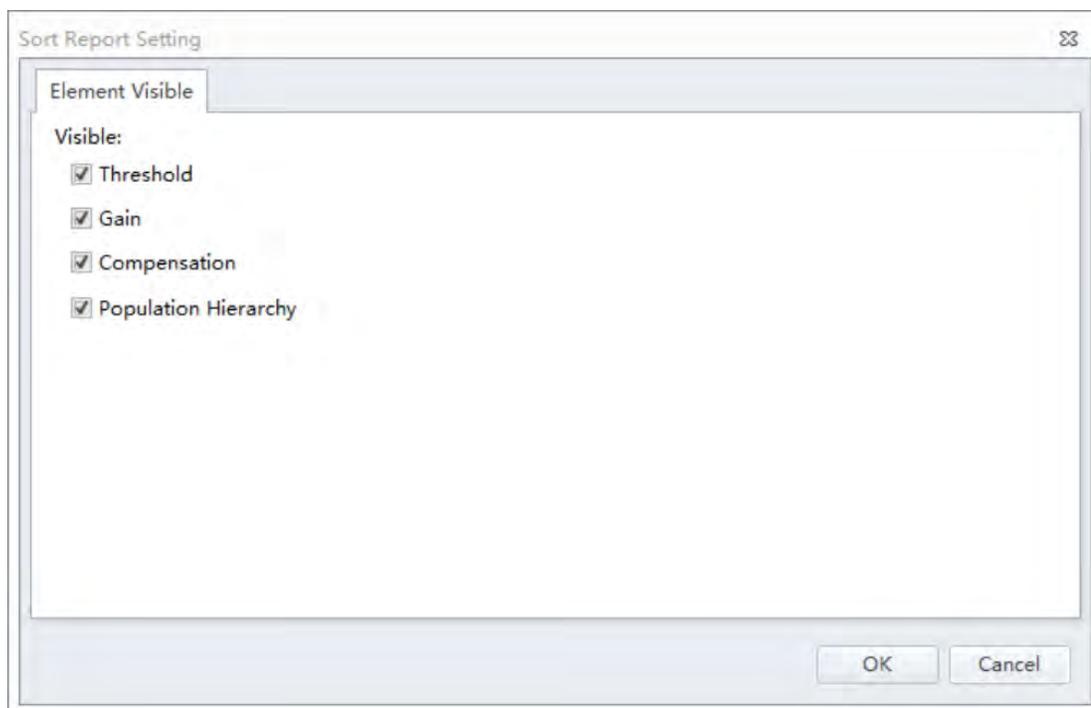
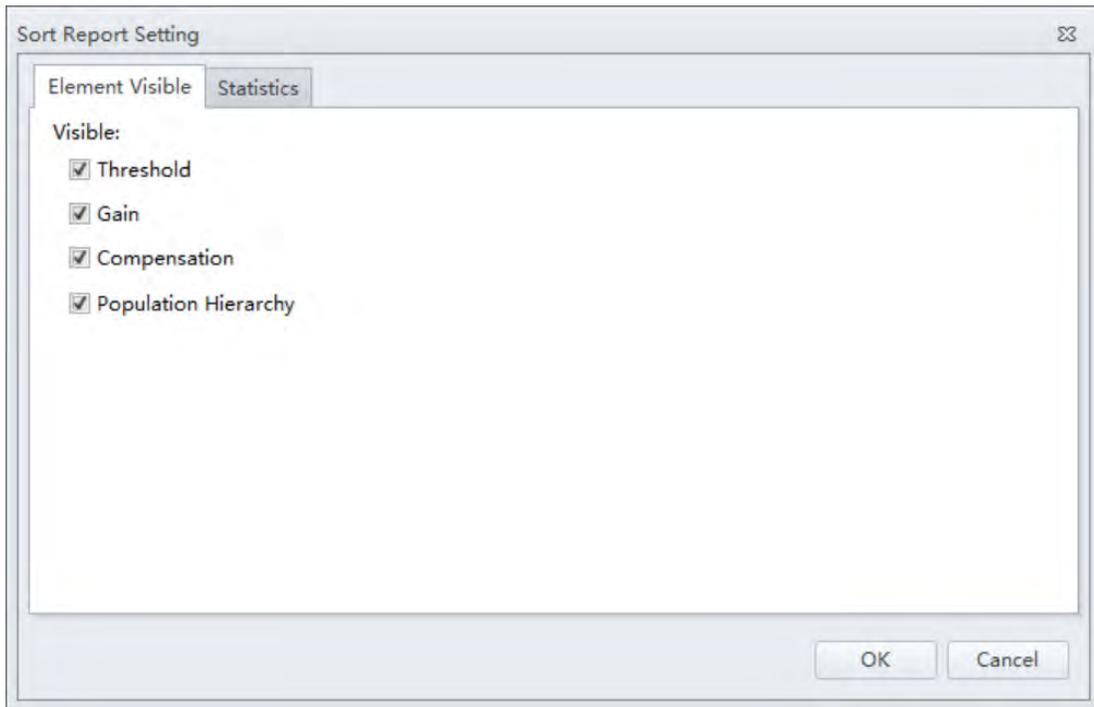
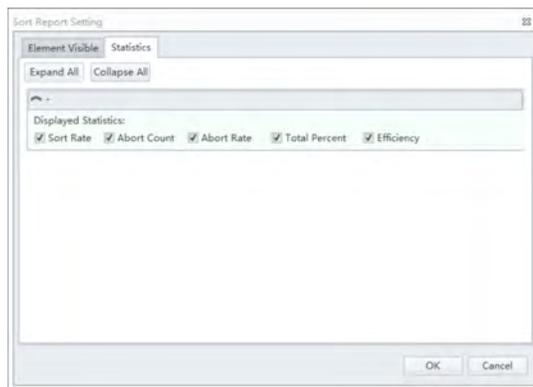


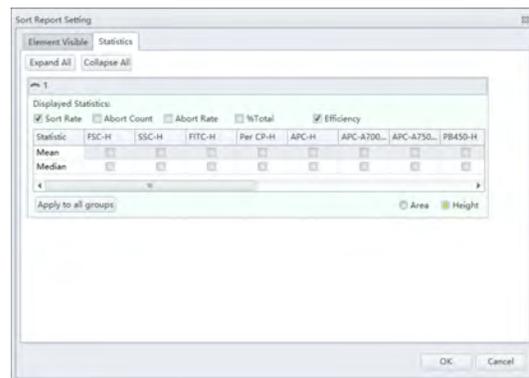
Figure 5.11 Sort Report Setting-Plate Experiment



Statistics-[With Index Sorting Disabled]



Statistics-[With Index Sorting Enabled]



Report Output

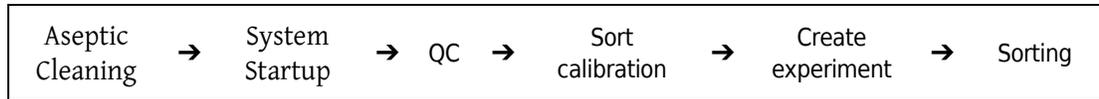
Reports can be either be printed or exported as a PDF or CSV file via the output icons on the report tool-bar. Refer to [Figure 5.12](#).

Figure 5.12 Output Options on Sort Report Toolbar



Additional Information for Aseptic Sorting

Aseptic Sorting Workflow:

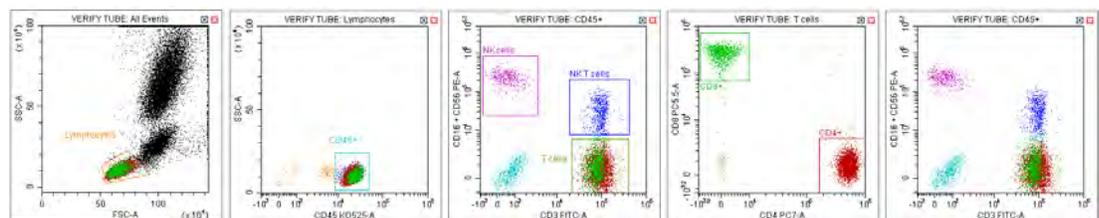


For instructions on the Aseptic Cleaning, refer to [Aseptic Clean Program](#) in [CHAPTER 10, Cleaning Procedures](#).

Exporting Data

1 Select the sample tube to be analyzed.

2 Establish new gates or adjust the position of existing gates. Refer to [Creating Plots and Gates](#).



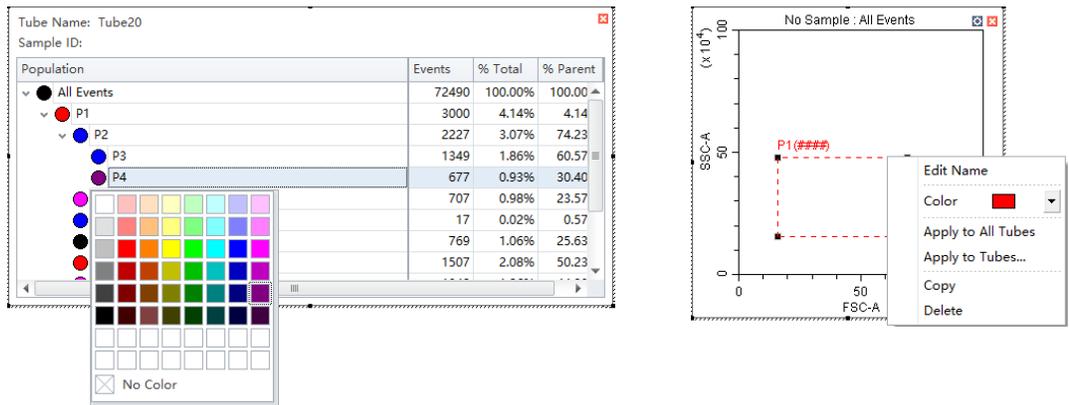
NOTE Changing a gate's position does not affect the positions of other gates already established on a given sample tube. Each test tube individually records the positions of its associated gates. If you need to make a change that concerns all the tubes, you must select the gate, then right-click the correctly positioned gate and select **Apply to All Tubes**.

3 Select . The Gate Hierarchy screen appears.

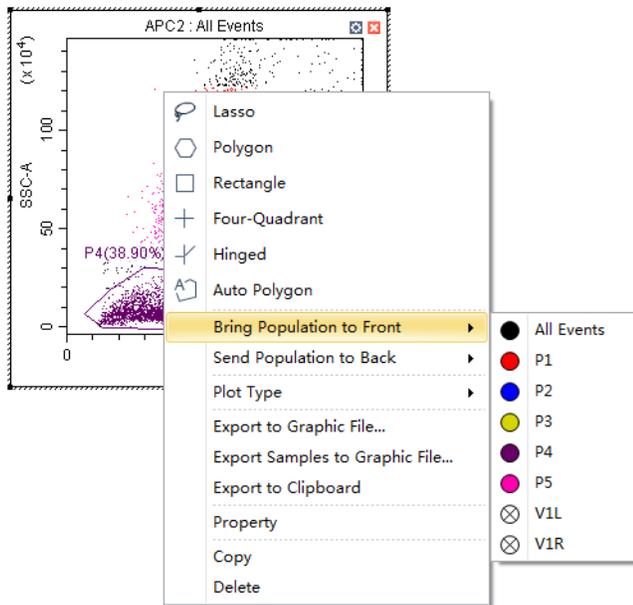
4 Check the relationship between the parent and daughter gates in the Gate Hierarchy window.

NOTE Newly added gates become subsets of populations displayed in plots with existing gates. The name and display color can be modified. Right-click directly on a gate plot to change the name and color.

NOTE Select **No Color** to leave the gated events uncolored while retaining the color of the parent populations. By default, the populations defined by a vertical gate, hinged gate, or four-quadrant gate are uncolored.



- 5 Right-click the plot and select **Bring population to front** to make the display color of the specified gate appear in front of all other colors, or select **Send population to back** to hide the display color of the specified gate behind all other colors.



- 6 Select  in the plot area to generate a statistical table.

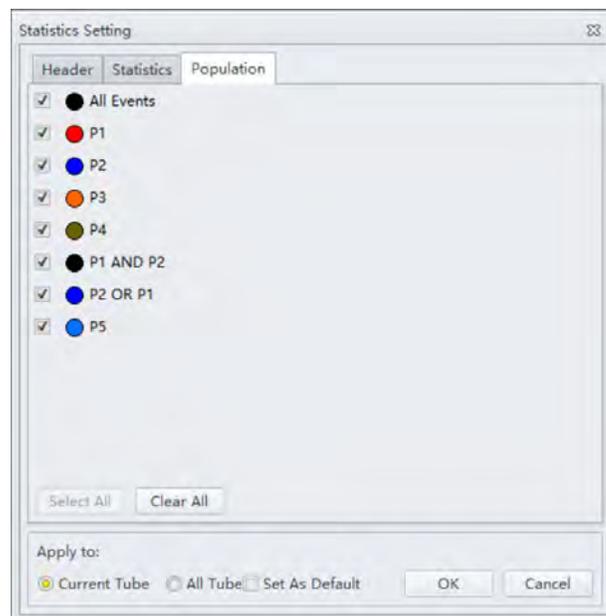
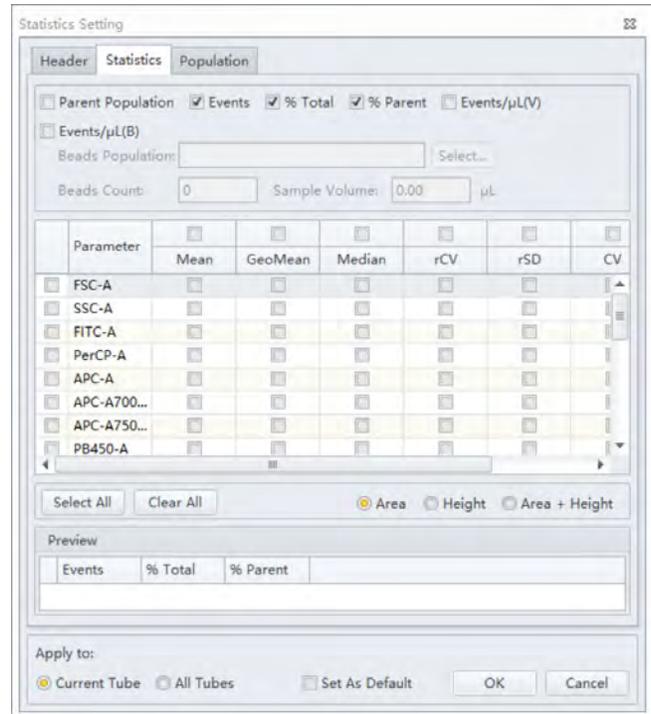
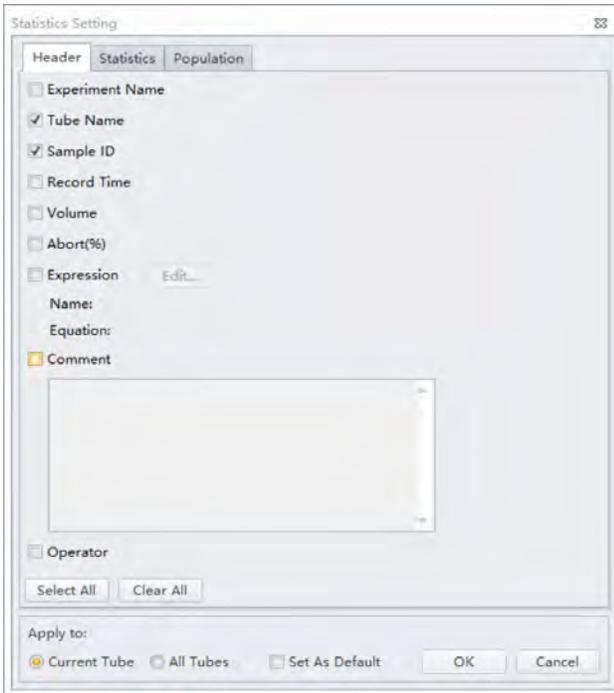
- 7 Right-click the table and select **Statistics Setting** to modify the settings of the statistics display parameters. The Statistics Setting window appears.

Tube Name: Tube31
Sample ID:

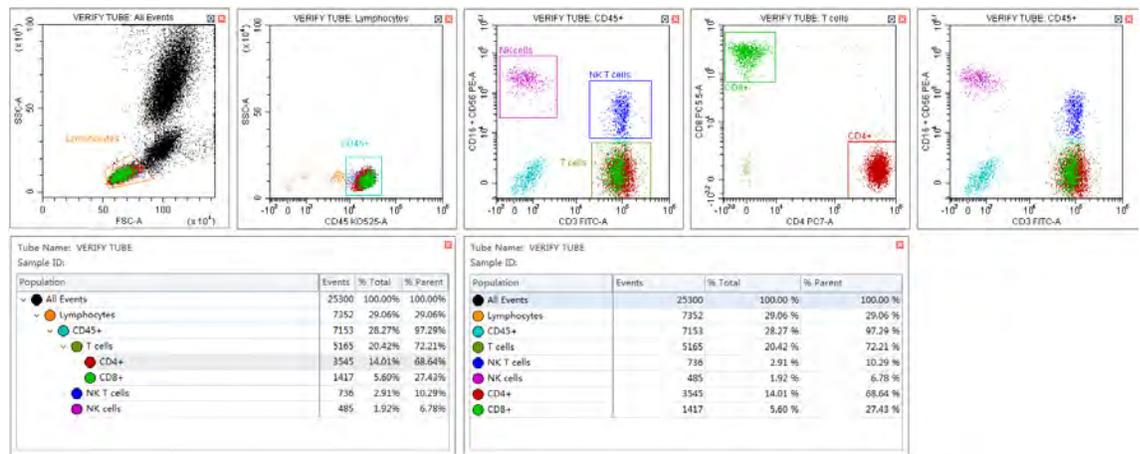
Population	Event	Parent
● All Events		100.00 %
● P1		24.68 %
● P2		2.04 %
● P3		7.73 %
● P4		0.00 %
● P1 AND P2		0.00 %
● P2 OR P1		26.72 %
● P5	887	22.34 %

- Export to CSV File...
- Export All Samples to CSV File...
- Export Samples to Graphic File...
- Export to Clipboard
- Export All Samples to Clipboard
- Statistics Setting
- Copy
- Delete

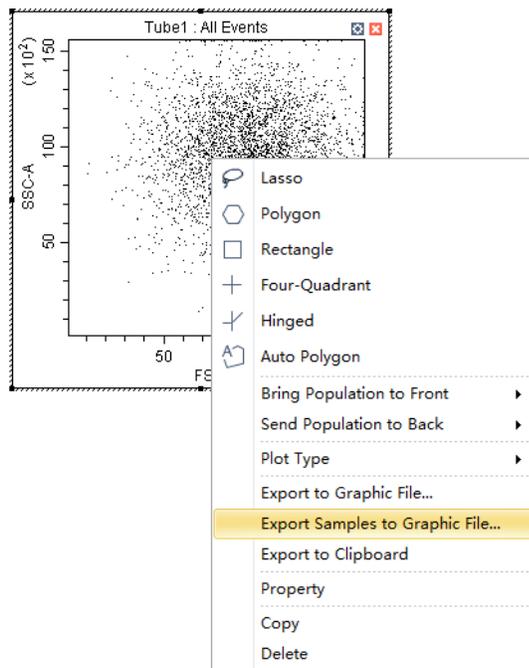
The Statistics Setting window allows you to change the display of the header, statistical elements and cell populations included.



The final generated plots appear as shown below.



- Right-click a plot and select **Export to Clipboard** or **Export to Graphic File** from the drop-down menu to select an image to export.



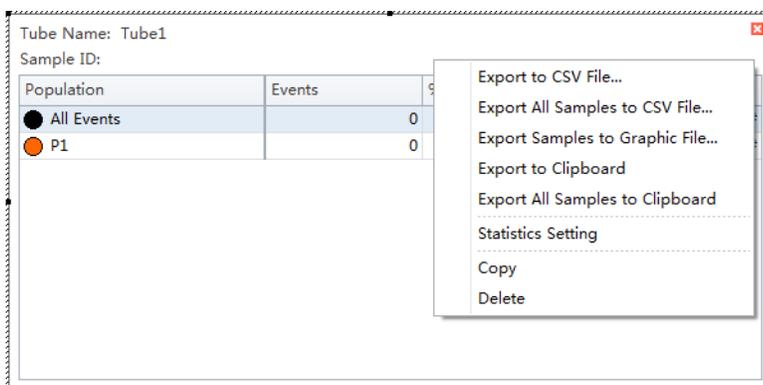
- Export to Clipboard** copies the plot to the clipboard, allowing you to paste it directly into documents in common file formats.

NOTE Multiple plots can only be copied and pasted into Microsoft® Word. If a single plot is copied, this can be pasted into both Microsoft® Word or Microsoft® PowerPoint.

- **Export to Graphic File** saves the plot as an image file.

NOTE **Export to Graphic File** can export plots in two selectable file formats. BMP bitmap format and EMF vector format.

- 9 To export statistics, right-click a statistical table to select any one of the available export options.



- **Export to CSV File** exports individual tube statistics as a single CSV file.
- **Export All Samples to CSV File** exports all tube statistics as a single CSV file.
- **Export to Clipboard** copies the statistics of an individual sample to the clipboard, allowing you to paste them directly into a Microsoft® Excel file or other file formats.
- **Export All Samples to Clipboard** assembles the statistics for all the sample tubes of an experiment and copies them together to the clipboard. From there they can be pasted as a group into a Microsoft® Excel file or other file formats.
- **Copy** converts a statistical table into an image format that can be pasted into documents.

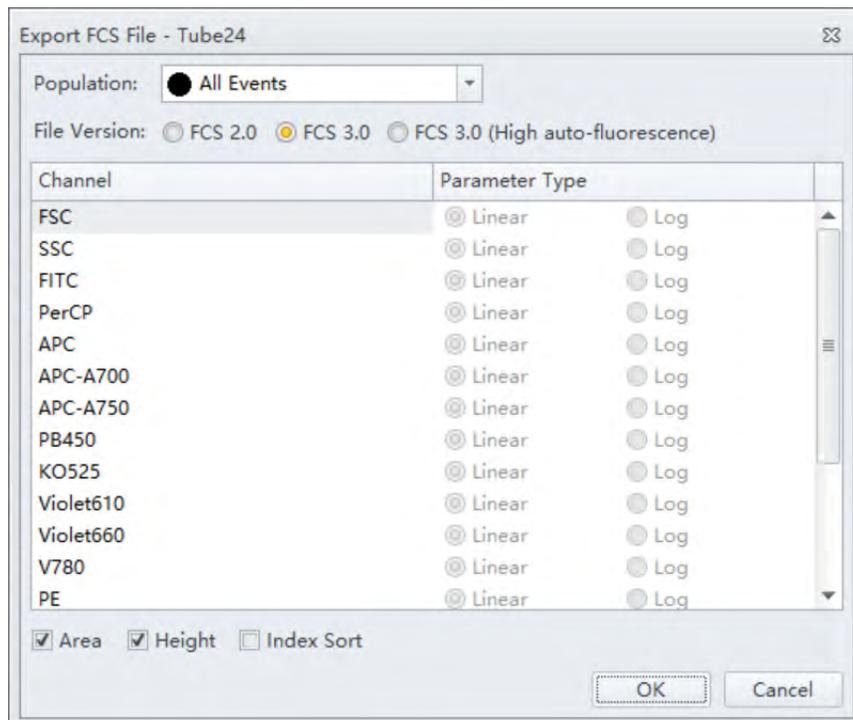
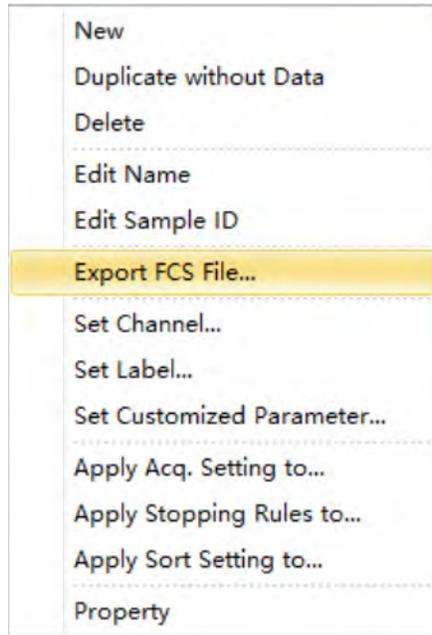
- 10 Export the FCS file if necessary. Refer to [Exporting FCS Files](#).

NOTE Ensure that any storage devices used with the instrument are free from viruses. To guard against data loss, Beckman Coulter recommends backing up data on a regular basis. Beckman Coulter is not liable for any loss of data resulting from computer viruses or damage to hardware.

Exporting FCS Files

Exporting Single Tube Files

- 1 Right-click the desired tube from the test tube section of the screen and select **Export FCS File**. The Export FCS File window appears.



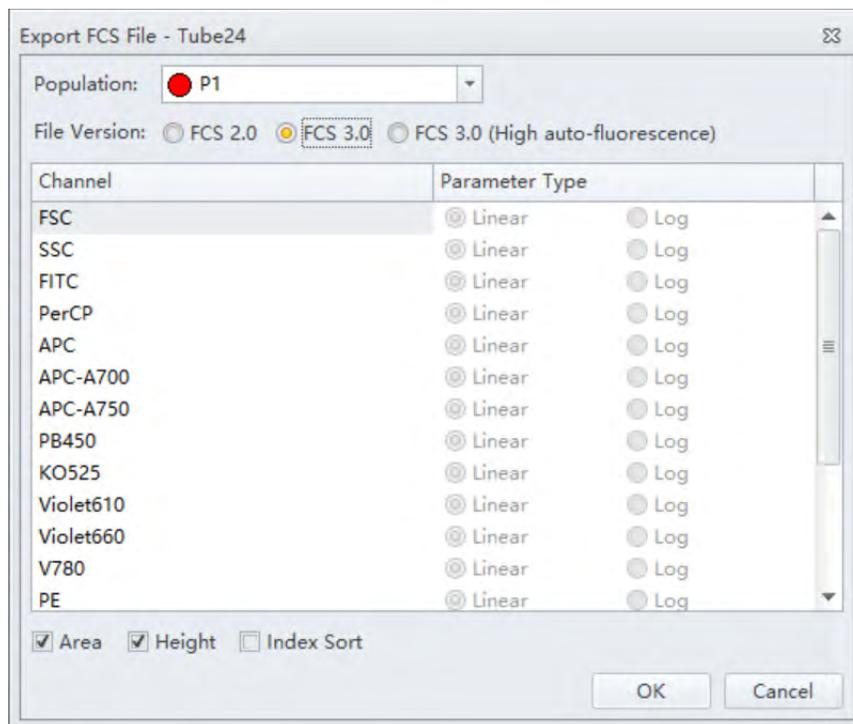
2 Select the population from the Population dropdown menu.

3 Select either **Area** or **Height**.

NOTE Select **Index Sort** to export the Index Sort data. The two parameters **Sort Index-X** and **the Sort Index-Y** will be added in the exported FCS file, which can be viewed by other analysis software.

4 Select the FCS format next to File Version.

NOTE The default setting is FCS 3.0. If FCS 2.0 is selected, select the parameter type (linear or log) from the parameter type section of the window.



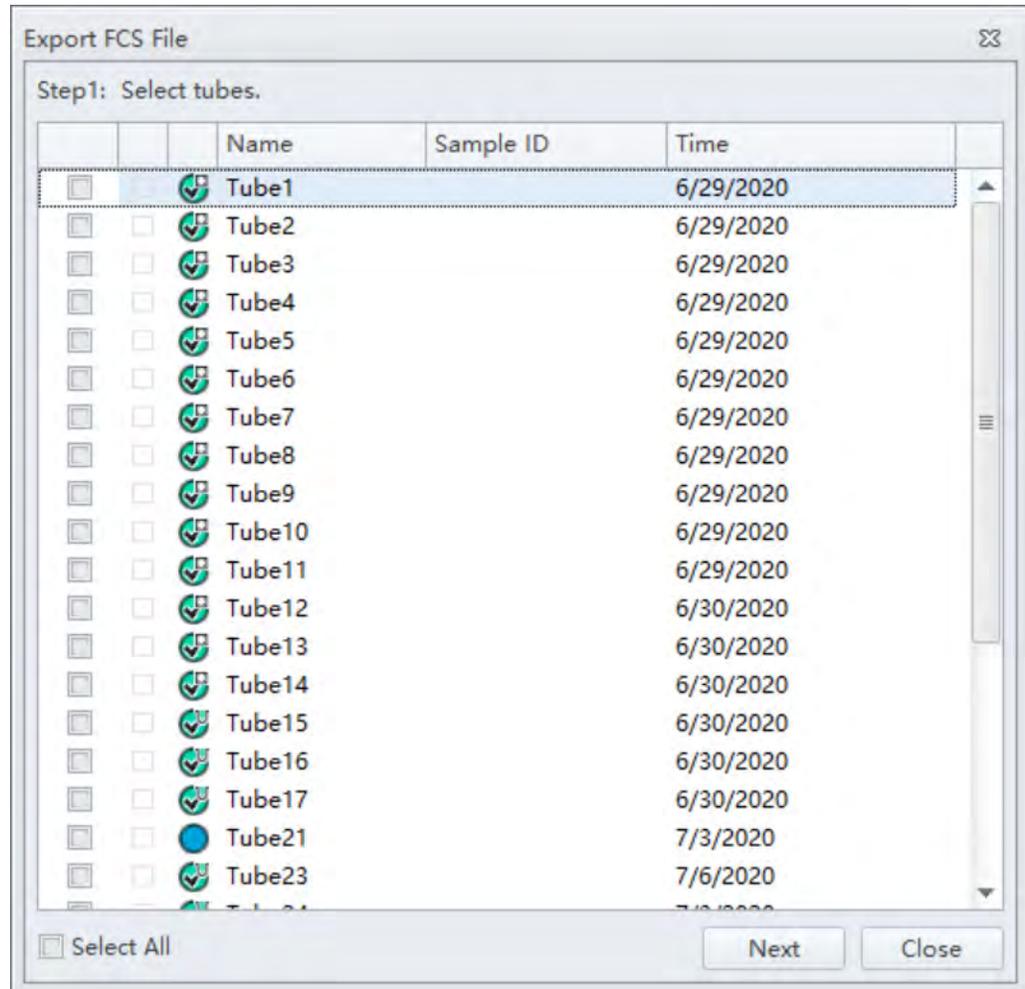
NOTE The default CytExpert SRT FCS file contains high auto-fluorescence vector values that may not be recognized by third party software. Therefore, the data displays differently in third party software packages than in CytExpert SRT. Auto-fluorescence values are added for the FCS 3.0 (High auto-fluorescence) export option to accommodate the use of third party software. Since both FCS 3.0 options have the same .fcs file extension, ensure that you save the FCS 3.0 (High auto-fluorescence) files to a different folder than the FCS 3.0 files.

5 Select **OK** to export the file.

6 Select the path to save the FCS file and select **Save**.

Exporting Multiple FCS Files

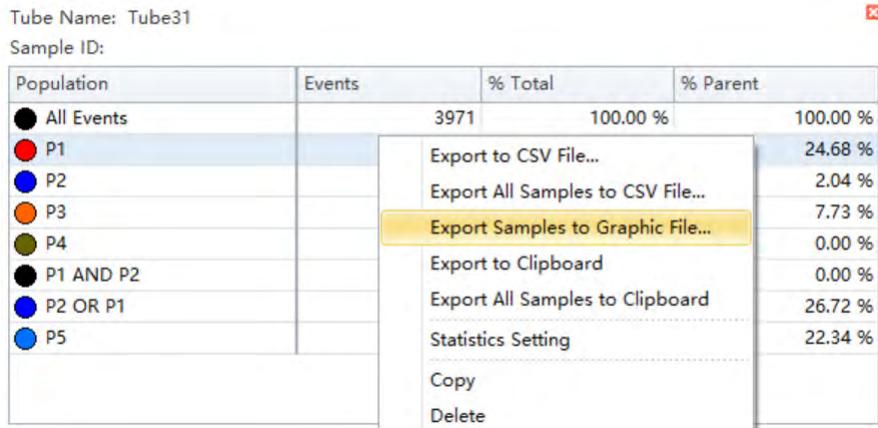
- 1 Select **Export FCS File** from the File menu. The Export FCS File window appears.



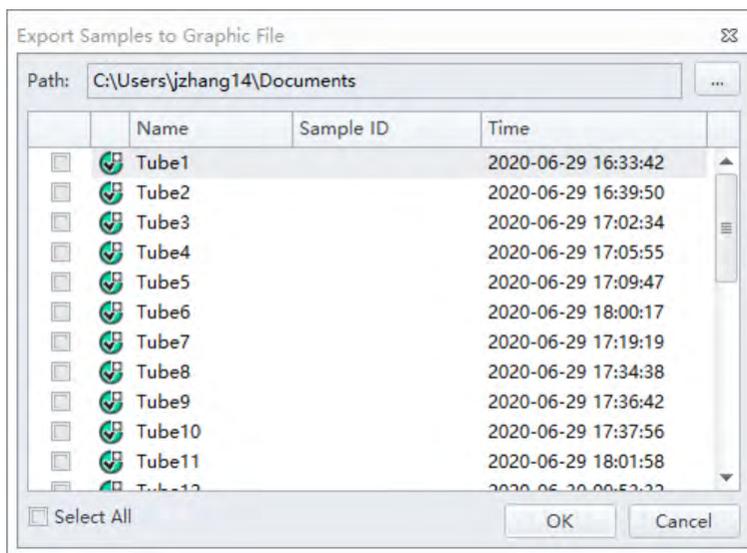
- 2 Select the tubes to export and select **Next**.
- 3 Repeat Steps 2-5 from [Exporting Single Tube Files](#).

Exporting Plots or the Statistics Table of Multiple Tubes as Picture Files

- 1 Right-click on the plot or statistics to export.



- 2 Select **Export Samples to Graphic File**. The Export Samples to Graphic Files window appears.



- 3 Select the desired tubes to export.

- 4 Select **OK**.

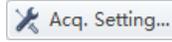
NOTE The plots of the selected tubes save as .bmp file.

Importing and Exporting Instrument Settings

The CytExpert SRT software supports importing and exporting instrument settings to facilitate the experiment process. Only instrument settings identical to the current configuration can be imported with current detector settings.

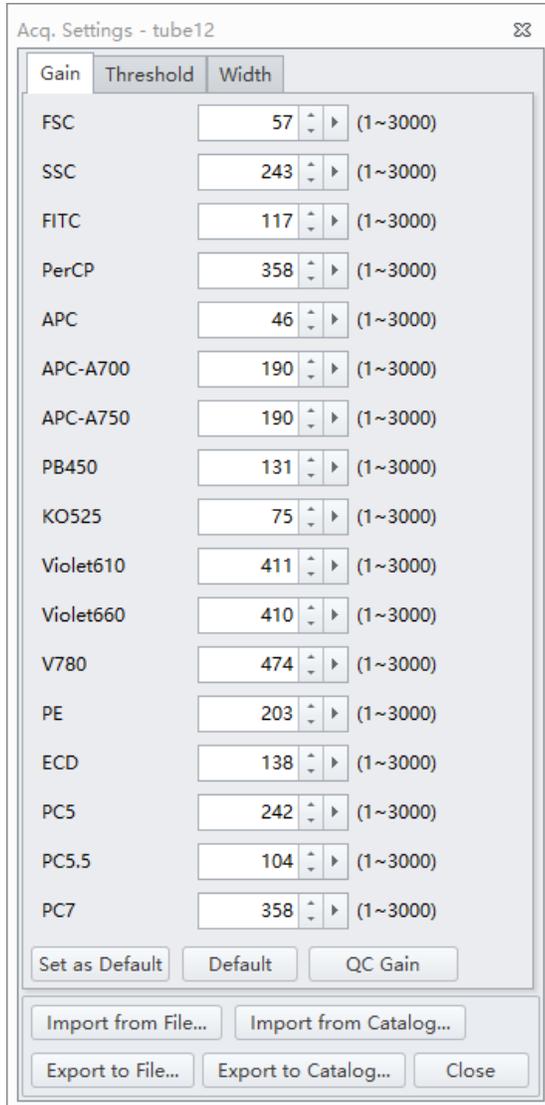
Select  to edit gain, threshold, and width. These can be imported from an experiment file or from a catalog of instrument settings.

Importing Instrument Settings

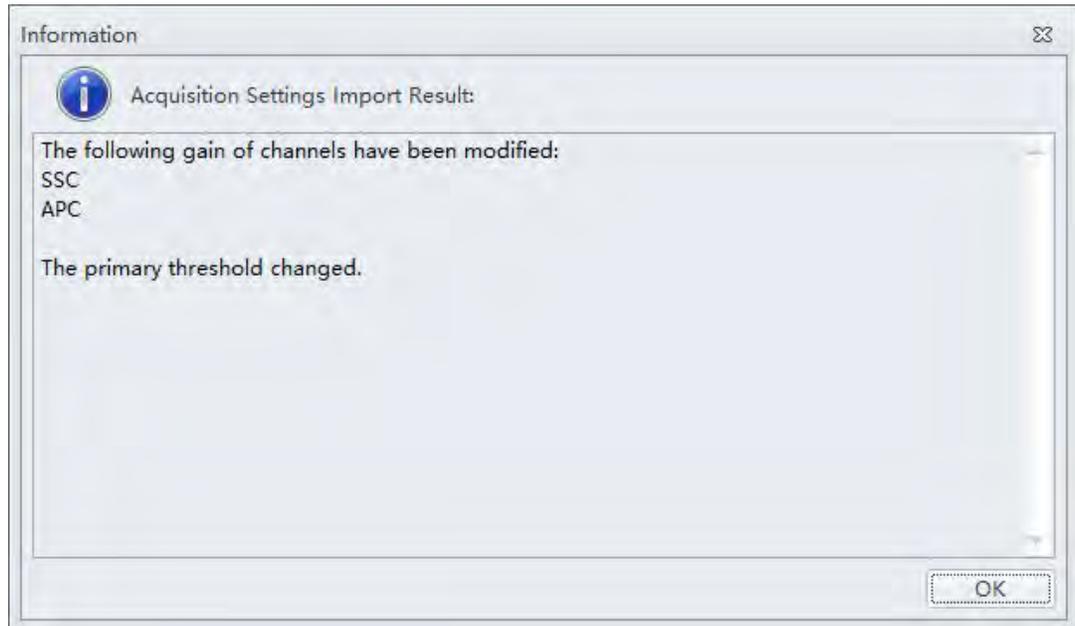
- 1 Select the desired sample tube to import. Then select .

NOTE Instrument settings can only be imported into tubes where data has not yet been recorded.

- 2 Select **Import From File**, locate the file with the required instrument settings, or select **Import From Catalog** to import the instrument settings.



Then the Information window displays.



- 3 Select **OK**.

Exporting Instrument Settings

- 1 Select the desired sample tube to export. Then select .
- 2 Select **Export To File** to export a current set of instrument settings, stored in a file ending in .acq.
Or
Select **Export To Catalog**, give a name to the settings to be exported, and export the file to the software's Acquisition Setting Catalog, then select **OK**.

- 3 Select **Close**.

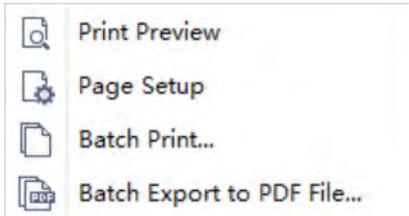
Importing and Exporting Compensation Settings

The software supports unrestricted importing and exporting of compensation data, regardless of whether the sample tube data has already been acquired. Imported compensation values only cover channels identical with the current instrument configuration. The software automatically adjusts compensation values according to differences in the gain level. Refer to [Importing and Exporting Compensation](#) in [CHAPTER 6, Compensation](#).

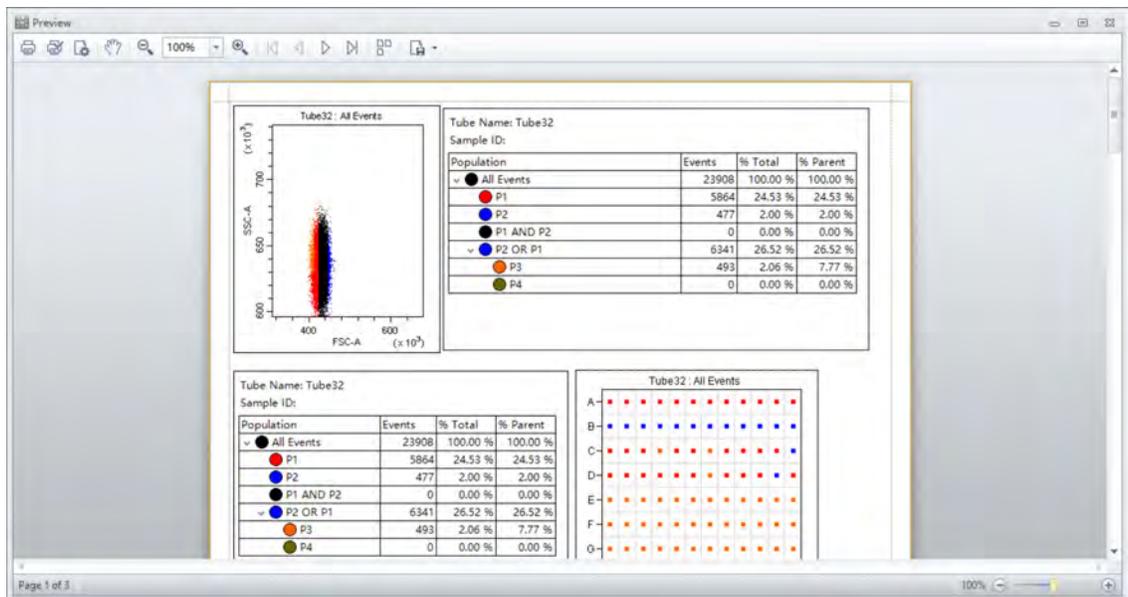
Printing Graphics

CytExpert SRT offers printing functionality for the plots and tables that appear in the plot area. The software also allows you to save these images by converting them into .jpg or .pdf files.

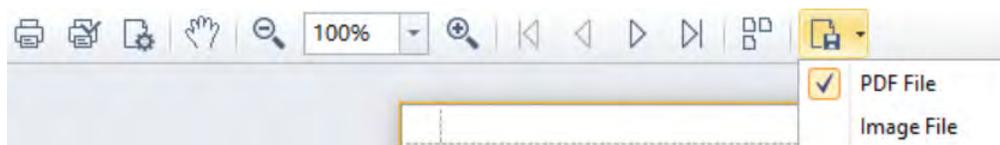
Select  in the printer control area to print directly. Or, select the print drop-down arrow for the following options:



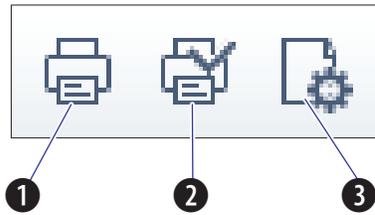
-  **Print Preview.** Used to access the Preview screen.



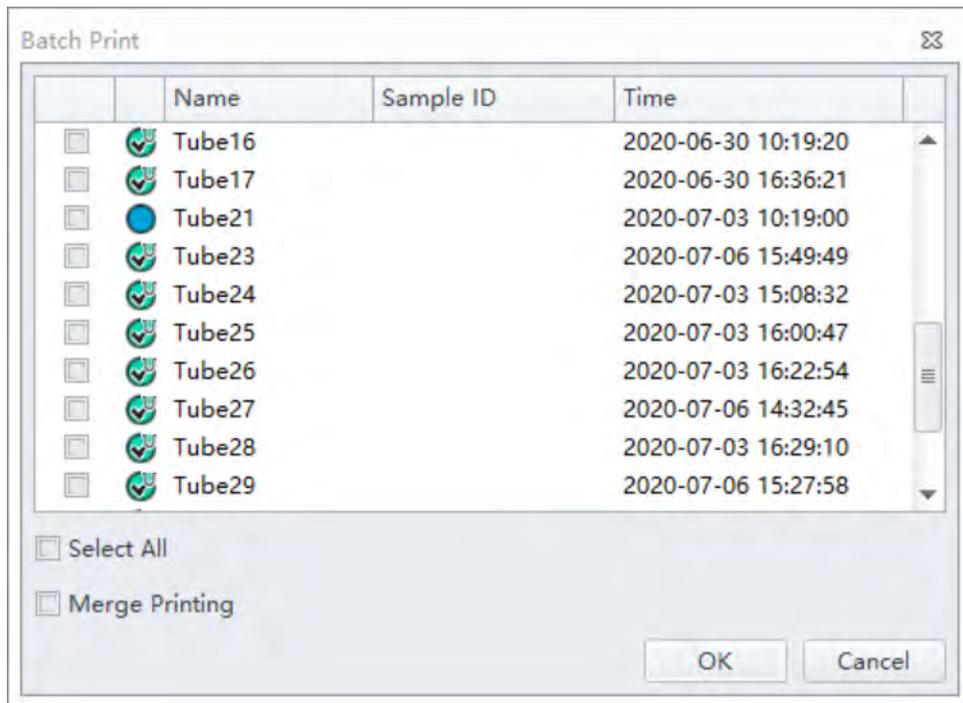
- Select  to select the required format of the file to be exported and to save the file in that format.



- Print preview also lets you choose between printing directly (1), modifying the printer configuration (2), or adjusting the page settings (3).

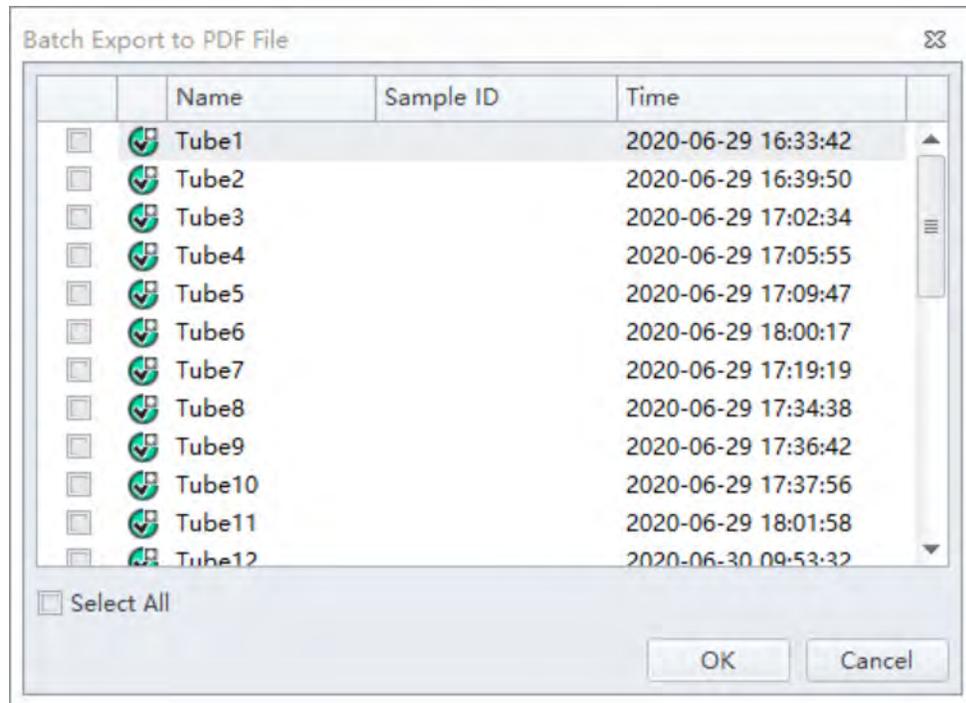


- **Page Setup.** Used to adjust the page settings.
- **Batch Print.** Used to print data for multiple tubes.
 1. Select **Batch Print.** The Batch Print window appears.



2. Select the tubes to print.
3. Select **OK.**

-  **Batch Export to PDF File.** Used to print a PDF of the data for multiple tubes.
 1. Select **Batch Export to PDF File**. The Batch Export to PDF File window appears.



2. Select the tube to print to PDF.
3. Select **OK**.

Saving the Experiment

Selecting **Save** in the File menu allows you to save the experiment.

Selecting **Save As** and saving the experiment under a different name allows you to create a backup.

Selecting **Save As Template** in the File menu allows you to save the experiment as a template.

Concluding the Experiment

Conclude the experiment as follows:

- Select **File > Close Experiment** to close the experiment and return to the Start Page.

NOTE If changes were made to the experiment, the software prompts you to save the latest changes in the experiment before returning to the Start Page.
- Shut down the system. Refer to [CHAPTER 8, Shutting Down the System](#).

Overview

This chapter describes how to create a compensation experiment and automatically calculate compensation values after acquiring the data. It also explains how to use these calculations for other experiments.

Compensation involves correction for fluorescence spillover emitted by the primary fluorochrome that is detected by the secondary fluorescent channels. For example, the excitation and the resulting fluorescence emission for the PE fluorochrome leads to the spillover fluorescence detected in the ECD, PC5.5, and PC7 channel. Compensation reduces the spillover fluorescence of the PE-positive population to match the background of the PE-negative population in the secondary channels. Compensation requires a single positive and a negative population for every single color sample.

Properly configured compensation minimizes false data interpretation caused by spillover fluorescence from another fluorochrome. Refer to [Figure 6.1](#) and [Figure 6.2](#) for an example of plots before and after compensation. Compensation adjustments can be completed during the data acquisition process or after the data acquisition process is complete.

Figure 6.1 Before Compensation

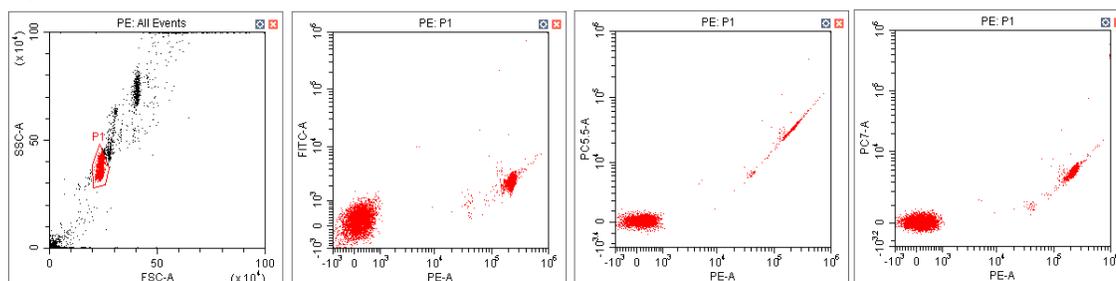
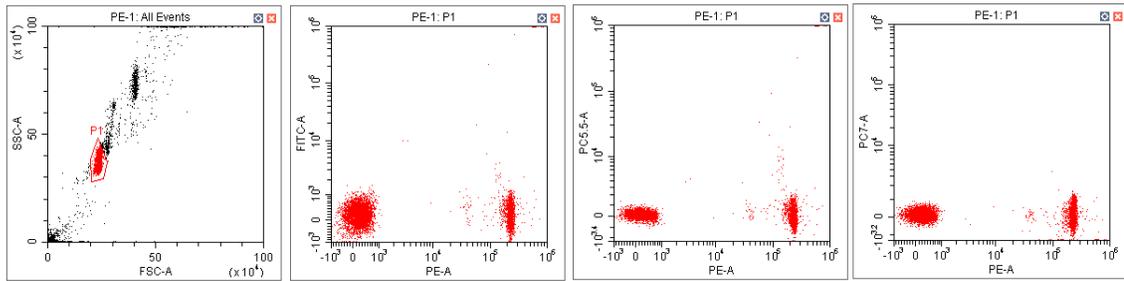


Figure 6.2 After Compensation



NOTE CytExpert SRT compensation allows full matrix compensation, manual, and automatic.

CytExpert SRT compensation also includes a novel Compensation Library for storage of spillover values of dyes to easily determine the correct compensation matrix with new gain settings.

Workflow:



This chapter contains information on:

- [Creating a Compensation Experiment](#)
- [Creating the Compensation Matrix from Previously Acquired Data](#)
- [Adjusting Compensation](#)

Creating a Compensation Experiment

Before creating a compensation experiment, you must verify the instrument’s detector configuration settings. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in [CHAPTER 5, Sorting](#).

- 1 Select **New Compensation** in the File menu or on the start page to create a new compensation experiment.

NOTE The file name of the newly created compensation experiment has a “.xipc” suffix.

- 2 Navigate to the desired file path and select **Save**. The Compensation Setup window appears.

Use	Tube	Label	Lot No.	Sample Type	
<input checked="" type="checkbox"/>	Unstained_Cell			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	Unstained_Bead			<input type="radio"/> Cell	<input checked="" type="radio"/> Bead
<input checked="" type="checkbox"/>	FITC			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	PerCP			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC-A700			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC-A750			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	PB450			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	KO525			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	Violet610			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	Violet660			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	V780			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	PE			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	ECD			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	PC5			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	PC5.5			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead
<input checked="" type="checkbox"/>	PC7			<input checked="" type="radio"/> Cell	<input type="radio"/> Bead

⚠ CAUTION

Risk of erroneous results. Select an unstained tube, according to which the fluorescence background will be set. If there is not an unstained tube available, then each single color tube must have a negative population.

It is important to specify the appropriate sample type. Otherwise, the background information could be incorrectly calculated and lead to erroneous compensation results.

- 3 Select the channel requiring compensation calculation and the sample type.

Compensation

Creating a Compensation Experiment

If a negative population is not present in each single color tube, then an unstained control tube is recommended.

NOTE The default selection is **Area**. The unstained negative control tube can be selected if needed.

NOTE Label and lot number information can be retained in the Compensation Library to facilitate future compensation calculations.

Compensation Setup

Compensation on:

Area Height

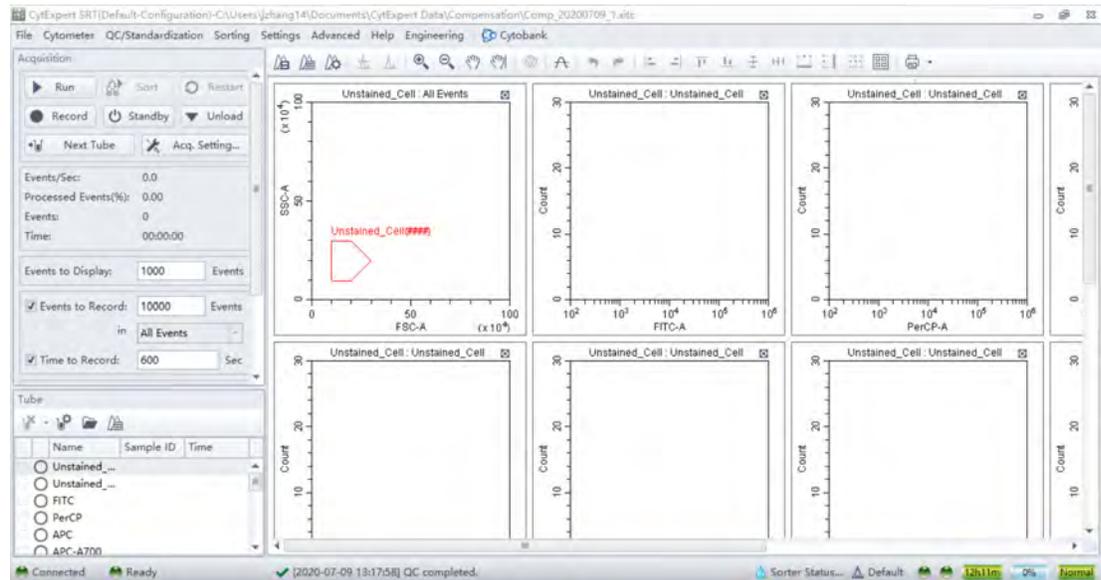
Use	Tube	Label	Lot No.	Sample Type
<input checked="" type="checkbox"/>	Unstained_Cell			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	Unstained_Bead			<input type="radio"/> Cell <input checked="" type="radio"/> Bead
<input checked="" type="checkbox"/>	FITC			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PerCP			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC-A700			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC-A750			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PB450			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	KO525			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	Violet610			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	Violet660			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PE			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	ECD			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PC5,5			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PC7			<input checked="" type="radio"/> Cell <input type="radio"/> Bead

Select All

OK Cancel

4 Select OK.

After confirmation, the software automatically generates the following compensation experiment.



NOTE Select **Area** to calculate compensation based on the Area measured. Alternatively, select **Height** to calculate compensation based on the Height measured.

Preparing the Compensation Sample

To perform a compensation experiment, prepare:

- A single positive control tube for each color
- A negative control tube (optional)

NOTE A negative control tube is required if a single positive control tube does not contain a negative population.

For the negative control sample and single positive control sample, you can use blood, cells, or dedicated compensation beads such as VersaComp Antibody Capture Beads. For details, refer to the appropriate reagent instructions for use. The negative control tube is used to determine the autofluorescence of the sample.

Using Control Samples to Generate the Compensation Matrix

Defining the Negative Population Using Unstained Samples

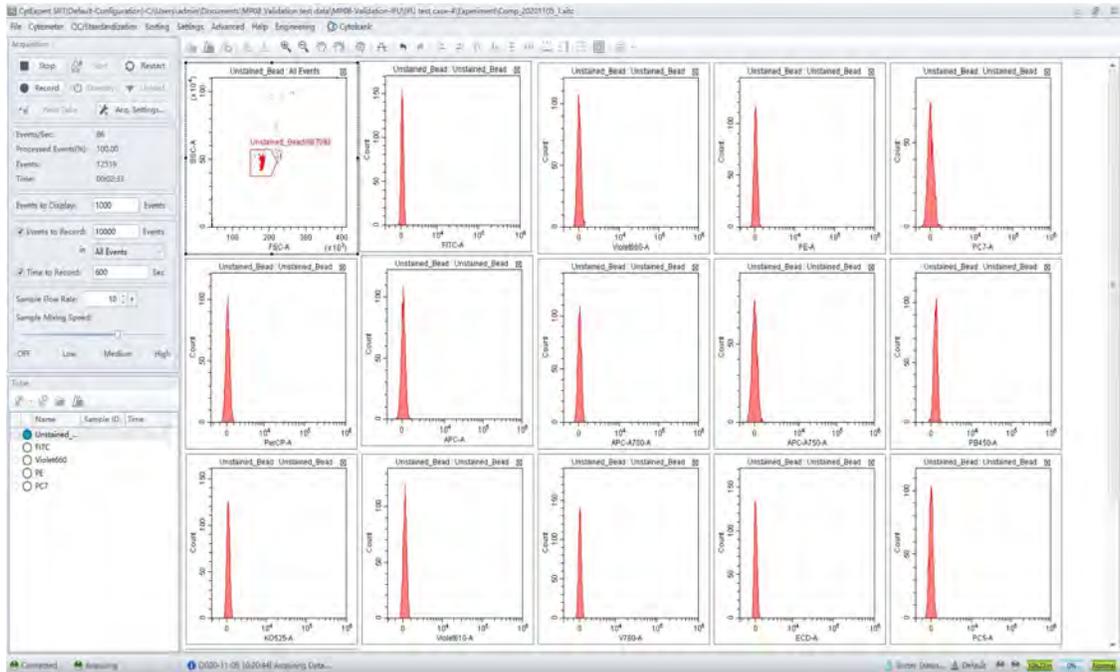
- 1 Select **Initialize** to put the instrument in the Ready state.

NOTE Skip this step if the instrument has already been initialized.

CAUTION

Risk of erroneous results. Calculations based on excessively small volumes of sampled data can be inaccurate. Ensure that more than 1,000 positive events and more than 1,000 negative events are sampled. If the ratio of positive cells is comparatively low, increase the number of acquisition events to a suitable amount.

- 2 Import the gain setting and apply the setting to all tubes. Refer to [Adjusting the Gain](#) in [CHAPTER 5, Sorting](#). Use the pan tool to adjust the axis scale so that the sample signal appears in a suitable position. Adjust the gate so that it encloses the target cell population. Refer to [Creating Plots and Gates](#) in [CHAPTER 5, Sorting](#).



- 3 Place the negative control tube in the sample tube holder.

- 4 Select the unstained tube.

5 Select **Run** to load the sample.

6 Set an appropriate number of cells to save in Events to Record located on the left side of the screen.



The screenshot shows a dialog box with the following settings:

- Events to Display: 1000 Events
- Events to Record: 10000 Events
- in All Events
- Time to Record: 600 Sec

7 Select **Record** to save the data.

Running the Single Positive Control Samples

1 Place the single positive tube in the sample station.

2 Select the appropriate, corresponding tube.

3 Select **Run** to load the sample.

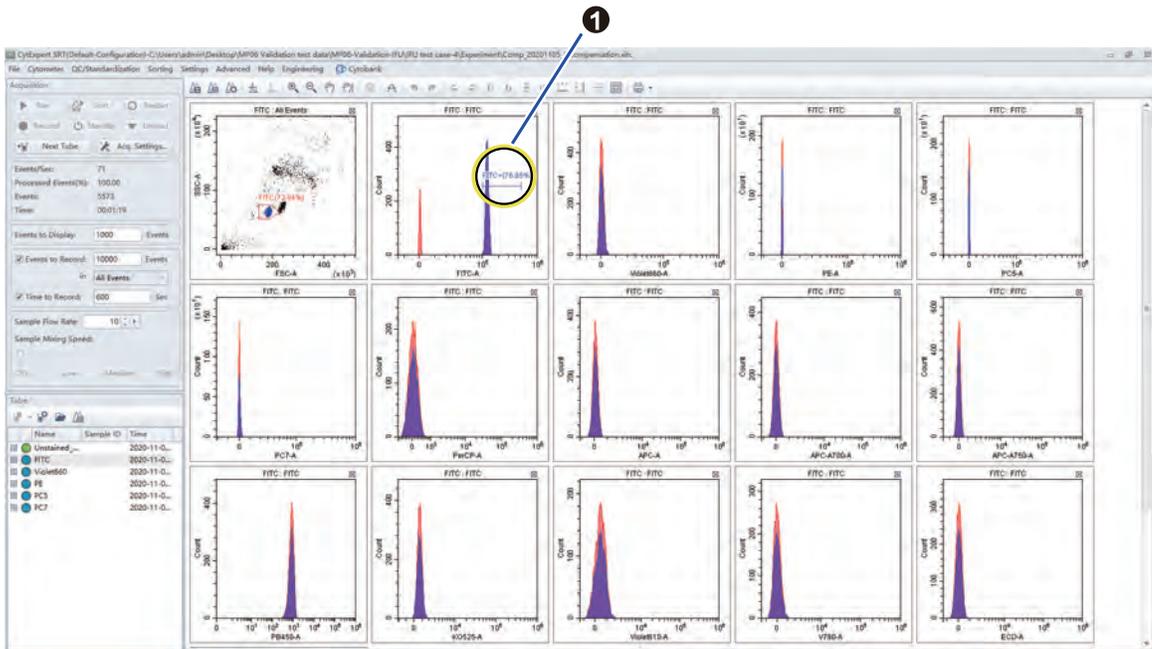
CAUTION

Risk of erroneous results. Calculations based on excessively small volumes of sampled data can be inaccurate. Ensure that more than 1,000 positive events and more than 1,000 negative events are sampled. If the number of positive cells is comparatively low, increase the number of acquisition events to a suitable amount.

- 4 Move the gate in the FSC/SSC plot so that it encloses the desired population. Move the positive gate in the plot so that it encloses the positive population.

NOTE Figure 6.3 shows an example of selecting the positive population when the negative population is defined by the unstained sample.

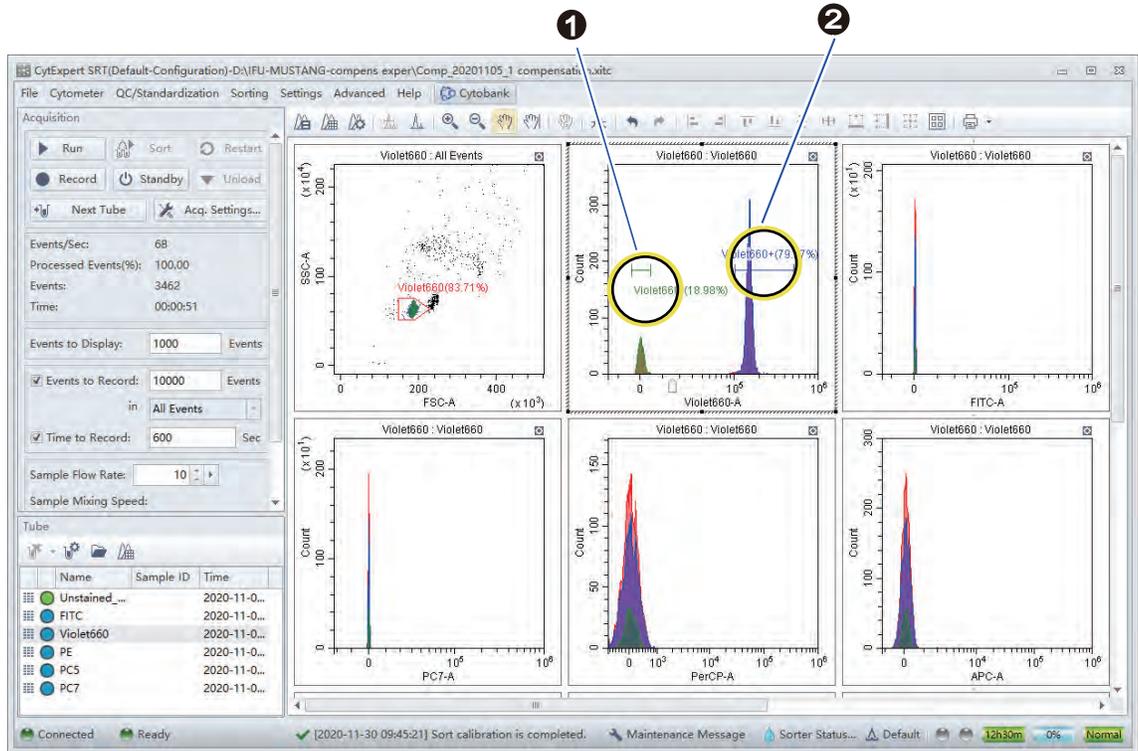
Figure 6.3 Positive Population Selected from the Single-Stained Sample



- 1. Positive population

NOTE Figure 6.4 shows an example of selecting both the positive and negative populations without an unstained sample.

Figure 6.4 Positive and Negative Populations Without an Unstained Sample



1. Negative population
2. Positive population

5 Select **Record**.

6 Repeat steps 1-5 to acquire data from subsequent single positive sample tubes.

CAUTION

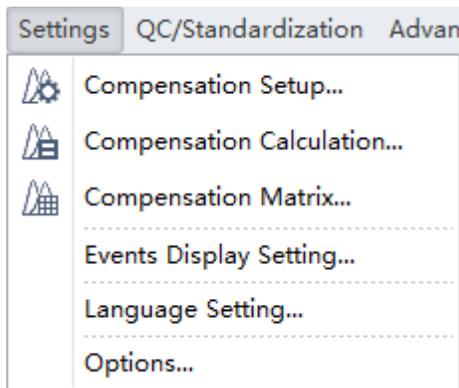
Risk of erroneous results. While the software automatically adjusts the compensation calculation according to gain, excessive manual adjustment of the fluorescence gain could lead to inaccurate results.

7 If necessary, adjust gain while acquiring data from single positive sample tubes. Refer to [Adjusting the Gain](#) in [CHAPTER 5, Sorting](#).

Calculating Compensation Values

1 Check all acquired sample tubes and confirm that the gating is appropriate.

2 Select  or select **Compensation Calculation** in the Compensation menu to calculate the compensation values.



The Compensation Matrix window appears, displaying the calculated compensation values.

Channel	-FITC%	-PE%	-ECD%	-PC5.5%	-PC7%	-APC%	-APC-A700...	-APC-A750...	-PB450%	-KO525%	-Violet610%	-Violet660%	-Violet780%	Area
FITC		0.66	0.11	0.02	1.43	0.00	0.08	0.00	0.00	0.91	0.11	0.00	0.02	
PE	35.08		18.23	1.13	2.39	0.00	0.10	0.00	0.00	1.14	4.10	0.07	0.01	
ECD	12.83	41.79		0.55	1.75	0.00	0.09	0.00	0.00	0.80	9.47	0.34	0.02	
PC5.5	4.96	18.15	65.09		1.64	0.52	0.88	0.03	0.00	0.49	9.15	2.04	0.07	
PC7	1.36	4.32	19.07	71.65		0.17	1.04	1.39	0.00	0.05	3.28	0.58	7.29	
APC	0.02	0.03	1.12	2.67	0.22		9.25	6.07	0.00	0.16	1.10	75.01	0.08	
APC-A700	0.03	0.15	0.45	43.95	0.00	28.94		2.48	0.00	0.20	0.50	36.76	0.36	
APC-A750	0.10	0.21	0.20	21.48	13.85	13.34	56.58		0.00	0.13	0.23	14.52	23.06	
PB450	0.10	0.22	0.00	0.04	0.00	0.00	0.15	0.00		8.26	8.62	6.12	2.94	
KO525	1.89	0.15	0.00	0.03	0.33	0.00	0.06	0.00	7.67		0.55	0.35	0.19	
Violet610	0.47	3.11	6.79	0.02	1.14	0.01	0.05	0.00	0.58	81.36		13.97	0.08	
Violet660	0.26	1.48	3.56	0.22	0.00	2.03	0.19	0.10	0.00	45.01	92.28		0.12	
Violet780	0.13	0.23	0.78	4.62	8.40	0.46	2.22	3.72	0.00	12.20	30.20	27.58		

NOTE The primary fluorescence channels are listed in columns; the secondary fluorescence channels are listed in rows.

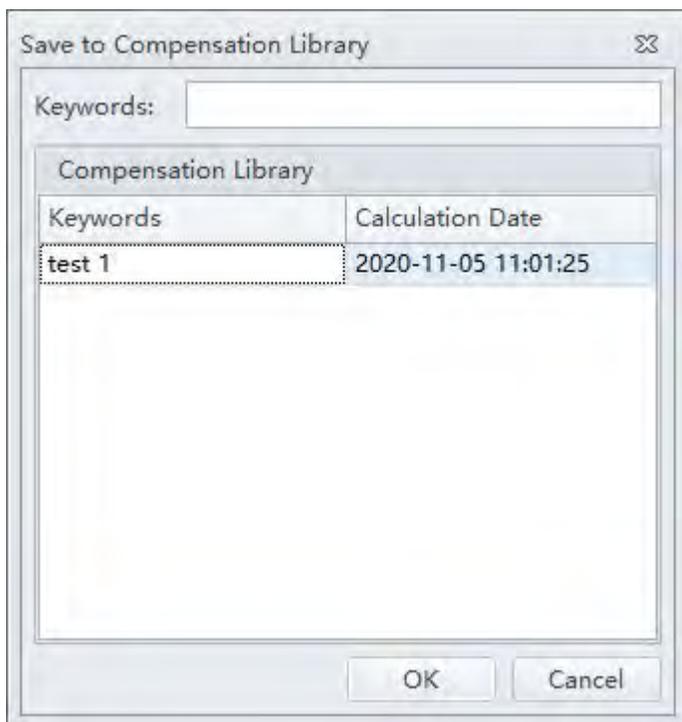
NOTE In the Compensation Matrix window:

- The *Use* checkbox applies the compensation to the selected sample.
- The *Show Autofluorescence* checkbox displays the vectors for the autofluorescence.

3 Select **Save As** to export the compensation matrix as a .comp file and specify where to save it.

NOTE The compensation matrix can also be imported for use in other experiments.

- 4 Select **Save To Compensation Library** to save the single color compensation values in the compensation library.
- 5 Specify the key words and select **OK**.



NOTE The settings stored in the compensation library are specific to the detector configuration. The compensation library can only be applied when the detector configurations are the same.

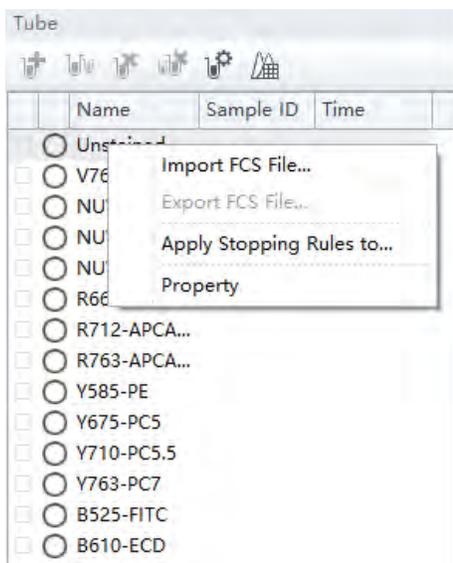
At any time, saved compensation experiments can be reopened and the compensation values recalculated.

- 6 Select **Close**.

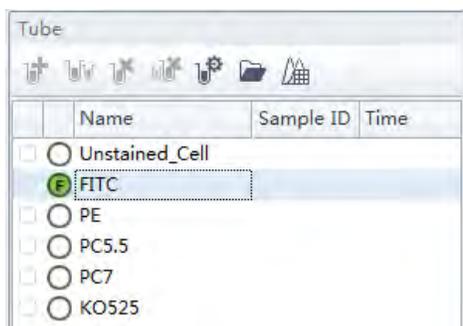
Creating the Compensation Matrix from Previously Acquired Data

The software supports importing single color data acquired from other experiments into a compensation experiment to perform compensation calculations. The data to be imported must match the active detector configuration at the time that the compensation experiment was created. Otherwise, the data cannot be imported. It is important to ensure that imported data comes from the same instrument and uses the same configuration and channels. Data originating from a different instrument will cause erroneous calculations.

- 1 Select **New Compensation** from the File menu or the start page.
- 2 To create a compensation experiment, select the required channels. Refer to [Setting the Channel and Label](#) in **CHAPTER 5, Sorting**.
- 3 Right-click on the appropriate test tube and select **Import FCS File**. Locate the corresponding data file and import the file. Only files compatible with the detector configuration are supported by the software for importing.



F in front of a test tube indicates that the corresponding data have been imported.



- 4 After importing the data, adjust the gates to properly identify the positive population and the negative population for each single-color sample.

-
- 5 Calculate the compensation values and export them. Refer to [Calculating Compensation Values](#).
-

Adjusting Compensation

Manually Adjusting Compensation

The compensation can be manually adjusted in an experiment in two ways:

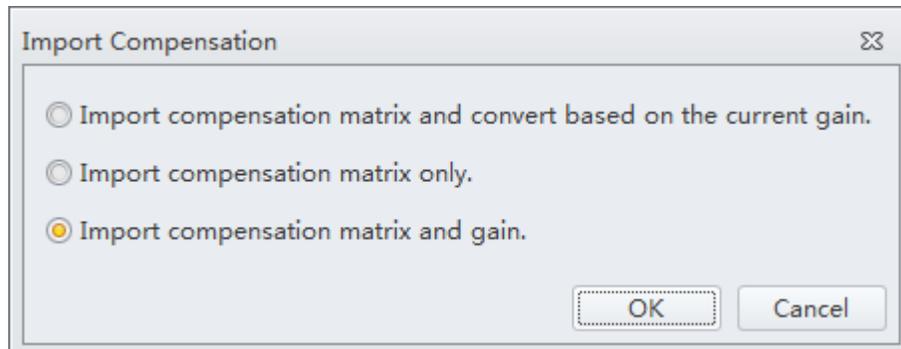
- Select the populations where needs to be adjusted in the bivariate plot. Select  from the graphic control area, then click and drag the mouse pointer up and down or left and right inside the plot to adjust compensation.
- Select **Compensation Matrix** in the Setting menu to open the compensation matrix. Adjust the compensation value between the primary channel and the secondary channel.

Importing and Exporting Compensation

Importing Compensation Settings from Compensation Matrix Files

- 1 Select the desired sample tube for importing compensation values.
-
- 2 Select **Compensation Matrix** in the Setting menu.
-
- 3 Select **Import** and locate the path where compensation matrix files are saved. Select the corresponding compensation matrix file (.comp) to import the compensation values. You can also select **Import from Library** to import compensation values from the compensation library. The Import from Compensation Library window appears. Refer to [Importing Compensation Settings from the Compensation Library](#). Both methods allow you to import the compensation values with or without the adjustment based on the gain settings.

- 4 After opening the desired compensation file, the Import Compensation window appears. Select one of the following:
 - Import compensation matrix and convert it with current gains.
 - Import compensation matrix only.
 - Import compensation matrix and gain.



NOTE

- If the tube does not have any data when importing compensation values calculated from other instrument settings, the software prompts you to select whether the gain settings must be imported as well. Select **Yes** to import fluorescence channel gains settings along with the rest of the data. Select **No** to allow the CytExpert SRT software to adjust the compensation matrix values based on the current gain settings.
- If the tube does have data when importing compensation values from other instrument settings, the software prompts you to select whether the compensation values are adjusted based on the current gain settings.
- It is important to note that automatic adjustments to compensation values calculated from other instrument gain settings could result in incorrect compensation. Always review the data after importing compensation values to ensure the sample is compensated properly.

- 5 Select **OK**.

- 6 If necessary, select **Apply to** to apply the compensation values to the selected test tubes.

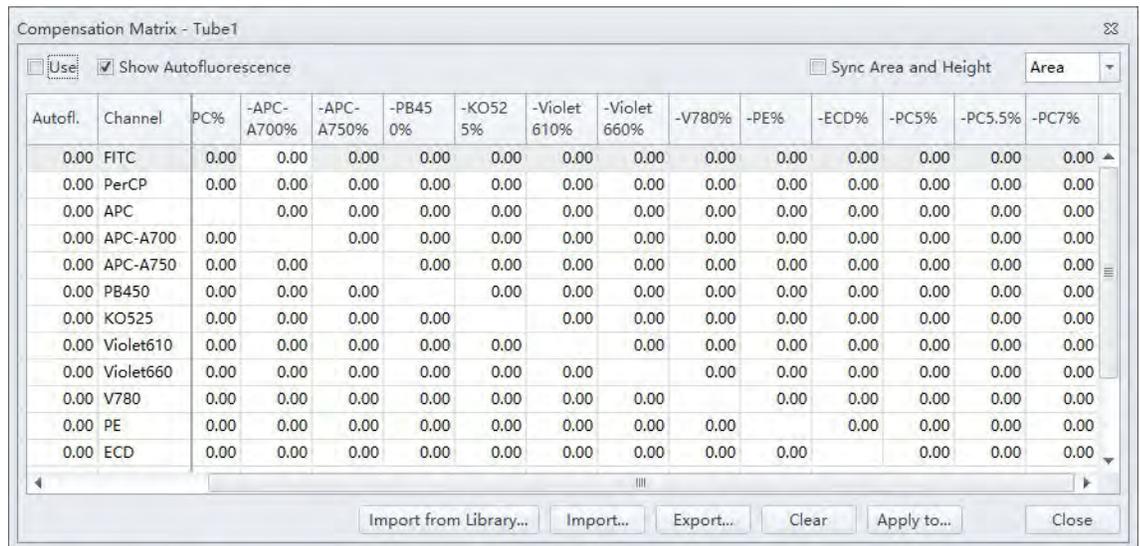
- 7 Select **Close**.

Importing Compensation Settings from the Compensation Library

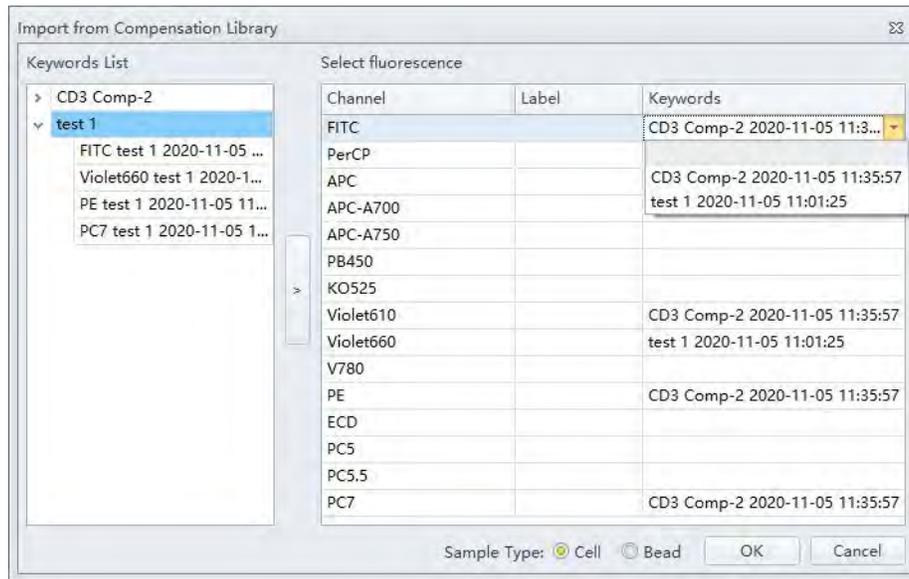
You can choose which single color data to include from the compensation library. Only single color data in the compensation library from the same detector configuration can be imported into the compensation matrix.

NOTE Files available in the compensation library are configuration-specific. The compensation library only displays the files created under the current default configuration.

- 1 Select **Import From Compensation Library** to select which compensation values to import from the compensation library.



- 2 In the Keywords column, the corresponding compensation values can be selected for each channel. The compensation values of the same keyword can also be selected using the drop-down menus in the Keywords column.



- 3 Select **OK** to import the compensation values.

Exporting Compensation Settings

- 1 Select the desired sample tube to export.
- 2 Select **Compensation Matrix** in the Setting menu.

3 Select **Export** to specify a path and file name for the compensation file you are saving.

Compensation Matrix - Versacomp test

Use Show Autofluorescence Area and Height in Sync

Autofl.	Channel	-AF79 0%	-PF840... 0%	-PB45 0%	-KOS2 5%	-Violet 610%	-Violet 660%	-Violet 780%	-DAPI%	-NUV5 25%	-HoechstRe...	-APC%	-APC- A700%	-APC- A750%	-PE%	-ECD%	-PC5.5%	-PEAF 680%	-PC7%	-FITC%	-ECDm Cherry...	-PerCP...
0.00	AF790		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PB440	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PB450	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	KOS25	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	Violet610	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	Violet660	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	Violet780	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	DAPI	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	NUV525	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	HoechstR...	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC-A700	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC-A750	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	ECD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00
0.00	PC5.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
0.00	PEAF680	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00
0.00	PC7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
0.00	FITC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00
0.00	ECDmCh...	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00
0.00	PerCP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

Import from Library... Import... Export... Clear Apply to... Close

4 Select **Save**.

NOTE The generated file ends in .comp.

Managing the Compensation Library

Compensation values can be managed in the Compensation Library.

- 1 Select **Compensation Library** from the Settings menu. The Compensation Library window appears.

The screenshot shows the 'Compensation Library' window. On the left, a tree view lists compensation libraries grouped by fluorescence channel: FITC, PerCP, APC, PB450, Violet610, and PC7. Each group contains a sample with a date and time stamp (2 2020-11-09 17:13:41). The 'FITC' channel is selected. On the right, configuration fields are visible: 'Fluorescence' is set to 'FITC', 'Compensation Keywords' is '2 2020-11-09 17:13:41', 'Label' and 'Lot No.' are empty, 'Keywords' is '2', and 'Gain' is '143'. Below these are sections for 'Cell Autofluorescence' (Height: 0.00, Area: 0.00) and 'Bead Autofluorescence' (Height: 2.97, Area: 0.91). At the bottom, a table shows compensation data for various channels.

Fluorescence	Height%	Area%	Gain	
PerCP		3.07	3.73	414
APC		0.01	0.00	378
PB450		0.02	0.00	6
Violet610		0.25	0.29	307
PE		0.00	0.00	372
PC7		0.00	0.00	655

Buttons at the bottom: Export..., Import..., Delete, OK, Cancel, Apply.

NOTE The Compensation Library is arranged by fluorescence detection channels.

- 2 Select the desired single color sample. The compensation information appears on the right side of the window.

NOTE Existing compensation values (height and area) can be modified by double-clicking the appropriate column in the Compensation Library window.

- 3 Enter the Label and Lot No. for the specified single color sample.

- 4 Select **OK**.

Adding Channels for Compensation

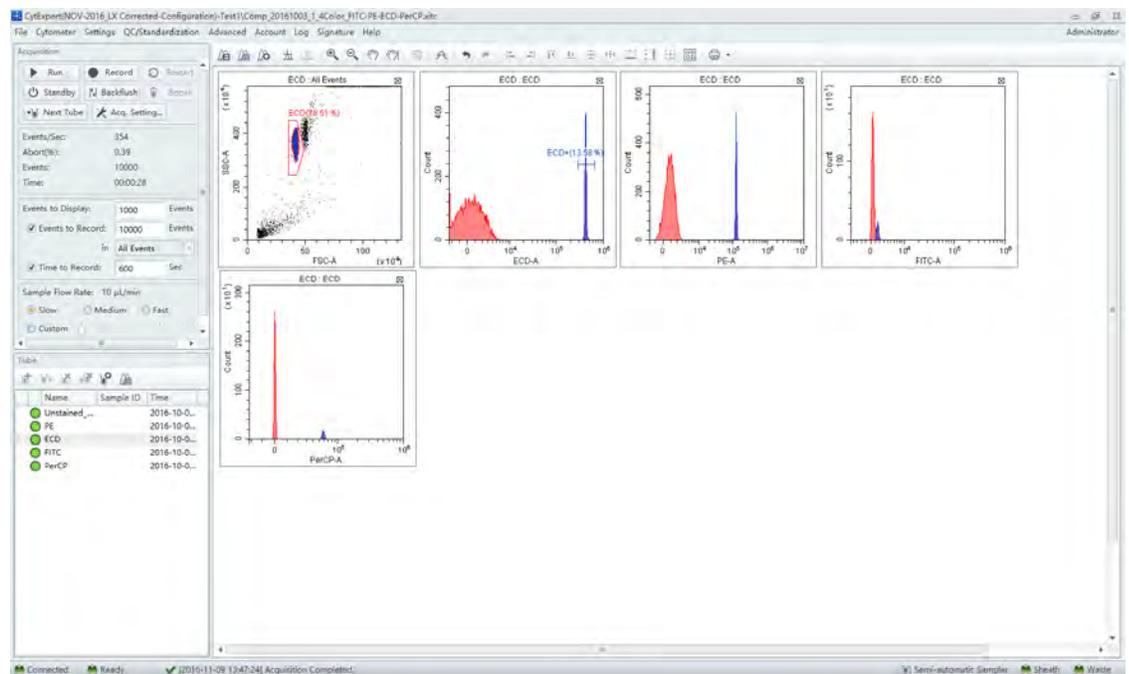
Channels requiring compensation calculations that have not been previously acquired can be added to the compensation experiment by acquiring the necessary positive tubes.

1 In the compensation experiment, select  in the compensation controls, or select **Compensation Setup** in the Settings menu. The Compensation Setup window appears.

2 Select the channel that needs to be added and select **OK**.

The software automatically adds a new single positive tube to the compensation experiment. It also adds a plot with appropriate parameters in the negative control tube.

NOTE It is important to ensure that the data for the previously acquired negative control now includes the data of the newly added channel and that the settings are correct. Otherwise, you must reacquire the negative control tube and adjust the gain.



3 Repeat 1-2 to detect and acquire newly added single positive sample data.

4 Repeat [Calculating Compensation Values](#) to recalculate and export the compensation results.

Overview

This chapter discusses how to use the Analysis screen to analyze data. Data can be analyzed using any computer equipped with the CytExpert SRT software. No online connection is required.

Workflow:

Import experiment or data → Plot and configure statistics → Export results

This chapter contains information on:

- [Copying Experiments and Importing Data](#)
- [Setting the Plots and Statistics](#)
- [Calculating Sample Concentration](#)
- [Adjusting Compensation Settings](#)
- [Exporting Results](#)

Copying Experiments and Importing Data

Copying a Previously Acquired Experiment

Experiments acquired by other CytoFLEX instruments using CytExpert software can be imported to your computer for analysis, provided your computer also uses CytExpert SRT software.

Select **Convert CytExpert Experiment** in the File menu. Then select **Browse** to open the folder where the experiment data files are stored and select a path to save the converted experiment. Then, select **OK**.

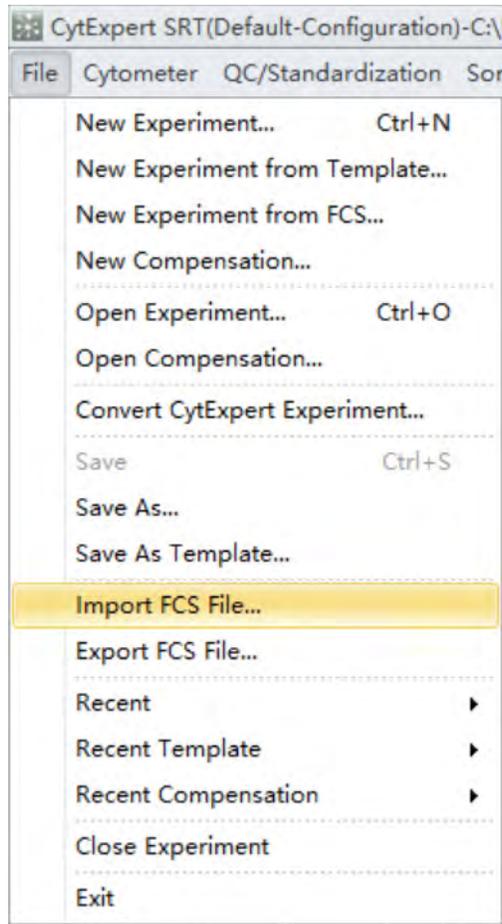
NOTE The .xits and data folder must be stored in the same path.

Importing Previously Acquired Data

The CytExpert SRT software can import and analyze compatible FCS data files acquired by other Sorters or cytometers.

- 1 Create a new experiment or open a saved experiment. Refer to [Creating an Experiment](#) in [CHAPTER 5, Sorting](#).

- 2 In the new or opened experiment, select **Import FCS File** in the File menu to import the data files.



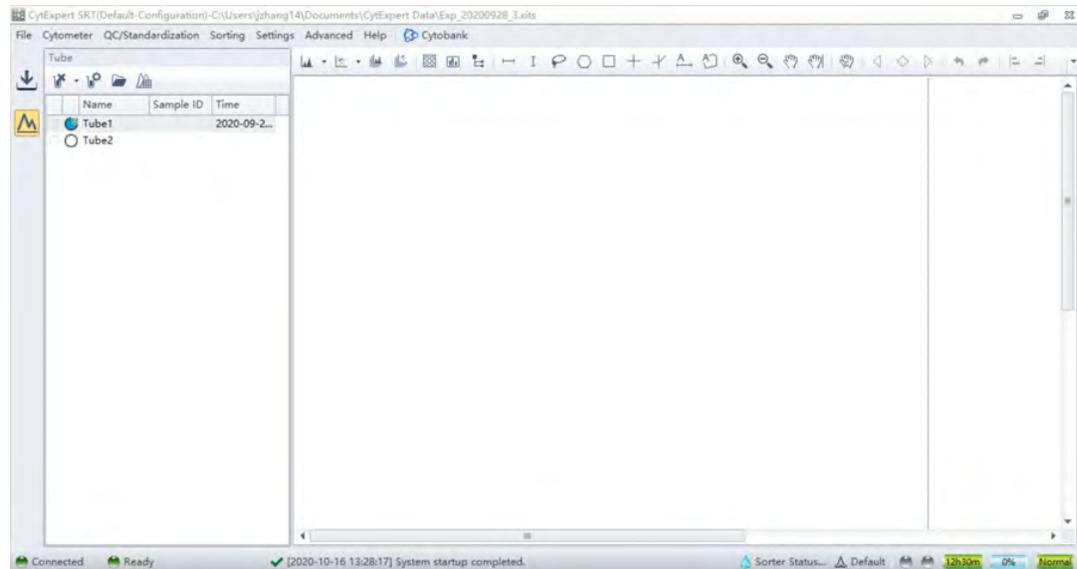
Imported data files appear in the Tube screen.

The **F** symbol in front of each data tube indicates that the data tube is an imported data file. Imported data files are copied and saved in the folder where the current experiment data files are saved.

Setting the Plots and Statistics

Opening the Analysis Screen

- 1 Select  on the left to enter the Analysis screen.



- 2 Copy plots obtained during data acquisition.

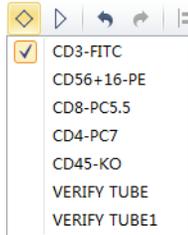
- a. If you need original plots used during data acquisition, select  to access the Acquisition screen.
- b. Select the appropriate plots.
- c. Right-click the selected plots and select **Copy** from the drop-down menu or press Ctrl+C to copy.
- d. Select  to return to the Analysis screen.
- e. Select the required test tube from the tube list on the left side of the screen.
- f. Right-click the plot area and select **Paste** from the drop-down menu or press Ctrl+V to paste the plot.

NOTE Pasted plots include all gates, but the gate names are reassigned.

- 3 New plots can be created according to need. After selecting the test tubes requiring analysis, use the plotting control buttons at the top of the screen to create a new plot.

NOTE Each graph in the **Analysis** screen may correspond to different data. Pay special attention to each plot's heading to avoid mistakes during analysis.

- 4 Use the sample selection controls in the graphics controls tool bar at the top of the page (see Figure 2.1) to change the data displayed in a plot.



- a. Select the plot requiring a change to the data displayed. By pressing and holding the Ctrl key while selecting plots, you can select several plots at one time.
- b. Select one of the two triangular sample selection buttons (◀ or ▶) to choose between the previous sample and the next sample, or select ◊ to specify which data to display.

Creating Histogram and Dot Plot Overlays

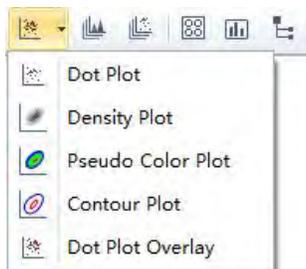
The CytExpert SRT software supports histogram and dot plot data overlay functionality, allowing you to combine data from differing sources onto the same histogram or dot plot.

- 1 Select **Histogram Overlay** under the histogram icon drop-down list to create a new multi-data histogram.



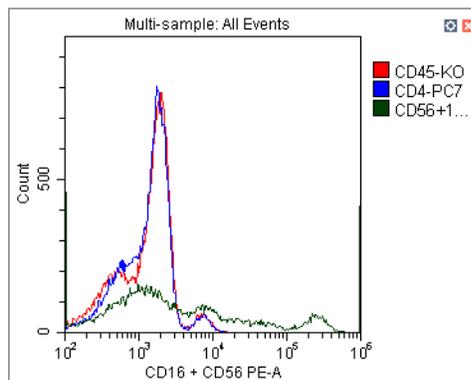
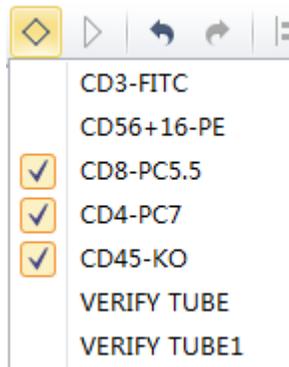
Or

Select **Dot Plot Overlay** under the dot plot icon drop-down list to create a new dot plot overlay.



IMPORTANT A maximum of 10 samples can be overlaid.

- 2 Select  to select samples for overlay display. Or, drag and drop samples from the tube list on the left into the histogram or dot plot overlay. The software automatically assigns different colors to different data.



To remove a sample, select  and uncheck the sample. Or, right-click the color legend and select **Remove [sample name]** or **Remove All Sample**. The corresponding data will no longer appear on the graph.

- 3 To change the color selections, right-click on the sample name in the legend located on the right side of the plot and select **Color** from the drop-down menu. A color pallet appears.

For configuring gates and generating statistics, refer to [CHAPTER 5, Sorting](#).

Calculating Sample Concentration

The CytoFLEX SRT instrument supports the calculation of the sample concentration based on the known concentration of the reference beads. The system does not support the direct calculation of concentration based on the volume.

NOTE If the Events/ $\mu\text{L}(V)$ checkbox is selected, the Events/ $\mu\text{L}(V)$ column displays #### in the statistics table. However, the system is compatible with the experiments using other CytExpert software. Select the Events/ $\mu\text{L}(V)$ checkbox to display the Events/ $\mu\text{L}(V)$ value if needed.

- Select **Statistics** in the graphic controls tool bar to create a statistics table.
- Right-click on the statistics table and select **Statistics Setting**. The Statistics Setting window appears.
- Select the Statistics tab. Refer to [Figure 7.1](#).
- Select the *Events/ $\mu\text{L}(B)$* checkbox and select the gated **Beads Population**.
- Enter the total number of reference beads as the **Beads Count**, as well as the **Sample Volume** in total.

NOTE You can also enter the reference bead concentration directly in the Beads Count field and set the Sample Volume as 1.

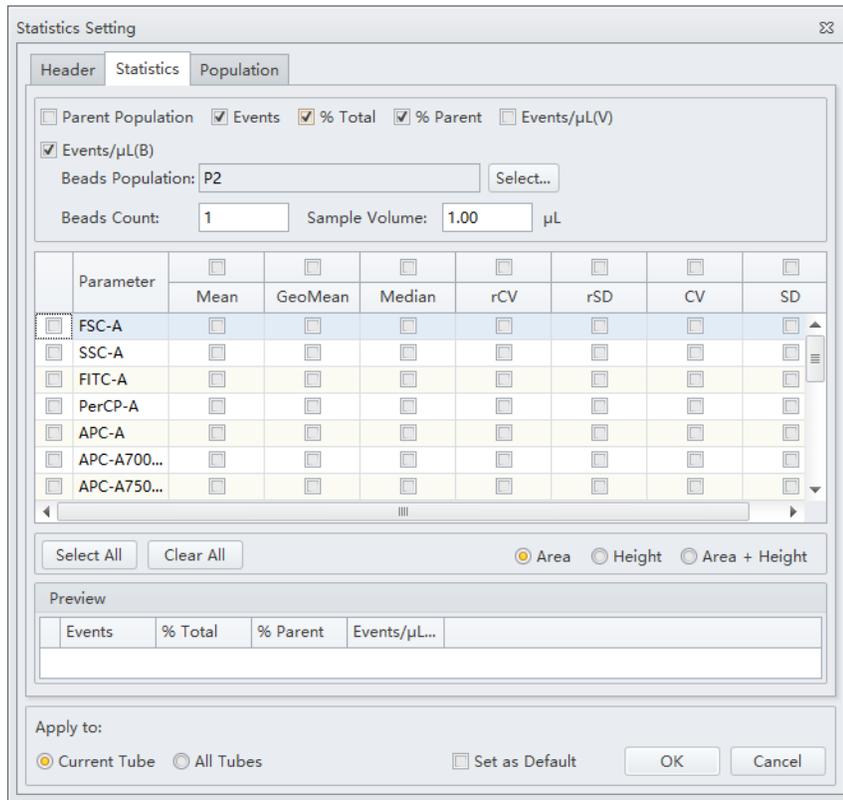
- The software automatically calculates the original sample concentration based on the input values.

To obtain accurate calculations, throughout the data acquisition process, ensure that:

- The sample concentration is 2×10^4 - 10^7 units/mL.
- Samples are thoroughly mixed before loading and that they exhibit no apparent subsidence throughout the testing process.
- The detection rate is maintained at less than 10,000 events/second throughout the sampling process.
- A constant sampling rate is maintained when recording data.
- You acquire at least 10 μL of sampling volume.

NOTE While collecting samples, instantaneous data calculation can appear inaccurate. Regard the calculation as accurate only after data acquisition has been completed.

Figure 7.1 Statistics Setting



Experiment Name: Exp_20210702_1
 Tube Name: Tube4
 Absolute Count: Beads Population=P2, Beads Count=1, Sample Volume=1μL

Population	Events	% Total	% Parent	Events/μL(V)	Events/μL(B)
● All Events	2585	100.00%	100.00%	####	1.30
● P1	2470	95.55%	95.55%	####	1.24
● P2	1984	76.75%	76.75%	####	####

Adjusting Compensation Settings

Data compensation can be carried out at any time. You can select the desired tube in the tube list on the left side of the screen and select  in the compensation controls, or select **Compensation Setup** in the Compensation menu. Refer to [Adjusting Compensation](#) in [CHAPTER 6, Compensation](#), for detailed instructions on adjusting compensation settings.

Exporting Results

Refer to [Exporting Data](#) in CHAPTER 5, [Sorting](#).

Shutting Down the System

Overview

CAUTION

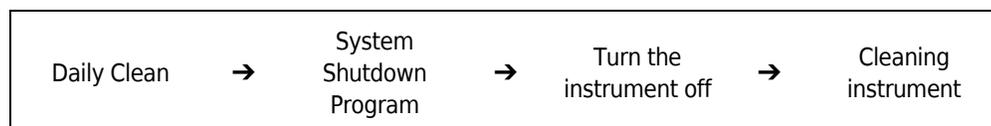
Risk of saline buildup in the fluidics system. Do not use the Urgent Stop button for routine shutdown. Urgent stop skips the important cleaning steps and may cause high carryover.

CAUTION

Risk of corrupting the embedded controller's file system. Do not skip the System Shutdown program to shut down the system. Turning off the main power switch located on the Sorter directly may corrupt the system.

This chapter provides procedures for shutting down the CytoFLEX SRT instrument.

Shutdown Workflow:



This chapter contains information on:

- [Running the Daily Clean](#)
- [Running Shutdown Program](#)
 - [System Shutdown](#)
 - [Long Term Shutdown](#)
- [Cleaning during Shutdown](#)
- [Turning Off the Power](#)

Running the Daily Clean

- 1 Select **Initialize** to put the instrument in the Ready state.

NOTE Skip this step if the instrument has already been initialized.

- 2 Run Daily Clean to clean the sample line. Refer to [Daily Clean Program](#) in CHAPTER 10, [Cleaning Procedures](#).
- 3 Select **Standby** to Turn off Sheath.

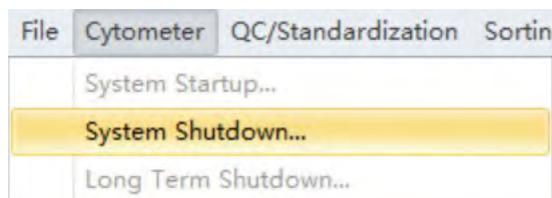
Running Shutdown Program

The System Shutdown Program is to rinse the sample line and perfuse the flow cell with the CytoFLEX SRT Shutdown fluid to prevent saline accumulation.

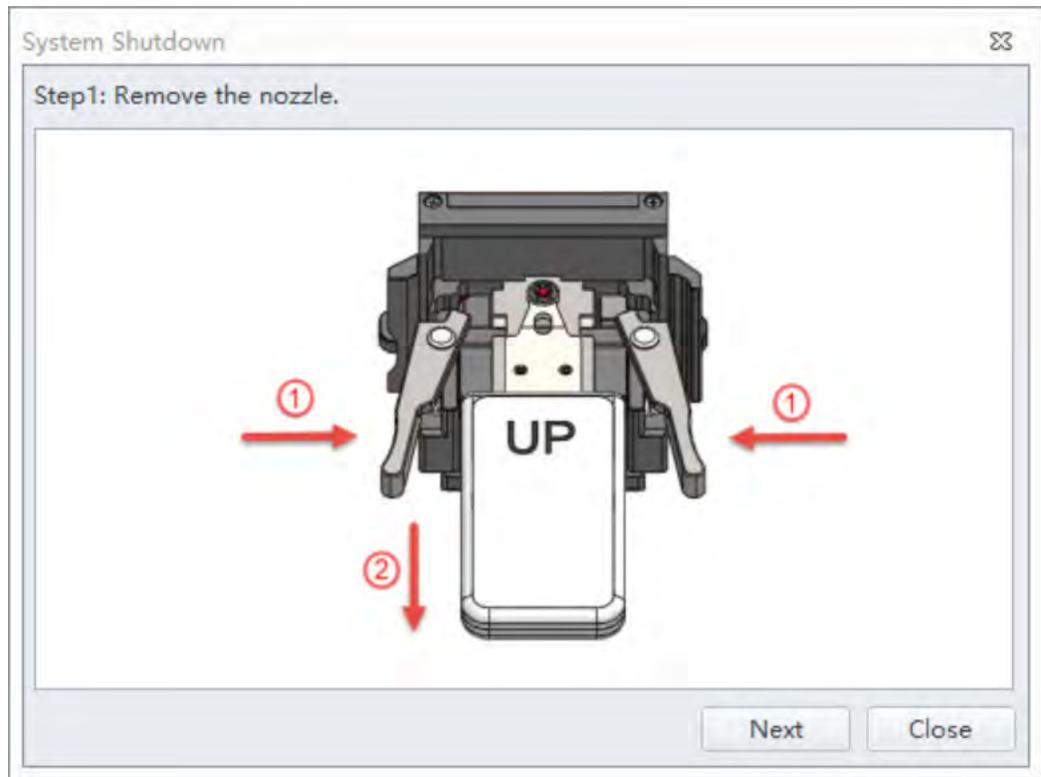
It is required to run the System Shutdown Program every time you shut down the instrument. Refer to [System Shutdown](#). However, if the instrument will not be used for more than 7 days, it is recommended to perform the Long Term Shutdown program. Refer to [Long Term Shutdown](#).

System Shutdown

- 1 Select **Standby** to turn off the sheath.
- 2 Select **System Shutdown** from the Cytometer menu.



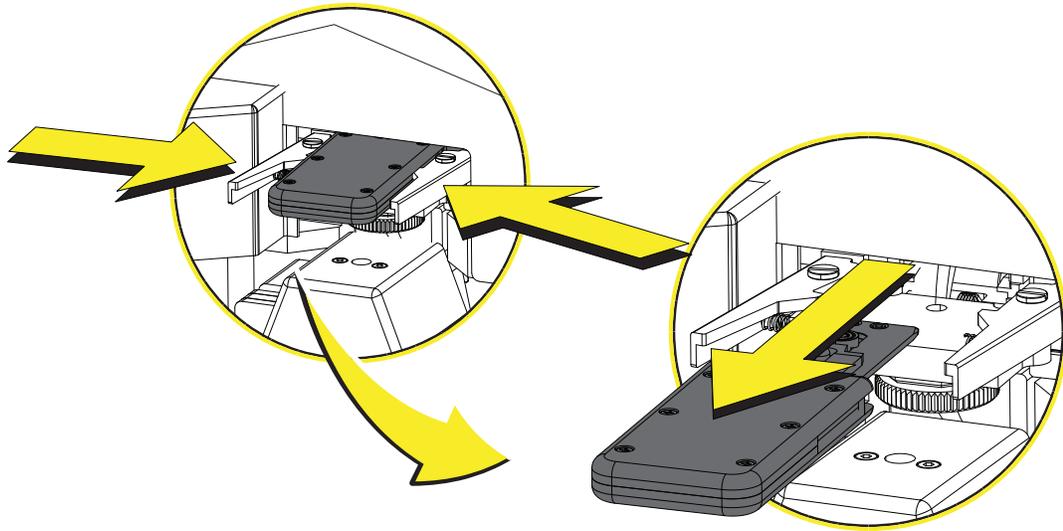
The System Shutdown window appears.



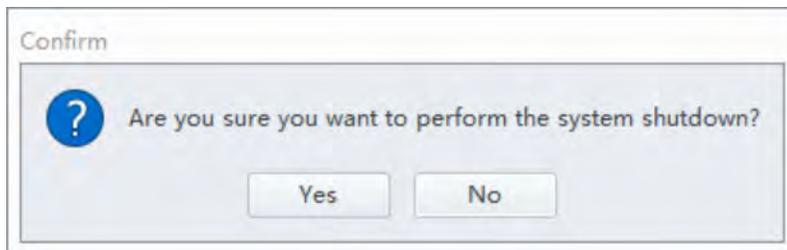
CAUTION

Risk of damage to the nozzle. When cleaning or replacing the nozzle, always handle with care to prevent the nozzle module from falling.

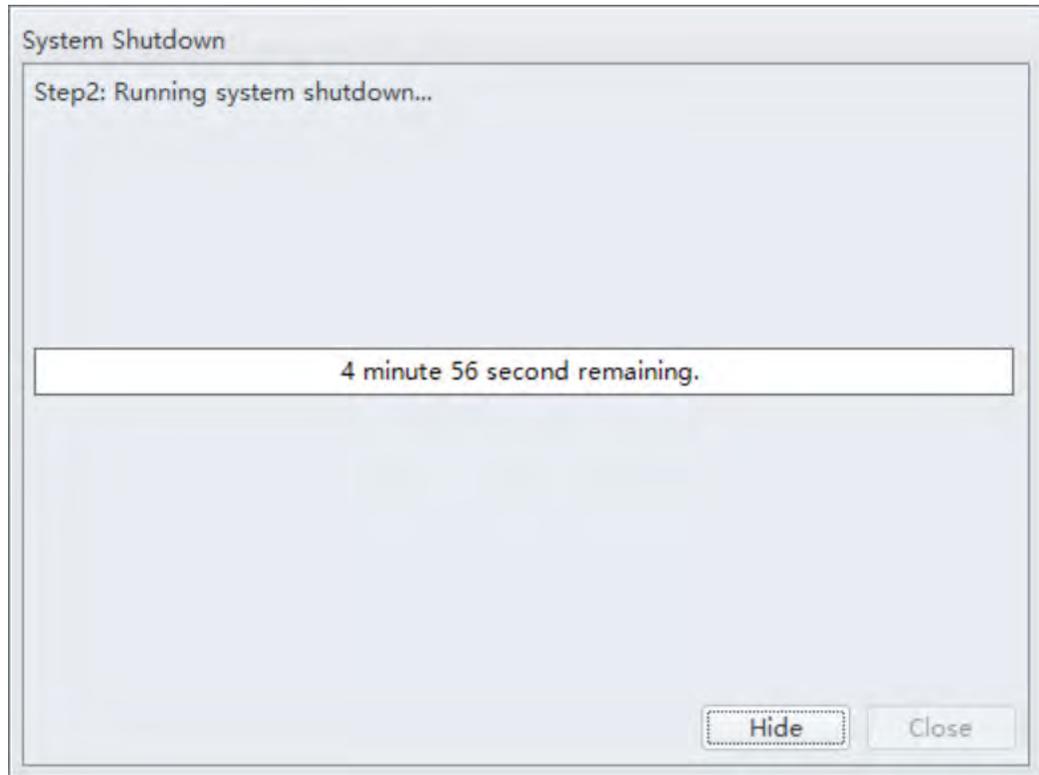
- 3 Remove the nozzle module by pushing the metal release clamps inwards.



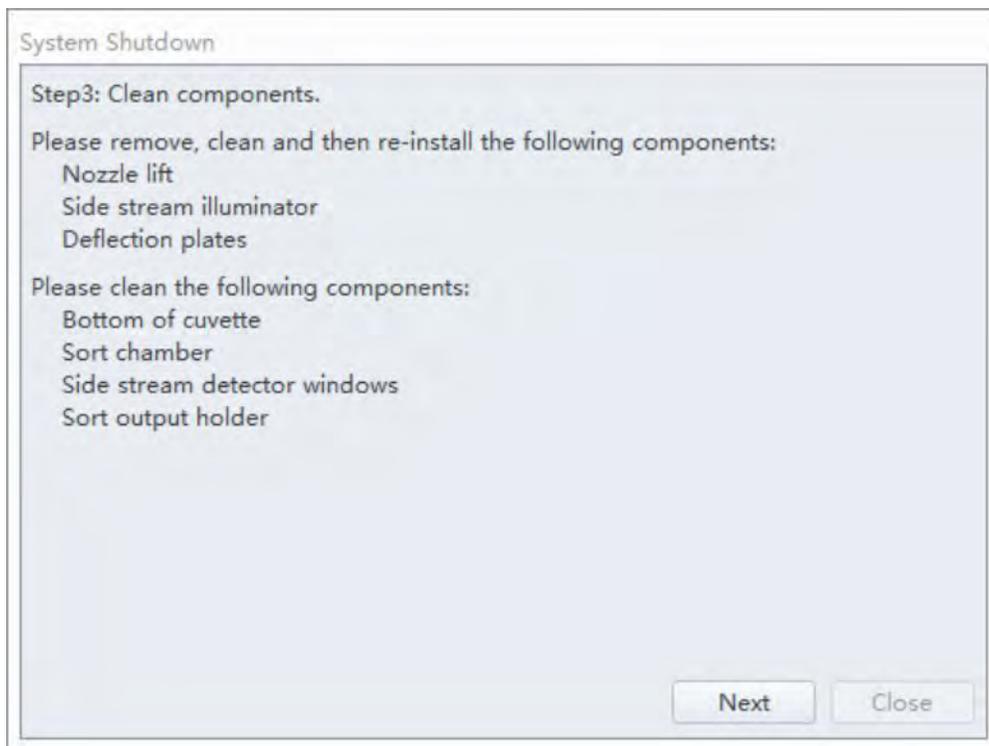
- 4 Select **Next**. The Confirm window displays.



- 5 Select **Yes** to start the System Shutdown program. The System Shutdown program takes about five minutes.

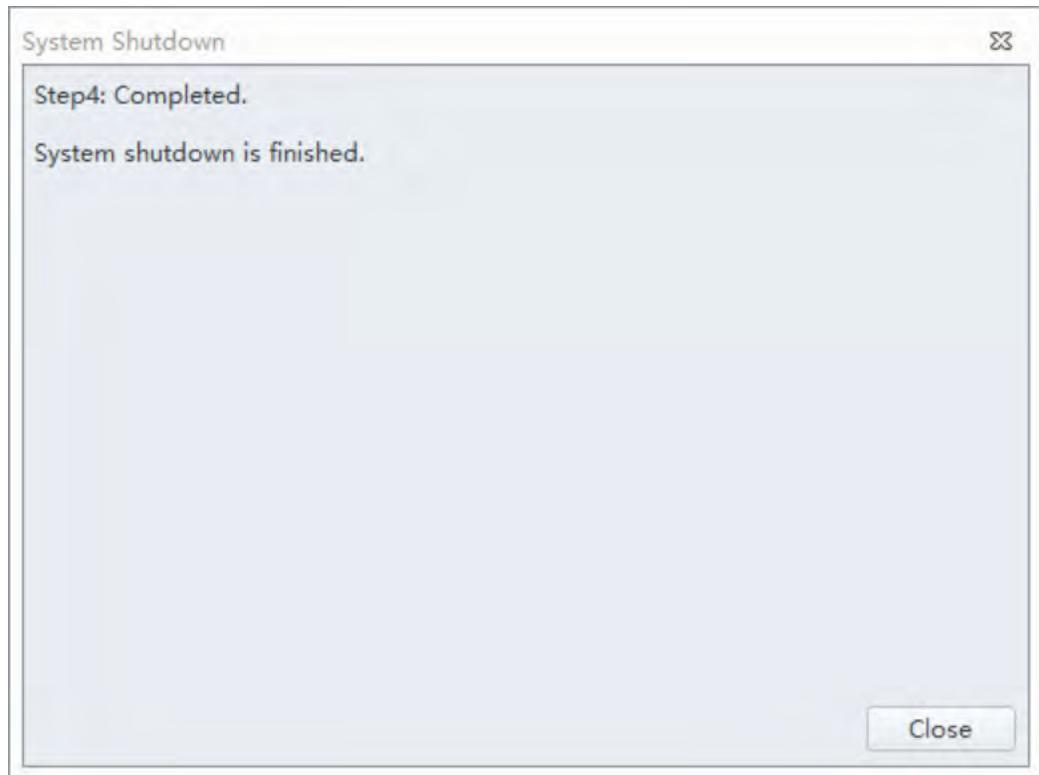


Then the following window displays.



-
- 6** Perform the cleaning. Refer to [Cleaning during Shutdown](#).

7 Select **Next**.



8 Select **Close**.

The system enters into the idle state.



Long Term Shutdown

The **Long Term Shutdown** is only available when the system is in the idle state.

IMPORTANT Once you begin, ensure that you complete the entire Long Term Shutdown procedure before leaving the instrument. The entire process takes about 30 minutes.

IMPORTANT If the Long Term Shutdown procedure was terminated by accident, perform the following:

- Empty the sheath tank and refill the sheath tank with IsoFlow sheath.
- Switch the aseptic cleaning filter back to the sheath fluid filter
- Restart the instrument
- Run the System Startup Program.

Required materials

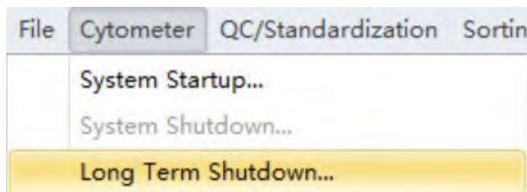
- 1.5 L 70% ethanol in deionized water
- An aseptic clean solution filter
- A new waste air filter

1 Run System Shutdown program to put the system in idle state. Refer to [System Shutdown](#).

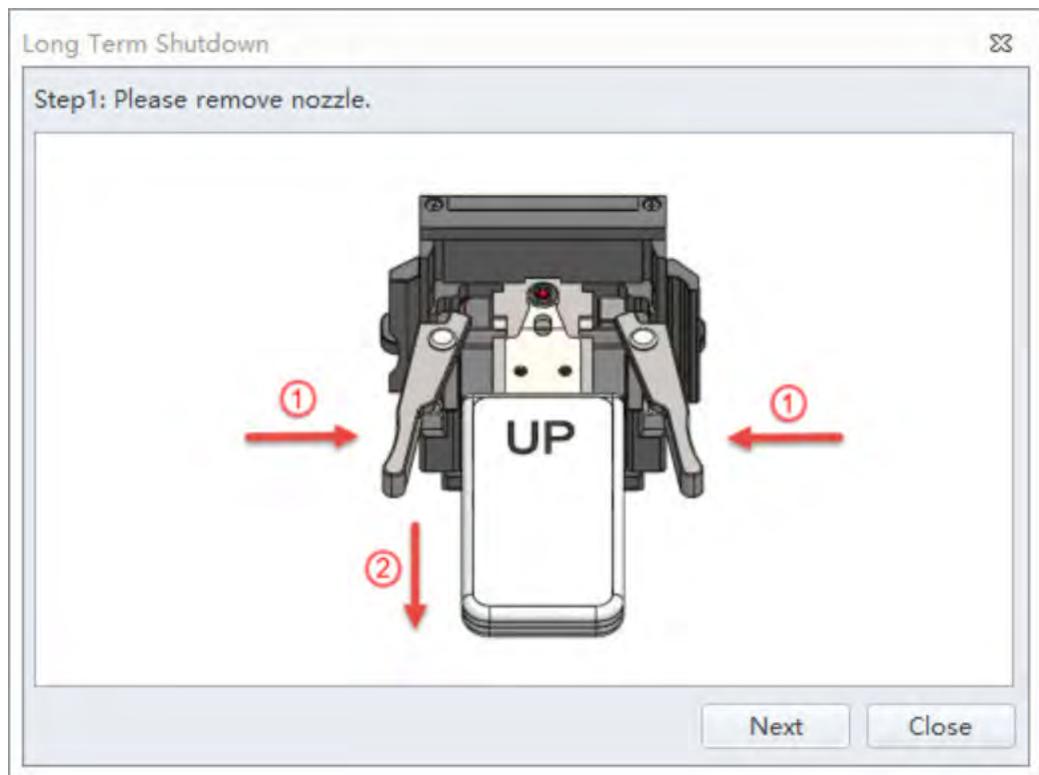
IMPORTANT Ensure that the sheath tank you used matches with the sheath tank type you selected. Otherwise, you could encounter System Startup failure, Aseptic Cleaning failure, Long-term Shutdown failure, or sheath flow indication error.

2 Verify that the sheath tank you used are matched with the sheath tank type you selected. Select Sheath Tank Scale Reset from the Advanced menu to display the current sheath tank type.

3 Select **Long Term Shutdown** in the Cytometer menu.



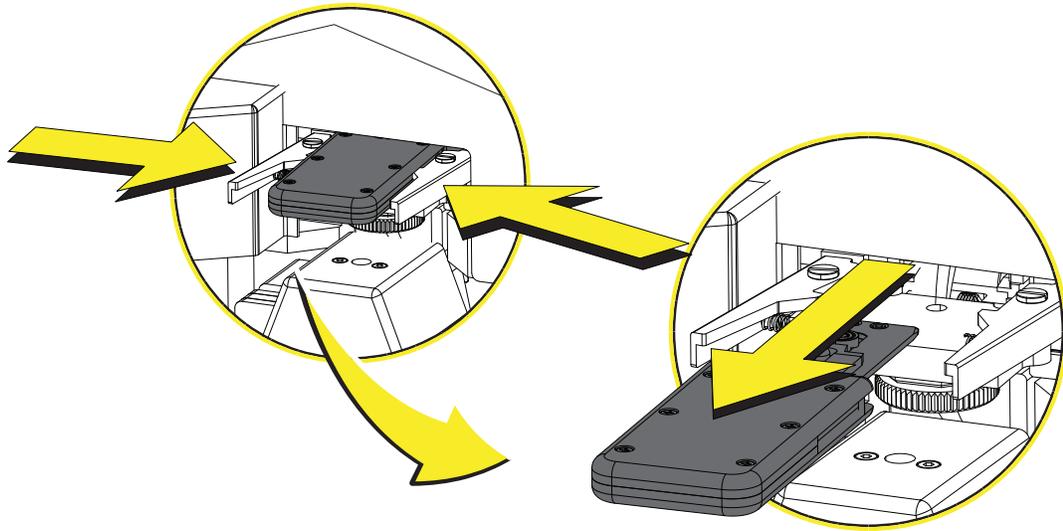
The Long Term Shutdown window appears.



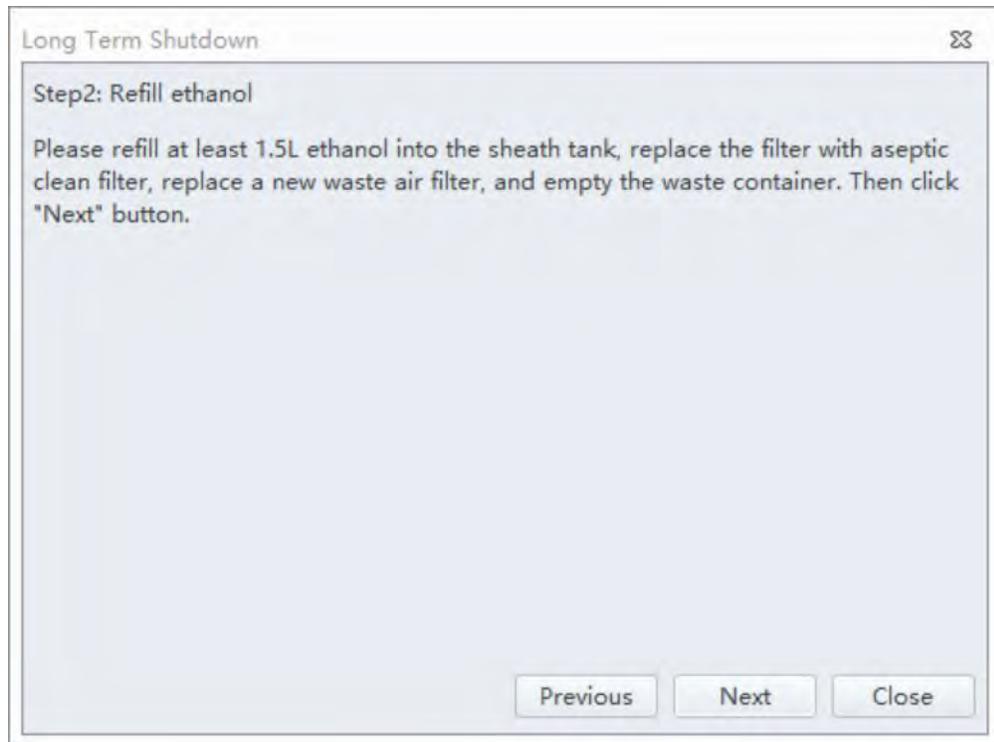
⚠ CAUTION

Risk of damage to the nozzle. When cleaning or replacing the nozzle, always handle with care to prevent the nozzle module from falling.

- 4 Remove the nozzle module by pushing the metal release clamps inwards.



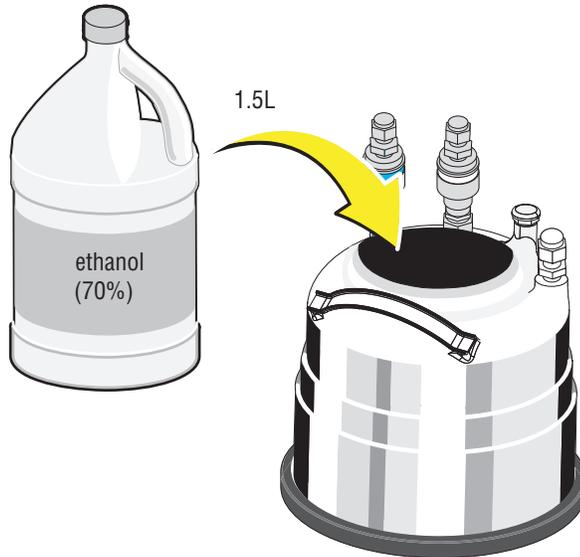
- 5 Select **Next**. The following window appears.



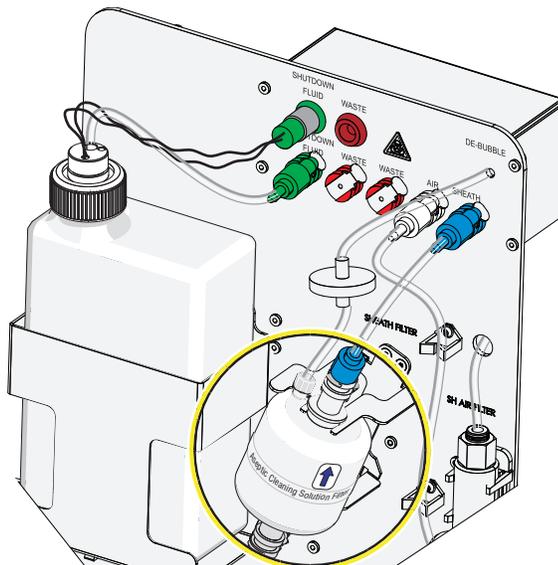
WARNING

Risk of fire hazard. Ethanol is a volatile liquid that cannot be used near a fire source.

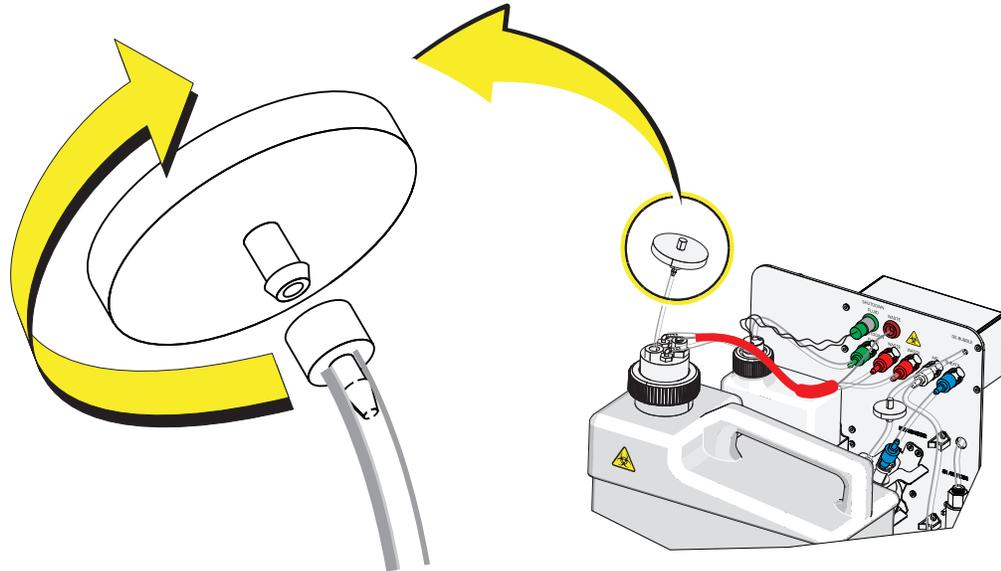
- 6 Empty the sheath tank and refill the sheath tank with at 1.5 L 70% ethanol. For instructions, refer to [Filling the Sheath Tank](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)



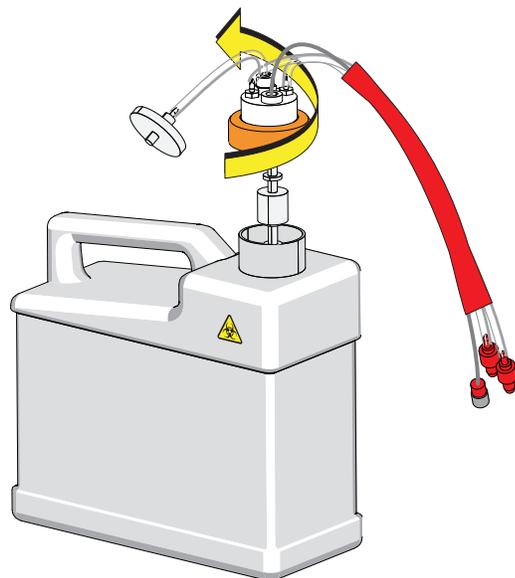
- 7 Replace the sheath fluid filter with an aseptic cleaning filter. For instructions, refer to [Replacing the Aseptic Cleaning Solution Filter](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).



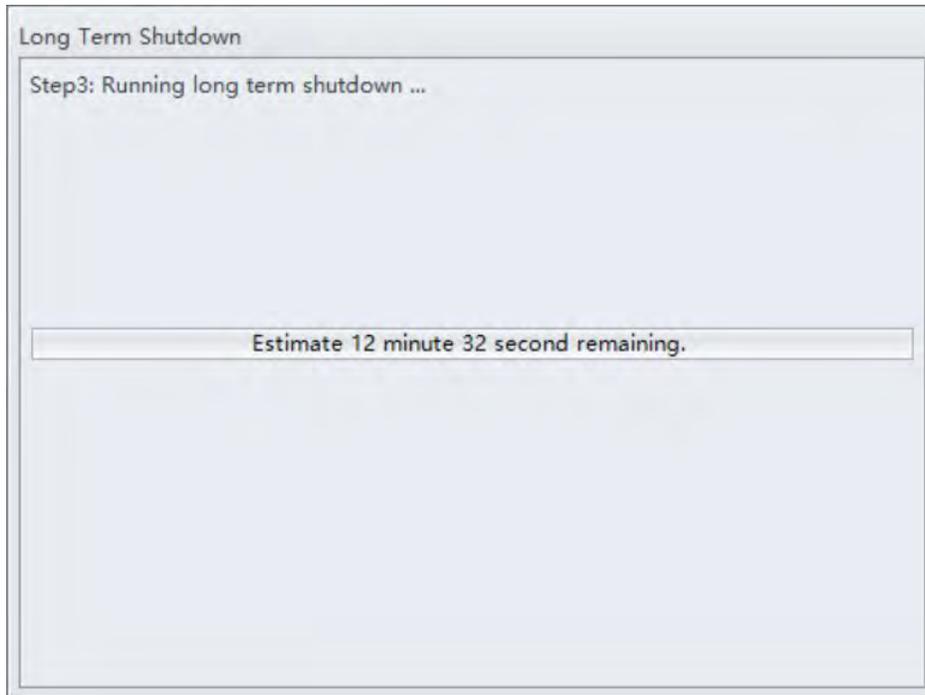
- 8 Replace the waste air filter with a new one. For instructions, refer to [Replacing the Waste Air Filter](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).



- 9 Empty the waste container. For instructions, refer to [Emptying the Waste Container](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).



- 10 Select **Next**. The system starts cleaning the sheath line, flow cell, and sample probe with the 70% ethanol solution. This process takes about 15 minutes.

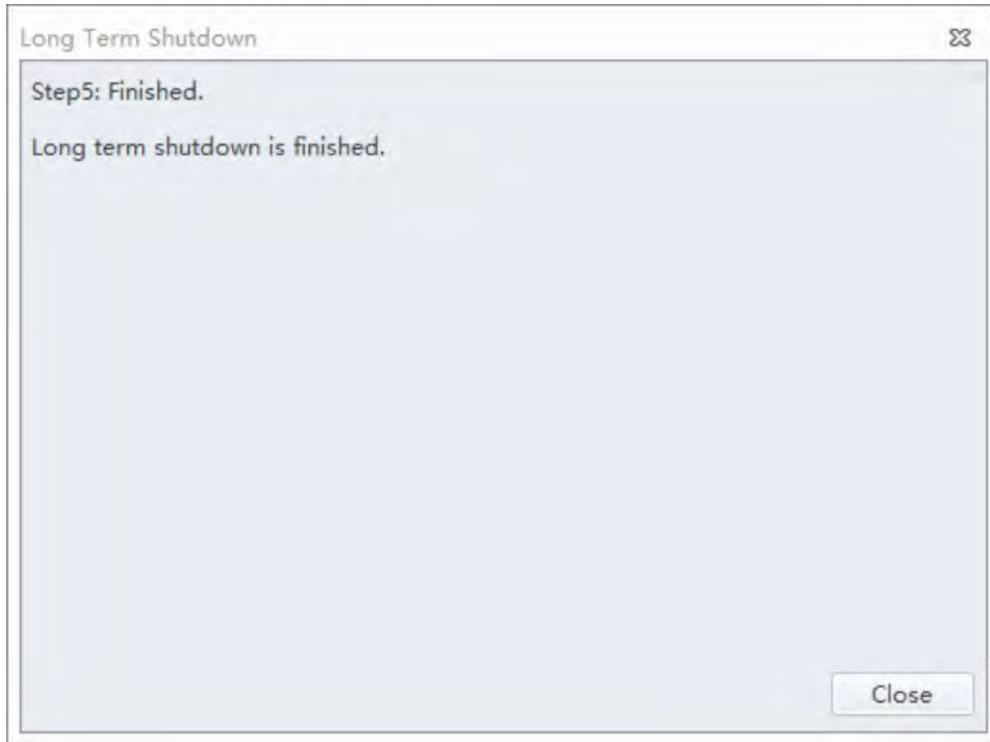


Wait for the process to finish. The following window appears.



11 Perform the cleaning. Refer to [Cleaning during Shutdown](#).

12 Select **Next**.



13 Select **Close**. The flow cell becomes empty.

NOTE The Long Term Shutdown program might introduce air bubbles into the system. Refer to [Removing Trapped Air Bubbles in CHAPTER 11, Replacement/Adjustment Procedures](#).

Cleaning during Shutdown



Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

WARNING

Use barrier protection, including protective eye wear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using chemicals.

Before you leave the instrument, clean the following instrument components:

- Deflection plates
- Nozzle
- Nozzle lift
- Bottom of cuvette
- Side stream illumination source
- Stream detection window
- Sort output holder
- All surfaces inside sort chamber
- Auxiliary door
- Sample station

WARNING

Risk of chemical injury from bleach. To avoid contact with the bleach, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

If a hazardous substance such as blood is spilled on the instrument, clean up the spill by using a 10% high-quality, fragrance-free, gel-free bleach solution, or use your laboratory decontamination solution. Then follow your laboratory procedure for disposal of hazardous materials. If the instrument needs to be decontaminated, [contact us](#).

- 1 Clean the nozzle. For instructions, refer to [Cleaning the Nozzle](#) in [CHAPTER 10, Cleaning Procedures](#).

-
- 2 Clean the deflection plates and side stream illumination source. For instructions, refer to [Cleaning the Side Stream Illumination Source and the Deflection Plates](#) in CHAPTER 10, [Cleaning Procedures](#).

 - 3 Clean the nozzle lift. Refer to [Daily Decontamination During Shutdown](#) in CHAPTER 10, [Cleaning Procedures](#).

 - 4 Clean the side stream illumination source, the bottom of cuvette, the sort output holder, side stream detection window and sort chamber. Refer to [Daily Decontamination During Shutdown](#) in CHAPTER 10, [Cleaning Procedures](#).

 - 5 **Optional:** Clean and refill the sheath tank. Refer to [Filling the Sheath Tank](#) in CHAPTER 11, [Replacement/Adjustment Procedures](#) and [Cleaning the Sheath Tank](#) in CHAPTER 10, [Cleaning Procedures](#).

NOTE Cleaning the sheath tank can be done daily or weekly as deemed appropriate by your laboratory manager.

 - 6 **Optional:** Empty and clean the waste container. Refer to [Emptying the Waste Container](#) in [Replacement/Adjustment Procedures](#), and [Cleaning the Waste Container](#) in CHAPTER 10, [Cleaning Procedures](#).
-

Turning Off the Power



Risk of corrupting the embedded controller's file system. Do not skip the System Shutdown program to shut down the system. Turning off the main power switch located on the Sorter or unplug the Sorter's power cable directly may corrupt the system.

NOTE Powering down is optional for daily shutdown but mandatory for the Long Term Shutdown.

-
- 1 Turn off the Sorter's main power switch located on the lower left side.

NOTE **Turn off** in the Cytometer menu can be used to remote power off the Sorter.

-
- 2 Exit the software.

3 Turn the computer off.

4 **Optional:** Turn the Biosafety Cabinet off.

NOTE It is recommended to leave the Biosafety Cabinet on to save time for the thermal equilibrium if the Sorter will be used the next day.

Overview

IMPORTANT In addition to the information stated, never disassemble the instrument or have it repaired by unauthorized personnel. Beckman Coulter bears no responsibility for any problems arising from the unauthorized repair of the instrument.

This chapter introduces solutions to common problems. If there is a problem, follow the information in this chapter to carry out self-inspection. If the problem cannot be resolved, [contact us](#).

This chapter contains information on:

- [Laser Related Hazards](#)
- [Hazard Labels and Locations](#)
- [Disposal Precaution](#)
- [Troubleshooting Table](#)
- [Backup and Restore](#)

Laser Related Hazards

Beckman Coulter design and manufacture of the instrument complies with the requirements governing the use and application of a laser specified in regulatory documents issued by the:

- U.S. Department of Health and Human Services
- Center for Devices and Radiological Health (CDRH)
- International Electrotechnical Commission (IEC)

In compliance with these regulatory documents, every measure has been taken to ensure the health and safety of users and laboratory personnel from the possible dangers of laser use.

Use the instrument according to the information in the manuals.

Use controls or adjustments or performance of procedures other than those specified herein might result in hazardous radiation exposure.

To ensure your safety, the lasers are covered with protective shields. Do not remove these shields.

No user-serviceable assemblies are accessible. Do not attempt to remove the laser or open it. The instrument has components that are dangerous to the operator. If any attempt has been made to defeat a safety feature, or if the instrument fails to perform as described in its manuals, disconnect the power and [contact us](#).

Laser Beam Hazards

The CytoFLEX SRT instrument contains 4 solid-state diode lasers that are capable of producing laser light at the following levels:

- 405-nm, 90-mW solid-state diode laser
- 488-nm, 50-mW solid-state diode laser
- 561-nm, 30-mW solid-state diode laser
- 638-nm, 100-mW solid-state diode laser

A laser beam is a unique light source that shows characteristics different from conventional light sources. The safe use of the laser depends upon familiarity with the instrument and the properties of coherent, intense beams of light.



Risk of personal injury. The laser beam can cause eye damage if viewed either directly or indirectly from reflective surfaces (such as a mirror or shiny metallic surfaces). To prevent eye damage, avoid direct exposure to the laser beam. Do not view it directly or with optical instruments.

The laser beam can cause eye or skin damage, as well as instrument damage. The laser has enough power to ignite substances placed in the beam path, even at a distance. Indirect contact with the laser beam from reflective surfaces (such as jewelry or a screwdriver) is called specular reflection and might also cause damage.

For these reasons, use the following precautions when working near an exposed laser:

- Never look directly into the laser light source or at scattered laser light from any reflective surface. Never look down the beam into the source.
- As a precaution against accidental exposure to the output beam or its reflection, wear proper laser safety glasses (as required by the wavelength being used) when performing service or maintenance procedures on the system.
- Do not use lasers in the presence of flammable material or explosives; these include volatile substances such as alcohol, solvents, and ether.
- Avoid direct exposure and indirect reflection of the laser beam to your skin. The intensity of the beam can cause flesh burns.
- Assure that any spectators are not potentially exposed to a hazardous condition.
- Do not leave the laser unattended if there is a chance that an unauthorized person may try to use it.
- Make the following recommendations to the laboratory:
 - Limit access to the instrument. Keep the instrument out of hands of inexperienced and untrained personnel.
 - Never attempt to remove a shield housing a laser.
 - Post warning signs at the site to alert personnel that lasers are being used.
 - Never remove a warning label from the instrument.
 - [Contact us](#) if a label is missing or unclear.

Laser Warning Labels



Risk of personal injury from radiation exposure. Never remove the shield surrounding a laser. Never remove covers.

CDRH-approved and IEC compliant labels are also placed near or on those covers that when removed might expose laser radiation. If necessary, a cover with a CDRH-approved or IEC compliant label must be removed by a qualified Beckman Coulter Representative only.

Refer to the following figures for the locations of the CDRH-approved and IEC compliant labels:

Refer to [Figure 9.1](#) for the Laser Warning Label on the Sorter optical bench.

Refer to [Figure 9.2](#) for the Laser Warning Label on the optical bench (located Inside the Sorter).

Refer to [Figure 9.3](#) for the Laser Warning Labels on the Sorter Back Cover.

The laser product is classified as CLASS 1 when all protective measures are in place. This product complies with 21 CFR Parts 1040.10 and 1040.11 as well as EN60825-1. Refer to [Figure 9.1](#).

Figure 9.1 Laser Warning Label on the Laser Optical Bench

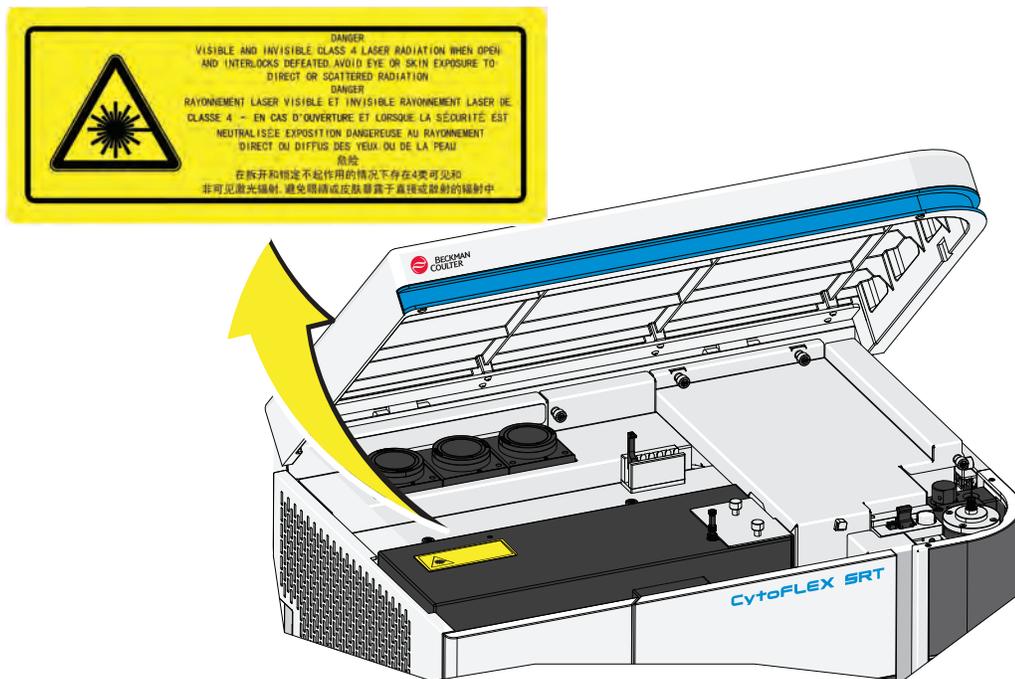


Figure 9.2 Laser Warning Label within the Optical Bench (Located Inside the Sorter)

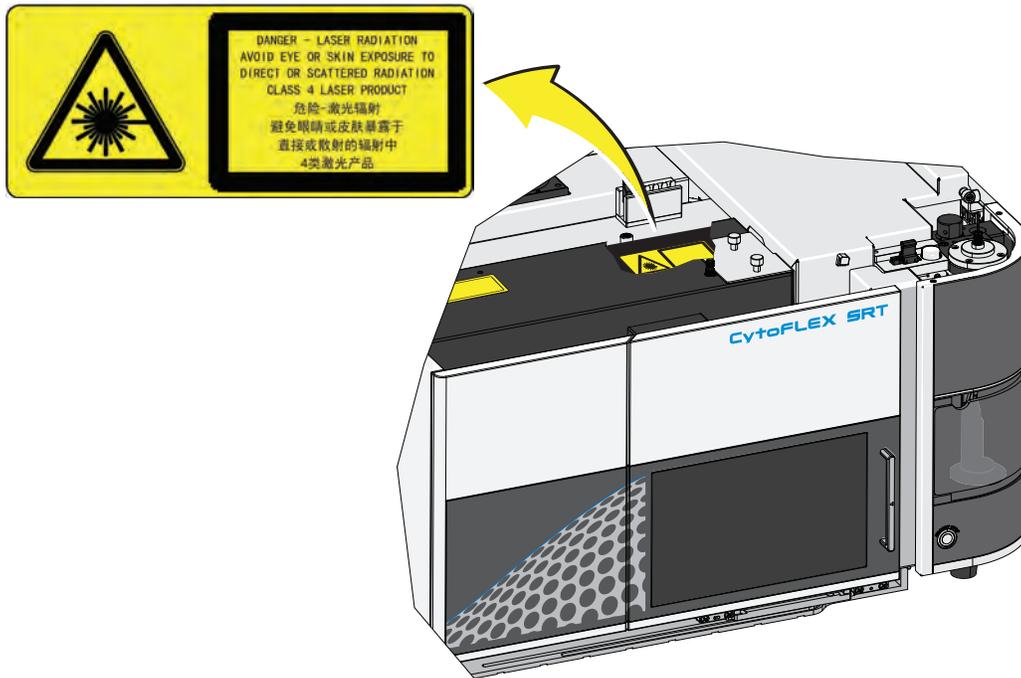
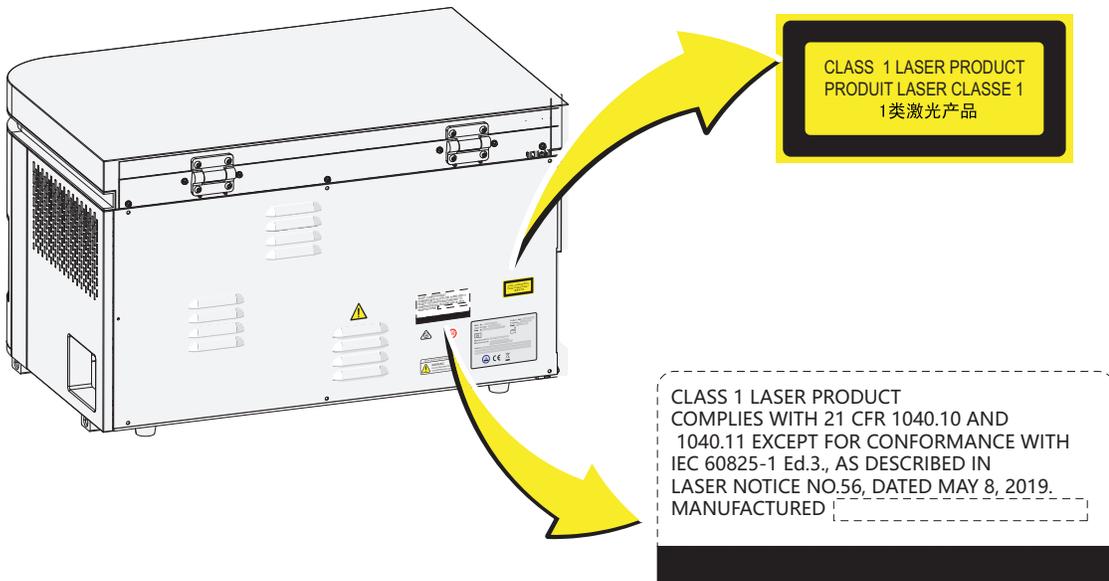


Figure 9.3 Laser Warning Labels on the Sorter Back Cover



Hazard Labels and Locations

Carefully read the hazard warning labels on the instrument. The hazard labels are located on the instrument as indicated.

NOTE If a label is missing or unclear, [contact us](#).

Biohazard Label and Location

Figure 9.4 Biohazard Label on the Fluidics Cart and Waste Container

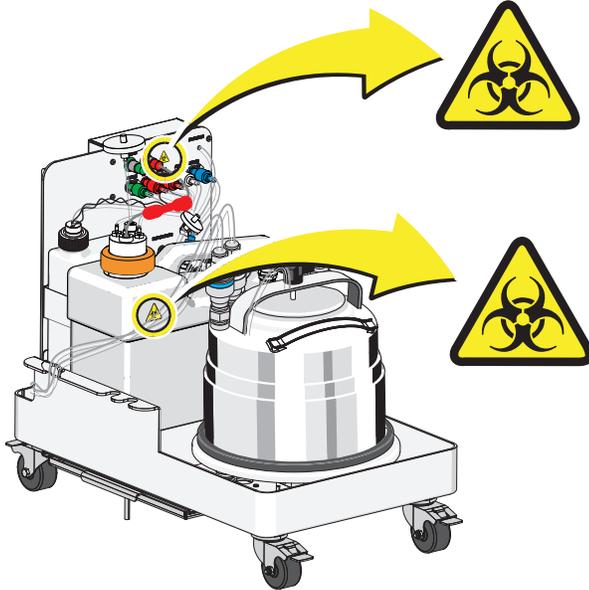
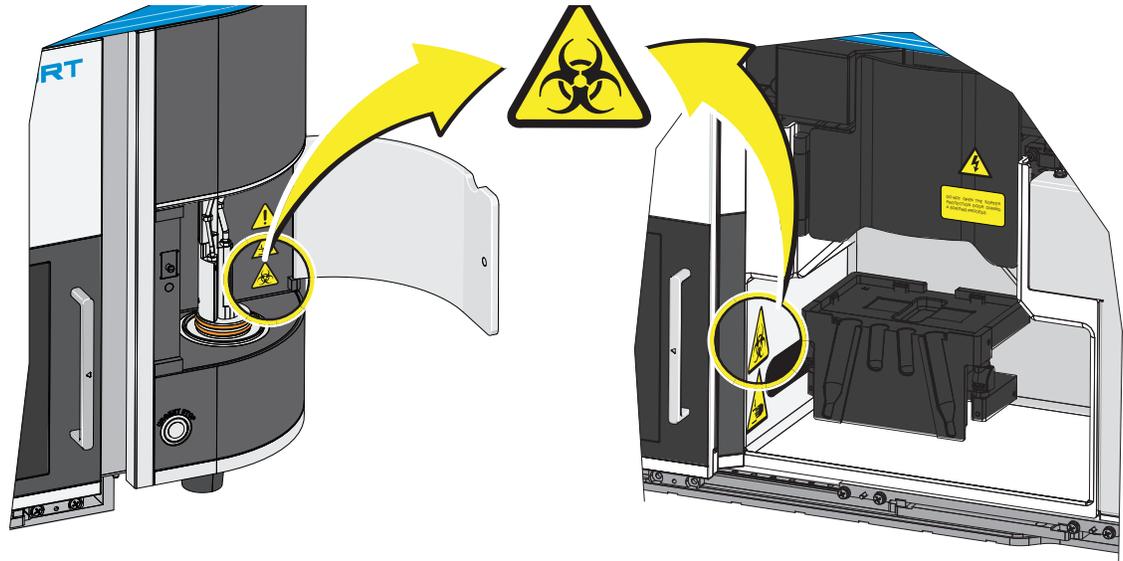
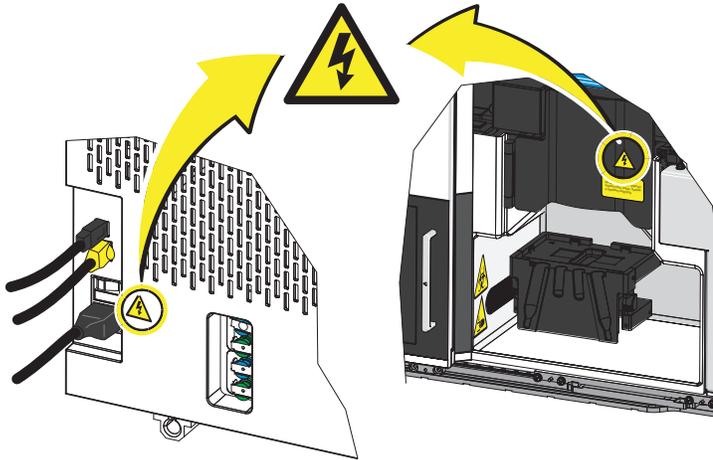


Figure 9.5 Biohazard Label on the Sample Station and Sort Chamber



Electrical Shock Hazard Label and Location

Figure 9.6 Electrical Shock Hazard Label



Caution Labels and Location

Figure 9.7 Caution Label on the Sorter Back Cover

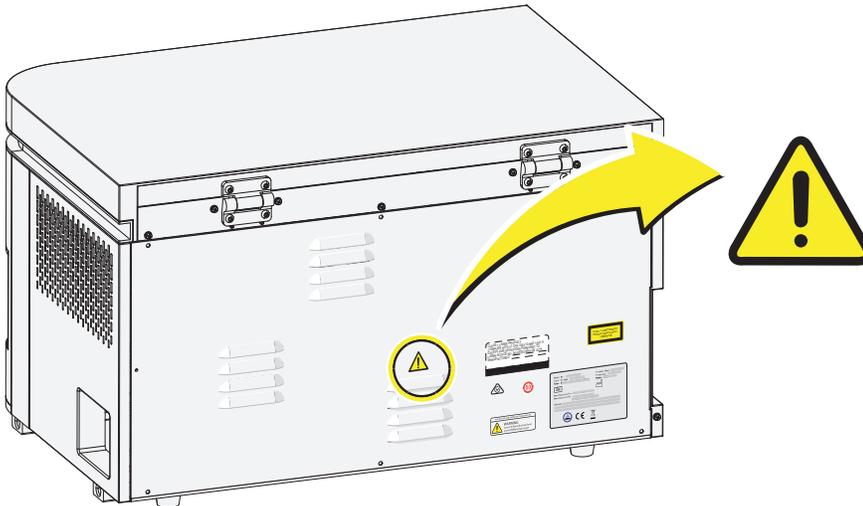


Figure 9.8 Label on the Sort Protection Door

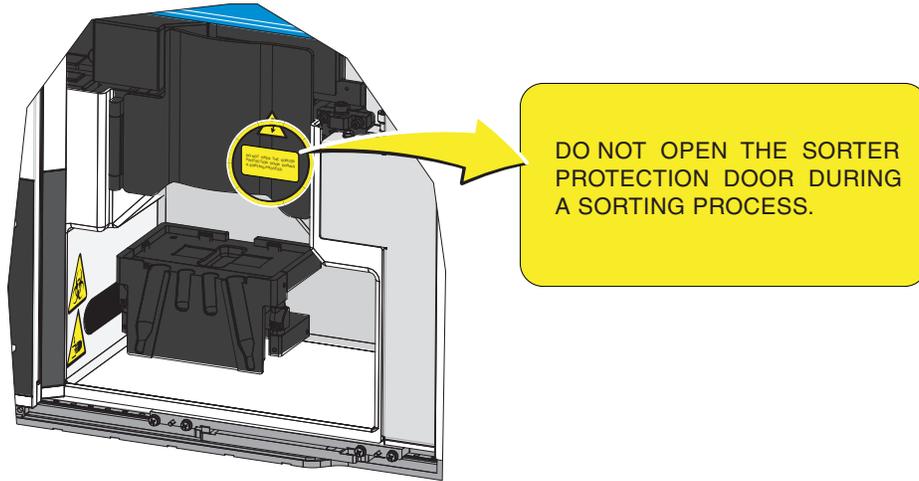
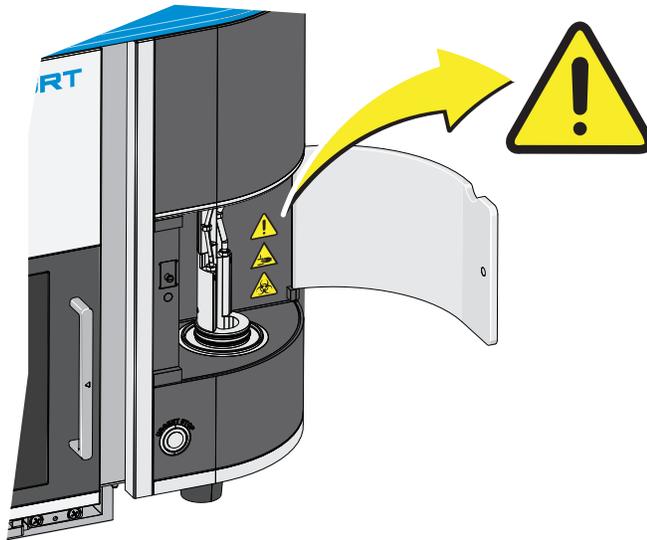


Figure 9.9 Caution Label on the Sample Station



Pinch Hazard Labels and Location

Figure 9.10 Pinch Hazard Label on the Sample Station

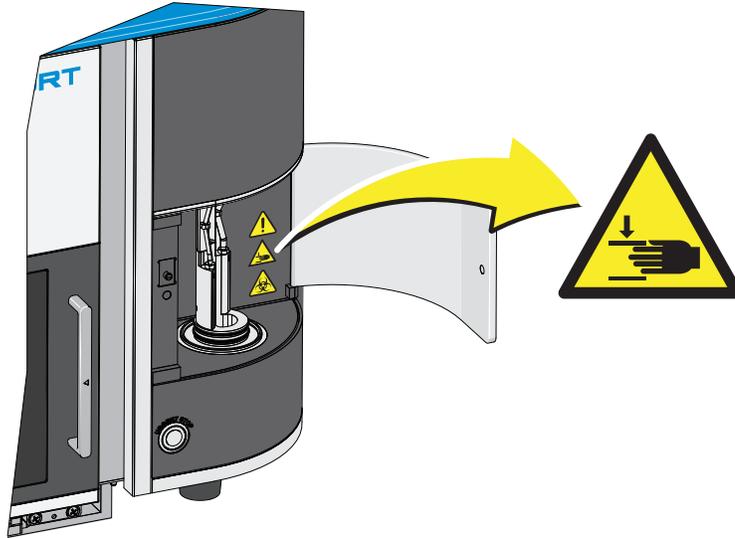
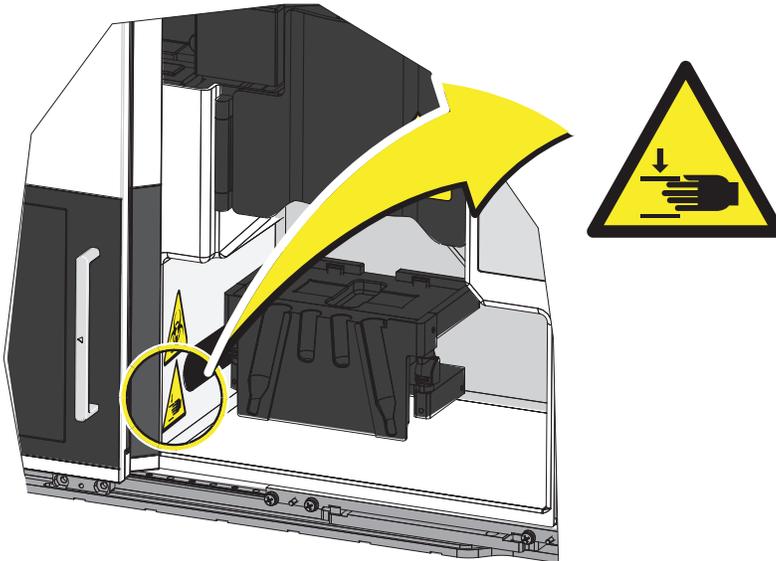


Figure 9.11 Pinch Hazard Label on the Sort Chamber



Disposal Precaution



WARNING

Risk of biohazardous contamination if you have skin contact with the waste container, its contents, and its associated tubing. The waste container and its associated tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of the waste container in accordance with your local regulations and acceptable laboratory procedures.

Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

Troubleshooting Table

Table 9.1 and Table 9.2 list problems that you could encounter while running the Sorter, the probable causes of each problem, and the corrective actions. These problems are listed alphabetically in the Index, under the primary entry “troubleshooting.”

Table 9.1 Troubleshooting-[Error Codes]

Problem	Probable Cause	Corrective Action
[Error code 010037] <i>Bubble detector error.</i>	<ul style="list-style-type: none"> • The silicone tubing is not completely seated in the slot of the sample line bubble detector. • Tube holder contains water. • The sample probe height is not accurate. • The bubble detector is defective. • Bubble Detector Calibration is not run after replacing the sample line. 	<ol style="list-style-type: none"> 1. Verify that the joint between the silicone tubing and the PEEK tubing is completely in the slot of bubble detector. Refer to Steps 19 -21 in Replacing the Sample Line. 2. Verify that the tube holder is clean and dry. 3. Verify that the sample probe height is accurate. Refer to Steps 10 - 14 in CHAPTER 11, Replacement/Adjustment Procedures. 4. Calibrate the bubble detector again. Refer to Calibrating the Sample Line Bubble Detector. 5. If the problem persists, contact us.
[Error code 060048] <i>Failed to set the charge phase. No droplet is detected.</i>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The side stream illumination is defective or not installed. • The charge drive board is defective. • The acquisition circuit board is defective. • The photodiodes are defective. • High-voltage components are broken. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 3. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 4. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 5. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 7. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 8. Clean the side stream illumination source. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 9. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (Continued)

Problem	Probable Cause	Corrective Action
<p>[Error code 060049] <i>The charge phase value is out of range.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • The charge drive board is defective. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 3. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 4. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 5. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 7. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 8. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 9. Wipe the side stream detection window. Refer to Figure 1.23. 10. Verify that the nozzle lift is clean and dry. Refer to Steps 4 - 6 in CHAPTER 10, Cleaning Procedures. 11. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (*Continued*)

Problem	Probable Cause	Corrective Action
<p>[Error code 060050] <i>Failed to set the charge voltage of R1 stream.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • The charging drive board is defective. • Electric leakage of high-voltage component. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 3. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 4. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 5. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 7. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 8. Wipe the side stream detection window. Refer to Figure 1.23. 9. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 10. Verify that the nozzle lift is clean and dry. Refer to Steps 4 - 6 in CHAPTER 10, Cleaning Procedures. 11. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (Continued)

Problem	Probable Cause	Corrective Action
<p>[Error code 060051] Charge phase verification failed.</p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • The charging drive board is defective. • Electric leakage of high-voltage component. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 3. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 4. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 5. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 7. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 8. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 9. Wipe the side stream detection window. Refer to Figure 1.23. 10. Verify that the nozzle lift is clean and dry. Refer to Steps 4 - 6 in CHAPTER 10, Cleaning Procedures. 11. Verify that the ambient temperature in the lab is within the range (15-27 °C), and the fluctuation is within <math>\pm 2</math> °C. Use appropriate air condition if necessary. 12. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (*Continued*)

Problem	Probable Cause	Corrective Action
<p>[Error code 060052] <i>Charge phase optimization failed.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • Airflow interference. • The narrow slot mask of the PD is partly or completely blocked. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 3. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 4. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 5. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 7. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 8. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 9. Wipe the side stream detection window. Refer to Figure 1.23. 10. Verify that the nozzle lift is clean and dry. Refer to Steps 4 - 6 in CHAPTER 10, Cleaning Procedures. 11. Verify that the ambient temperature in the lab is within the range (15-27 °C), and the fluctuation is within $< \pm 2$ °C. Use appropriate air condition if necessary. 12. Verify that the Sorter is not exposed directly to a ventilation outlet of an air condition. 13. Rerun Sort Calibration. Refer to Sort Calibration (Auto Drop Delay) in CHAPTER 5, Sorting. 14. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (Continued)

Problem	Probable Cause	Corrective Action
<p>[Error code 060053] <i>The charge voltage of L2 stream is out of range.</i></p> <p>[Error code 060054] <i>The charge voltage of L1 stream is out of range.</i></p> <p>[Error code 060056] <i>The charge voltage of R1 stream is out of range.</i></p> <p>[Error code 060057] <i>The charge voltage of R2 stream is out of range.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • Airflow interference. • Electric leakage of high-voltage component. • Leakage of the fluidics system. • The narrow slot mask of the PD is partly or completely blocked. • The core stream or the side stream Hardware is not straight enough. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 3. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 4. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 5. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 7. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 8. Wipe the side stream detection window. Refer to Figure 1.23. 9. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 10. Verify that the nozzle lift is clean and dry. Refer to Steps 4 - 6 in CHAPTER 10, Cleaning Procedures. 11. Ensure that the ambient temperature in the lab is stable and within the range (15-27 °C), and the fluctuation is within <math>\pm 2\text{ }^\circ\text{C}</math>. Use appropriate air condition if necessary. 12. Verify that the Sorter is not exposed directly to a ventilation outlet of an air condition. 13. Rerun Sort Calibration. Refer to Sort Calibration (Auto Drop Delay) in CHAPTER 5, Sorting. 14. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (*Continued*)

Problem	Probable Cause	Corrective Action
<p>[Error code 060058] <i>Failed to optimize the charge voltage for L2 stream.</i></p> <p>[Error code 060059] <i>Failed to optimize the charge voltage for L1 stream.</i></p> <p>[Error code 060060] <i>Failed to optimize the charge voltage for R1 stream.</i></p> <p>[Error code 060061] <i>Failed to optimize the charge voltage for R2 stream.</i></p> <p>[Error code 060062] <i>Failed to optimize the charge voltage for central stream.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • Airflow interference. • Electric leakage of high-voltage component. • Leakage of the fluidics system. • The narrow slot mask of the PD is partly or completely blocked. • The core stream or the side stream Hardware is not straight enough. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 3. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 4. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 5. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 7. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 8. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 9. Wipe the side stream detection window. Refer to Figure 1.23. 10. Verify that the nozzle lift is clean and dry. Refer to Steps 4 - 6 in CHAPTER 10, Cleaning Procedures. 11. Ensure that the ambient temperature in the lab is stable and within the range (15-27 °C), and the fluctuation is within $< \pm 2$ °C. Use appropriate air condition if necessary. 12. Verify that the Sorter is not exposed directly to a ventilation outlet of an air condition. 13. Rerun Sort Calibration. Refer to Sort Calibration (Auto Drop Delay) in CHAPTER 5, Sorting. 14. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (Continued)

Problem	Probable Cause	Corrective Action
<p>[Error code 060063] Failed to determine the Detector Delay for L2 stream.</p> <p>[Error code 060064] Failed to determine the Detector Delay for L1 stream.</p> <p>[Error code 060065] Failed to determine the Detector Delay for R1 stream.</p> <p>[Error code 060066] Failed to determine the Detector Delay for R2 stream.</p> <p>[Error code 060067] Failed to determine the Detector Delay for Gap detector.</p> <p>[Error code 060068] Failed to determine the Detector Delay for Central stream.</p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • Airflow interference. • Electric leakage of high-voltage component. • Leakage of the fluidics system. • The narrow slot mask of the PD is partly or completely blocked. • The core stream or the side stream module is not aligned. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 3. Wipe the side stream detection window. Refer to Figure 1.23. 4. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 5. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 7. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 8. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 9. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 10. Verify that the Sorter is not exposed directly to a ventilation outlet of an air condition. 11. Rerun Sort Calibration. Refer to Sort Calibration (Auto Drop Delay) in CHAPTER 5, Sorting. 12. Restart the system. Refer to CHAPTER 3, Daily Startup. 13. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (*Continued*)

Problem	Probable Cause	Corrective Action
<p>[Error code 060069] <i>Scan defanning value error.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • Airflow interference. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 3. Wipe the side stream detection window. Refer to Figure 1.23. 4. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 5. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 7. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 8. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 9. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 10. Verify that the Sorter is not exposed directly to a ventilation outlet of an air condition. 11. Rerun Sort Calibration. Refer to Sort Calibration (Auto Drop Delay) in CHAPTER 5, Sorting. 12. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (Continued)

Problem	Probable Cause	Corrective Action
<p>[Error code 060070] <i>The Central stream is not tight.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • Airflow interference. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 3. Wipe the side stream detection window. Refer to Figure 1.23. 4. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 5. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 7. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 8. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 9. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 10. Verify that the Sorter is not exposed directly to a ventilation outlet of an air condition. 11. Rerun Sort Calibration. Refer to Sort Calibration (Auto Drop Delay) in CHAPTER 5, Sorting. 12. Restart the system. Refer to CHAPTER 3, Daily Startup. 13. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (*Continued*)

Problem	Probable Cause	Corrective Action
<p>[Error code 060075] <i>The defanning value is out of range.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • Airflow interference. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Clean the side stream illumination and the deflection plates. Refer to Cleaning the Side Stream Illumination Source and the Deflection Plates in CHAPTER 10, Cleaning Procedures. 3. Wipe the side stream detection window. Refer to Figure 1.23. 4. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 5. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 7. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 8. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 9. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 10. Verify that the Sorter is not exposed directly to a ventilation outlet of an air condition. 11. Rerun Sort Calibration. Refer to Sort Calibration (Auto Drop Delay) in CHAPTER 5, Sorting. 12. Restart the system. Refer to CHAPTER 3, Daily Startup. 13. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (Continued)

Problem	Probable Cause	Corrective Action
<p>[Error code 060093] <i>The central stream is not aligned with the detectors.</i></p>	<ul style="list-style-type: none"> • The fluid stream is unstable. • The photodiodes are defective. • The nozzle is clogged or damaged. • Air bubbles are in the system. • Airflow interference. • Leakage of the fluidics system. • Electric leakage of high-voltage components. • The narrow slot mask of the PD is partly or completely blocked. • The side stream detector or gap detector are not aligned with the core stream. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Wipe the side stream detection window. Refer to Figure 1.23. 3. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 4. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 5. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 7. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 8. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 9. Verify that the Sorter is not exposed directly to a ventilation outlet of an air condition. 10. If the problem persists, contact us.

Table 9.1 Troubleshooting-[Error Codes] (*Continued*)

Problem	Probable Cause	Corrective Action
<p>[Error code 070004] <i>No stream is detected.</i></p>	<ul style="list-style-type: none"> • The camera USB cable is not securely connected. • Some quick connectors for the sheath line are not securely connected. • The clogged nozzle blocks the fluid stream. • The O-ring is missing or damaged. • Air bubbles get into the sheath filter, or flow cell. • The fluid leaks around the nozzle. • The abnormal sheath pressure causes the break-off point to descend. • The sheath tank gets moved. • The fluid stream is not in the center of camera view after the nozzle is replaced. • The stream camera does not work properly. • The strobe LED is defective. 	<ol style="list-style-type: none"> 1. Select Standby to turn off the sheath. 2. Verify that the camera USB on the Sorter is securely connected to the workstation. Refer to Figure 1.34. 3. Verify that the quick connectors on the sheath tank, the fluidics cart, and the Sorter are securely connected. Refer to Figure 1.6, Figure 1.8, Figure 1.11. 4. Verify that the nozzle is clean and O-ring is present. <ul style="list-style-type: none"> • Clean the nozzle if needed. Refer to Cleaning the Nozzle in CHAPTER 10, Cleaning Procedures. • If necessary, replace the O-ring. Refer to Replacing the O-Ring in CHAPTER 11, Replacement/Adjustment Procedures. • If the nozzle is damaged, replace the nozzle. Refer to Replacing the Nozzle in CHAPTER 11, Replacement/Adjustment Procedures. 5. Reinstall the nozzle module. Refer to Removing/Installing the Nozzle Module in CHAPTER 11, Replacement/Adjustment Procedures. 6. Run Sheath Filter debubble. Refer to Performing the Sheath Filter De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 7. Run Flow Cell Debubble. Refer to Performing the Flow Cell De-bubble in CHAPTER 11, Replacement/Adjustment Procedures. 8. Run Daily Clean. Refer to Daily Clean Program in CHAPTER 10, Cleaning Procedures. 9. Run Flow cell Clean. Refer to Flow Cell Clean Program in CHAPTER 10, Cleaning Procedures. 10. Restart the system. 11. If the problem persists, contact us.