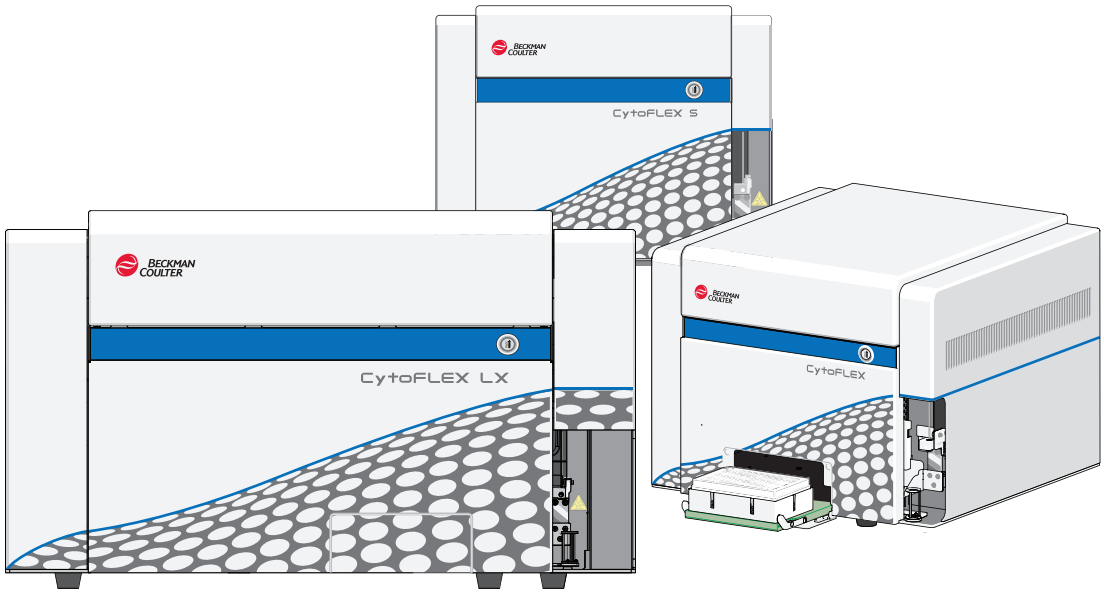
Instructions for Use

CytoFLEX Series

CytoFLEX, CytoFLEX S, and CytoFLEX LX Flow Cytometers

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



B49006AL
December 2017

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

CytoFLEX Series
Flow Cytometer
PN B49006AL (December 2017)

Contact Us
If you have any questions, contact our Customer Support Center.

- Worldwide, find us via our website at *www.beckmancoulter.com/customersupport/support.*
- In the USA and Canada, call us at 1-800-369-0333.
- Outside of the USA and Canada, contact your local Beckman Coulter Representative.

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Revision History

Initial Issue AA, 09/2014
Software Version 1.0

Issue AB, 12/2014
Software Version 1.0

Updates were made to the following sections: Symbol Explanations, Figure 1.10, Figure 1.11, Figure 9.3, Figure 9.5, Figure 9.6, Figure 9.7, Disposal Of Electrical Instrumentation, Lifting and Carrying Instructions, Replacing the Sheath Fluid Harness and/or Waste Harness, and Installing the Instrument and Connecting the Equipment.

Issue AC, 02/2015
Software Version 1.1

Updates were made to the following sections:

Safety Notices

- Symbol Explanations

CHAPTER 1, System Overview

- Optical Fiber
- Fluidics Module
- Plate Loader Components
- System Configuration
- Instrument Specifications
- Performance Characteristics

CHAPTER 2, Using the CytExpert Software

- Start Page
- Acquisition Screen
- Test Tubes
- Plot area
- Status Bar
- Analysis Screen
- Compensation Experiment Screen
- QC Experiment Screen
- Software Menu
- Graphic and Gating Styles
- Software Settings

CHAPTER 3, Daily Startup

- Opening the Software
- Selecting the Plate Loader Sample Injection Mode [With Plate Loader]
- Running the System Startup Program
- Running the System Startup Program [With Plate Loader]

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CHAPTER 4, Instrument Quality Control

- Preparing the QC Sample [With Plate Loader]
- Collecting QC Data
- Confirming Results
- Collecting QC Data [With Plate Loader]
- Creating Levey-Jennings Charts
- QC Result Manager

CHAPTER 5, Data Acquisition and Sample Analysis

- Creating an Experiment
- Creating an Experiment [With Plate Loader]
- Sampling and Collecting Data
- Setting the Channel and Label
- Adjusting the Threshold
- Creating Plots and Gates
- Setting Customized Parameters
- Setting Custom Statistics
- Analyzing and Exporting Data
- Exporting FCS Files
- Exporting Plots or the Statistics Table of Multiple Tubes as Picture Files
- Printing Graphics

CHAPTER 6, Compensation

- Creating a Compensation Experiment
- Creating a Compensation Experiment [With Plate Loader]
- Manually Adjusting Compensation
- Managing the Compensation Library

CHAPTER 7, Data Review

- Calculating Sample Injection Volume and Concentration
- Importing Previously Acquired Data

CHAPTER 9, Troubleshooting

- Plate Loader Hazard Labels and Location
- Table 9.2, Troubleshooting [With Plate Loader]

CHAPTER 10, Cleaning Procedures

- Daily Clean [With Plate Loader]
- Preparing the Instrument for Transport or Storage

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CHAPTER 11, Replacement/Adjustment Procedures

- Adding the Deep Clean Solution
- Replacing the Sample Probe Assembly [With Plate Loader]
- Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [With Plate Loader]
- Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [With Plate Loader]
- Replacing the Plate Holder [With Plate Loader]
- Plate Loader Module Removal and Reinstallation [With Plate Loader]
- Calibrating the Sample Flow Rate
- Calibrating the Sample Flow Rate [With Plate Loader]
- Changing Sample Mixing and Backflush Settings
- Calibrating the Plate Position [With Plate Loader]

APPENDIX A, Instrument Installation

- Power Source
- Unpacking the Instrument and Inspecting the Materials for Defects or Omissions
- Installing the CytExpert Software

Issue AD, 04/2015
Software Version 1.1

Updates were made to the following sections:

CHAPTER 1, System Overview

- Cytometer

CHAPTER 2, Using the CytExpert Software

- Analysis Screen

CHAPTER 8, Daily Shutdown

- Shutting Down the Instrument

CHAPTER 11, Replacement/Adjustment Procedures

- Changing the Event Rate Setting
- Calibrating the Plate Position [With Plate Loader]

Issue AE, 11/2015
Software Version 1.1

Updates were made to the following sections:

CHAPTER 1, System Overview

- Plate Loader Components
- Plate Holder Components
- Dimensions [CytoFLEX]
- Acoustic Noise Level
- Performance Characteristics

CHAPTER 4, Instrument Quality Control

- Confirming Results

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CHAPTER 9, Troubleshooting

Plate Loader Hazard Labels and Location

CHAPTER 10, Cleaning Procedures

Daily Clean
Daily Clean [With Plate Loader]
Preparing the Instrument for Transport or Storage

CHAPTER 11, Replacement/Adjustment Procedures

Replacing the Sample Probe Assembly [With Plate Loader]
Replacing the Plate Holder [With Plate Loader]
Plate Loader Module Removal and Reinstallation [With Plate Loader]

APPENDIX A, Instrument Installation

Installing the Instrument and Connecting the Equipment

Issue AF, 10/2016
Software Version 1.1

Updates were made to the following sections:

Safety Notices

Safety Precautions

Introduction

About this Manual

CHAPTER 11, Replacement/Adjustment Procedures

Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [With Plate Loader]
Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [With Plate Loader]

CytoFLEX Plate Loader Upgrade Kit

CytoFLEX Plate Loader Upgrade Kit Components
Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [With CytoFLEX Plate Loader Upgrade Kit]
Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [With CytoFLEX Plate Loader Upgrade Kit]

Issue AG, 1/2017
Software Version 2.0

Complete Revision

Issue AH, 3/2017
Software Version 2.0

Updates were made to the following sections:

Introduction

About this Manual

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CHAPTER 1, System Overview

- Performance Characteristics [CytoFLEX]
- Performance Characteristics [CytoFLEX LX]

CHAPTER 3, Daily Startup

- Selecting the Plate Loader Sample Injection Mode [With Plate Loader]

CHAPTER 11, Replacement/Adjustment Procedures

- Managing the Maintenance Reminder
- Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing
- Replacing the Sample Probe Assembly [With Plate Loader]
- Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [CytoFLEX With Plate Loader]
- Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [CytoFLEX With Plate Loader]
- Changing Sample Mixing and Backflush Settings

APPENDIX A, Instrument Installation

- Installing the Software [CytoFLEX]
- Upgrading the CytExpert Software
- Reinstalling the CytExpert Software

Added the following Chapter:

APPENDIX C, Sample Injection Mode Control Kit

Issue AJ, 5/2017
Software Version 2.0

Updates were made to the following sections:

CHAPTER 1, System Overview

- Consumables and Supplies

CHAPTER 4, Instrument Quality Control and Standardization

- Preparing the QC Sample
- Preparing the QC Sample [With Plate Loader]
- Importing Lot-Specific Target Values

CHAPTER 5, Data Acquisition and Sample Analysis

- Setting Sample Wells

CHAPTER 9, Troubleshooting

- Table 9.1

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Issue AK, 8/2017
Software Version 2.1

Updates were made to the following sections:

CHAPTER 1, System Overview

- Product Description
- Wavelength Division Multiplexer (WDM)
- WDM Optical Filter Mount Color Codes [CytoFLEX LX]
- Optical Fiber
- System Configuration
- Performance Characteristics

CHAPTER 2, Using the CytExpert Software

- Start Page
- Acquisition Screen
- Software Menu
- Acquisition and Analysis Screen Menu
- Advanced Menu
- Account Menu
- Log Menu
- Signature Menu
- Backup/Restore Menu
- Viewing and Exporting User Logs

CHAPTER 3, Daily Startup

- Loggig Into the Software
- Selecting the Proper Sample Injection Mode

CHAPTER 4, Instrument Quality Control and Standardization

- Overview
- Importing Lot-Specific Target Values
- Confirming Results

CHAPTER 5, Data Acquisition and Sample Analysis

- Creating an Experiment [With Plate Loader]
- Add Plate from Layout
- Setting Sample Wells
- Creating a Heat Map
- Modifying Existing Heat Map Settings

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Creating Plots and Gates
Sampling and Collecting Data
Setting the Channel and Label
Setting Collection Conditions
Laser Settings
Analyzing and Exporting Data
Printing Graphics

CHAPTER 6, Compensation

Creating a Compensation Experiment
Manually Adjusting Compensation

CHAPTER 8, Daily Shutdown

Shutting Down the Instrument

CHAPTER 9, Troubleshooting

Laser Beam Hazards
Laser Warning Labels
Backup and Restore

CHAPTER 11, Repalcement/Adjustment Procedures

Calibrating the Sample Flow Rate

APPENDIX A, Instrument Installation

Unpacking the Instrument and Inspecting the Materials for Defects or Omissions [CytoFLEX]

APPENDIX B, CytExpert Electronic Record Management

Figure B.3
Printing an Experiment Signature

APPENDIX D, Table of Hazardous Substances

APPENDIX D, Table of Hazardous Substances Name and Concentration [CytoFLEX 355]

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Issue AL, 11/2017
Software Version 2.2

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.beckmancoulter.com and download the latest version of the manual or system help for your instrument.

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.beckmancoulter.com and download the latest version of the manual or system help for your instrument.

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 Danger, Warning, and Caution

DANGER

DANGER indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury.

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.**
- **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 software 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.

⚠ WARNING

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 Explanations

Symbol meanings	
	Caution / Warning
	Electrical shock hazard. Operate with caution!
	The laser radiation symbol indicates that there can be laser light radiation in the area. Take precautions to prevent exposure.
	Biohazard
	Potential pinch point.[a]
	Potential puncture hazard.[a]
	CE = Conformité Européenne A “CE” mark indicates that a product has been assessed before being placed on the market, and has been found to meet European Union safety, health, and/or environmental protection requirements. It is a mandatory marking for various product categories that are placed into service within the European Economic area.
	RCM Symbol.The RCM is a symbol signifying that a supplier has taken the necessary steps to have the product comply with the electrical safety and/or electromagnetic compatibility (EMC) legislative requirements.
	This symbol indicates that this electronic information product contains certain toxic or hazardous substances or elements, and can be used safely during its environmental protection use period.

Symbol meanings	
	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	Date of Manufacture indicates the date when the medical device was manufactured.
	Laser Compliance
	Class 3B Laser Caution, do not stare into the beam.
	Class 1 Laser Caution
	Class 3B Laser Caution, do not stare into the beam.
	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.

a. This label only appears on the Plate Loader Module.

Contents

Illustrations

Tables

Introduction

Overview

This introduction contains the following information:

How to Use Your Manual

Your CytoFLEX flow cytometer system includes the manuals listed below:

- Use this **Instructions for Use** manual for information on the day-to-day operation of your CytoFLEX flow cytometer. You can find detailed step-by-step procedures for Daily Startup and Quality Control, configuring settings, running samples, analyzing data, and performing Startup and Shutdown. This manual also contains physical and system specifications, safety and troubleshooting information, as well as information about what your CytoFLEX flow cytometer does and the methods it uses. It also contains procedures for cleaning and replacement.
- The **CytoFLEX Setup Guide** provides instructions for unpacking and setting up the CytoFLEX flow cytometer system.
- The **CytoFLEX S Special Configuration Specifications** package insert contains the sections and procedures that are specific to the CytoFLEX S instrument. Use the CytoFLEX S Special Configuration Specifications package insert along with your Instructions for Use manual.

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 flow cytometer and the corresponding functions of these components.

CHAPTER 2, Using the CytExpert Software

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

CHAPTER 3, Daily Startup
Provides instructions for starting your CytoFLEX flow cytometer 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 flow cytometer 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, Data Acquisition and Sample Analysis
Provides instructions for operating the CytoFLEX instrument, including data acquisition, 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, Daily Shutdown
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, Instrument Installation
Provides the instrument installation procedures for your CytoFLEX flow cytometer.

APPENDIX B, CytExpert Electronic Record Management
Provides the instructions for using the CytExpert Electronic Record Management software option.

APPENDIX C, Sample Injection Mode Control Kit
Provides the instructions for using the CytoFLEX Sample Injection Mode Control Kit.

APPENDIX D, Deep Well Plate
Provides a list of deep well plates suggested for use on the Plate Loader DW.

APPENDIX E, 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.
- 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.
- The information in your Instructions for Use manual applies to the CytoFLEX and CytoFLEX S instruments equipped with and without a plate loader, and the CytoFLEX LX, unless otherwise specified.

  NOTE When information in this document only applies to the plate loader configuration, it is marked [With Plate Loader]. When information in this document only applies to the configuration not equipped with a plate loader, it is marked [Without Plate Loader]. When information in this document only applies to one instrument in the series, it is marked either [CytoFLEX] or [CytoFLEX LX].

  NOTE There are two kinds of plate loaders, the standard CytoFLEX Plate Loader and the CytoFLEX Plate Loader Deep Well (DW). The CytoFLEX Plate Loader DW (hereinafter called as Plate Loader DW) is compatible with CytoFLEX, CytoFLEX S, and CytoFLEX LX instruments. When information in this document only applies to one plate loader, it is marked either [Plate Loader DW] or [Standard Plate Loader].
- Sections that contain entirely new content are flagged with a New Section icon  at the end of the section title.
- The CytExpert Default software installation screens are shown in all instances unless otherwise specified.

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 system in the background may not depict the latest production version of the system.

CHAPTER 1

System Overview

Overview

This chapter describes the individual components of the CytoFLEX flow cytometer and the corresponding functions of these components.

This chapter contains information on:

Product Description

The CytoFLEX and CytoFLEX LX flow cytometers are used for the qualitative and quantitative measurement of biological and physical properties of cells and other particles. These properties are measured when the cells pass through one or multiple laser beams in a single-file. The CytoFLEX flow cytometer can perform up to 13 color marker analysis. The system can be ordered in various configurations. The CytoFLEX S may be ordered in various configurations from 2 lasers, 4 colors to a maximum of 4 lasers, 13 colors. The CytoFLEX LX flow cytometer can perform up to 21 color marker analysis. The system can be ordered in various configurations from a 4 laser, 14 colors, to a maximum of 6 lasers, 21 colors. Many of the configurations can be upgraded in the field. For Research Use Only. Not for use in diagnostics procedures.

NOTE A CytoFLEX instrument cannot be upgraded to a CytoFLEX S cytometer.

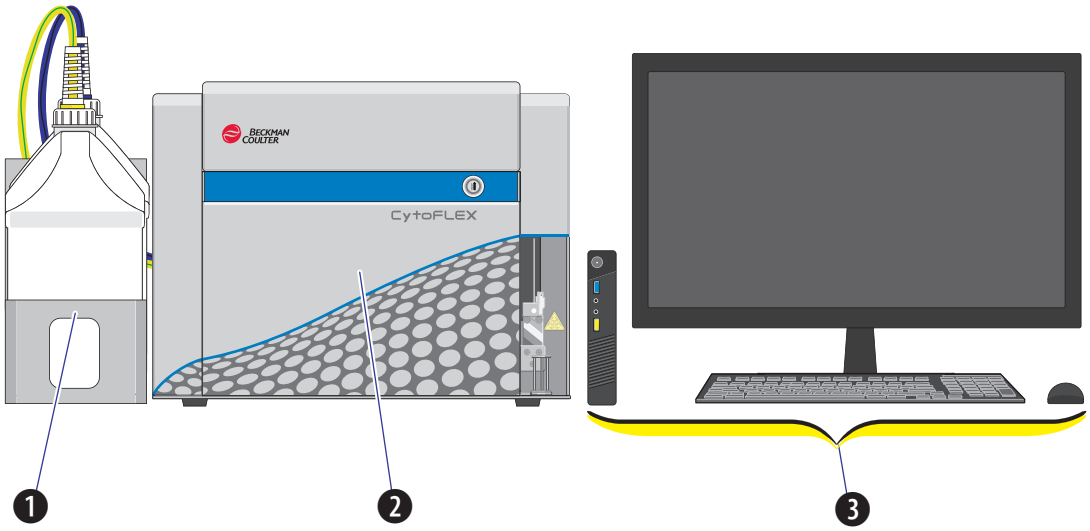
Main Components

⚠ CAUTION

Risk of instrument damage and/or instrument stability. Do not place any objects on top of the instrument, as this could cause warping of the top cover or affect the stability of the optical path.

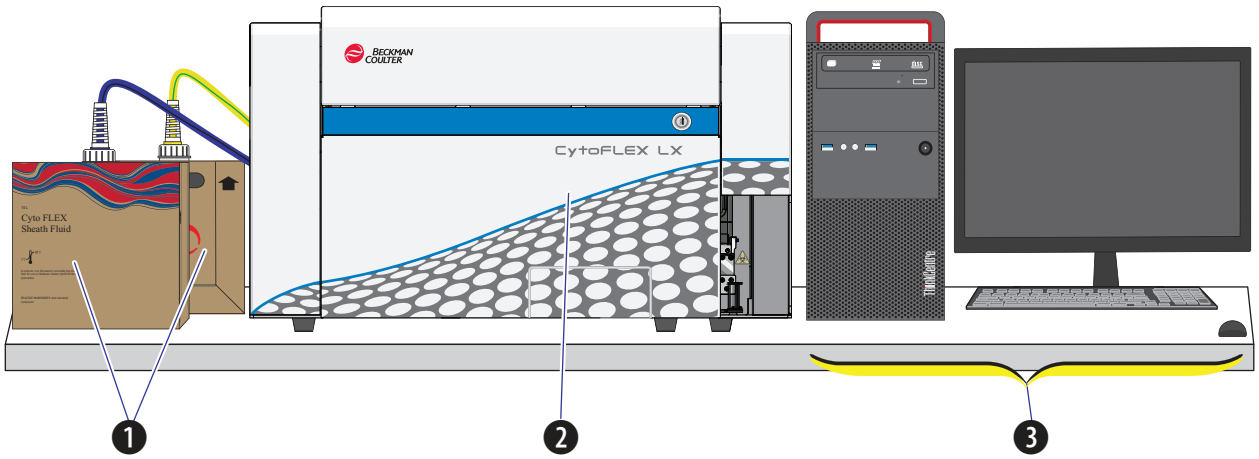
The instrument consists of three main components: Fluid Containers/Cubitainers, Cytometer, and the Workstation.

Figure 1.1 Main Components [CytoFLEX Without Plate Loader]



1. **Fluid Containers.** Accommodates sheath fluid and waste liquids as required for operation of the instrument.
2. **Cytometer.** Provides signal generation and collection.
3. **Workstation.** Displays the content of the workstation and data generated by the Cytometer.

Figure 1.2 Main Components [CytoFLEX LX]



1. **Fluid Cubitainers.** Accommodates sheath fluid and waste liquids as required for operation of the instrument.

NOTE The CytoFLEX LX does not have a fluid container holder.

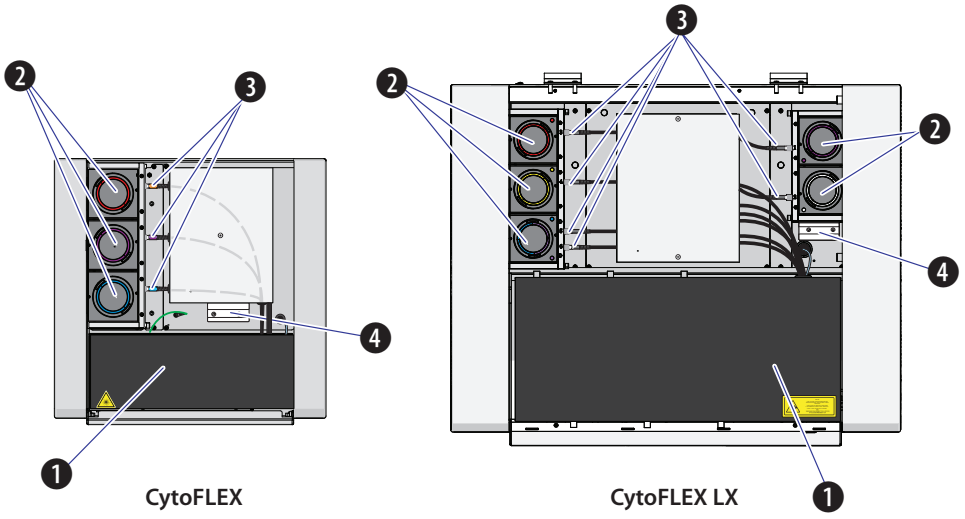
2. **Cytometer.** Provides signal generation and collection.
3. **Workstation.** Displays and manipulates the contents of the Workstation and displays data generated by the Cytometer.

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 Cytometer 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.



1. **Optical bench.** Includes laser light sources, an optical beam combiner, 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. **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.
3. **Optical fiber.** Transmits emitted fluorescence to the specific WDM.

⚠ CAUTION

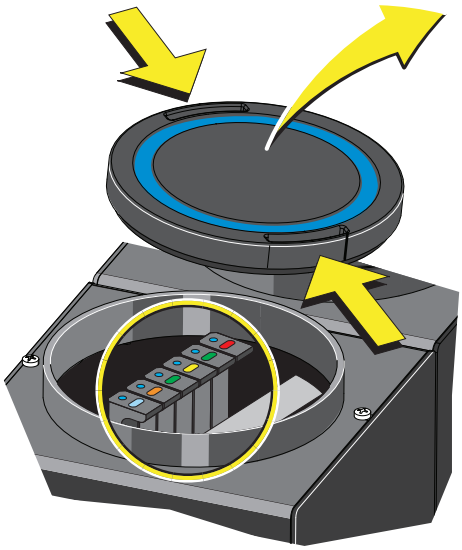
Risk of instrument damage. Do not place sample tubes in the optical filter holder. Liquid spills can damage instrument components. Use a tube rack to hold any sample tubes.

4. **Optical filter holder.** Securely holds additional CytoFLEX optical filters.

Wavelength Division Multiplexer (WDM)

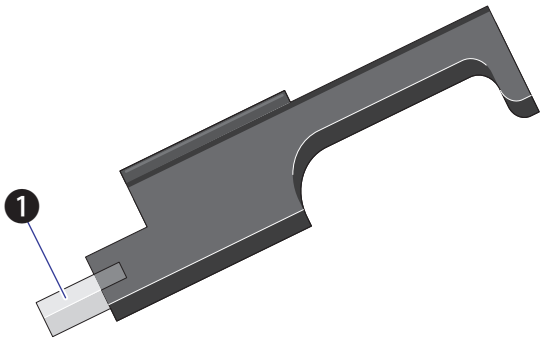
Each WDM corresponds to a different laser, or in some cases two lasers. The color of the ring on each cap corresponds to the color of the respective laser. Pressing the two release buttons on opposite edges of the cap allows you to open the WDM and replace the filters inside. See Figure 1.3. All optical filters are designed to be interchangeable. Refer to Replacing the Optical Filter in CHAPTER 11, Replacement/Adjustment Procedures to replace an optical filter.

Figure 1.3 Optical Filter Mounts



Each optical filter mount has an optical filter glass piece. See Figure 1.4.

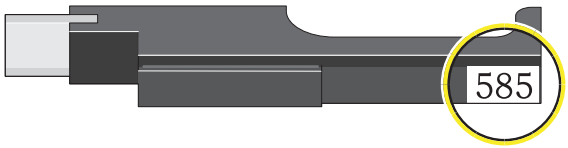
Figure 1.4 Optical Filter Mount with Optical Filter



1. Optical filter glass piece

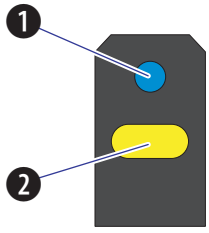
Each optical filter mount is labeled with the corresponding laser and band-pass information. See Figure 1.5.

Figure 1.5 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. See Figure 1.6. The color of the line **(2)** indicates the wavelength range of the optical band-pass filter. See Figure 1.6.

Figure 1.6 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; Pink indicates a 808 nm laser; White indicates either 375 nm or 355 nm.
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 ranges of wavelength. These ranges are designed to measure fluorescence from fluorochromes such as those listed in Table 1.1. (with red and violet laser upgrades installed). 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 [CytoFLEX]

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
488 nm	525/40 BP	FITC	FITC, Alexa Fluor™ 488, CFSE, Fluo-3
	585/42 BP	PE	PE, PI
	610/20 BP	ECD	ECD, PE-Texas Red®, PE-CF594, PI
	690/50 BP	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI, DRAQ7™
	780/60 BP	PC7	PC7, DRAQ7™
638 nm	660/10 BP	APC	APC, Alexa Fluor™ 647, eFluor™ 660, Cy5
	712/25 BP	APC-A700	APC-A700, Alexa Fluor™700, Cy5.5, DRAQ7™
	780/60 BP	APC-A750	APC-A750, APC-Cy7, APC-H7, APC- eFluor™ 780, DRAQ7™
405 nm	450/45 BP	PB450	Pacific Blue™dye, V450, eFluor™ 450, BV421
	525/40 BP	KO525	Krome Orange, AmCyan, V500, BV510
	610/20 BP	Violet610	BV605, Qdot® 605
	660/10 BP	Violet660	BV650, Qdot® 655
	780/60 BP	Violet780	BV785, Qdot® 800

NOTE The following are additional BP filters supplied in an inactive position within the CytoFLEX and CytoFLEX S WDMs:

- 405/10 BP
- 638/6 BP
- 561/6 BP
- 488/8 BP

Table 1.2 WDM Optical Filter Mount Color Codes [CytoFLEX LX]

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
355 nm	405/30 BP	UV405	BUV395
	525/40 BP	UV525	BUV496
	675/30 BP	UV675	Hoescht Red, BUV661
	450/45 BP	N/A[a]	DAPI
375 nm	450/45 BP	NUV450	BUV395, DAPI
	525/40 BP	NUV525	BUV496
	675/30 BP	NUV675	Hoescht Red, BUV661
405 nm	450/45 BP	V450-PB	Pacific Blue™ dye, V450, eFluor™ 450, BV421
	525/40 BP	V525-KrO	Krome Orange, AmCyan, V500, BV510
	610/20 BP	V610	BV605, Qdot® 605
	660/10 BP	V660	BV650, Qdot® 655
	763/43 BP	V763	BV785, Qdot® 800
488 nm	525/40 BP	B525-FITC	FITC, Alexa Fluor™ 488, CFSE, Fluo-3
	610/20 BP	B610-ECD	ECD, PE-Texas Red®, PE-CF594, PI
	690/50 BP	B690-PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI, DRAQ7™
561 nm	610/20 BP	Y610-mCherry	mCherry, ECD, PE-CF594
	763/43 BP	Y763-PC7	PC7
	585/42 BP	Y585-PE	PE, DsRed
	675/30 BP	Y675-PC5	PC5, mPlum
	710/50 BP	Y710-PC5.5	PC5.5, PE-AF680

Table 1.2 WDM Optical Filter Mount Color Codes [CytoFLEX LX] *(Continued)*

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
638 nm	763/43 BP	R763-APCA750	APC-A750, APC-Cy7, APC-H7, APC- eFluor™ 780, DRAQ7™
	660/10 BP	R660-APC	APC, Alexa Fluor™ 647, eFluor™ 660, Cy5
	712/25 BP	R712-APCA700	APC-A700, Alexa Fluor™700, Cy5.5
808 nm	840/20 BP	IR840-A790	Alexa Fluor®790
	885/40 BP	IR885	PromoFluor-840, IR fixable viability dye

a. Only used as an alterative for other BPs like 405/30 BP.

NOTE The following are additional BP filters supplied in an inactive position within the CytoFLEX LX WDMs:

- 405/10 BP
- 638/6 BP
- 561/6 BP
- 488/8 BP

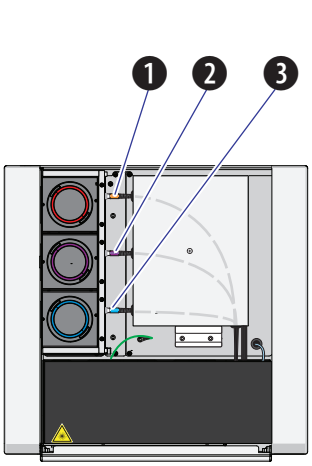
Optical Fiber

⚠ CAUTION

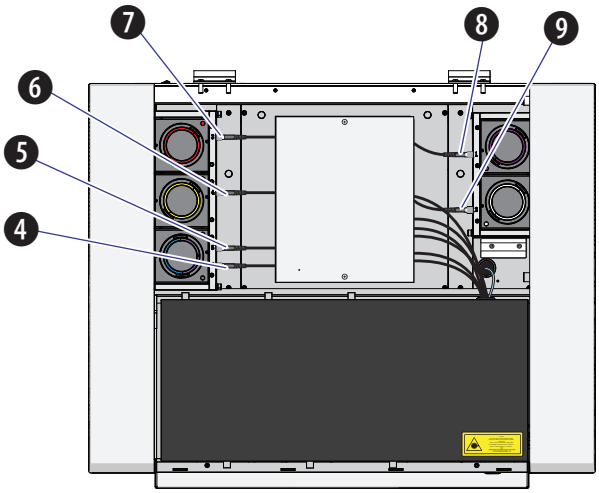
Risk of data integrity damage.

- **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 a colored ring on the end that connects to the WDM, indicating the color of the corresponding laser. Ensure that the correct fiber is properly connected to the corresponding WDM.



CytoFLEX



CytoFLEX LX

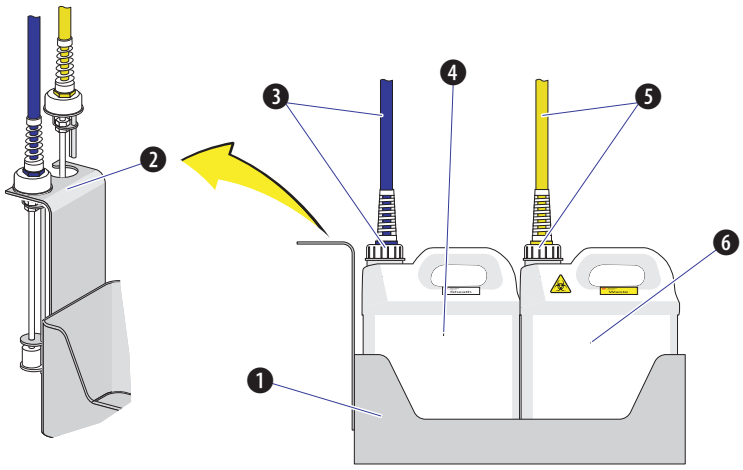
1. Red laser fiber
2. Violet laser fiber
3. Blue laser fiber
4. Infrared laser fiber
5. Blue laser fiber
6. Yellow laser fiber
7. Red laser fiber
8. Violet laser fiber
9. NUV or UV laser fiber

Fluidics System

The fluidics system consists of two parts: the Fluid Containers/Cubitainers and the Fluidics module. The Fluidics module is located on the right side of the Cytometer. You need to open the right-side cover of the instrument (see Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures) to perform maintenance operations. The fluidics system helps to transmit the sheath fluid at a stable rate into the flow cell, forming a laminar fluidics system that ensures that the tested particles go through the detection area sequentially.

NOTE The 10 L sheath fluid and waste cubitainers are available from Beckman Coulter as an alternative to the 4 L Fluid Containers (refer to Figure 1.7) provided with your CytoFLEX and CytoFLEX S Flow Cytometers. Contact us to order the 10 L Sheath/Waste Line Kit. The CytoFLEX LX is only available with the 10 L sheath fluid and waste cubitainers. Refer to Figure 1.8.

Figure 1.7 Fluid Containers [CytoFLEX]



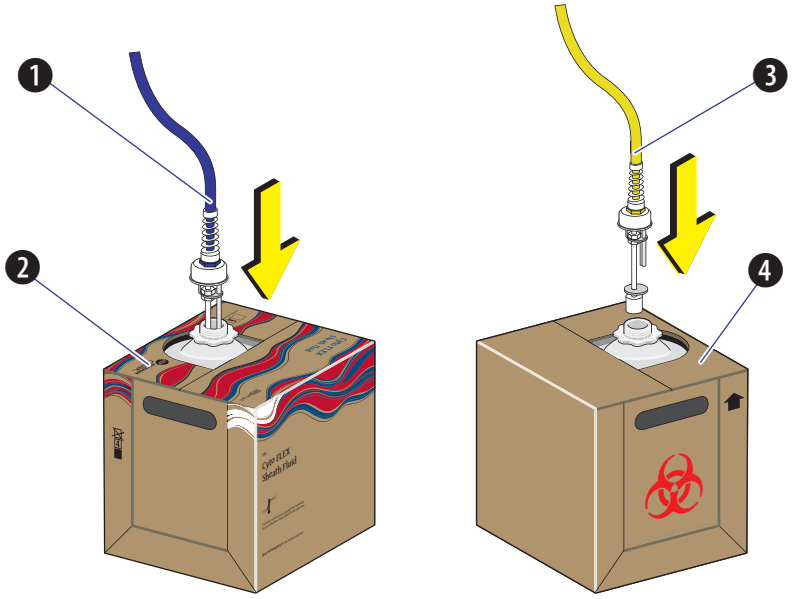
1. **Fluid Container holder.** Holds the Fluid Containers.
2. **Fluid sensor holder cutout.** Holds the sheath fluid harness and the waste harness when removed from their respective containers to protect the sensors from damage and/or contamination.
3. **Sheath fluid harness.** Connects to the sheath fluid container; conveys the sheath fluid into the instrument. The sheath fluid harness includes a level sensor, sheath fluid tubing, and backflush tubing. The other end of the harness is connected to the Fluidics module in the Cytometer. When the sheath fluid container is near empty, a warning notice is transmitted to the instrument and an audible signal sounds as a warning.
4. **Sheath fluid container.** 4 L capacity, for holding sheath fluid. Beckman Coulter recommends using CytoFLEX Sheath Fluid or a similar nonionic sheath fluid to ensure system performance.

   NOTE The sheath fluid container must be on the same level as the Cytometer.

5. **Waste harness.** Connects to the waste fluid container; conveys the waste fluid from the instrument to the waste container. The waste harness includes a level sensor. The other end of the harness is connected to the Fluidics module in the Cytometer. When the waste fluid container is near full, a warning notice is transmitted to the instrument and audible signal sounds as a warning.
6. **Waste container.** 4 L capacity, for holding waste liquids. Attention to biosafety and waste labeling is required.

   NOTE The waste container must be on the same level as the Cytometer.

Figure 1.8 Fluid Cubitainers [CytoFLEX LX]



1. **Sheath fluid harness.** Connects to the sheath fluid cubitainer; conveys the sheath fluid into the instrument. The sheath fluid harness includes a level sensor, sheath fluid tubing, and backflush tubing. The other end of the harness is connected to the Fluidics module in the Cytometer. When the sheath fluid cubitainer is near empty, a warning notice is transmitted to the instrument and an audible signal sounds as a warning.
2. **Sheath fluid cubitainer.** 10 L capacity, for holding sheath fluid. Beckman Coulter recommends using CytoFLEX Sheath Fluid or a similar nonionic sheath fluid to ensure system performance.

   NOTE The sheath fluid cubitainer must be on the same level as the Cytometer.

3. **Waste harness.** Connects to the waste fluid cubitainer; conveys the waste fluid from the instrument to the waste cubitainer. The waste harness includes a level sensor. The other end of the harness is connected to the Fluidics module in the Cytometer. When the waste fluid cubitainer is near full, a warning notice is transmitted to the instrument and audible signal sounds as a warning.
4. **Waste cubitainer.** 10 L capacity, for holding waste liquids. Attention to biosafety and waste labeling is required.

   NOTE The waste cubitainer must be on the same level as the Cytometer.

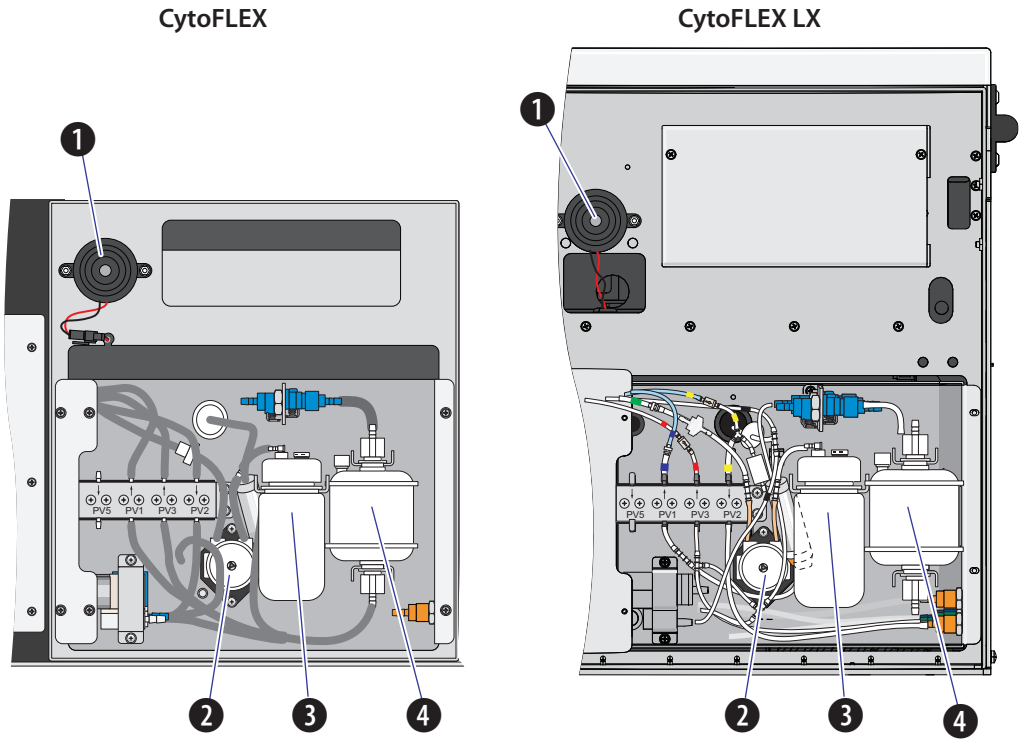
Fluid Containers/Cubitainers

On the CytoFLEX, two Fluid Containers are placed in the Fluid Container holder: a sheath fluid container and a waste container. See Figure 1.7. On the CytoFLEX LX, two 10-L Fluid Cubitainers are placed to the left side of the instrument: a sheath fluid cubitainer and a waste cubitainer. Each container/cubitainer cap is fitted with a harness and a level sensor. The blue harness connects to the sheath fluid container/cubitainer while the yellow harness connects to the waste container/cubitainer. The containers/cubitainers do not require additional pressure inside. Take all necessary biosafety precautions and use proper personal protective equipment when handling the Fluid Containers/Cubitainers.

Fluidics Module

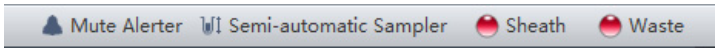
The Fluidics module is on the right side of the Cytometer. To access it, you must first open the right-side cover. Refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures. Inside the module, in addition to working pumps, valves, and tubing, there is a sheath filter and a Deep Clean solution bottle. During maintenance, it may be necessary to replace the filter (see Replacing the Sheath Fluid Filter in CHAPTER 11, Replacement/Adjustment Procedures) or to add Deep Clean solution (see Adding the Deep Clean Solution in CHAPTER 11, Replacement/Adjustment Procedures).

Figure 1.9 Fluidics Module View



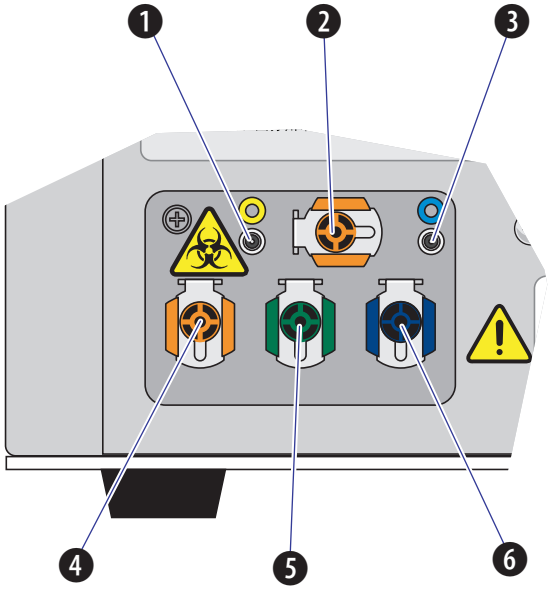
1. **Alarm.** Emits a warning sound when there is a problem with the Fluid Container/Cubitainer capacity or with the performance of certain operations.

   NOTE When the alarm sounds, the Mute Alerter icon appears in the status bar. The alarm continues for about 30 seconds. To mute the alarm temporarily select the **Mute Alerter** in the status bar. The icon disappears when the waste container/cubitainer is emptied and/or the sheath container/cubitainer is filled/replaced.



2. **Deep Clean solution peristaltic pump.** Transfers cleaning solution to the flow cell.
3. **Deep Clean solution bottle.** Contains the diluted cleaning solution that helps to clean the flow cell.
4. **Sheath fluid filter.** 0.2 µm filter, for filtering sheath fluid.

Figure 1.10 Fluidic Connections



1. **Waste level sensor connector.** Connects to the waste liquid sensor cable.
2. **Flow cell waste out.** Connects to the waste tubing of the wash station.
3. **Sheath fluid level sensor connector.** Connects to the sheath fluid sensor cable.
4. **Waste out.** Connects to the waste liquid tubing from the flow cell.
5. **Sheath return.** Connects to the sheath fluid tubing.

   NOTE The sheath fluid is pressurized by a diaphragm pump. To improve pressure stability, a bypass line returns some of the sheath fluid back into the sheath fluid container/cubitainer.

6. **Sheath fluid in.** Connects to the sheath fluid tubing.

NOTE Use CytoFLEX Sheath Fluid or other filtered nonionic sheath fluid. Using unfiltered sheath fluid can shorten the service life of the sheath fluid filters and increase noise and debris detection.

Sample Station

⚠ 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 13 mm.

Figure 1.11 Sample Station with Original Tube Holder

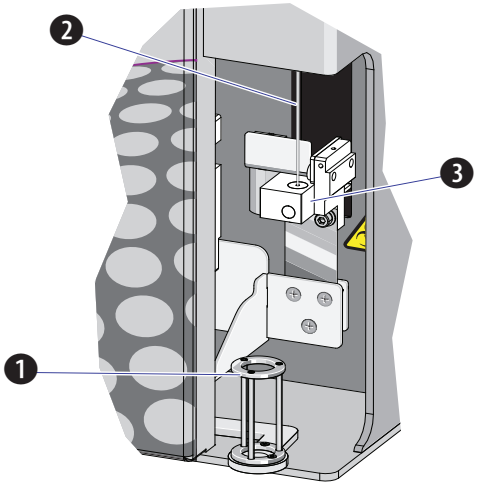
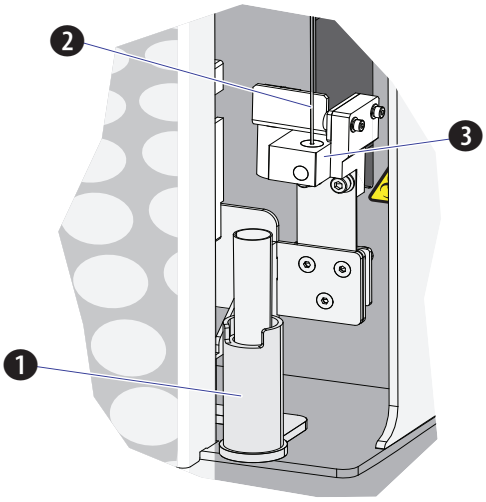


Figure 1.12 Sample Station with New Tube Holder



1. **Sample tube holder.**Supports sample tubes for testing, such 12 x 75 mm, 1.5-mL, and 2-mL microtubes.
2. **Sample probe.** Draws and transfers samples into the flow cell.
3. **Wash station and mixer.** During the sampling process, samples are automatically shaken and mixed for a default time of 1 second. The sample probe is automatically cleaned when the instrument performs a backflush.

Sample Tube Holder Positions

Three of the sample tube holder positions are shown in Figure 1.13: sample loading position **(1)**, standby position **(2)**, and sample acquisition position **(3)**. You can only distinguish the mixing position from the sample acquisition position if you are looking directly at the sample tube holder while the Cytometer is processing a sample. The mixing position is about 6 mm lower than the sample acquisition position.

Figure 1.13 Sample Tube Holder Positions

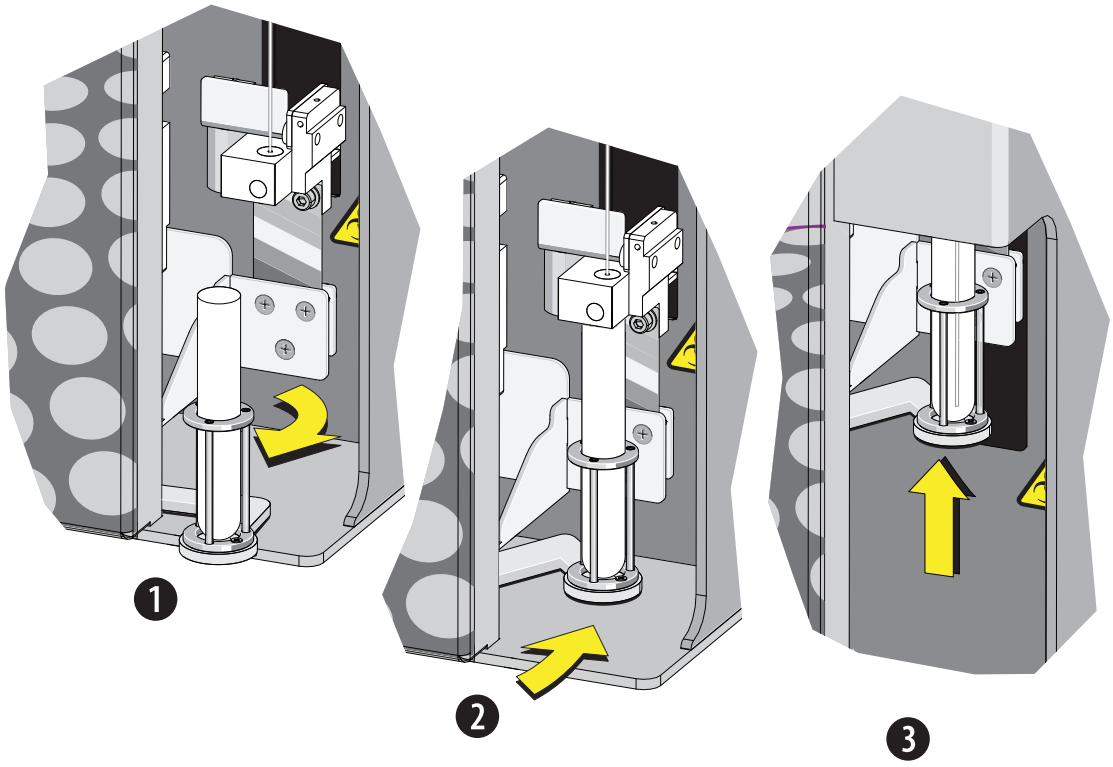


Figure 1.14 Sample Tube Holder Positions

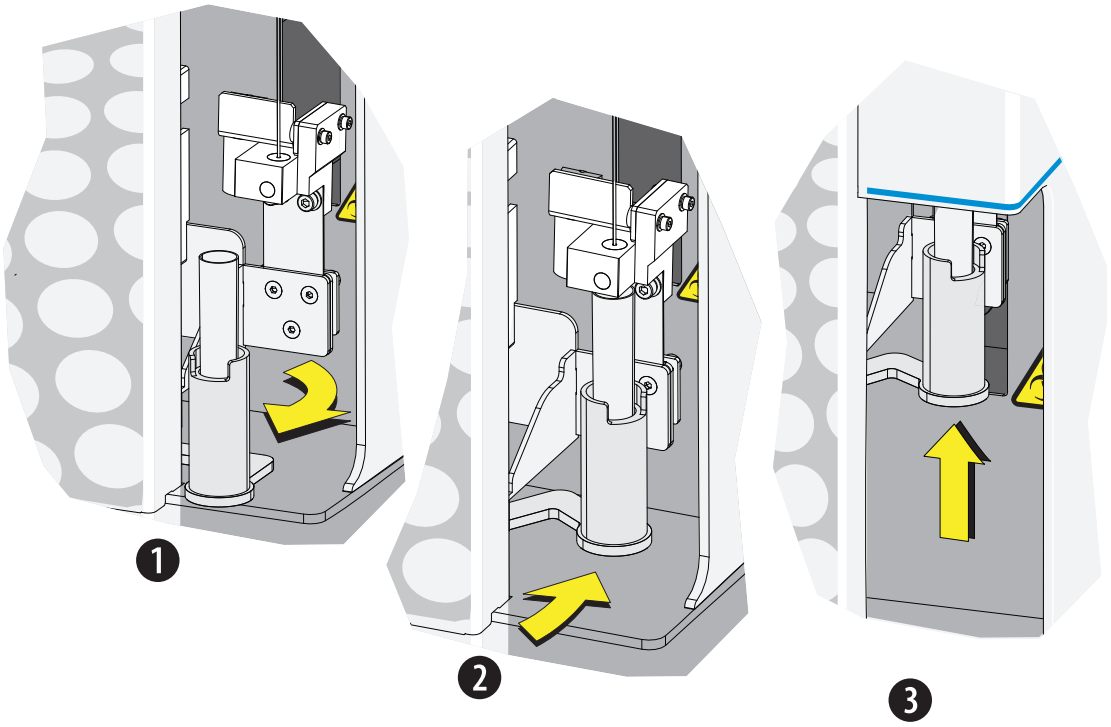
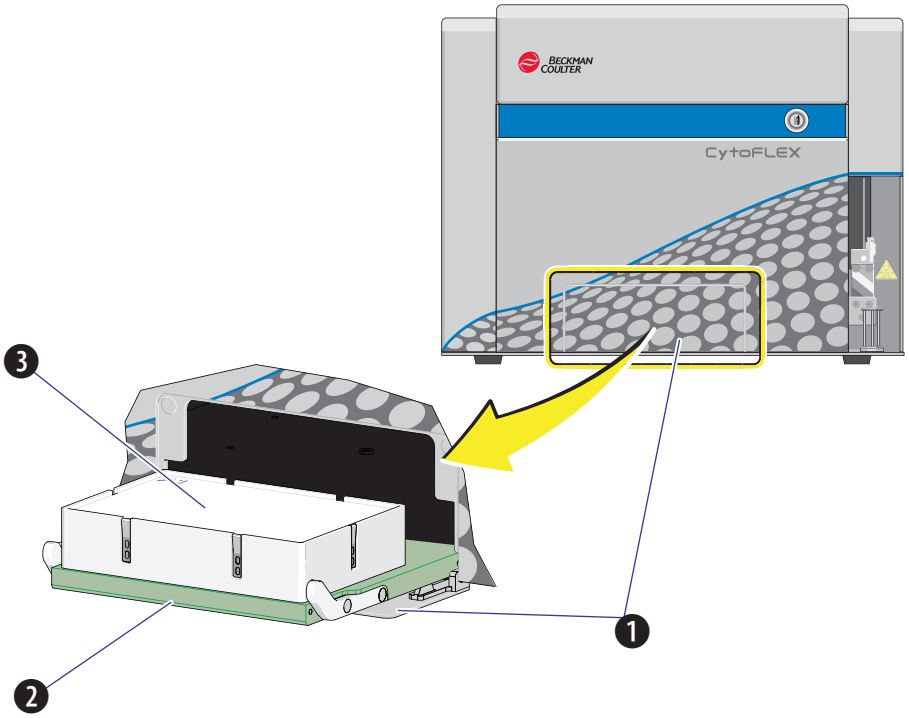


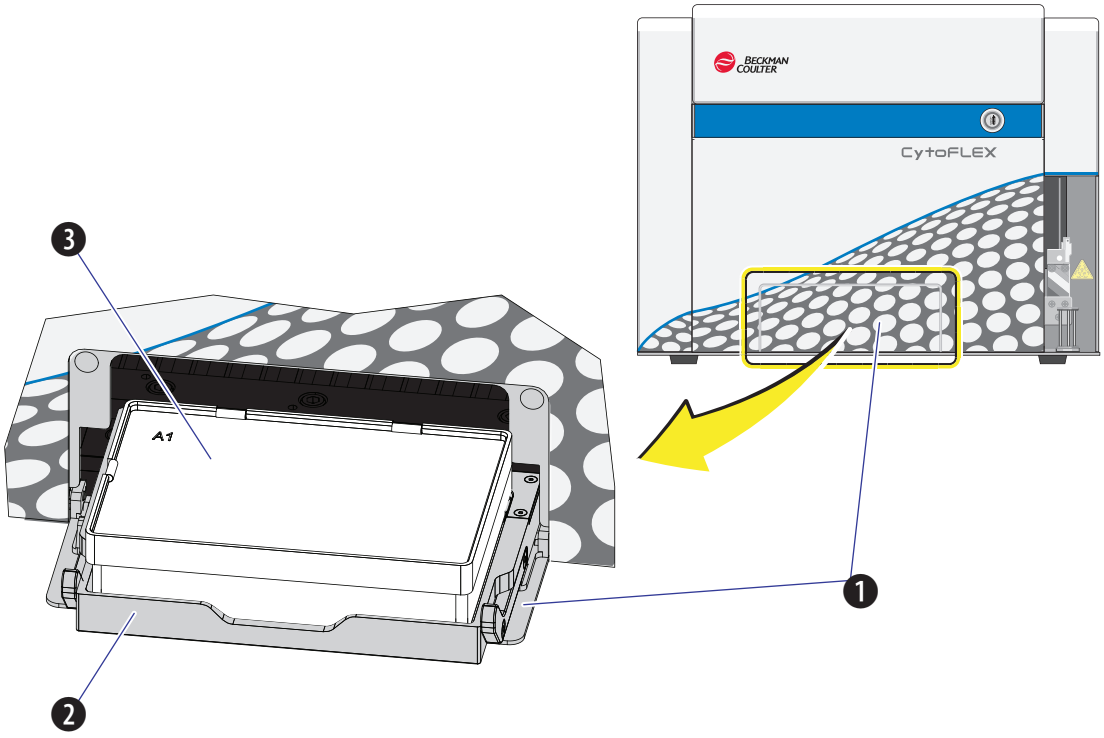
Plate Loader Components

Figure 1.15 Standard Plate Loader [CytoFLEX Shown]



1. Plate loader door
2. Plate holder stage
3. Plate holder (removable)

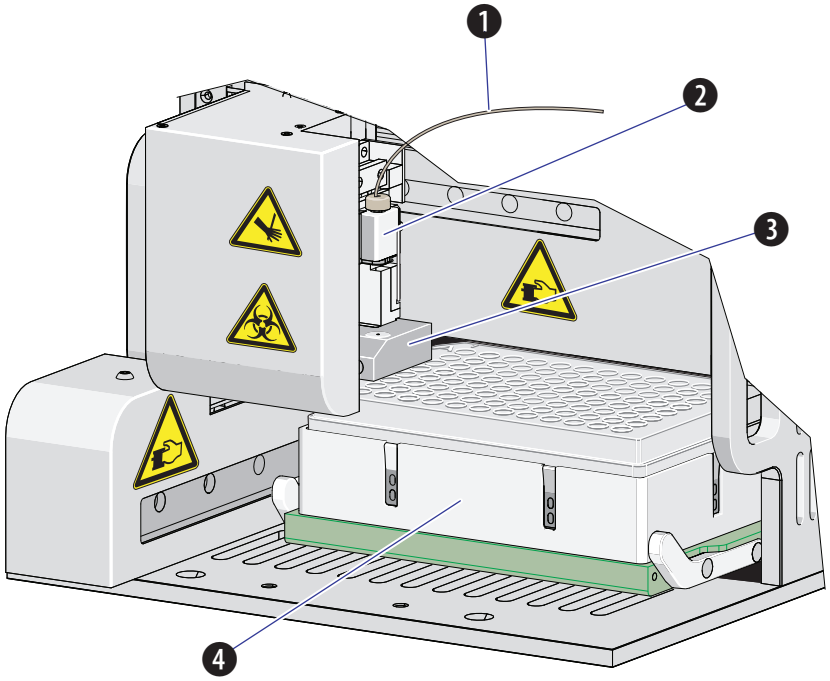
Figure 1.16 Plate Loader DW [CytoFLEX Shown]



1. Plate loader door
2. Plate holder stage
3. Plate holder (removable)

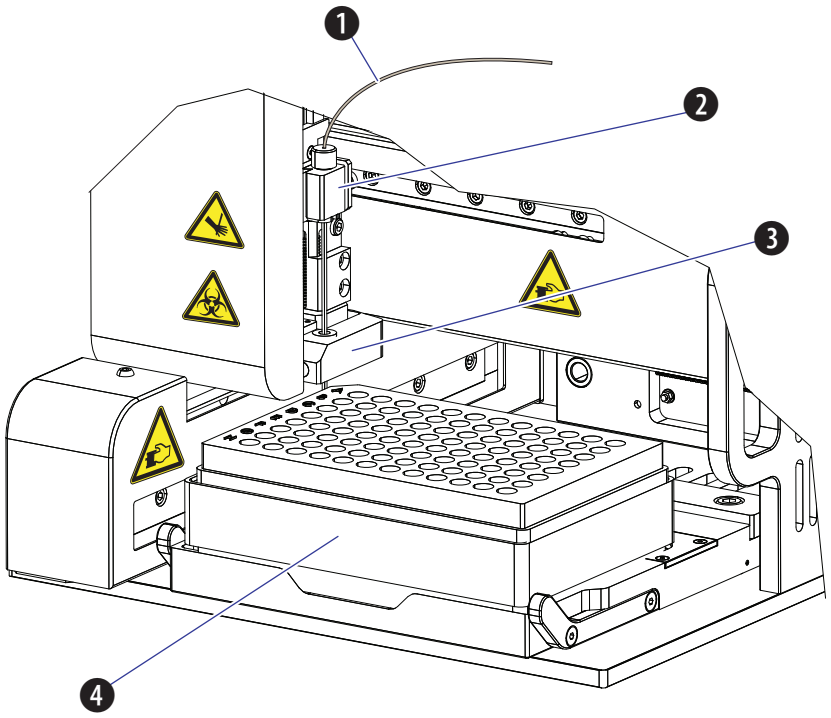
NOTE The Plate Loader DW supports both standard 96-well plates, and 96-well deep well plates.

Figure 1.17 Standard Plate Loader (Front Cover Removed)



1. Plate loader PEEK tubing
2. Plate loader sample probe assembly
3. Plate loader wash station
4. Plate holder

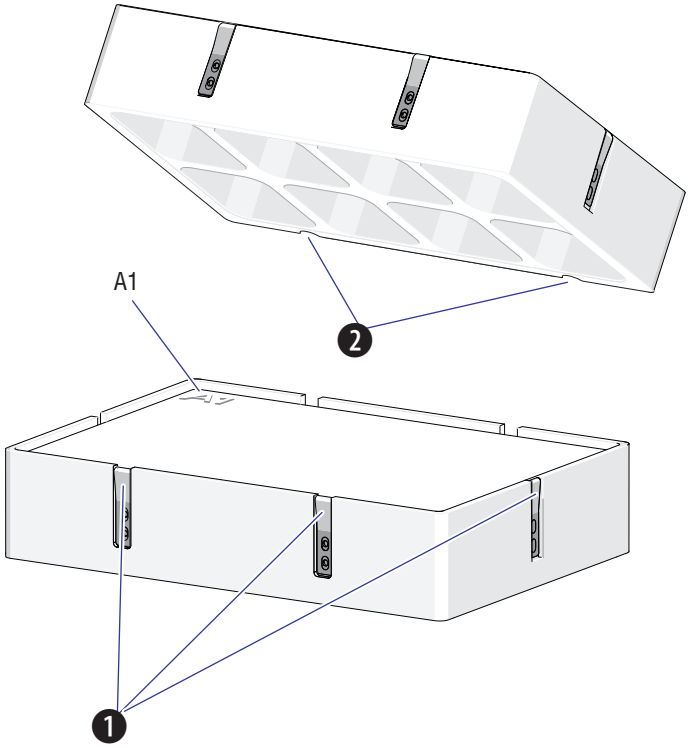
Figure 1.18 Plate Loader DW (Front Cover Removed)



1. Plate loader PEEK tubing
2. Plate loader sample probe assembly
3. Plate loader wash station
4. Plate holder

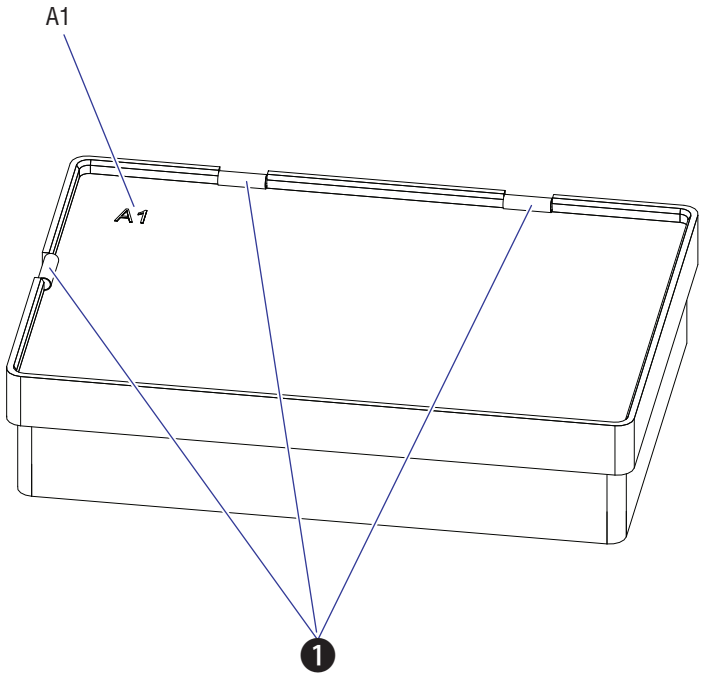
Plate Holder Components

Figure 1.19 Standard Plate Holder without Groove (Standard Plate Loader)



1. Spring leaves to hold plate
2. Plate holder notches

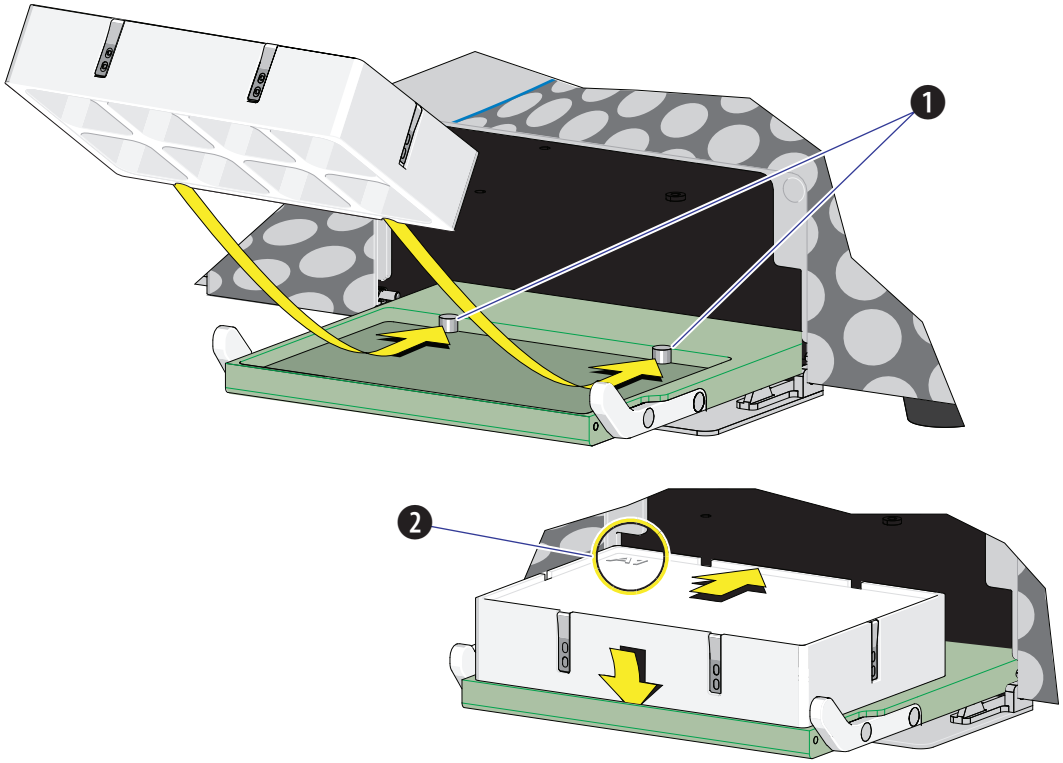
Figure 1.20 Plate Holder with Groove (Plate Loader DW)



1. Spring leaves to hold plate

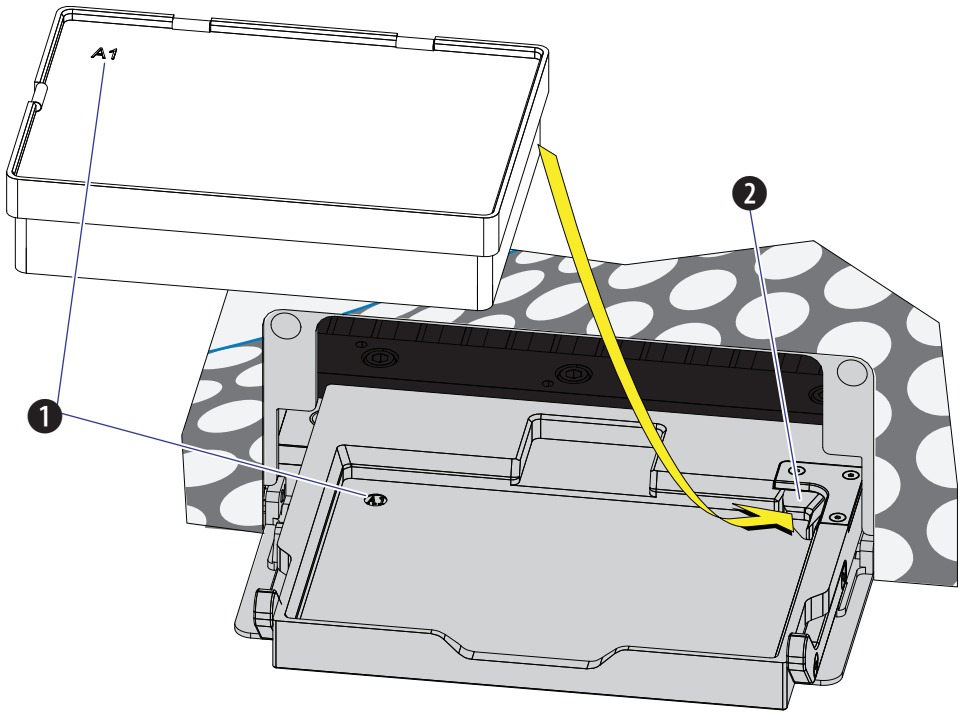
NOTE The plate holder is removable and replaceable. Refer to Replacing the Plate Holder [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.
The plate holder is only used to hold standard 96-well plates. Note that 96-well deep well plates do not use a plate holder.

Figure 1.21 Plate Holder Stage (Standard Plate Loader)



1. Pins
2. Position A1

Figure 1.22 Plate Holder Stage (Plate Loader DW)



1. Position A1
2. Spring Lock

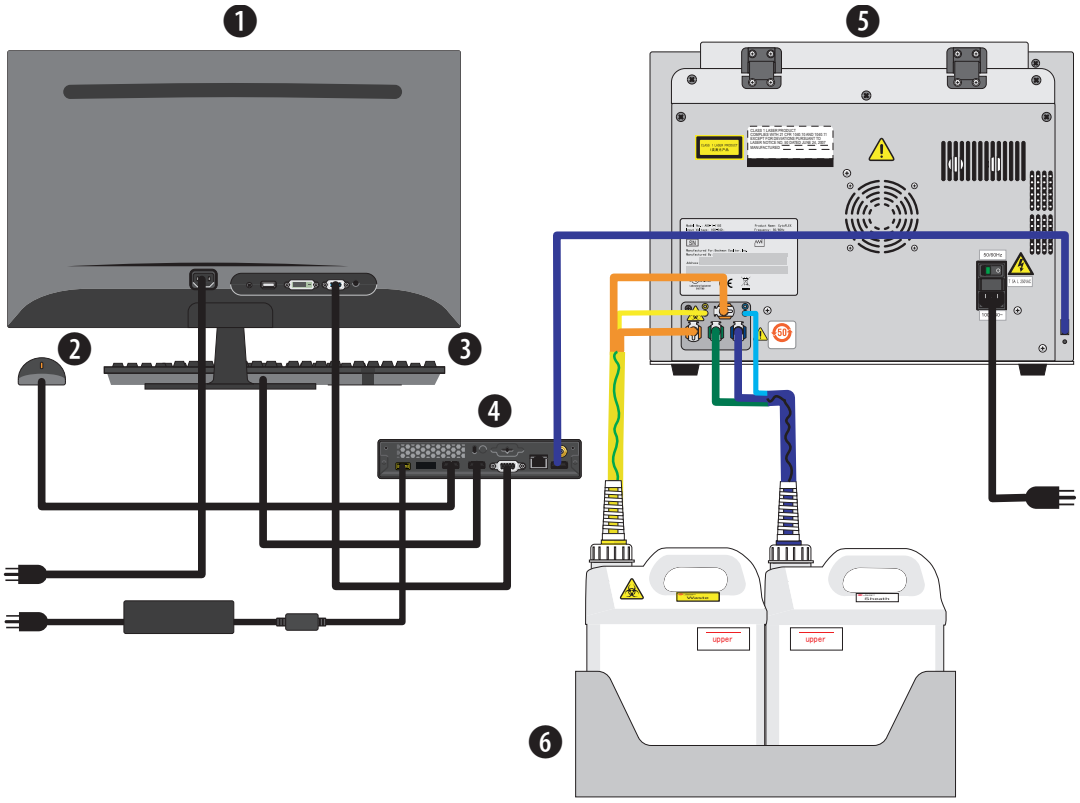
System Configuration

⚠ CAUTION

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

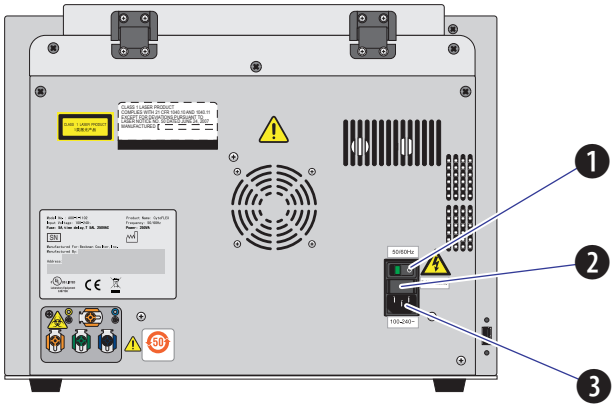
System Configuration [CytoFLEX and CytoFLEX S]

Figure 1.23 System Connections



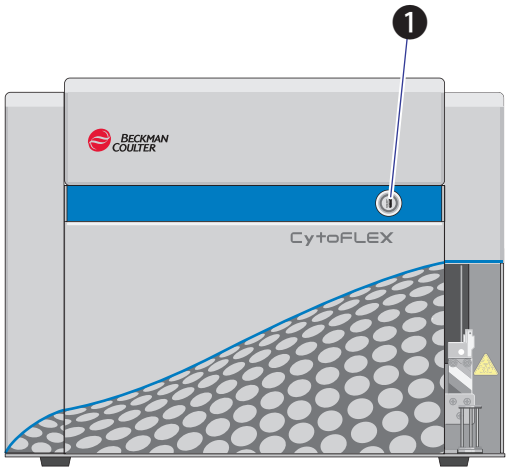
1. Monitor
2. Mouse
3. Keyboard
4. Computer
5. Cytometer
6. Fluid Container holder

Figure 1.24 Back Cover Connections



1. **Power switch.** Turns Cytometer on and off. An indicator light glows when the power is on.
2. **Fuse.** Protects the internal system from damage by high electrical current.
3. **Power line socket.** Supplies the power to the Cytometer.

Figure 1.25 Front of Cytometer [CytoFLEX Without Plate Loader Shown]



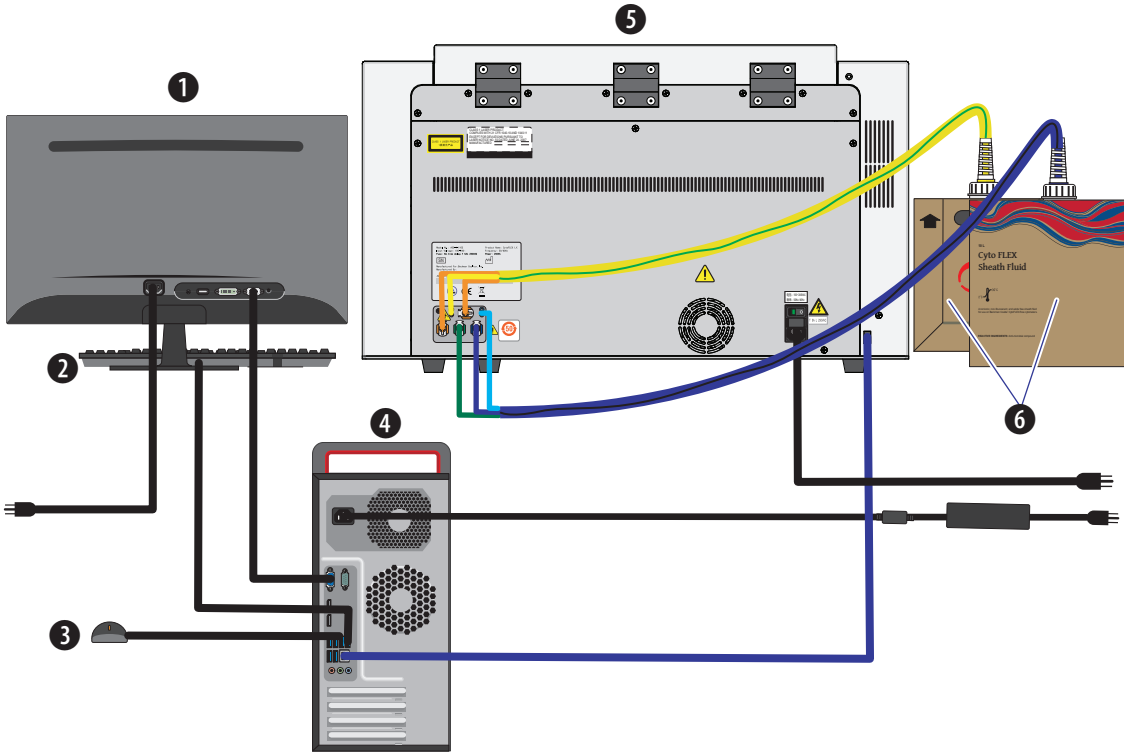
1. **Load button.** In addition to the software controls, this button can be used for automatic sample loading and data recording.

   NOTE This function is not available in the Plate Loader sample injection mode.

System Configuration [CytoFLEX LX]

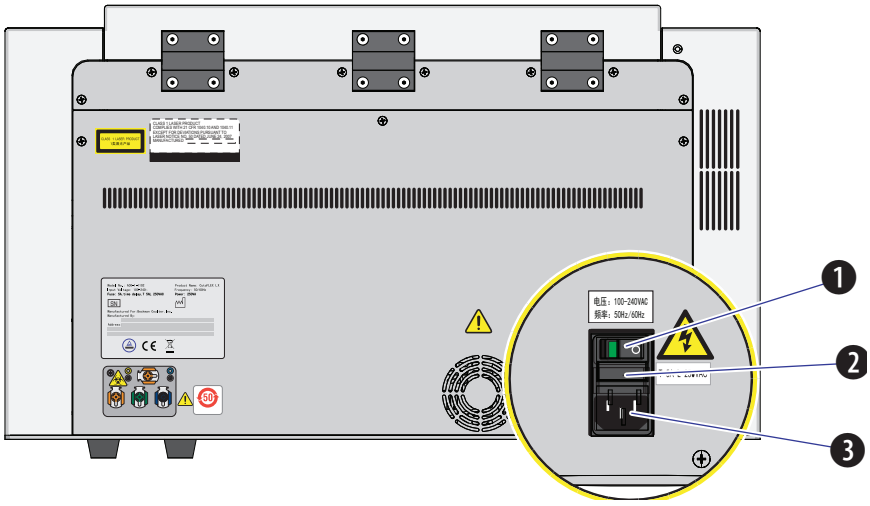
IMPORTANT The fluid cubitainers must be on the same level as the Cytometer. Do not place the fluid cubitainers on the floor.

Figure 1.26 System Connections



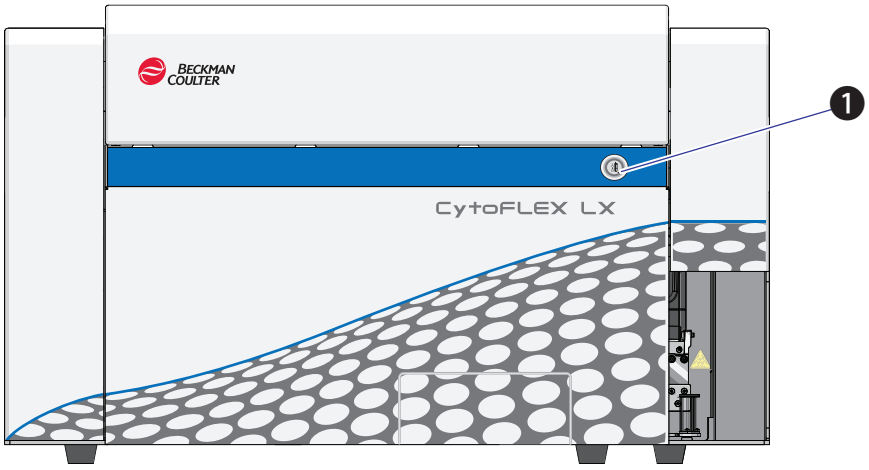
1. Monitor
2. Keyboard
3. Mouse
4. Computer
5. Cytometer
6. Fluid Cubitainers

Figure 1.27 Back Cover Connections



1. **Power switch.** Turns Cytometer on and off. An indicator light glows when the power is on.
2. **Fuse.** Protects the internal system from damage by high electrical current.
3. **Power line socket.** Supplies the power to the Cytometer.

Figure 1.28 Front of Cytometer



1. **Load button.** In addition to the software controls, this button can be used for automatic sample loading and data recording.

   NOTE This function is not available in the Plate Loader sample injection mode.

Consumables and Supplies

Reagents

The following reagents are available for the CytoFLEX and CytoFLEX LX instrument:

CytoFLEX Daily QC Fluorospheres
CytoFLEX Daily QC Fluorospheres is a suspension of fluorescent microspheres which may be used for daily verification of the CytoFLEX flow cytometer's optical alignment and fluidics system.

NOTE Not for daily verification of the Infrared optical alignment.

CytoFLEX Sheath Fluid
A nonionic, non-fluorescent, and azide-free sheath fluid for use on Beckman Coulter CytoFLEX flow cytometers.

Contrad® 70 Reagent
Diluted 1:1 with DI water for use in the Deep Clean solution bottle.

FlowClean
For use as a cleaning agent for flow cytometer components that come in contact with blood samples.

The following reagents are available for the IR laser QC:

CytoFLEX Daily IR QC Fluorospheres
CytoFLEX Daily IR QC Fluorospheres is a suspension of fluorescent microspheres which may only be used for daily verification of the CytoFLEX flow cytometer's Infrared optical alignment.

Material Safety Data Sheets (SDS/MSDS)

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

- On the Internet, go to **www.beckmancoulter.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.

Ordering Information

Your instrument may be upgraded to a more highly configured model. For information on specific upgrades, replacement parts, or supplies, visit:

- www.beckman.com/coulter-flow-cytometry/cytoflex-quoter for CytoFLEX
- www.beckman.com/coulter-flow-cytometry/cytoflex-s-quoter for CytoFLEX S
- www.beckman.com/coulter-flow-cytometry/cytoflex-lx-quoter for CytoFLEX LX

Otherwise, contact us.

Instrument Specifications

Dimensions [CytoFLEX]

Dimensions		
Instrument dimensions (Length x Width x Height)	**Cytometer [With or Without Plate Loader]**	42.5 cm x 42.5 cm x 34 cm
	Fluid Containers and Fluid Container holder	14 cm x 35.6 cm x 35.6 cm
Weight	**Cytometer [Without Plate Loader]**	23.4 kg
	Cytometer [With Standard Plate Loader]	28 kg
	Cytometer [With Plate Loader DW]	28.4 kg

Dimensions [CytoFLEX LX]

Dimensions		
Instrument dimensions (Length x Width x Height)	**Cytometer [With or Without Plate Loader]**	60.5 cm x 73.3 cm x 45.1 cm
	Fluid Cubitainers	25 cm x 25 cm x25 cm
Weight	**Cytometer [Without Plate Loader]**	79 kg
	Cytometer [With Standard Plate Loader]	83.6 kg
	Cytometer [With Plate Loader DW]	84 kg

Installation Category

Installation Category 2

Pollution Degree

Pollution Degree 2

Acoustic Noise Level

Measure Level: <65 dBA

Electrical Ratings

Voltage: 100-240 VAC, 50/60 Hz, 250 VA

Cytometer

Optics		
Excitation Optics	The CytoFLEX system can be configured with up to three 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. The CytoFLEX LX system can be configured with up to six 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.	
Emission Optics	Patent-pending alignment-free integrated optics quartz flow cell design with >1.3 NA. Flow Cell dimensions: 430-µm x 180-µm internal diameter.	
Laser devices	**Standard wavelengths**	**Blue laser** • Wavelength: 488 nm, 50 mW • Beam spot size: 5 µm x 80 µm **Red laser** • Wavelength: 638 nm, 50 mW • Beam spot size: 5 µm x 80 µm **Violet laser** • Wavelength: 405 nm, 80 mW • Beam spot size: 5 µm x 80 µm
	Additional standard wavelengths [CytoFLEX S or CytoFLEX LX]	**Yellow laser** • Wavelength: 561 nm, 30 mW • Beam spot size: 5 µm x 80 µm **Near Ultraviolet (NUV) laser** • Wavelength: 375 nm, 60 mW • Beam spot size: 5 µm x 80 µm **Infrared (IR) laser** • Wavelength: 808 nm, 60 mW • Beam spot size: 5 µm x 80 µm
Forward scatter detection	Silicon photodiode with built-in 488/8 band-pass filter.	

Optics	
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 loading speed	**Defaults**	Slow 10 µL/min Medium 30 µL/min Fast 60 µL/min
Fluid capacity	**CytoFLEX:** Standard 4-L sheath fluid and waste containers; Optional 10 L sheath fluid and waste cubitainers **CytoFLEX LX:** Standard 10-L sheath fluid and waste cubitainers	
Automated maintenance cycles	Startup (initialize), system startup program, sample mix, backflush, prime, Shutdown (Daily Clean), Deep Clean	
Sample input formats	**Single Tube Loader format**	5 mL (12 x 75 mm) polystyrene and polypropylene sample tubes 1.5 mL and 2 mL micro-centrifuge sample tubes

Fluidics System [With Standard 96-well Plate]		
Sample input formats	**Plate Loader format**	Flat/U/V bottom standard 96-well plate
Dead Volume	**96-well flat bottom plate**	20 µL
	96-well U bottom plate	10 µL
	96-well V bottom plate	10 µL
Minimum Sample Volume	50 µL/well	
Maximum Sample Volume	250 µL/well	

Fluidics System [With 96-well Deep Well Plate][a]		
Sample input formats	**Plate Loader format**	U/V bottom 96-well deep well plate
Dead Volume	20 µL	
Minimum Sample Volume	50 µL/well[b]	
Maximum Sample Volume	1000 µL/well (for circular well) or 2000 µL/well (for square well)	

a. The 96-well deep well plates are only available for use if the Plate Loader DW is installed. The information below is based on the deep well plates manufactred by Beckman Coulter. Refer to APPENDIX D, Specimen Collection Plate Specifications.

b. To mix sample throughly, it is recommended to add at least 200 µL sample per well for one time.

Electronics	
Signal processing	7 decade data display
Digital sampling rate	25 MHz
Signal	Pulse area and height for every channel, width for one selectable channel

Data Management		
Software	CytExpert software	
Language	English and Chinese	
FCS format	FCS 3.0	
Minimum Workstation/ computer requirements [CytoFLEX]	**Operating system**	Windows® 7, 8, 10 Professional 64-bit
	Processor	4th Gen Intel® Core™ i3 (3MB Cache, 2.90 GHz)
	Memory	4 GB RAM
	Storage	256 GB
	Port	1 GB Ethernet port
	USB	5 USB 2.0 and above ports
Minimum Workstation/ computer requirements [CytoFLEX LX]	**Operating system**	Windows®7, 8, 10 Professional 64-bit
	Processor	6th Generation Intel Core i7 (8MB cache 4.0 GHz)
	Memory	8 GB RAM
	Storage	256 GB
	Port	1 GB Ethernet port
	USB	5 USB 2.0 and above ports
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.	

Performance Characteristics

Performance Characteristics [CytoFLEX and CytoFLEX S]

Performance		
Sensitivity	**MESF**	FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE)
Fluorescence resolution	rCV <3% The CytoFLEX LX Flow Cytometer is capable of achieving <3% rCV. Using CytoFLEX Daily QC Fluorospheres and Daily IR QC Fluorospheres (for 808 nm Laser) for daily QC, the pass criteria is ≤5% for the Violet, Blue, Yellow, and Red lasers while the pass criteria is ≤7% for NUV, UV and IR lasers.	
Blue Side scatter resolution	<300 nm	
Violet Side scatter resolution	<200 nm	
Forward and side scatter resolution	Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes as well as nanoparticles.	
Carryover	**Single Tube Loader format**	≤1.0%
Signal acquisition speed	30,000 particles/second with 15 parameters	

Performance [With Standard Plate Loader]		
Carryover	**Plate Loader format**	<0.5%
Throughput [With Plate Loader][a]	10 second acquisition without mixing or backflush: <32 min.	
	10 second acquisition with 3 second mixing and 3 second backflush: <45 min.	

a. This performance characteristic is different if you have the Sample Injection Mode Control Kit installed on your CytoFLEX flow cytometer. Refer to APPENDIX C, Sample Injection Mode Control Kit.

Performance [With Plate Loader DW]		
Carryover	**Plate Loader format**	<0.5%
Throughput [With Plate Loader DW][a]	Standard 96-well plate, 10 second acquisition without mixing or backflush: <36 min. Deep-well 96-well plate, 10 second acquisition without mixing or backflush: <37 min	
	Standard 96-well plate, 10 second acquisition with 5 second mixing and 4 second backflush: <54 min. Deep-well 96-well plate, 10 second acquisition with 10 second mixing and 4 second backflush: <64 min.	

a. The Plate Loader DW is equipped with the Sample Injection Mode Control Kit. Refer to APPENDIX C, Sample Injection Mode Control Kit.

Performance Characteristics [CytoFLEX LX]

Performance		
Sensitivity	**MESF**	FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE)
Fluorescence resolution	rCV <3% The CytoFLEX LX Flow Cytometer is capable of achieving <3% rCV. Using CytoFLEX Daily QC Fluorospheres and Daily IR QC Fluorospheres (for 808 nm Laser) for daily QC, the pass criteria is ≤5% for the Violet, Blue, Yellow, and Red lasers while the pass criteria is ≤7% for NUV, UV and IR lasers.	
Blue Side scatter resolution	<300 nm	
Violet Side scatter resolution	<200 nm	
Forward and side scatter resolution	Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes as well as nanoparticles.	
Carryover	**Single Tube Loader format**	≤1.0%
Signal acquisition speed	30,000 particles/second with 23 parameters	

Performance [With Standard Plate Loader]		
Carryover	**Plate Loader format**	<0.5%
Throughput [With Standard Plate Loader][a]	10 second acquisition without mixing or backflush: <34 min.	
	10 second acquisition with 3 second mixing and 3 second backflush: < 47 min.	

a. This performance characteristic is different if you have the Sample Injection Mode Control Kit installed on your CytoFLEX LX flow cytometer. Refer to APPENDIX C, Sample Injection Mode Control Kit.

Performance [With Plate Loader DW]		
Carryover	**Plate Loader format**	<0.5%
Throughput [With Plate Loader DW][a]	Standard 96-well plate, 10 second acquisition without mixing or backflush: <39 min. Deep-well 96-well plate, 10 second acquisition without mixing or backflush: <40 min	
	Standard 96-well plate, 10 second acquisition with 5 second mixing and 6 second backflush: <60 min. Deep-well 96-well plate, 10 second acquisition with 10 second mixing and 6 second backflush: <69 min.	

a. The Plate Loader DW is equipped with the Sample Injection Mode Control Kit. Refer to APPENDIX C, Sample Injection Mode Control Kit.

Reagent Limitations

- Only use nonionic sheath fluid, like CytoFLEX Sheath Fluid. Do not use sheath fluid containing electrolytes.
- Do not use organic solvents in the system.

CHAPTER 2

Using the CytExpert Software

Overview

The CytExpert software is a full-feature software package that controls the instrument's operation, collection of experiment data, and analysis of the results. 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
- User Management Operation Log
- Graphic and Gating Styles
- Software Settings

Launching the Software

Select the desktop shortcut 

to launch the CytExpert software.

If there is no desktop shortcut, run the "CytExpert.exe" software directly from the software installation directory. The default installation path is C:/Program Files/CytExpert. Or, select  **> All Programs > CytExpert**.

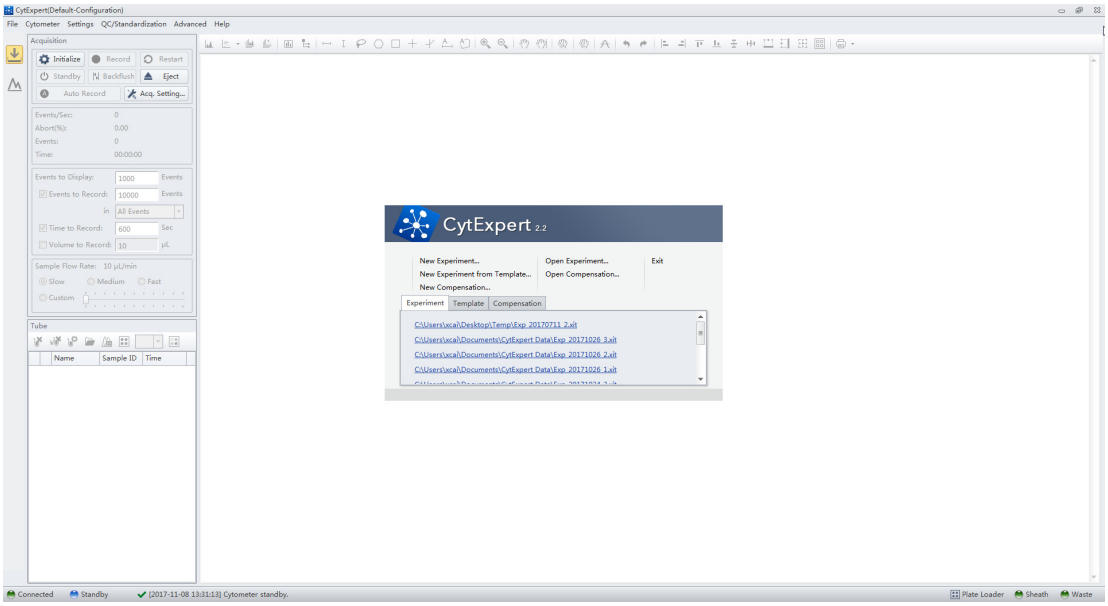
Refer to Logging Into the Software in CHAPTER 3, Daily Startup, for detailed instructions on opening the software and confirming the connection status.

Main Software Screen

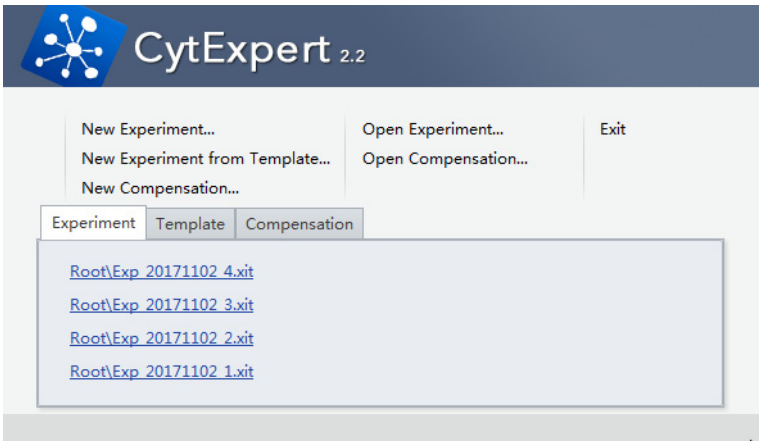
Hover your cursor over any button to display a text pop-up of the button's function.

Start Page

The start page automatically opens after the software has been launched.



The following operations can be selected from the start page:

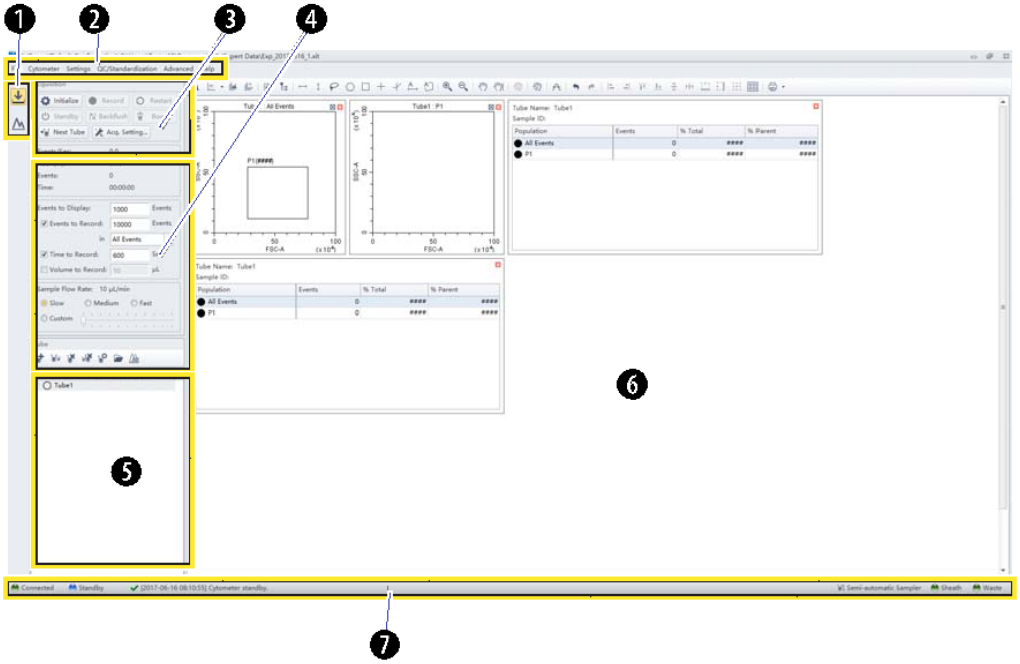


- **New Experiment**. For creating a new experiment. The process creates a file with the .xit extension and a folder with the same file name where the raw data (.fcs files) are kept.
- **New Experiment From Template.** For creating an experiment using a template saved from a previously saved experiment.
- **New Compensation.** For setting up compensation for an experiment.
- **Open Experiment**. For opening a previously created experiment.
- **Open Compensation.** For opening a previously created compensation experiment.
- **Exit**. For exiting CytExpert.

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.



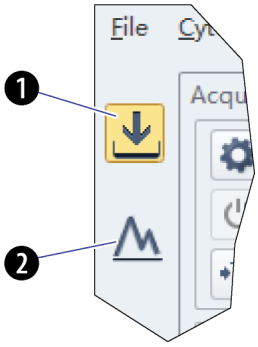
1. **Navigation.** Gives the option of accessing the Acquisition screen or Analysis screen.
2. **Menu.** Allows you to configure settings for sample acquisition, instrument operation, and software options.
3. **Instrument Operation Controls.** Controls sample loading/unloading and data acquisition and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

   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 to Display, Events/Sec, and the Acquisition buttons.

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

Acquisition Screen Navigation

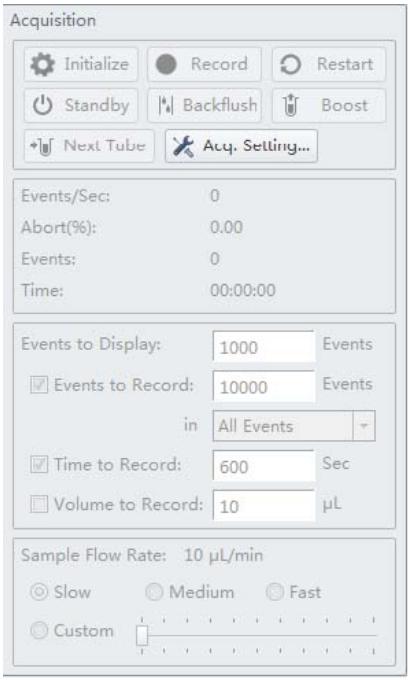
The Acquisition screens have two navigation icons, one for the Acquisition screen and the other for the Analysis screen.



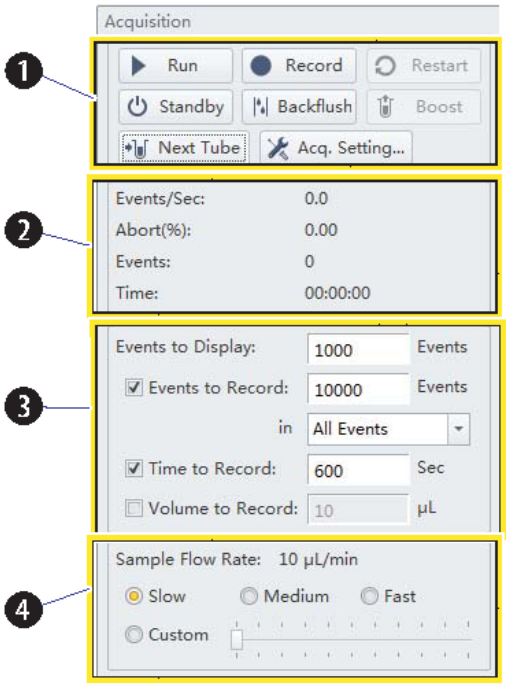
1. **Acquisition screen icon.** Accesses the Acquisition screen.
2. **Analysis screen icon.** Accesses the Analysis screen.

Collection

Standby state



Initialized state



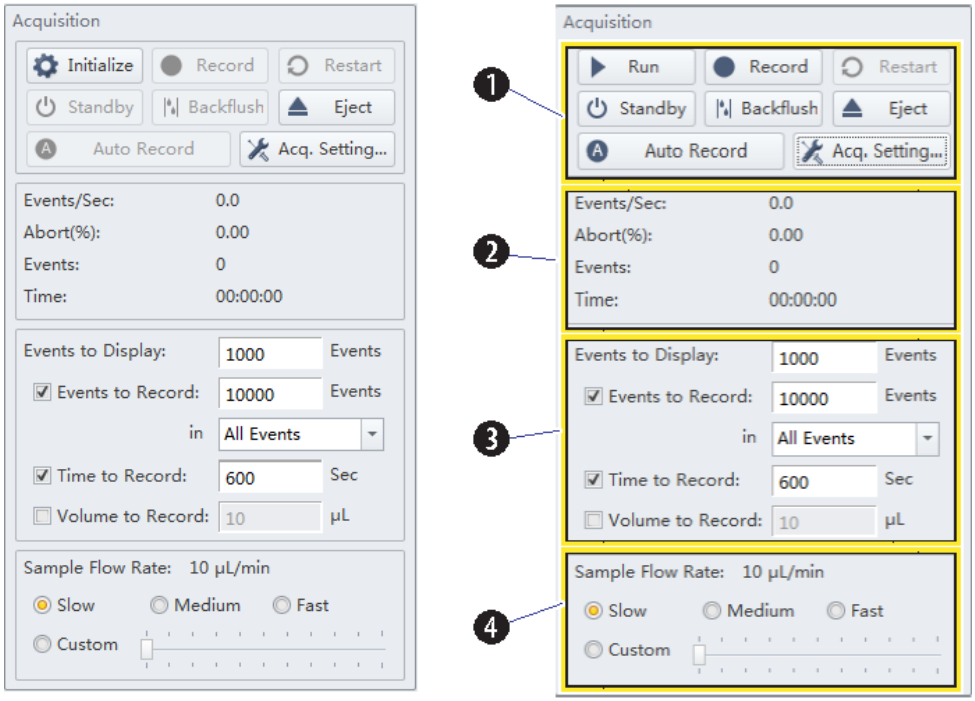
1. **Acquisition control.** Controls sample loading/unloading and data acquisition and recording.
2. **Acquisition status.** Displays such information as the acquisition rate (Events/Sec), event count, duration, and abort (%).
3. **Acquisition conditions.** Sets the necessary conditions for recording data.
4. **Sample flow rate.** Sets the acquisition rate for data collection.

NOTE High acquisition rate may increase the abort rate and measurement CVs.

Custom: The flow rate can be adjusted in 1 µL increments.

Collection [With Plate Loader]

Standby state **Initialized state**

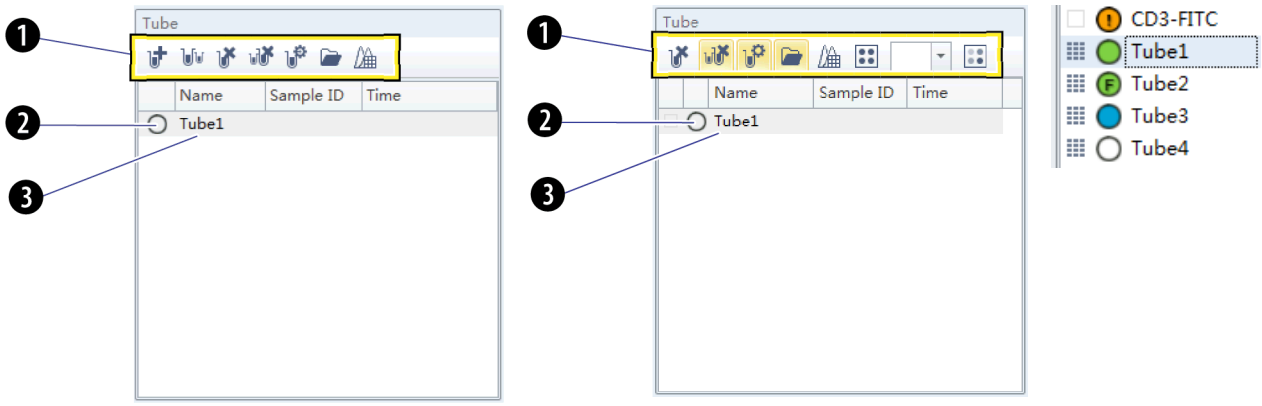


1. **Acquisition control.** Controls sample loading/unloading and data acquisition and recording.
2. **Acquisition status.** Displays such information as the acquisition rate (Events/Sec), event count, duration, and abort (%).
3. **Acquisition conditions.** Sets the necessary conditions for recording data.
4. **Sample flow rate.** Sets the acquisition rate for data collection.

NOTE High acquisition rate may increase the abort rate and measurement CVs.

Test Tubes

Manual/Semi-Automatic Sample Injection Mode | **Plate Loader Sample Injection Mode** | **Test Tube Status**



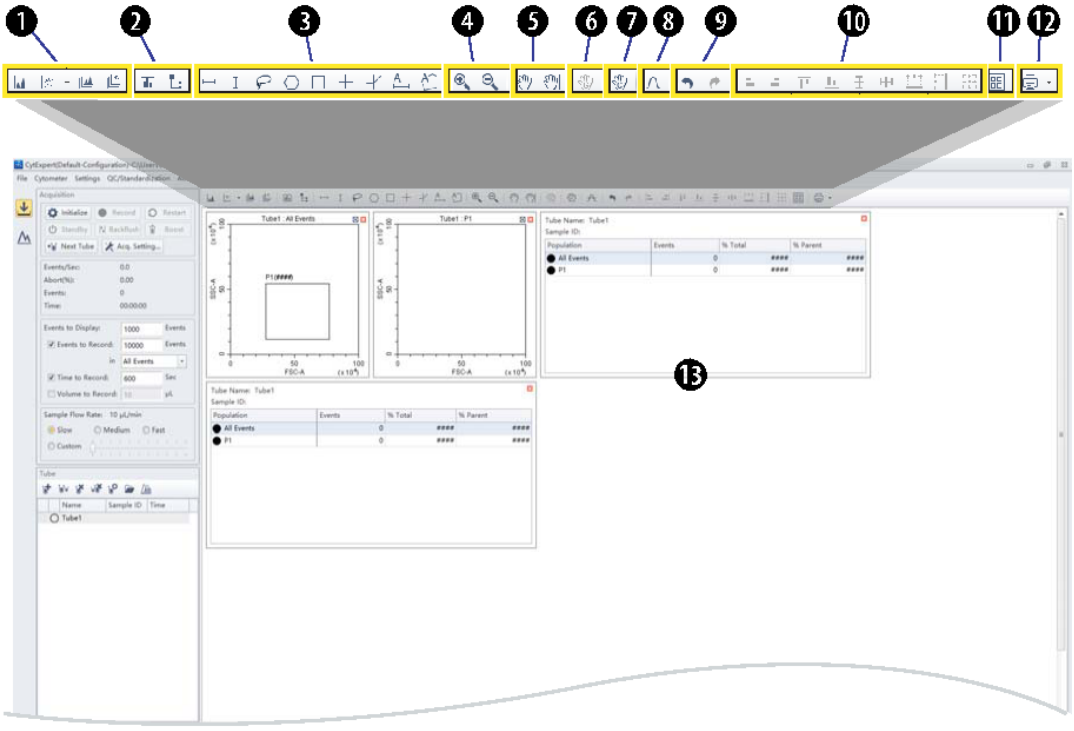
1. **Tube management controls.** Manages sample tubes. Used to add, copy, or delete attributes, open the tube property, and open the compensation matrix.
2. **Test tube status indication.** Displays a colored symbol in front of each tube indicating the status of the tube processing.
   - ○ indicates that the tube data was not collected.
   - ● indicates that the tube data was acquired by selecting **Run** but can be overwritten.
   - ● indicates that the tube data was saved by selecting **Record** or **Auto Record** and that 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 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.

   NOTE In the Plate Loader Sample Injection mode the well number displays at the end of the tube name.

Plot area

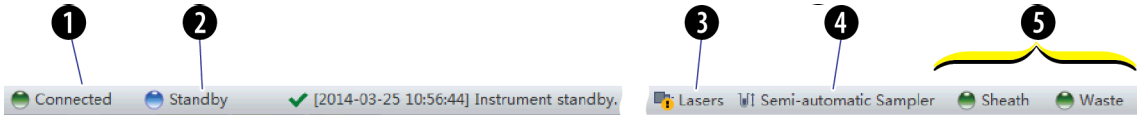


1. **Plot controls.** For creating single or multiple plots, such as dot plots, histograms, density plots, pseudo color plots, and contour plots.
2. **Statistics and hierarchy controls.** For creating statistical and hierarchical charts.
3. **Graphical gating controls.** For creating graphical gates.
4. **Zoom controls.** For zooming in and out within a plot.
5. **Pan axis display controls.** For scaling axis ranges in the plots.
6. **Gain adjustment control.** For increasing and lowering gain adjustments on the plots.

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

7. **Adjust compensation control.** For adjusting compensation of either of the parameters on a 2D histogram.
8. **Threshold control.** For setting the minimum particle size limit, scatter value, or fluorescence intensity that acquisition will allow.
9. **Undo and redo controls.** For undoing or redoing an action in the drawing area.
10. **Display controls.** For controlling how plots and tables are aligned and arranged.
11. **Rearrange.** For restoring the plots to the default positions.
12. **Printing controls.** For printing and previewing the plot area.
13. **Plot area.** For creating plots and displaying statistics and hierarchy tables.

Status Bar



1. **Communication connection status.** Displays whether the Cytometer and the Workstation are connected.
2. **Instrument status information.** Displays the status of the Cytometer.
3. **Laser status.** Displays the status of each laser.

   NOTE The laser status only displays when a required laser is disabled.

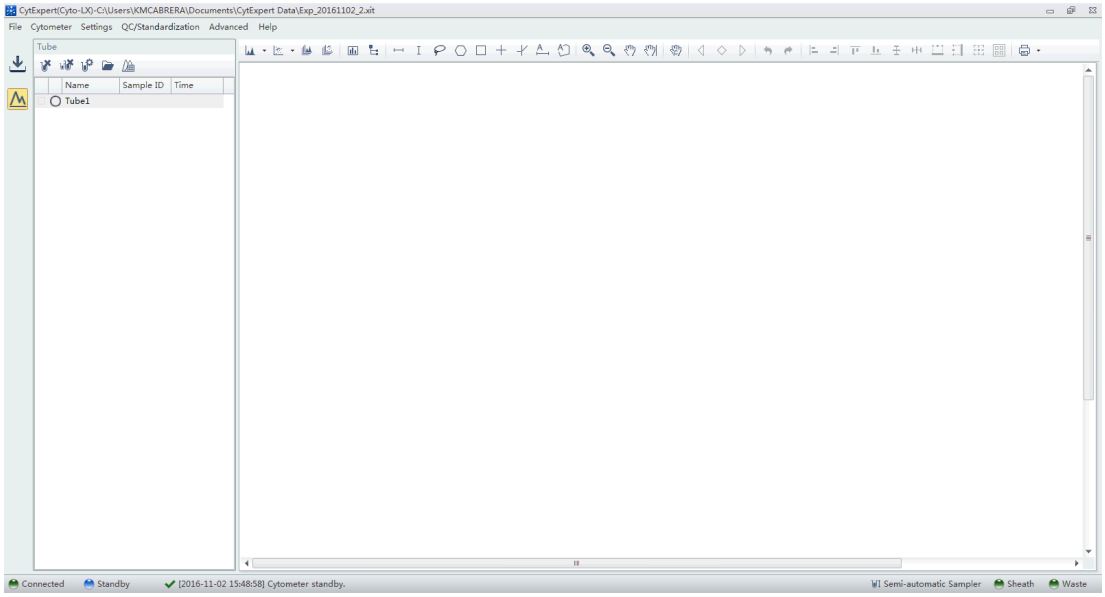
4. **Sampler status.** Displays the sample injection mode state. There are two sample injection modes: Semi-automatic sample injection mode and manual sample injection mode.

   NOTE CytoFLEX Cytometers equipped with a plate loader have three sample injection modes: Semi-automatic sample injection mode, manual sample injection mode, and plate loader sample injection mode.

5. **Fluid status information.** Displays the liquid level of the Fluid Containers/Cubitainers.

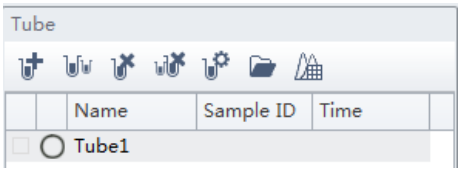
Analysis Screen

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



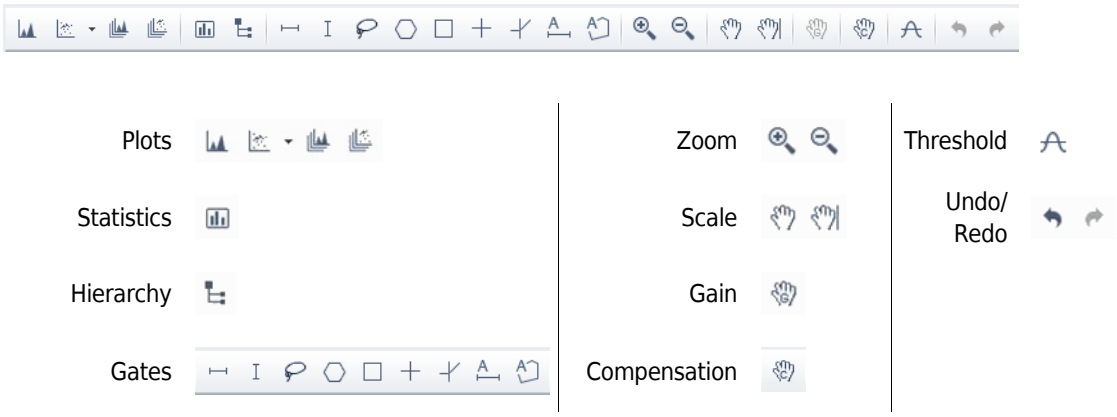
The Tube management module cannot add new sample tubes. Return to the Acquisition screen to add new sample tubes.

Semi-Automatic Sample Injection Mode Shown



Drawing controls (see Figure 2.1) include the multi-data histograms and graphical display data controls.

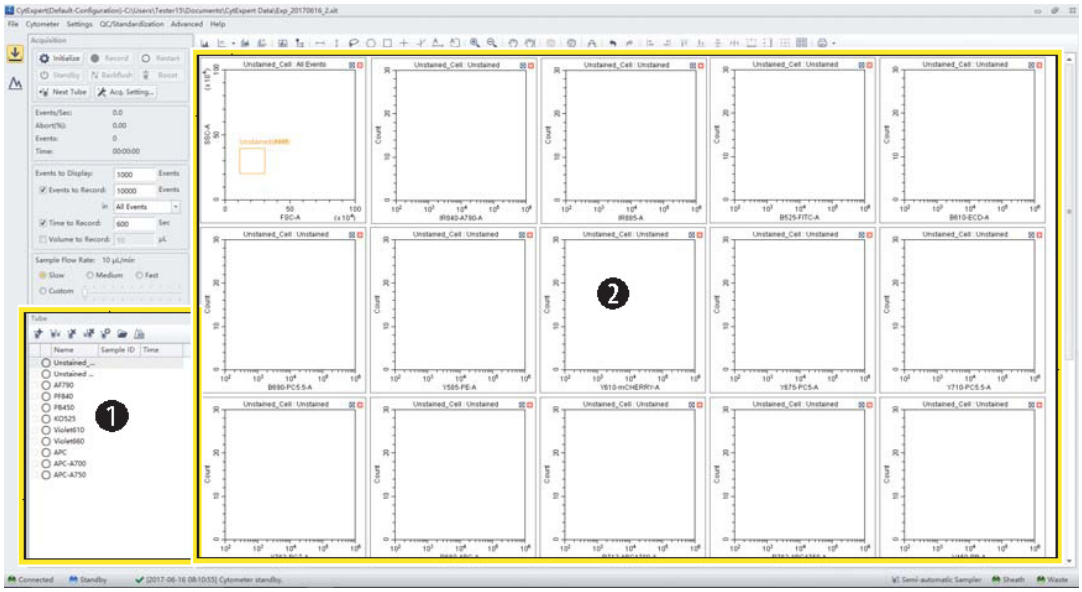
Figure 2.1 Drawing Controls Toolbar (Top of Screen)



Compensation Experiment Screen

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

Semi-Automatic Sample Injection Mode Shown



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 area includes 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		Print	
Pan			

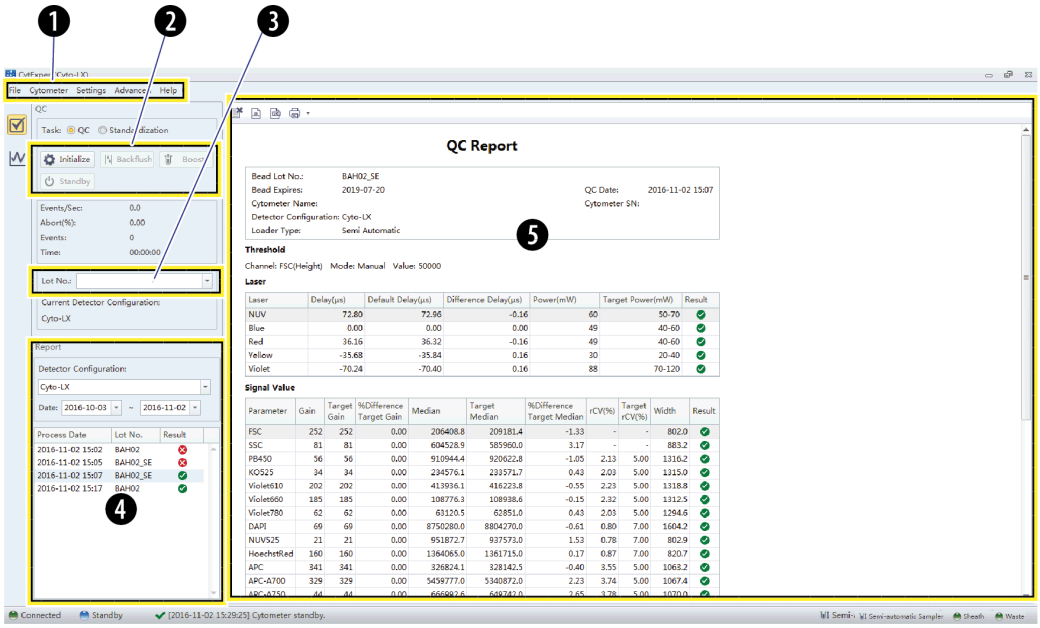
QC Experiment Screen

The Quality Control (QC) Experiment screen appears when you access a QC experiment.

QC Report Screen

Before starting the QC routine, a Settings screen appears.

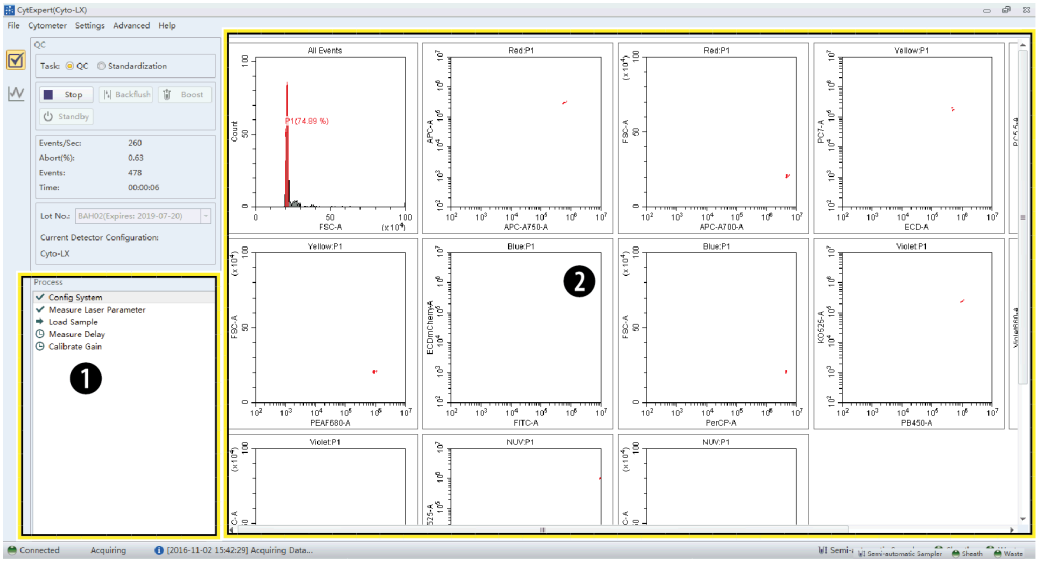
Figure 2.2 QC Report Screen [CytoFLEX LX Semi-Automatic Sample Injection Mode Shown]



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

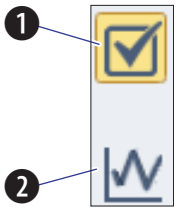
When acquiring QC samples, the software opens the QC screen.



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.



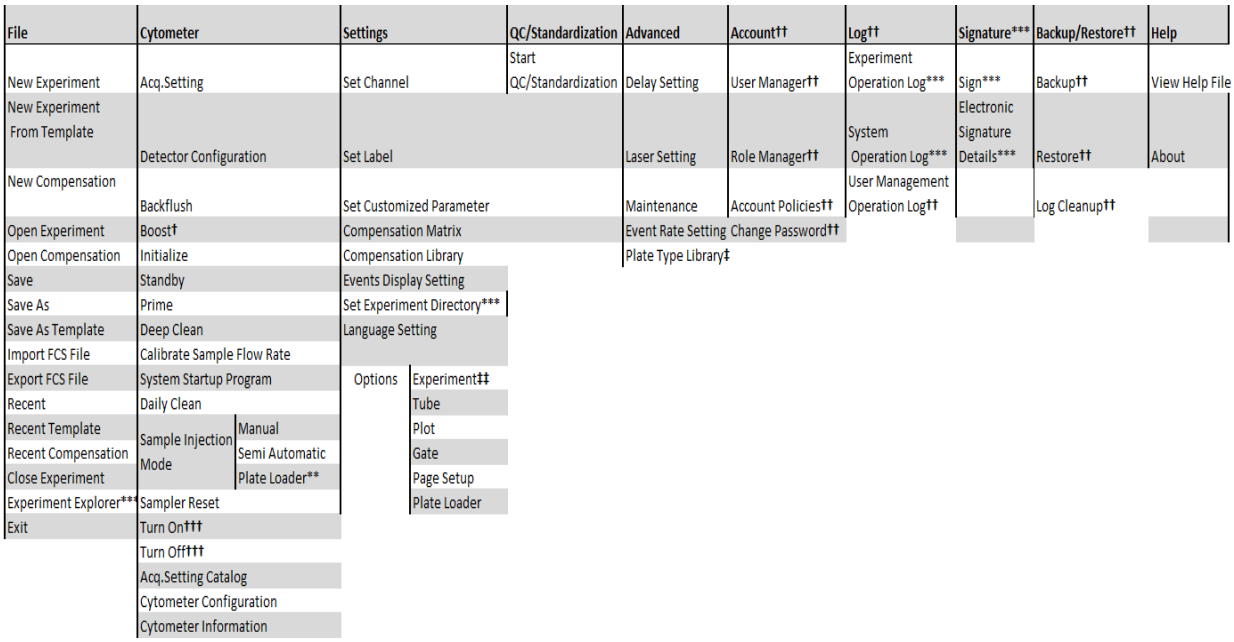
1. **QC screen icon.** Accesses the QC screen.
2. **LJ screen icon.** Accesses the Levey-Jennings (LJ) screen.

Software Menu

IMPORTANT All menu items apply to the CytExpert Default software option unless otherwise specified.

The CytExpert software contains the following selectable menu items:

Figure 2.3 Software Menu Tree*



* The menu options for **File**, **Cytometer**, **Settings**, and **QC/Standardization** change when you select Start QC/Standardization. Refer to Figure 2.4.

† **Boost** is only active in the Manual Sample Injection mode.

‡ **Plate Type Library** is only an option if the Plate Loader module is installed and the Plate Loader Sample Injection mode is selected.

** **Plate Loader** is only an option if the Plate Loader module is installed.

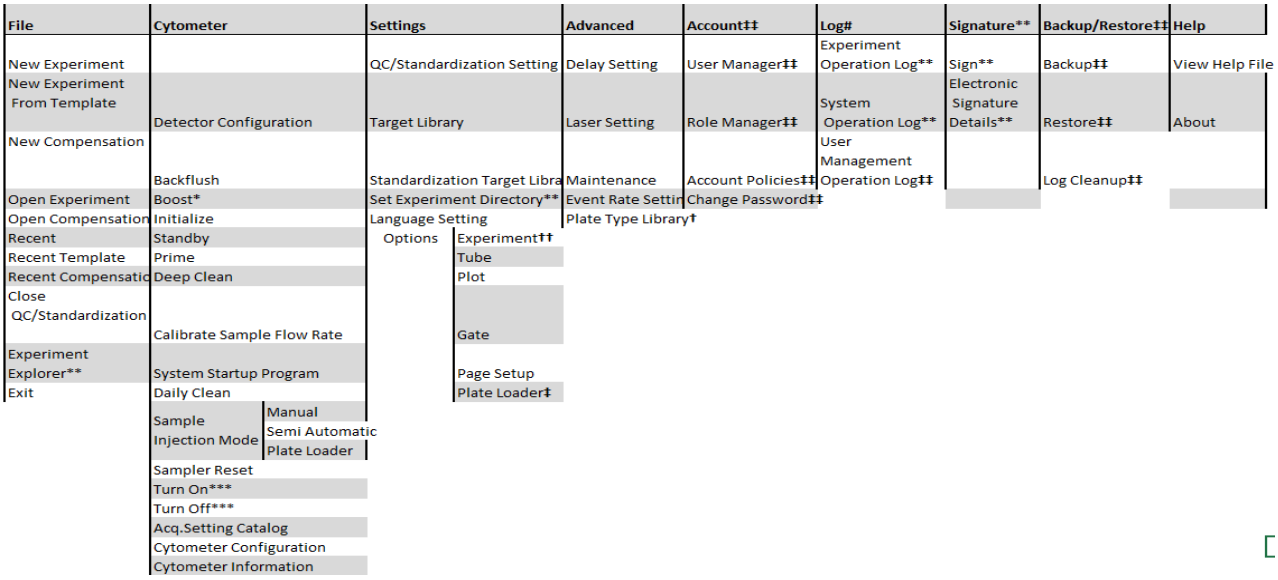
†† These options are only available if the CytExpert User Management or CytExpert Electronic Record Management software option is installed.

‡‡ **Experiment** is only an option if either the CytExpert Default or the CytExpert User Management software option is installed.

*** These options are only available if the CytExpert Electronic Record Management software option is installed.

††† These options are only available on the CytoFLEX LX flow cytometer.

Figure 2.4 QC Software Menu Tree



* **Boost** is only active in the Manual Sample Injection mode.

† Plate Type Library is only an option if the Plate Loader module is installed and the Plate Loader Sample Injection mode is selected.

‡ Plate Loader is only an option if the Plate Loader module is installed.

** These options are only available if the CytExpert Electronic Record Management software option is installed.

† †Experiment is only an option if either the CytExpert Default or the CytExpert User Management software option is installed.

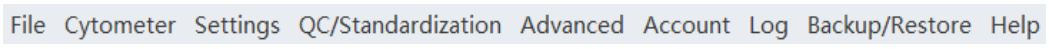
‡‡ These options are only available if either the CytExpert User Management or the CytExpert Electronic Record Management software option is installed.

***These options are only available on the CytoFLEX LX flow cytometer.

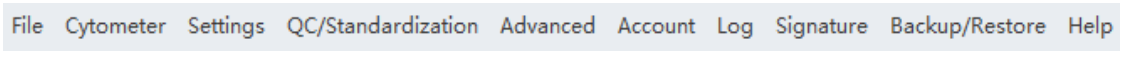
Acquisition and Analysis Screen Menu

CytExpert Default Software Option

CytExpert User Management Software Option



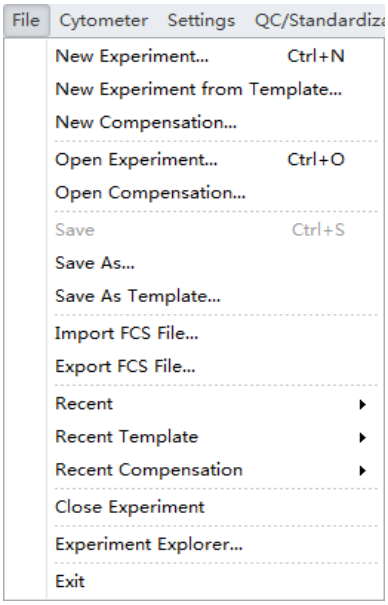
CytExpert Electronic Record Management Software Option



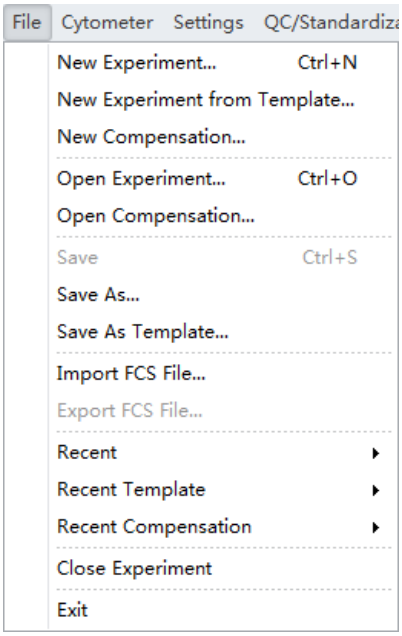
File Menu

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

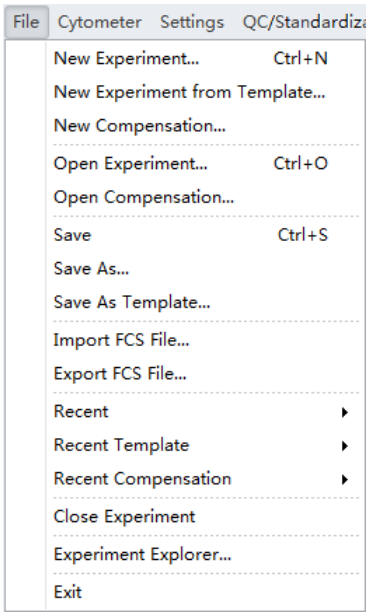
CytExpert Default Software Option



CytExpert User Management Software Option



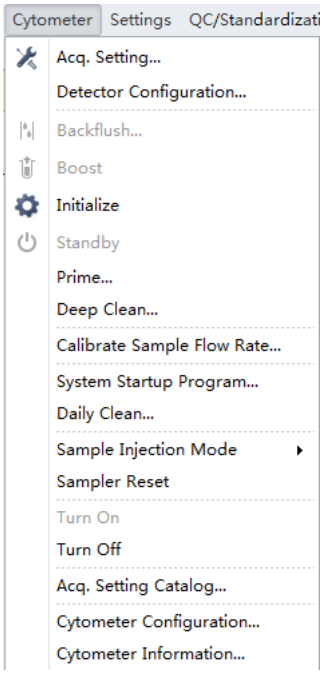
CytExpert Electronic Record Management Software Option



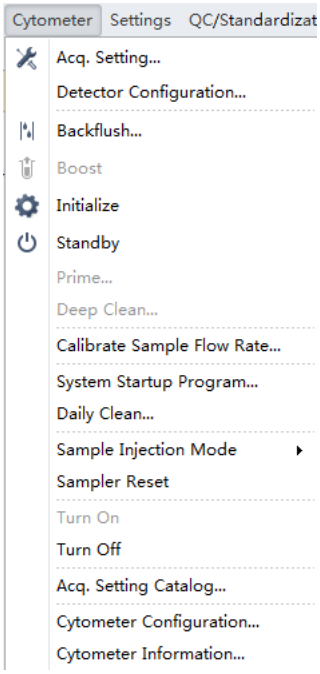
Cytometer Menu

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

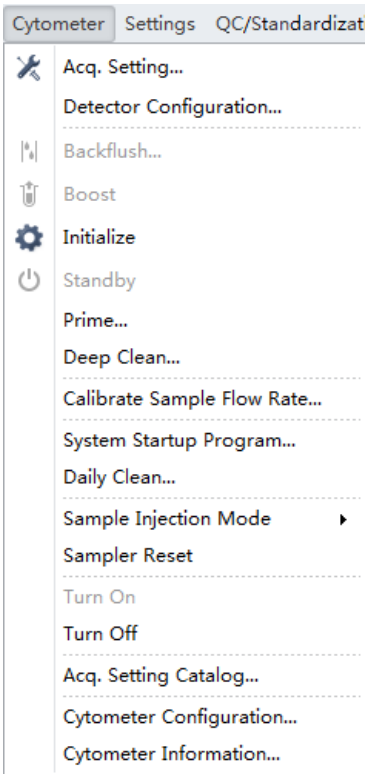
CytExpert Default Software Option - Standby state



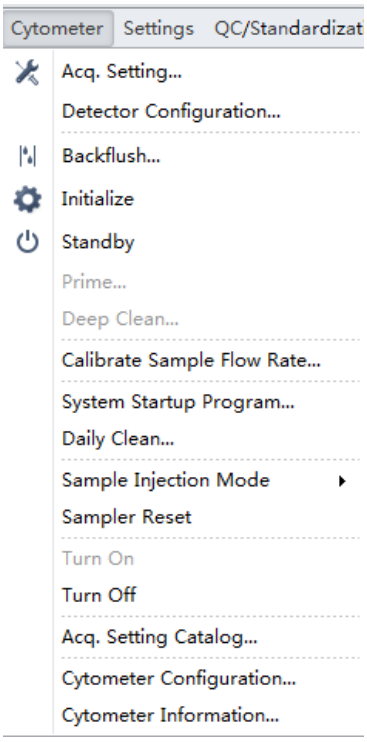
CytExpert Default Software Option - Initialized state



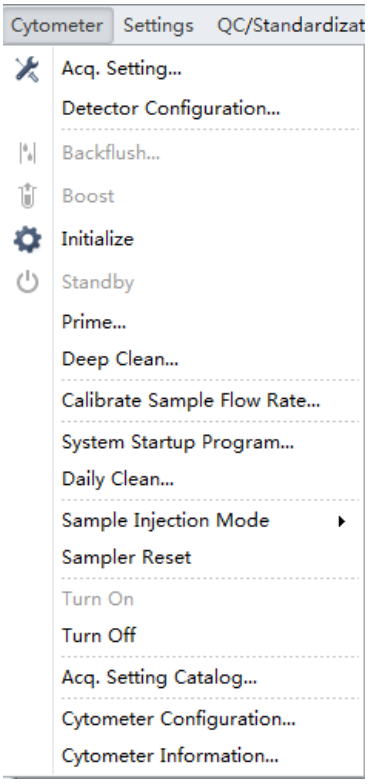
CytExpert User Management Software Option - Standby state



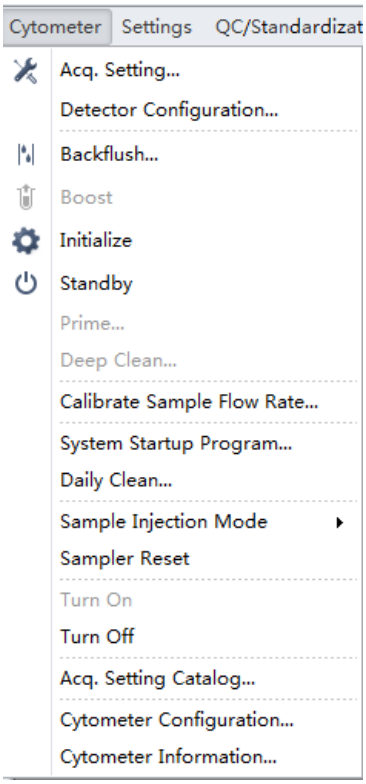
CytExpert User Management Software Option - Initialized state



CytExpert Electronic Record Management Software Option - Standby state



CytExpert Electronic Record Management Software Option - Initialized state

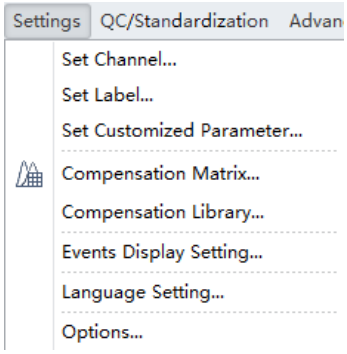


NOTE The **Turn On** and **Turn Off** selections are only available on the CytoFLEX LX.

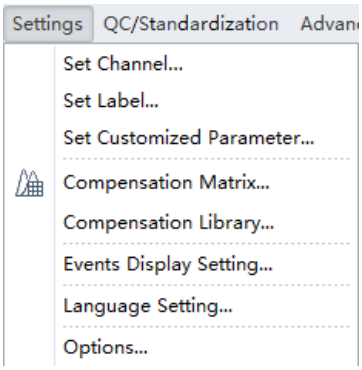
Settings Menu

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

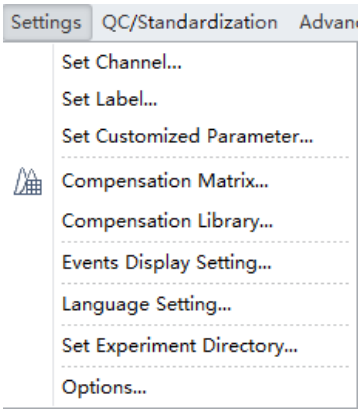
CytExpert Default Software Option



CytExpert User Management Software Option

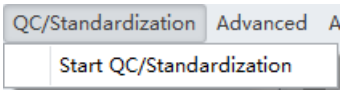


CytExpert Electronic Record Management Software Option



QC/Standardization Menu

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

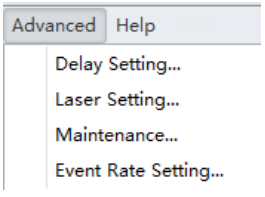


NOTE The QC/Standardization menu is the same for the CytExpert Default, CytExpert User Management, and the CytExpert Electronic Record Management software options.

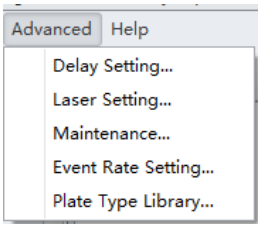
Advanced Menu

Used to access advanced settings for experienced users. Includes laser time delay settings.

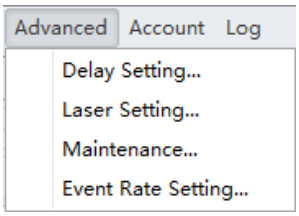
CytExpert Default Software Option - Semi-Automatic/Manual Sample Injection Mode



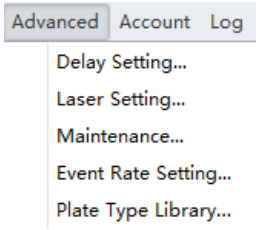
CytExpert Default Software Option - Plate Loader Sample Injection Mode



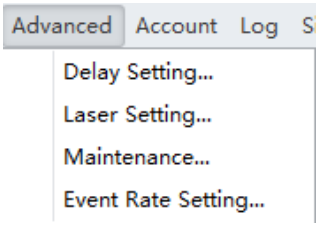
CytExpert User Management Option - Semi-Automatic/Manual Sample Injection Mode



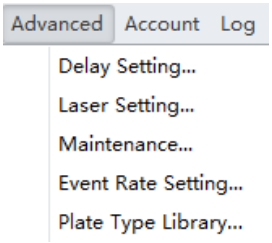
CytExpert User Management Software Option - Plate Loader Sample Injection Mode



CytExpert Electronic Record Management Software Option - Semi-Automatic/Manual Sample Injection Mode



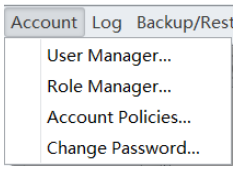
CytExpert Electronic Record Management Software Option - Plate Loader Sample Injection Mode



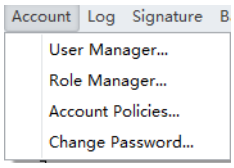
Account Menu

Used to for user account management settings.

CytExpert User Management Software Option



CytExpert Electronic Record Management Software Option

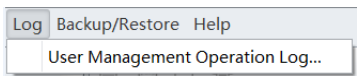


NOTE The Account menu is not available in the CytExpert Default software option.

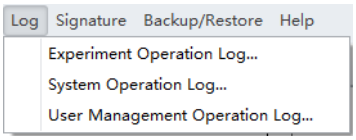
Log Menu

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

CytExpert User Management Software Option



CytExpert Electronic Record Management Software Option

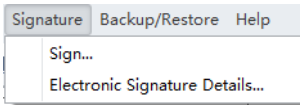


NOTE The Log menu is not available in the CytExpert Default software option.

Signature Menu

Used to sign experiment and view signature details.

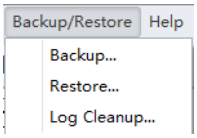
CytExpert Electronic Record Management Software Option



NOTE The Signature menu is only available in the CytExpert Electronic Record Management software option.

Backup/Restore Menu

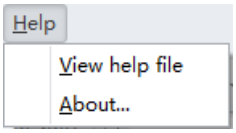
Used to backup/restore databases. Refer to Backup and Restore in CHAPTER 9, Troubleshooting.



NOTE The Backup/Restore menu is not available in the CytExpert Default software option.

Help Menu

For displaying software version information and system Instructions for Use.



NOTE The Help menu is the same for the CytExpert Default, CytExpert User Management, and CytExpert Electronic Record Management software options.

User Management

IMPORTANT Only an Administrator can manage users. You must have either the CytExpert User Management or CytExpert Electronic Record Management software option installed to use this feature. Refer to CytExpert Software Installation Options in APPENDIX A, 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)

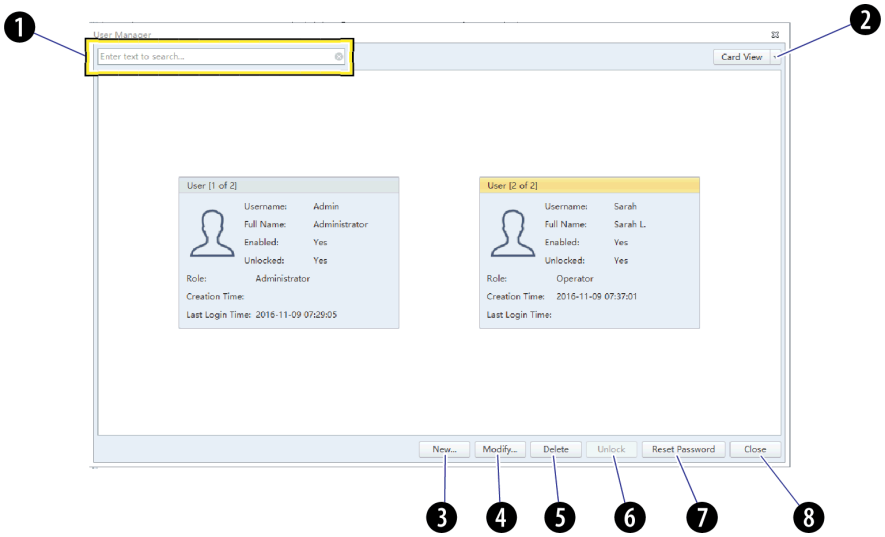
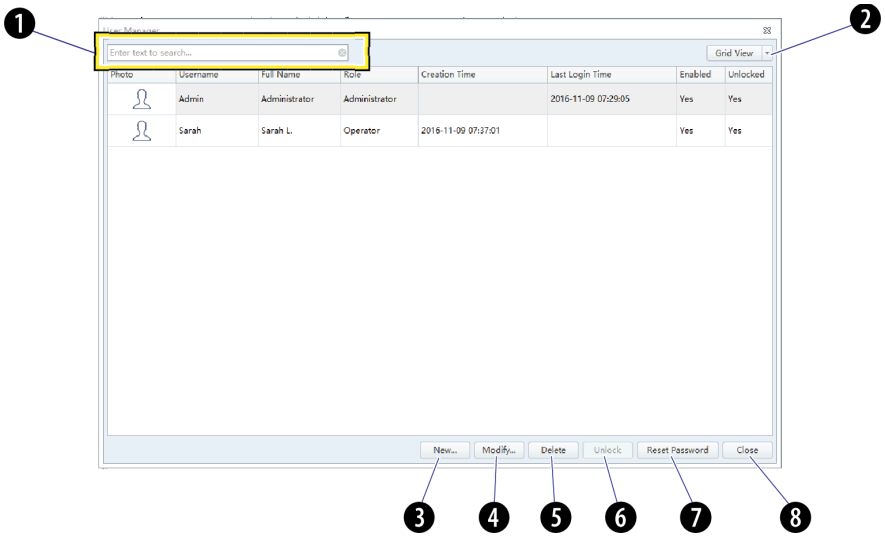


Figure 2.6 User Manager (Grid View)



1. **Search text box:** Filters users by username and display 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.

   NOTE An account locks after 3 failed password attempts. The number of attempts can be changed by the administrator. Refer to Account Policies.

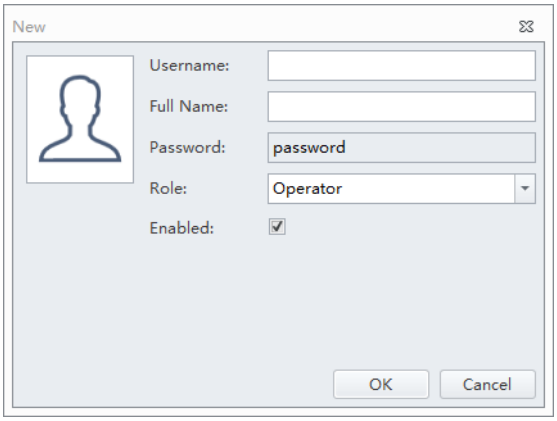
   NOTE An account automatically unlocks after 30 minutes. The duration can be changed by the administrator. Refer to Account Policies.

7. **Reset Password:** Used to reset an existing user password to the default password: *password*.
8. **Close:** Closes the User Manager window.

Creating, Deleting, and Modifying Users in User Manager

Creating a New User in User Manager

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



2 Fill in the new user information.

a. Enter the Username.

b. Enter the Full Name.

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.

3 Select OK. The new user displays in User Manager.

4 Select Close.

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 Delete.

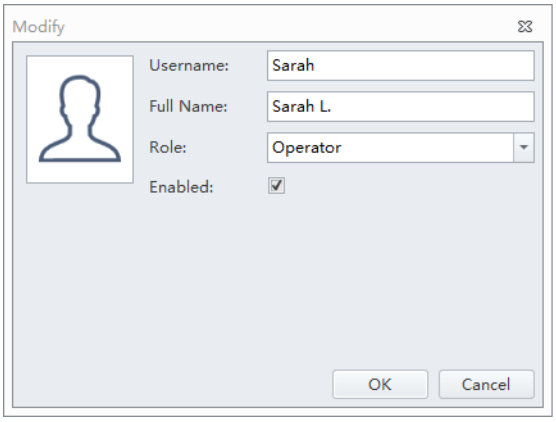
NOTE The user 'Admin' is a system default user and cannot be deleted.

2 Select Close.

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



NOTE The user 'Admin' is a system default user and cannot be modified.

2 Modify the user information as necessary.

NOTE Uncheck the enabled box to disable a user.

3 Select OK.

4 Select Close.

Unlocking a User Account

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

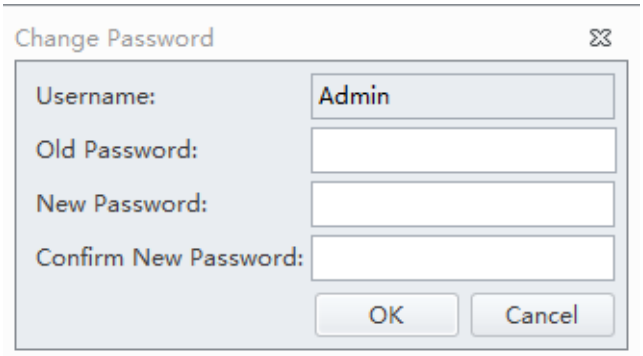
NOTE You cannot unlock an active user.

Resetting a User Passwords

Select a user in the User Manager window then select Reset Password. The user password is automatically reset as *password*.

Changing a User Password

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



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

3. Select OK.

Role Management

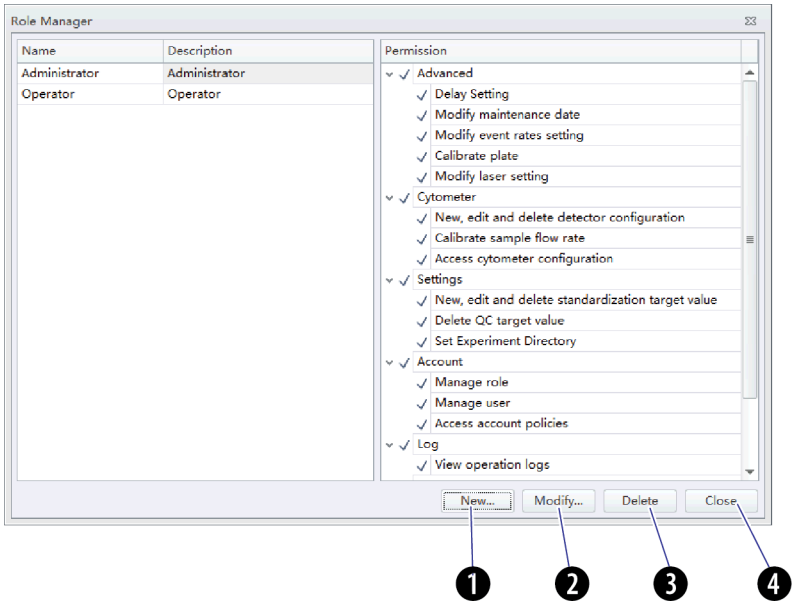
IMPORTANT Only an Administrator can manage users. You must have either the CytExpert User Management or CytExpert Electronic Record Management software option installed to use this feature. Refer to CytExpert Software Installation Options in APPENDIX A, Instrument Installation.

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

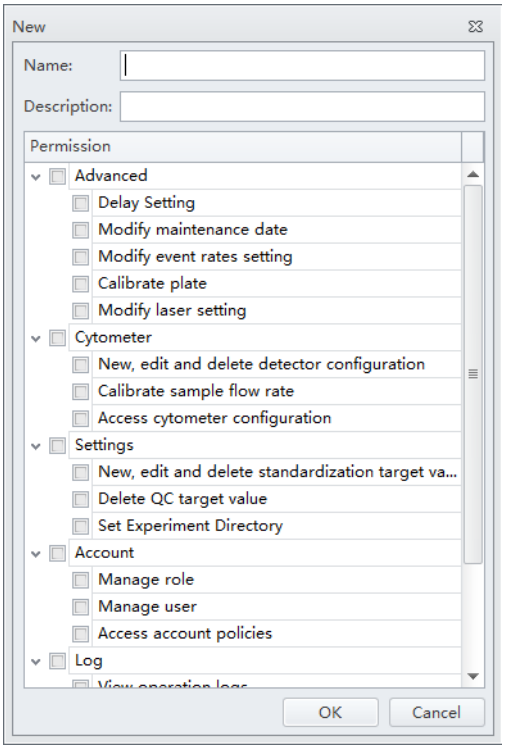


1. **New:** Used to create a new role profile.
2. **Modify:** Used to modify an existing role profile.
3. **Delete:** Used to delete an existing role profile.
4. **Close:** Closes the Role Manager window.

Creating, Deleting, and Modifying User Roles in Role Manager

Creating New User Roles in Role Manager

1 Select New... . The New window appears.



2 Fill in the new role information.

a. Enter the role name.

b. Enter the role description.

c. Select the permissions applicable to the new role.

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

4 Select Close .

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.

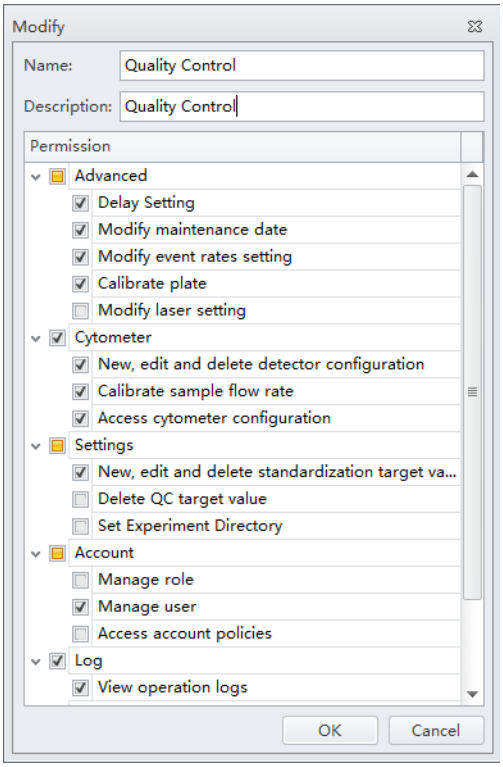
1 Select the Role to be deleted in Role Manager then select Delete.

2 Select Close.

Modifying User Roles in the Role Window

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

1 Select Modify... . The Modify window appears.



2 Modify the role information as necessary.

3 Select OK.

4 Select Close .

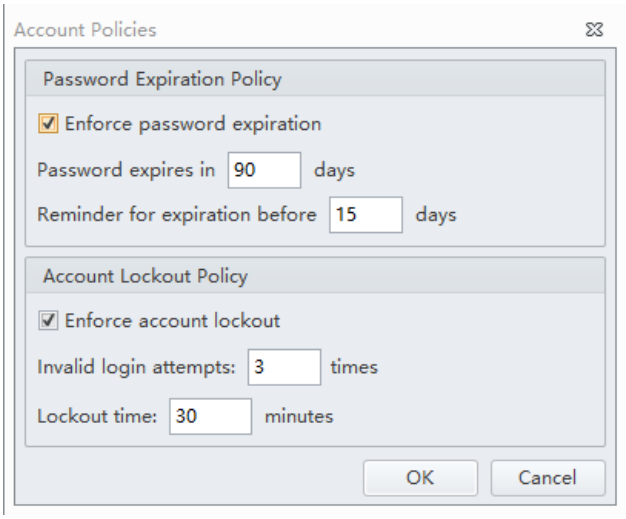
Account Policies

IMPORTANT Only an Administrator can manage users. You must have either the CytExpert User Management or CytExpert Electronic Record Management software option installed to use this feature. Refer to CytExpert Software Installation Options in APPENDIX A, Instrument Installation.

Account policies is used to manage user password expiration and account lockout policies.

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

Figure 2.8 Account Policies



NOTE The allowable range for each entry is as follows:

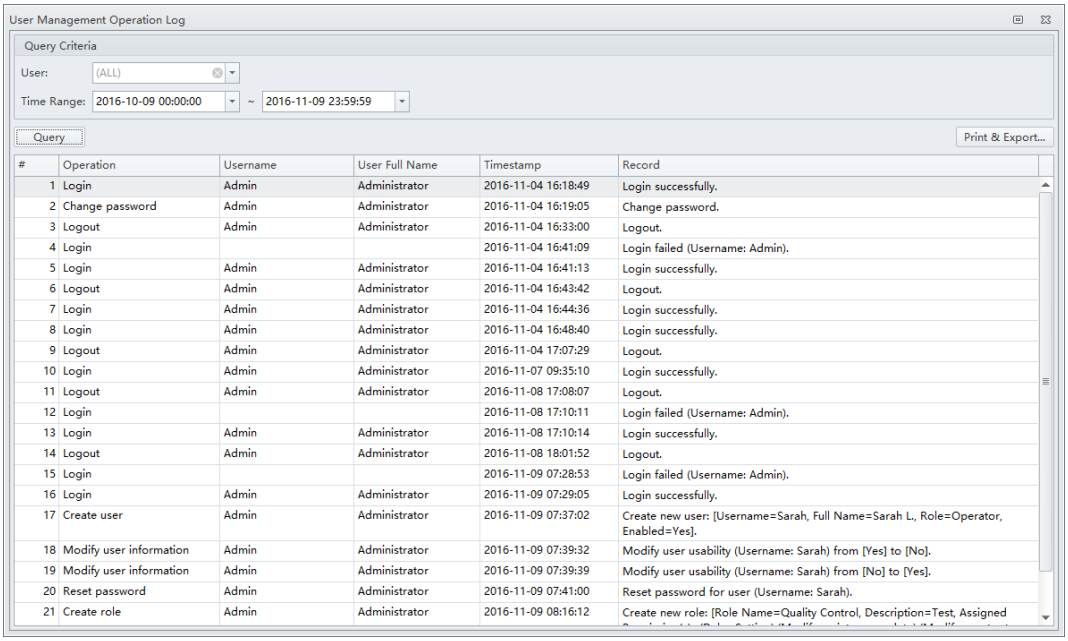
- Password Expiration: 1-999 days
- Reminder for Expiration: 1-90 days
- Invalid Login Attempts: 3-10 times
- Lockout Time: 15-1,440 minutes

User Management Operation Log

IMPORTANT You must have either the CytExpert User Management or CytExpert Electronic Record Management software option installed to use this feature. Refer to CytExpert Software Installation Options in APPENDIX A, Instrument Installation.

Viewing and Exporting User Logs

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



#	Operation	Username	User Full Name	Timestamp	Record
1	Login	Admin	Administrator	2016-11-04 16:18:49	Login successfully.
2	Change password	Admin	Administrator	2016-11-04 16:19:05	Change password.
3	Logout	Admin	Administrator	2016-11-04 16:33:00	Logout.
4	Login			2016-11-04 16:41:09	Login failed (Username: Admin).
5	Login	Admin	Administrator	2016-11-04 16:41:13	Login successfully.
6	Logout	Admin	Administrator	2016-11-04 16:43:42	Logout.
7	Login	Admin	Administrator	2016-11-04 16:44:36	Login successfully.
8	Login	Admin	Administrator	2016-11-04 16:48:40	Login successfully.
9	Logout	Admin	Administrator	2016-11-04 17:07:29	Logout.
10	Login	Admin	Administrator	2016-11-07 09:35:10	Login successfully.
11	Logout	Admin	Administrator	2016-11-08 17:08:07	Logout.
12	Login			2016-11-08 17:10:11	Login failed (Username: Admin).
13	Login	Admin	Administrator	2016-11-08 17:10:14	Login successfully.
14	Logout	Admin	Administrator	2016-11-08 18:01:52	Logout.
15	Login			2016-11-09 07:28:53	Login failed (Username: Admin).
16	Login	Admin	Administrator	2016-11-09 07:29:05	Login successfully.
17	Create user	Admin	Administrator	2016-11-09 07:37:02	Create new user: [Username=Sarah, Full Name=Sarah L., Role=Operator, Enabled=Yes].
18	Modify user information	Admin	Administrator	2016-11-09 07:39:32	Modify user usability (Username: Sarah) from [Yes] to [No].
19	Modify user information	Admin	Administrator	2016-11-09 07:39:39	Modify user usability (Username: Sarah) from [No] to [Yes].
20	Reset password	Admin	Administrator	2016-11-09 07:41:00	Reset password for user (Username: Sarah).
21	Create role	Admin	Administrator	2016-11-09 08:16:12	Create new role: [Role Name=Quality Control, Description=Test, Assigned

2 Enter the filter conditions: User and Time Range.

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

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

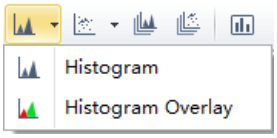
Graphic and Gating Styles

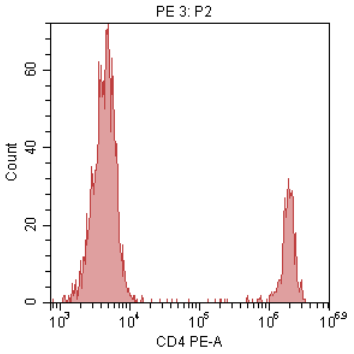
Plots

The CytExpert software offers a variety of plot formats including:

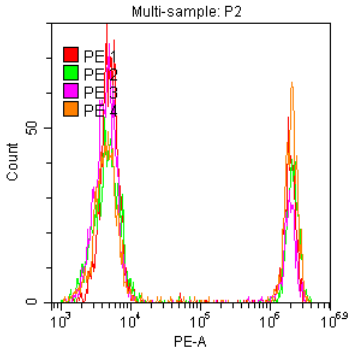
- Single-parameter plots and histogram overlays
- Dual-parameter plots: dot plots, density plots, pseudo color plots, contour plots, and dot plot overlays

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.

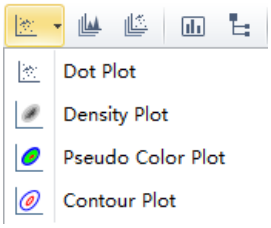


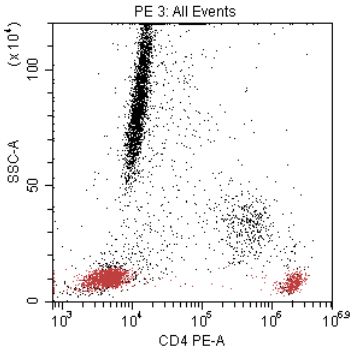


Histogram

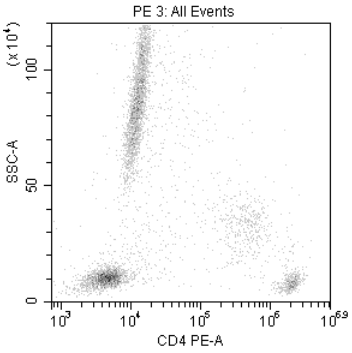


Multi-sample histogram overlays

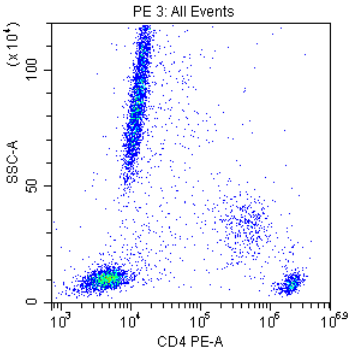




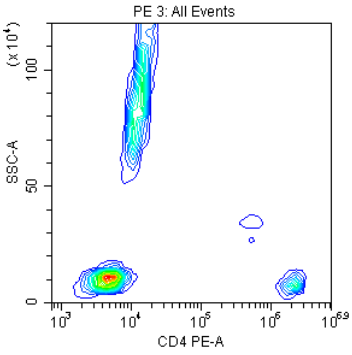
Dot plot



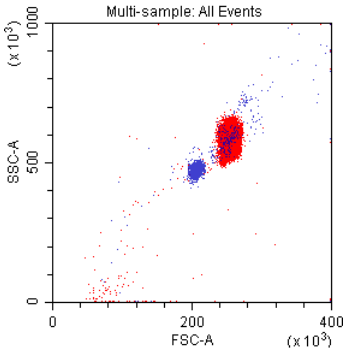
Density plot



Pseudo color plot



Contour plot



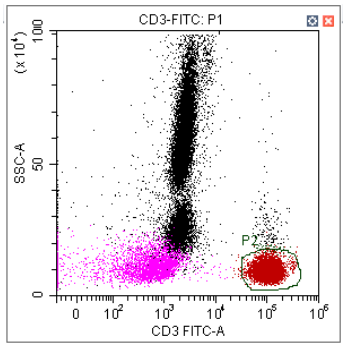
Dot plot overlay

Gates

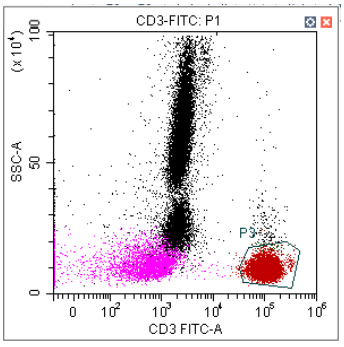
Various gating choices are available.

The software includes the following gate types:

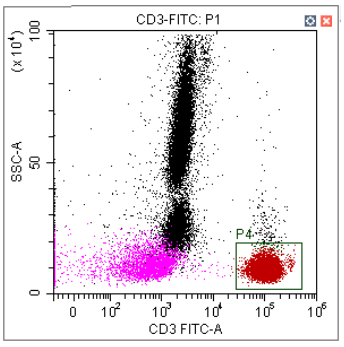
- For dual-parameter plots: lasso, polygon, rectangle, four-quadrant, hinged gates, and auto polygon
- For single-parameter plots: line-segment, vertical gates, and auto line segment



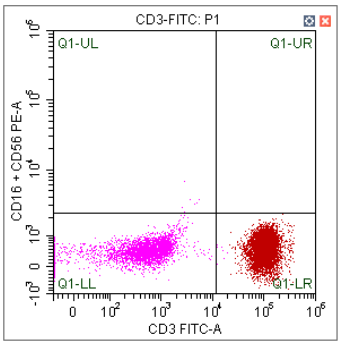
Lasso gate



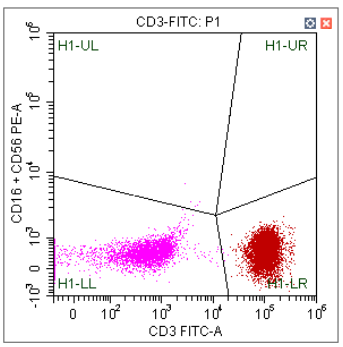
Polygon gate[a]



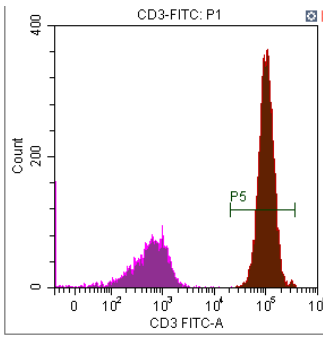
Rectangle gate



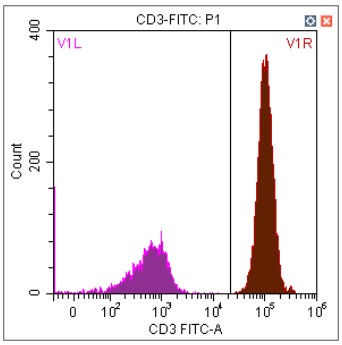
Four-Quadrant gate



Hinged gate



Line Segment gate[a]



Vertical gate

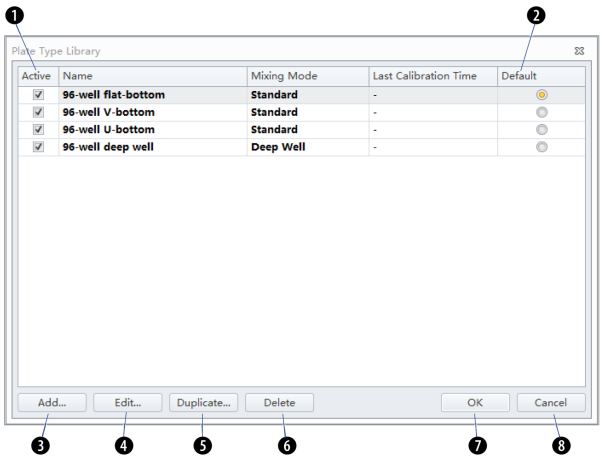
a. This gate can be created using the autogate functionality. Refer to Creating and Adjusting Auto Gates in CHAPTER 5, Data Acquisition and Sample Analysis

Plate Type Library

The Plate Type Library is used to manage and calibrate plates. Plates can be added, deleted, duplicated, and edited from the Plate Type Library. To calibrate a plate, refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.

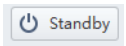
Select **Advanced > Plate Type Library** to access the Plate Type Library. Refer to Figure 2.9.

Figure 2.9 Plate Type Library



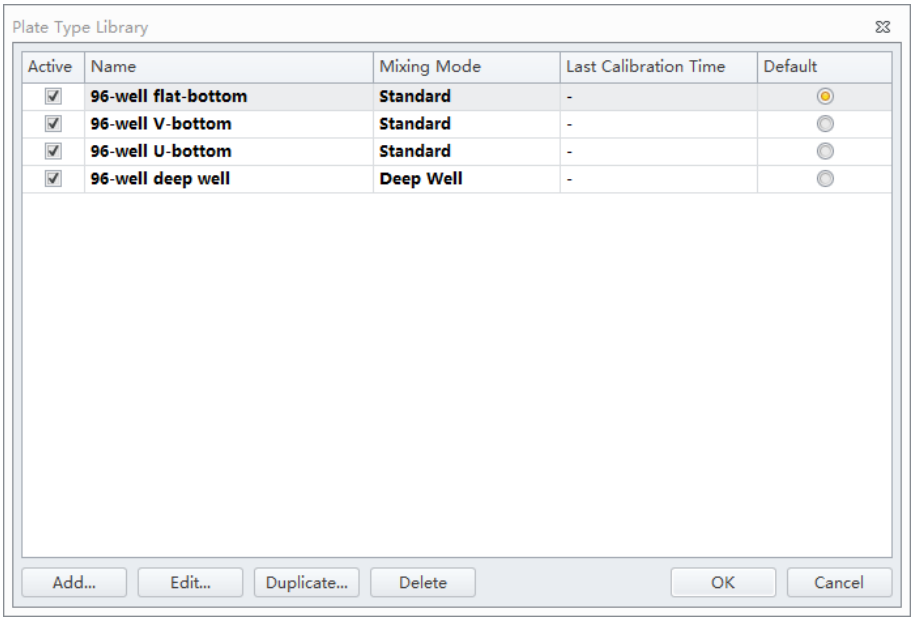
1. **Active.** Indicates that the plate type is available for use.
2. **Default.** Applies the default settings.
3. **Add.** Creates a new plate.
4. **Edit.** Edits a plate.
5. **Duplicate.** Duplicates a plate.
6. **Delete.** Deletes an existing plate.
7. **OK.** Saves the plate type.
8. **Cancel.** Cancels the settings.

Adding a Plate Type

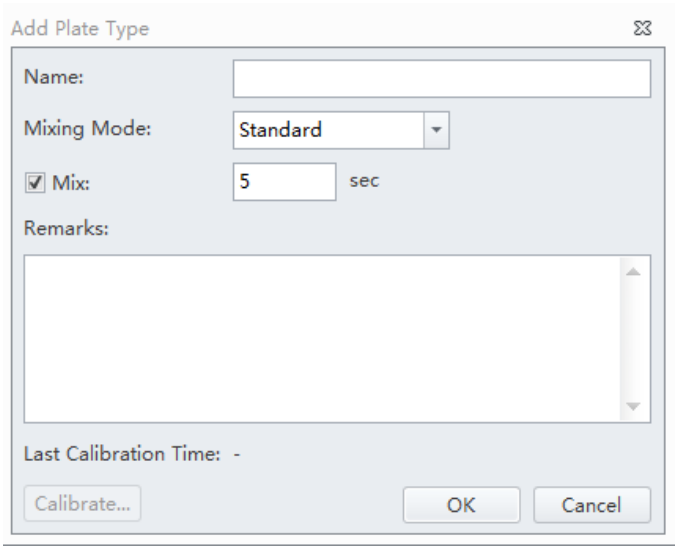
1 Select 

.

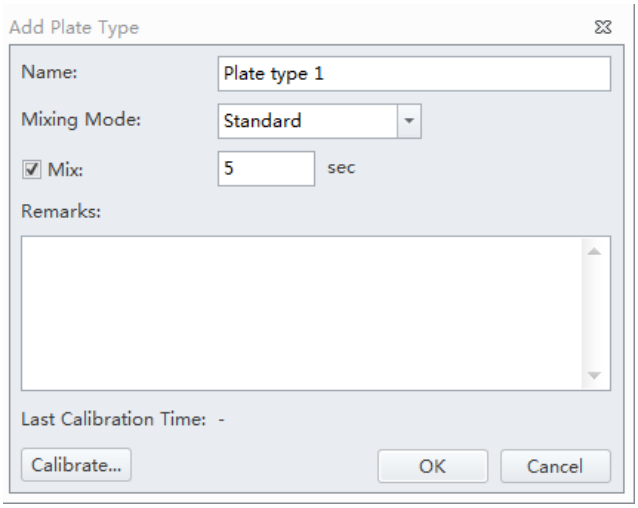
2 Select **Advanced > Plate Type Library.** The Plate Type Library window appears.



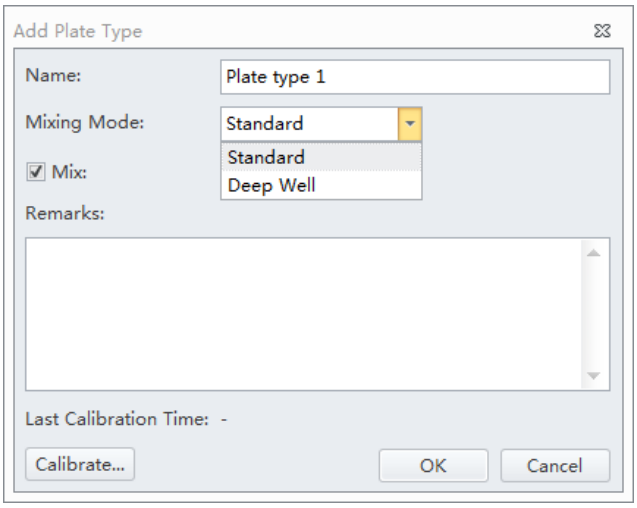
3 Select **Add.** The Add Plate Type window appears.



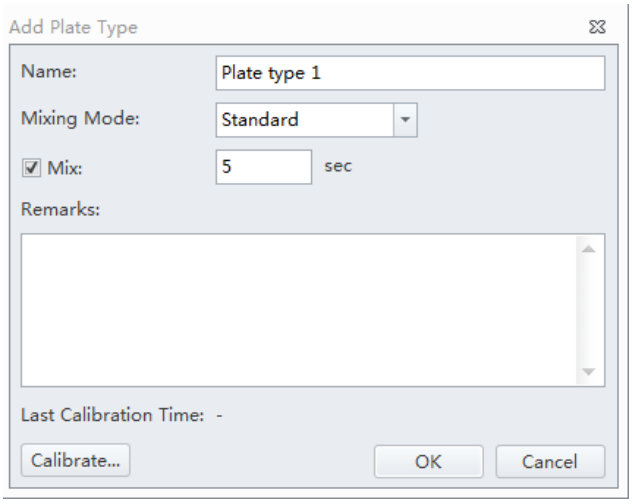
4 Enter the plate name in the Name section of the screen.



5 Select the mixing mode.



6 Enter the Mix time.



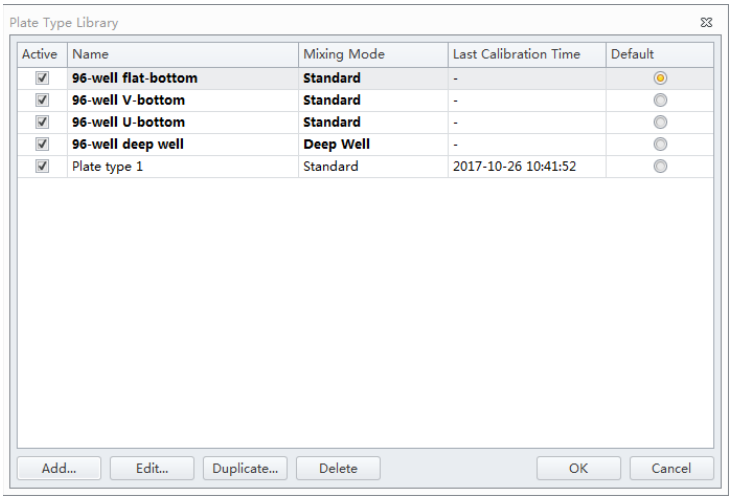
NOTE The default setting is 5 seconds in Standard Mixing Mode. The default setting is 10 seconds (for 0.5 mL sample) in Deep Well Mixing Mode. You might need custom the Mix time according to the sample volume.

7 Place the new plate on the plate holder.

8 Select **Calibrate** to calibrate the plate position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.

NOTE It is required to calibrate the plate position when adding a new plate.

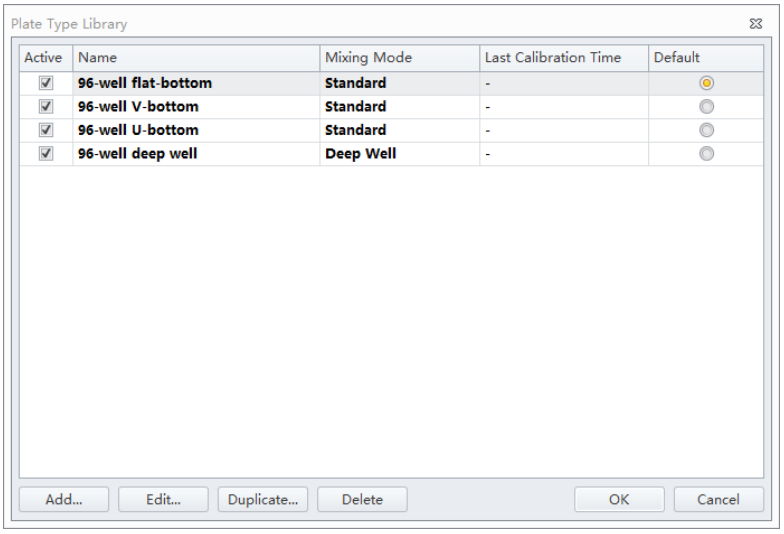
9 Select **OK**. The plate is added into the Plate Type Library.



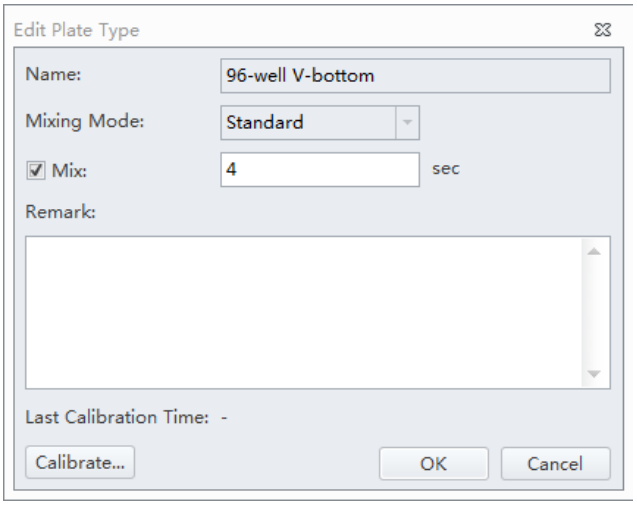
10 Select **OK**.

Editing a Plate Type

1 Select **Advanced > Plate Type Library**. The Plate Type Library window appears.



2 Select the plate type to be edited, and select **Edit**. The Edit Plate Type window appears.



3 Enter the Mix setting.

4 **Optional:** Enter any remarks.

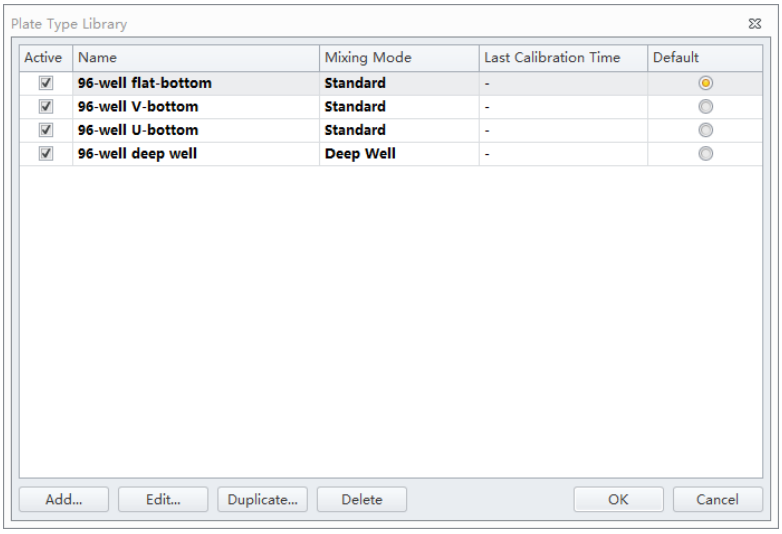
5 **Optional:** Select **Calibrate** to calibrate the plate position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures

The Calibration can be performed at any time.

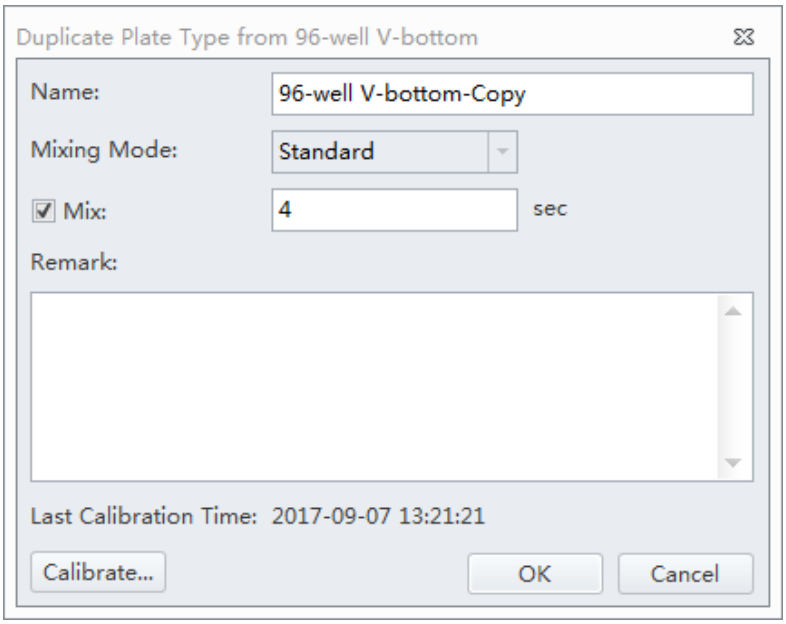
6 Select **OK**.

Duplicating a Plate Type

1 Select **Advanced > Plate Type Library**. The Plate Type Library window appears.



2 Select the plate type to duplicate, and select **Duplicate**. The Duplicate Plate Type window appears.



3 Enter the Mix setting.

4 **Optional:** Enter any remarks.

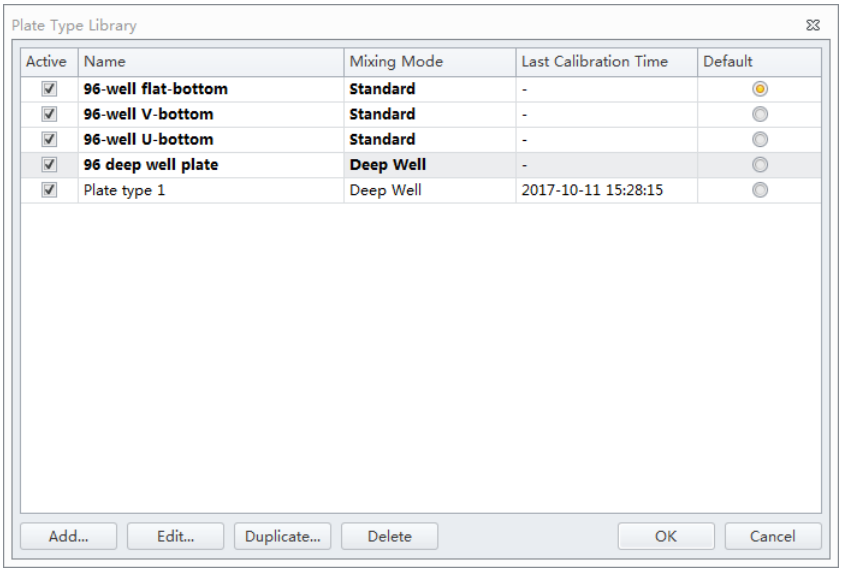
5 **Optional:** Select **Calibrate** to calibrate the plate position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures

The Calibration can be performed at any time. The duplication retains the calibration information if the original plate was already calibrated.

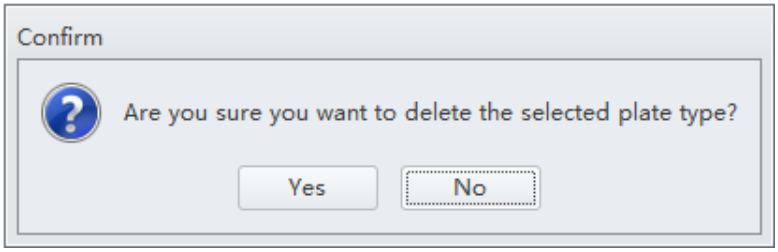
6 Select **OK**.

Deleting a Plate Type

1 Select **Advanced > Plate Type Library.** The Plate Type Library window appears.



2 Select the plate type to be deleted, and select **Delete.** The following message appears:



NOTE The default plate types, shown in bold, cannot be deleted from the Plate Type Library.

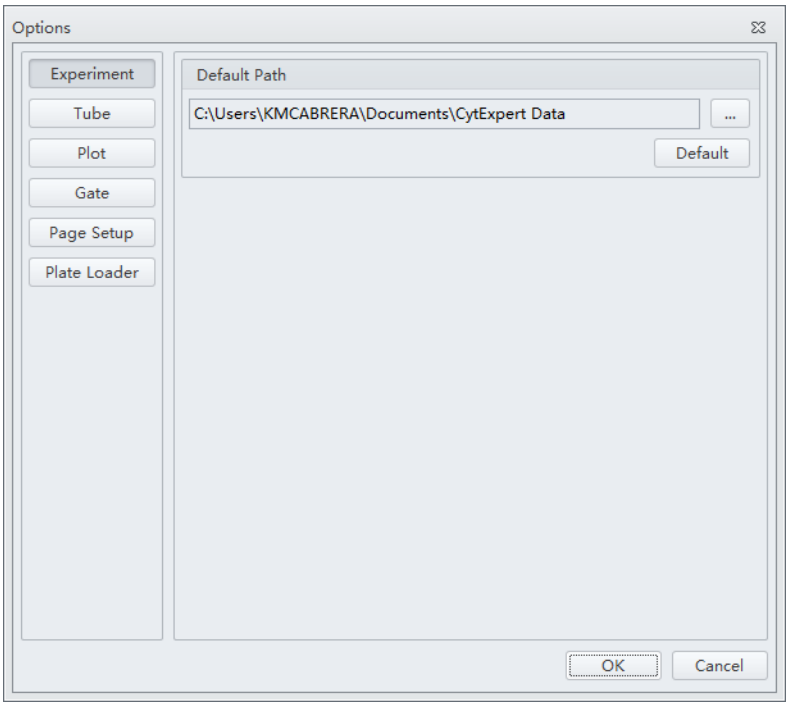
3 Select **Yes.** The selected plate type is removed from the Plate Type Library.

Software Settings

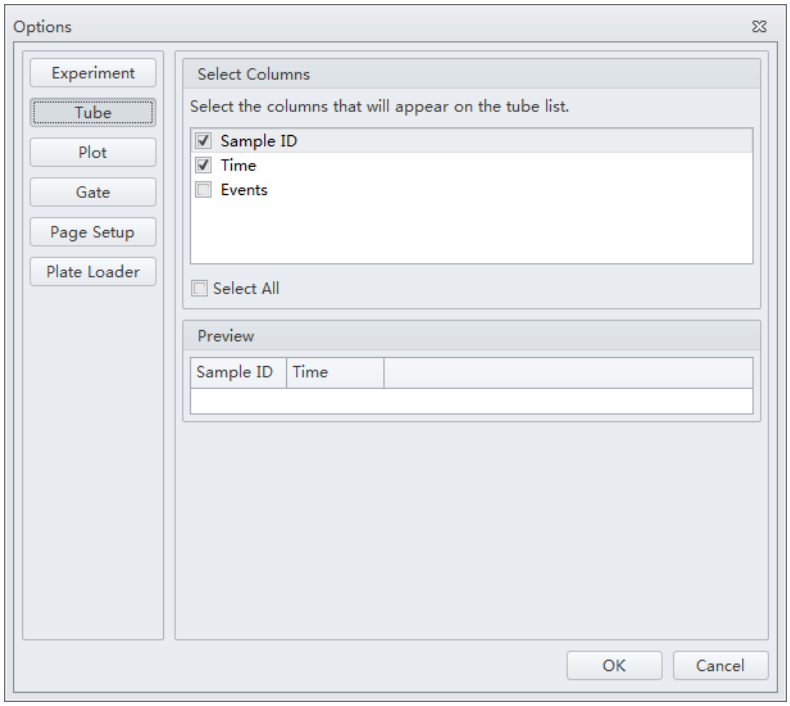
Select **Options** in the Settings menu to configure the software settings.

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

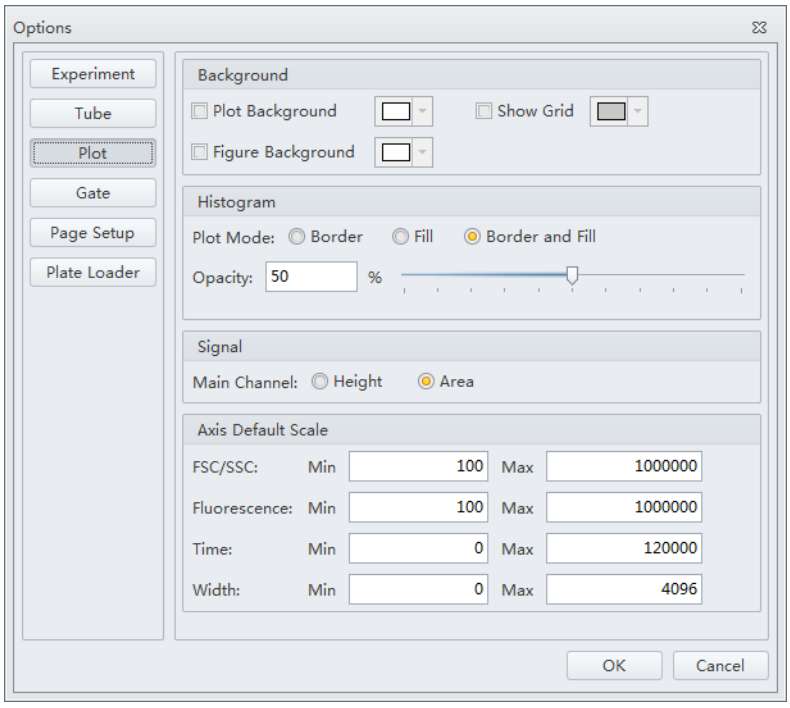
NOTE The Experiment setting is only available if either the CytExpert Default or the CytExpert User Management software option is installed.



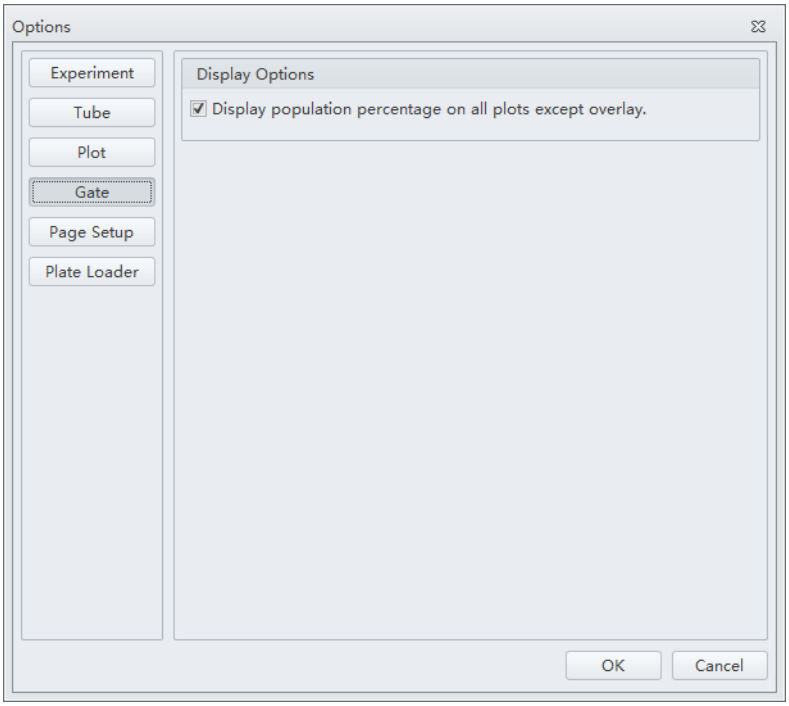
In the tube settings, you can select the columns that display in the tube section of the screen.



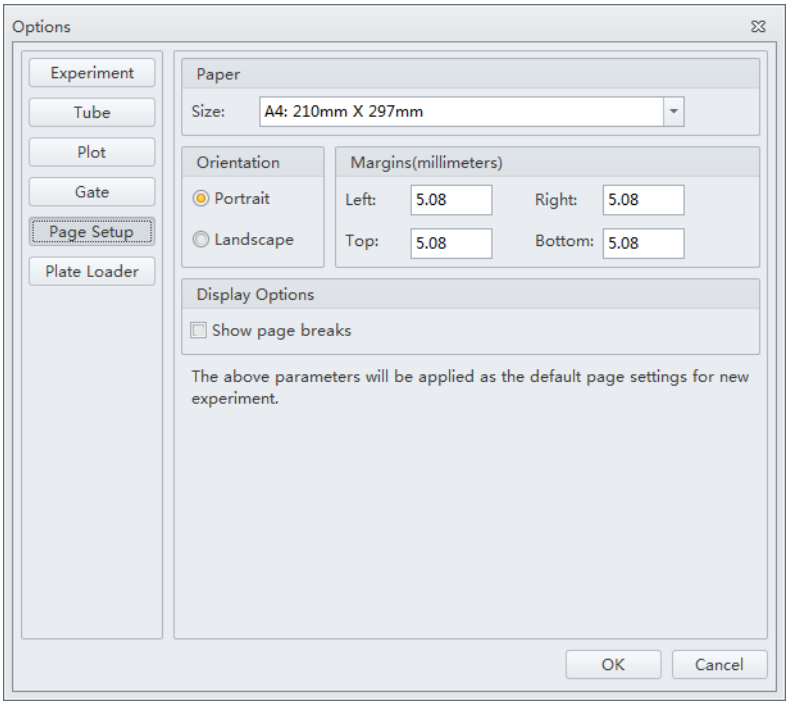
In the plot settings, you can define the background of the graphics display area, configure the histograms, and set the default signal parameters to either the channel's area or the channel's height. The default is area. You can also set the default axis display range.



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

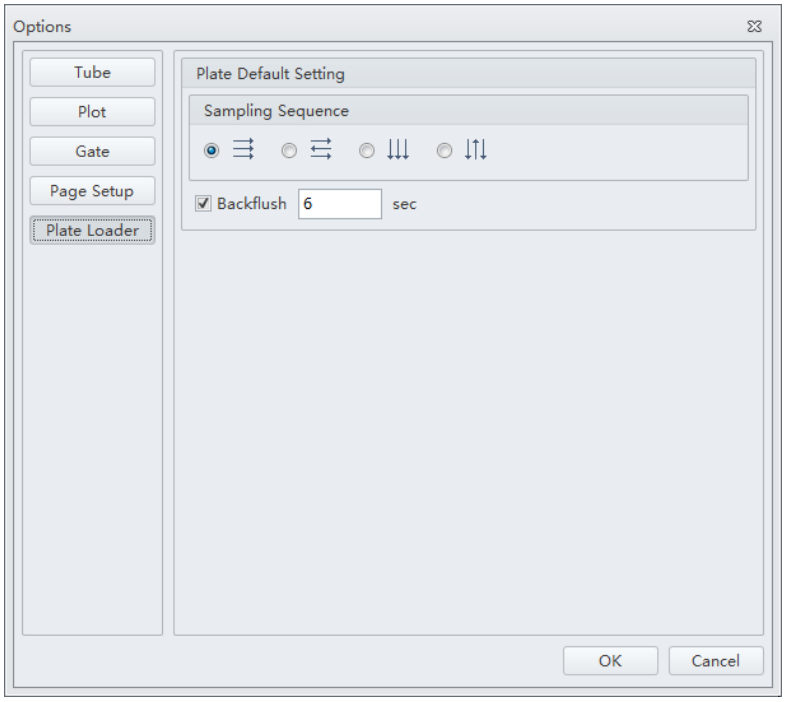


In the Page Setup settings, you can change the page size, orientation, margin size, and display options. Select **Show page breaks** to display page boundaries within the Acquisition or Analysis views for simplifying plot arrangement for printing.



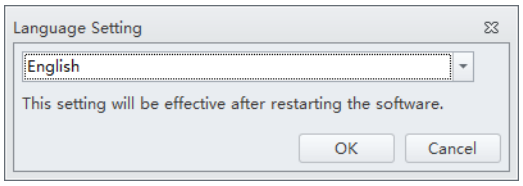
In the plate loader settings, you can select the plate type, sampling sequence, mix, and backflush settings for the plate loader.

NOTE This setting is only available in the Plate Loader sample injection mode.



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.



Setting Up CytExpert Application Programming Interface (API) Test Client

The CytExpert API is available for external software to control CytoFLEX series instruments. It allows external software to perform operations such as running methods and allows for basic control of the plate loader. It is also possible to report population statistics as each sample finishes. Contact us to request a copy of the CytExpert API Instructions for Use manual.

CHAPTER 3

Daily Startup

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:

Pre-startup inspection	→	Turn on power	→	Open software	→	Initialize instrument

This chapter contains information on:

Pre-Startup Inspection

Before using the CytoFLEX or CytoFLEX LX flow cytometer, perform the following system checks.

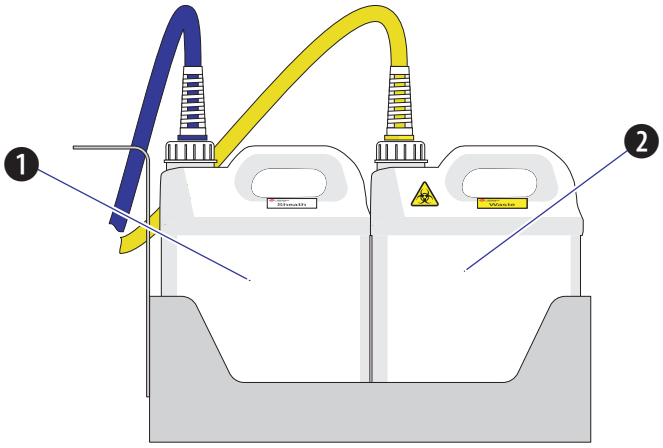
Check Waste and Reagent Levels [4 L Fluid Containers]

⚠ CAUTION

Risk of instrument damage. Do not use a saline-based sheath fluid on the CytoFLEX instrument. Saline-based sheath fluid could damage instrument components. Beckman Coulter recommends using CytoFLEX Sheath Fluid or a similar nonionic sheath fluid to ensure system performance.

1 Examine the sheath fluid and waste containers. Verify that there is sufficient sheath fluid in the sheath fluid container and that the waste container is empty.

NOTE When the sheath fluid container is near empty or the waste container is near full, a warning message is transmitted to the Workstation and audible signals sound as a warning.



1. Sheath fluid container
2. Waste container

⚠ CAUTION

Risk of instrument damage. Remove the sheath fluid container from the Fluid Container holder before filling the sheath fluid container to avoid damage to instrument electronics.

2 If necessary, fill the sheath fluid container with CytoFLEX Sheath Fluid or a similar nonionic sheath fluid while not exceeding the maximum volume indicated (4 L). Refer to Filling the 4 L Sheath Fluid Container [CytoFLEX] in CHAPTER 10, Cleaning Procedures.

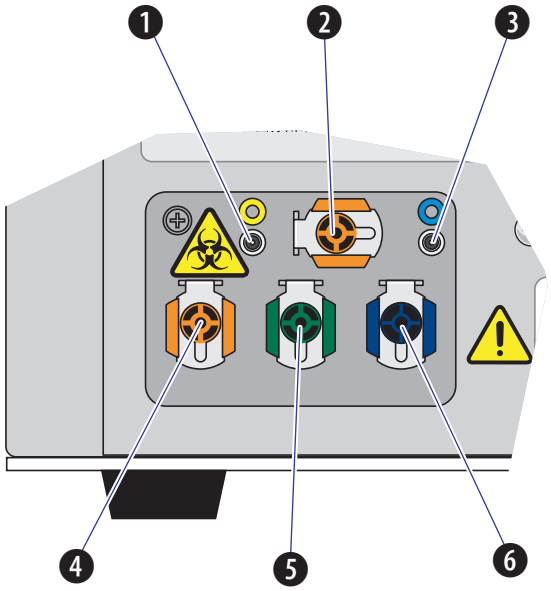
⚠ WARNING

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.

3 If necessary, empty all waste liquid from the waste container. If biohazardous samples are used for data collection, add 400 mL of 5 to 6% bleach to the waste container. Refer to Emptying the 4 L Waste Container [CytoFLEX] in CHAPTER 10, Cleaning Procedures.

4 Verify that the Fluid Containers and the Cytometer are on the same level.

5 Verify that all sheath fluid tubing, waste tubing, and sensor cables are properly connected, as shown in the figure:



1. **Waste level sensor connector.** Connects to the waste liquid sensor cable.
2. **Flow cell waste out.** Connects to the flow cell waste tubing.
3. **Sheath fluid level sensor connector.** Connects to the sheath fluid sensor cable.
4. **Waste out.** Connects to the waste liquid tubing.
5. **Sheath return.** Connects to the sheath fluid tubing.
6. **Sheath fluid in.** Connects to the sheath fluid tubing.

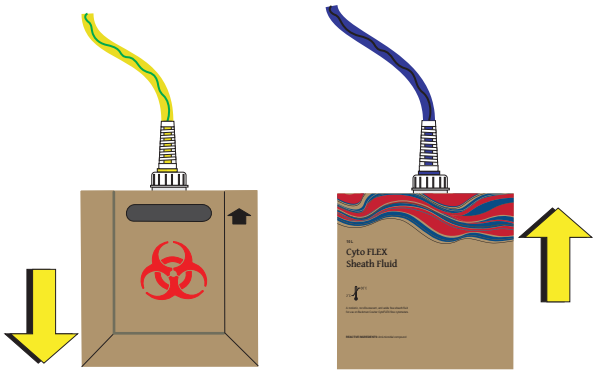
Check Waste and Reagent Levels [10 L Fluid Cubitainers]

⚠ CAUTION

Risk of instrument damage. Do not use a saline-based sheath fluid on the CytoFLEX Series instrument. Saline-based sheath fluid could damage instrument components. Beckman Coulter recommends using CytoFLEX Sheath Fluid or a similar nonionic sheath fluid to ensure system performance.

1 Examine the sheath fluid and waste cubitainers. Verify that there is sufficient sheath fluid in the sheath fluid container and that the waste container is empty.

NOTE When the sheath fluid container is near empty or the waste container is near full, a warning message is transmitted to the Workstation and audible signals sound as a warning.



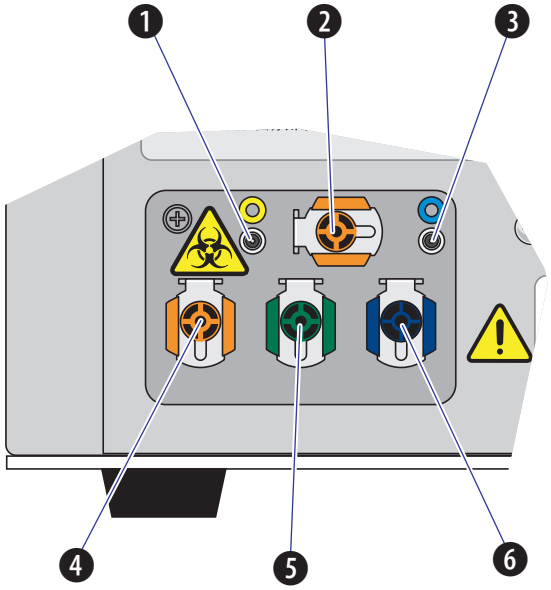
2 Confirm that the instrument is in the standby state.

3 If necessary, replace the sheath fluid cubitainer with CytoFLEX Sheath Fluid or a similar nonionic sheath fluid. Refer to Replacing the 10 L Sheath Fluid Cubitainer in CHAPTER 10, Cleaning Procedures.

4 If necessary, empty all waste liquid from the waste container. Refer to Emptying the 10 L Waste Cubitainer in CHAPTER 10, Cleaning Procedures.

5 Verify that the Fluid Cubitainers and the Cytometer are on the same level.

6 Verify that all sheath fluid tubing, waste tubing, and sensor cables are properly connected, as shown in the figure:



1. **Waste level sensor connector.** Connects to the waste liquid sensor cable.
2. **Flow cell waste out.** Connects to the flow cell waste tubing.
3. **Sheath fluid level sensor connector.** Connects to the sheath fluid sensor cable.
4. **Waste out.** Connects to the waste liquid tubing.
5. **Sheath return.** Connects to the sheath fluid tubing.
6. **Sheath fluid in.** Connects to the sheath fluid tubing.

Power Source Inspection

Check the power cable located below the power switch on the back of the Cytometer, and verify it is securely connected to both the Cytometer and the power source.

Workstation Connections Inspection

Check that the monitor, mouse, keyboard, and the Cytometer are properly connected to the computer. Refer to Figure 1.23.

Turning On the Instrument

⚠ CAUTION

1. **If the Cytometer or Workstation fails to start properly, check first to see whether the power cable and connection cables are properly connected.**
2. **Never shut off the power or disconnect a data cable while the Cytometer is performing a task. Doing so can result in data loss or damage to the system.**

1 Turn on the main power switch located on the back of the Cytometer.

2 Wait for the Cytometer to finish powering on, then turn on the Workstation.

Logging Into the Software

1 Log in to the Windows operating system and double-click the CytExpert desktop icon 

to open the software.

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

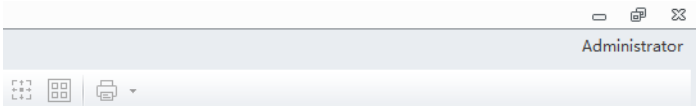
If you are running either the CytExpert User Management or the CytExpert Electronic Record Management software installation, the login window appears.

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. Double-click CytExpert.exe to run the software.

2 Enter your username and password.

3 Select  .

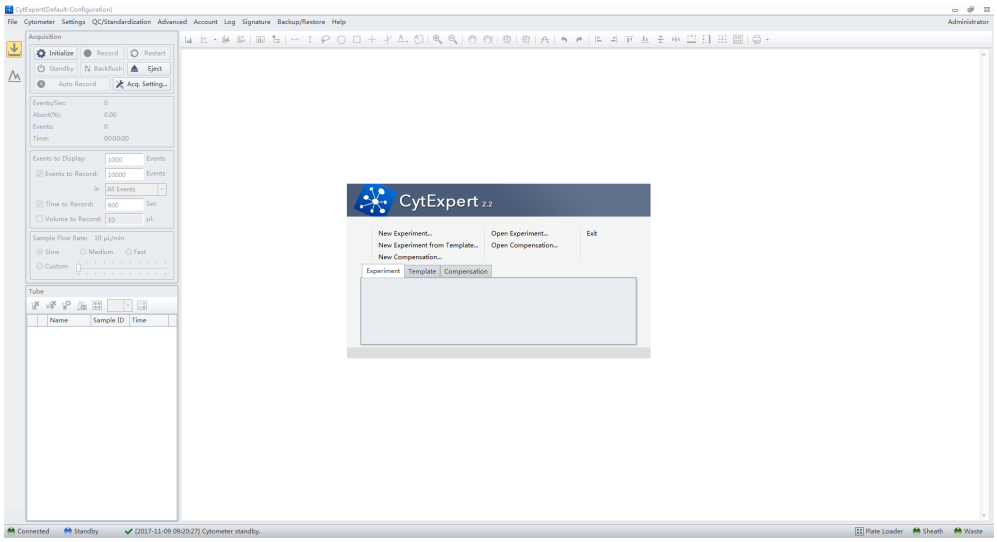
NOTE The display name of the user that is currently logged in displays in the top, right corner of the software screen.



4 Confirm that the software and the Cytometer are properly connected.

a. Open the software. The Startup screen appears.

[CytExpert Electronic Record Management Software Option Shown]

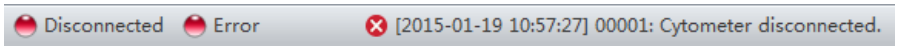


b. Verify that the connection indicator light in the lower left corner of the software screen is green, and *Connected* is displayed. The left side shows the connection status, the middle shows the instrument status, and the right side shows the status details.

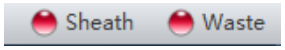
c. Verify that the *Sheath* and *Waste* flow indicators in the lower right corner of the software screen are green indicating that the fluidics system is normal.

NOTE

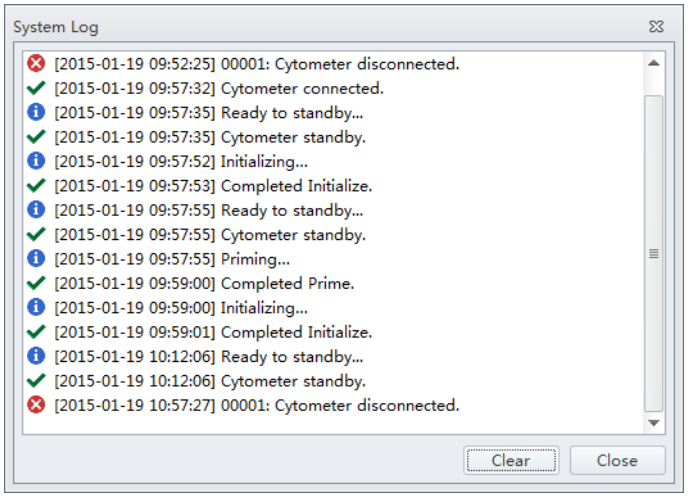
- A red connection indicator light indicates that there is a faulty connection. Ensure that the instrument is properly turned on and connected. If necessary, restart both the Cytometer and the Workstation.



- After the instrument initializes, a warning beep sounds if there is a problem with the fluidics system. If a flow indicator is red and blinking, it means that the fluidics system requires attention.

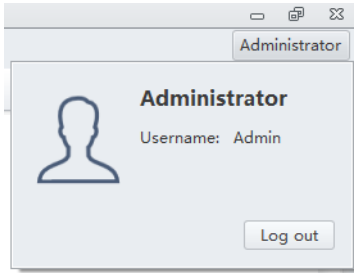


- When the waste fluid sensor is disconnected, the waste flow indicator shows that the waste container is full or nearly full.
- Select the status information in the lower left to open the system log. Send a copy of the system log to your Beckman Coulter Representative for support if a service call is requested.



Logging Out of the Software

If you have the CytExpert User Management or the CytExpert Electronic Record Management software option installed, select the username displayed in the top-right corner of the software screen and select Log out.

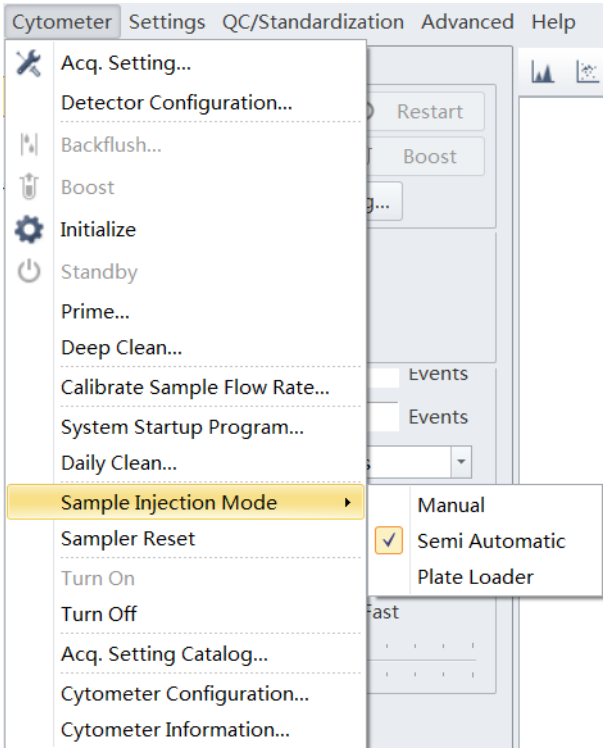


If you have the CytExpert Default software option installed, log out is not required. Select **File** > **Exit** to close the CytExpert software.

Selecting the Proper Sample Injection Mode

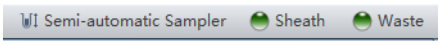
Select **Sample injection Mode** in the Cytometer Menu to change between the Semi-Automatic Injection mode and the Manual Injection mode. The Semi-Automatic Injection mode is recommended under most circumstances. The Manual Injection mode can be used for two purposes:

running 1.5-mL and 2-mL microcentrifuge sample tubes and a backup mode that allows you to continue to collect data if the Semi-Automatic Injection mode is not working correctly.



Using Semi-Automatic Injection Mode

1 Select **Sample Injection Mode >Semi-Automatic** in the Cytometer menu to change the Sample Injection mode selection. The sampler status icon located in the bottom right side of the screen changes to display *Semi-automatic Sampler*.



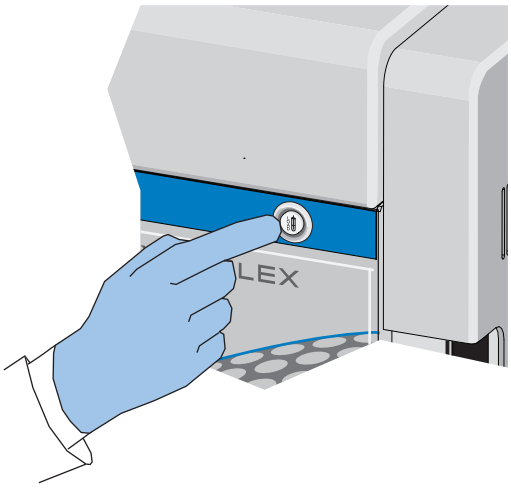
2 Select **Initialize**. The sample tube holder swings out from the standby position to the sample loading position (see Figure 1.13) so that you can load the sample tube.

NOTE You can also swing out the sample tube holder manually, load the sample tube, then select **Initialize**.

3 Select **Run**. The sample tube holder automatically swings back to the standby position and raises the sample tube to the sample acquisition position (see Figure 1.13), where the instrument mixes the sample and transfers the sample to the flow cell.

At the flow cell, the sample runs at the designated flow rate and the Cytometer begins to acquire data.

NOTE You can also push the load button on the front of the instrument to automatically start the run and record the data.



4 When you are satisfied with the data, select **Record** to record the data.

5 Wait for the data acquisition to finish or select **Stop**. The sample tube holder automatically lowers the sample tube and moves it to the sample loading position (see Figure 1.13) and the Cytometer backflushes the sample probe.

⚠ WARNING

Risk of biohazardous contamination. When using 1.5-mL and/or 2-mL sample tubes, always cut the cap off and do not exceed 300-µL sample volume. Running samples with a cap attached to the sample tube or with volumes exceeding 300 µL can result in sample splashing.

Using the Manual Injection Mode

1 Select **Sample Injection Mode > Manual** in the Cytometer menu to change the Sample Injection mode selection. The sampler status icon located in the bottom right side of the screen changes to display *Manual Sampler*.

2 Manually swing the sample tube holder out from the standby position to the sample loading position (see Figure 1.13).

3 Select **Initialize**.

4 Load the sample tube.

NOTE The sample tube holder accommodates 1.5-mL, 2.0-mL, and 12 x 75 mm sample tubes.

5 Manually swing the sample tube holder gently back to the standby position (see Figure 1.13).

6 Manually raise the sample tube holder gently to the sample acquisition position (see Figure 1.13) and hold the tube in that position.

7 Select **Boost** to transfer the sample to the flow cell.

8 Select **Run**.

The sample runs at the designated flow rate and the Cytometer begins to acquire data.

9 When you are satisfied with the data, select **Record** to record the data.

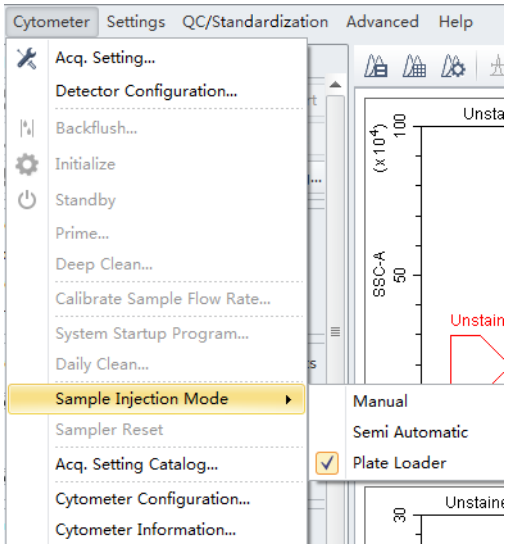
10 Wait for the data acquisition to finish or select **Stop**. Then, manually lower the sample tube holder and move it to the sample loading position (see Figure 1.13).

11 Select **Backflush** to clean the probe.

Selecting the Plate Loader Sample Injection Mode [With Plate Loader]

Select **Sample injection Mode** in the Cytometer Menu to change between the Semi-Automatic Injection mode, the Manual Injection mode, and the Plate Loader Injection mode. The Plate Loader Injection mode can be used for running small volumes using the following plates: 96-well flat-bottom, 96-well V-bottom, and 96-well U-bottom.

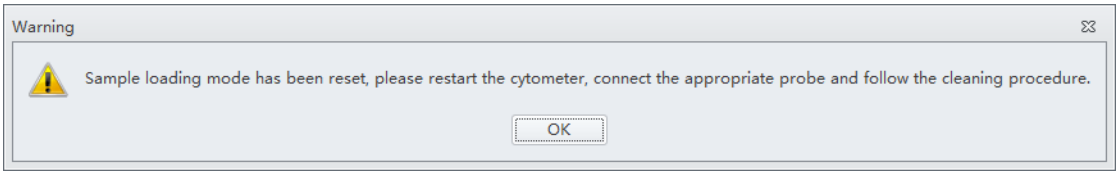
[CytoFLEX Shown]



Using Plate Loader Injection Mode

1 Select **Sample Injection Mode** > **Plate Loader** in the Cytometer menu to change the Sample Injection mode selection.

2 The restart warning prompt appears on screen. Select **OK**.



NOTE The restart warning only appears when switching to and from the Plate Loader sample injection mode.

3 Turn the Cytometer's main power switch off.

NOTE If you have a CytoFLEX LX instrument, you can turn the Cytometer's power off by selecting **Cytometer** > **Turn Off**.

IMPORTANT If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, refer to APPENDIX A, Instrument Installation for detailed instructions on switching from the single tube sample probe to the Plate Loader.

4 Remove the single tube sample probe and replace it with the plate loader PEEK tubing. Refer to Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [CytoFLEX With Plate Loader] in CHAPTER 10, Cleaning Procedures.

5 Turn the Cytometer's main power switch on.

The sampler status icon located in the bottom right side of the screen changes to display *Plate Loader*.

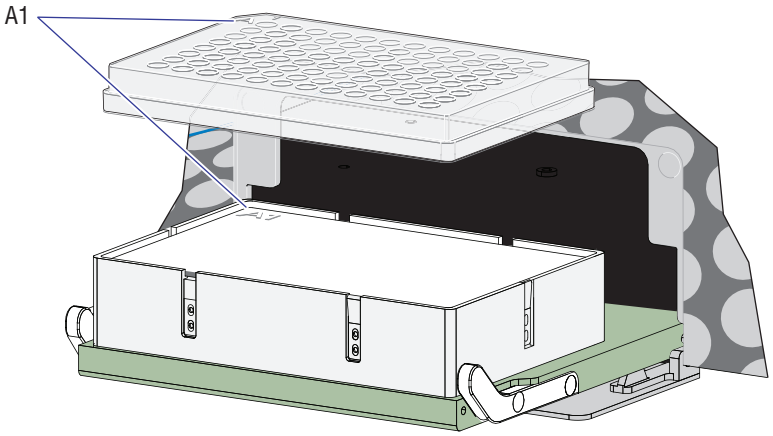
NOTE If you have a CytoFLEX LX instrument, you can turn the Cytometer's power on by selecting **Cytometer** > **Turn On**.

6 Select **Initialize**.

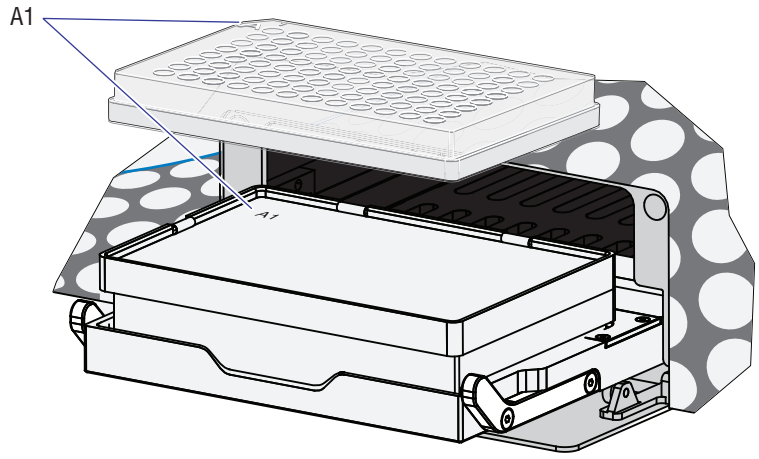
7 Select **Eject**.

8 Place the plate flat on the plate holder and ensure that it is secure.

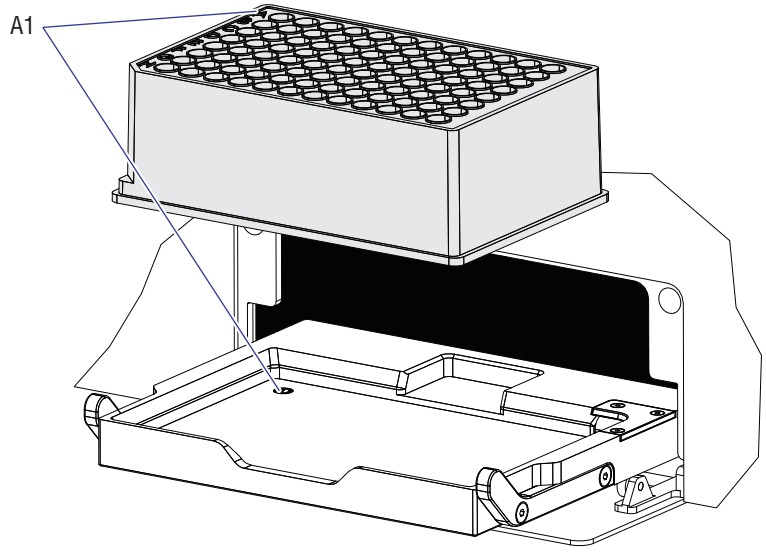
[Standard 96-Well Plate in the Plate Holder (Without Groove)]



[Standard 96-Well Plate in the Plate Holder (With Groove)]



[96-Well Deep Well Plate Only]



NOTE Ensure that plate well A1 aligns with position A1 on the plate holder.

9 Select **Load** to load the plate.

10 Select **Run**. The plate loader automatically loads the plate holder stage and begins to acquire data.

11 When you are satisfied with the data, select **Record** to record the data.

12 Wait for the data acquisition to finish or select **Stop**. The Cytometer backflushes the sample probe.

13 Select **Eject** to eject the sample loader.

Running the System Startup Program [with the Single Tube Loader]

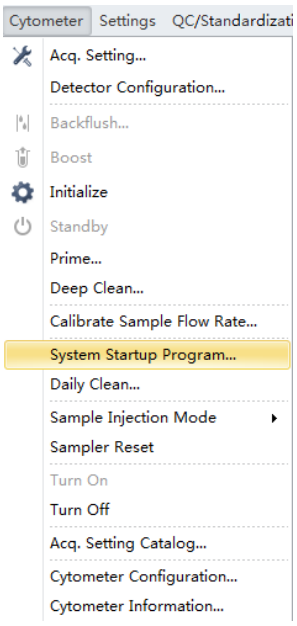
IMPORTANT Instructions on the software window vary depending on whether you are in semi-automatic injection mode or manual injection mode.

The system startup program takes approximately 10 minutes.

1 Select **Initialize**.

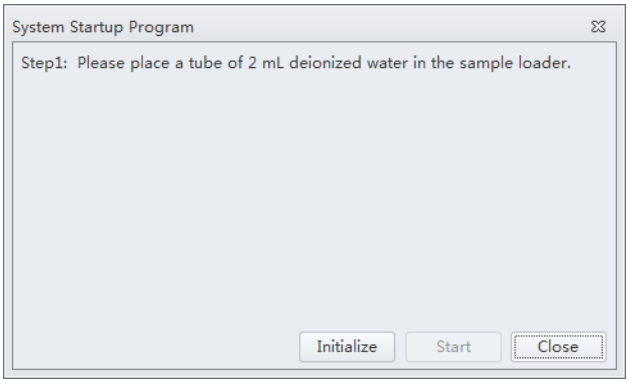
2 Select **System Startup Program** in the Cytometer menu.

[CytoFLEX LX Shown]

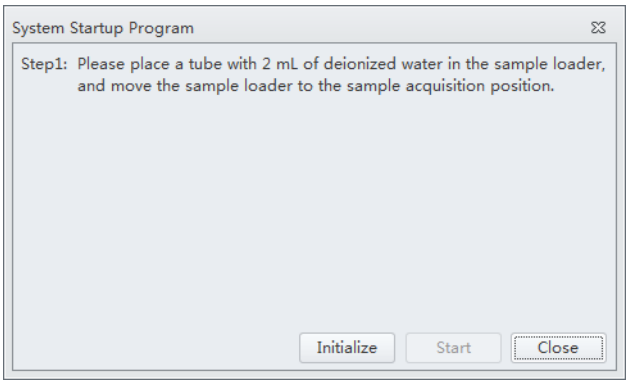


3 The System Startup Program window appears. Select **Initialize.**

System Startup Program Window in Semi-Automatic Injection Mode

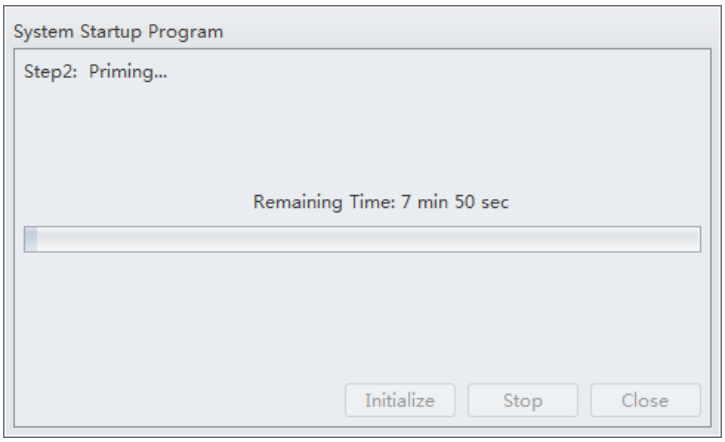


System Startup Program Window in Manual Injection Mode

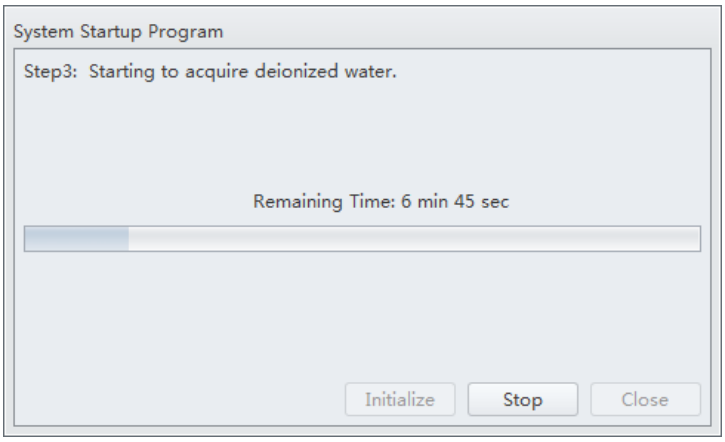


4 Wait for the system to initialize. Follow the on screen software prompts, then select **Start.**

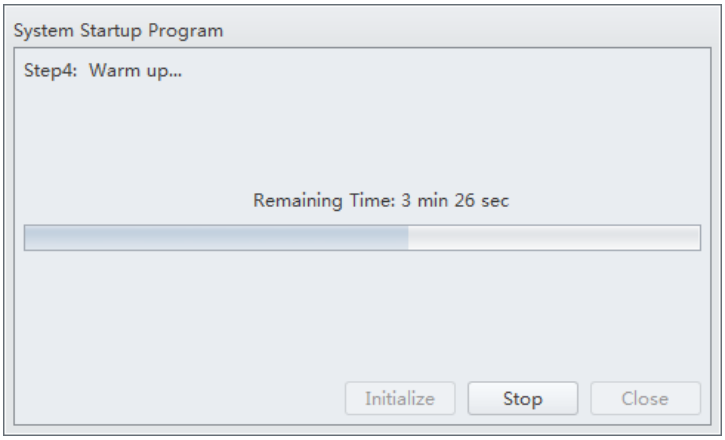
The instrument begins priming. This process takes about 4 minutes.



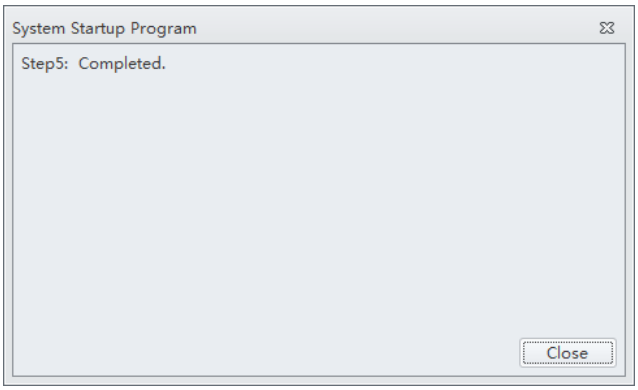
After priming, the system initializes again. The sample is loaded automatically. This process takes about 3 minutes.



The sample tube is unloaded after sample acquisition has finished. The system uses the remaining time to warm up.



5 When warm up is finished, select **Close** to quit the startup program. The system is now initialized.



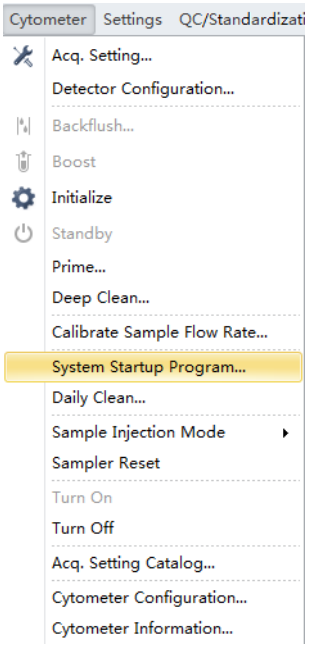
Running the System Startup Program [With Plate Loader]

The system startup program takes approximately 10 minutes.

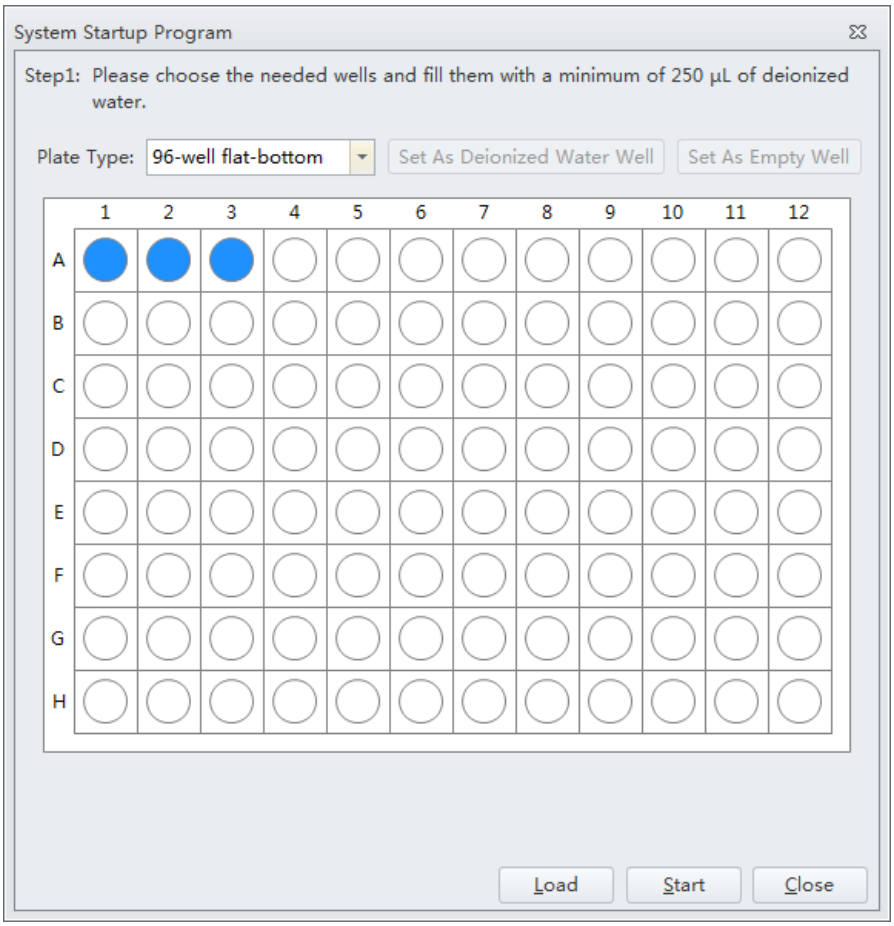
1 Select **Initialize**.

2 Select **System Startup Program** from the Cytometer menu to open the System Startup Program window.

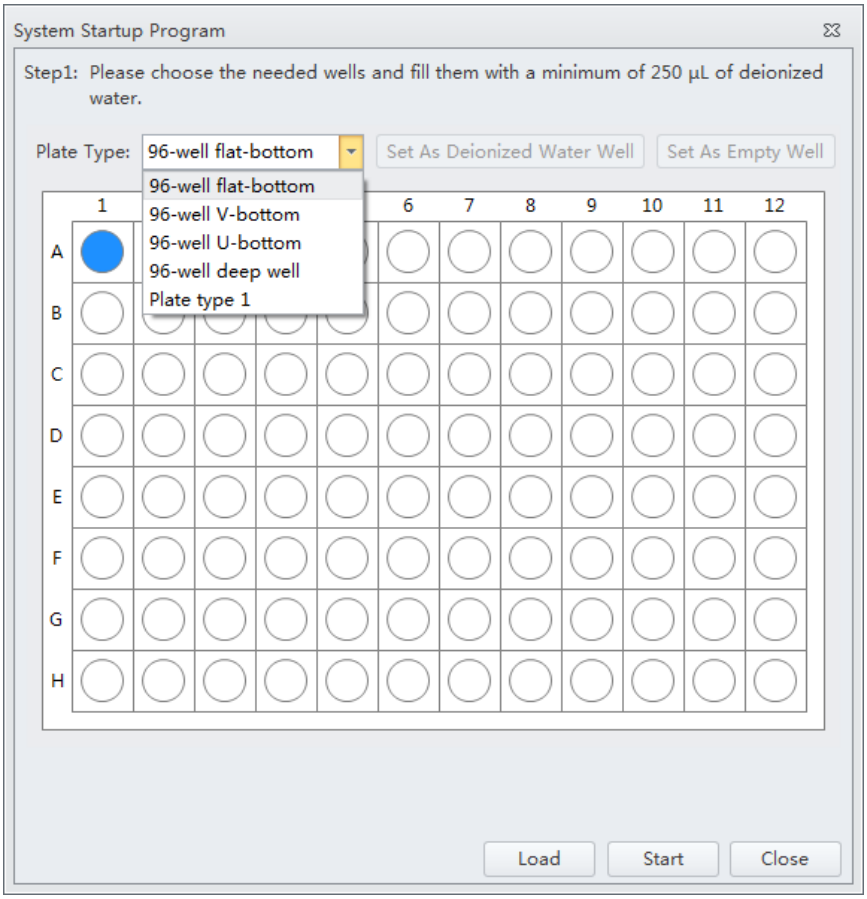
[CytoFLEX LX Shown]



The plate loader automatically ejects the plate holder stage and the System Startup Program window appears.

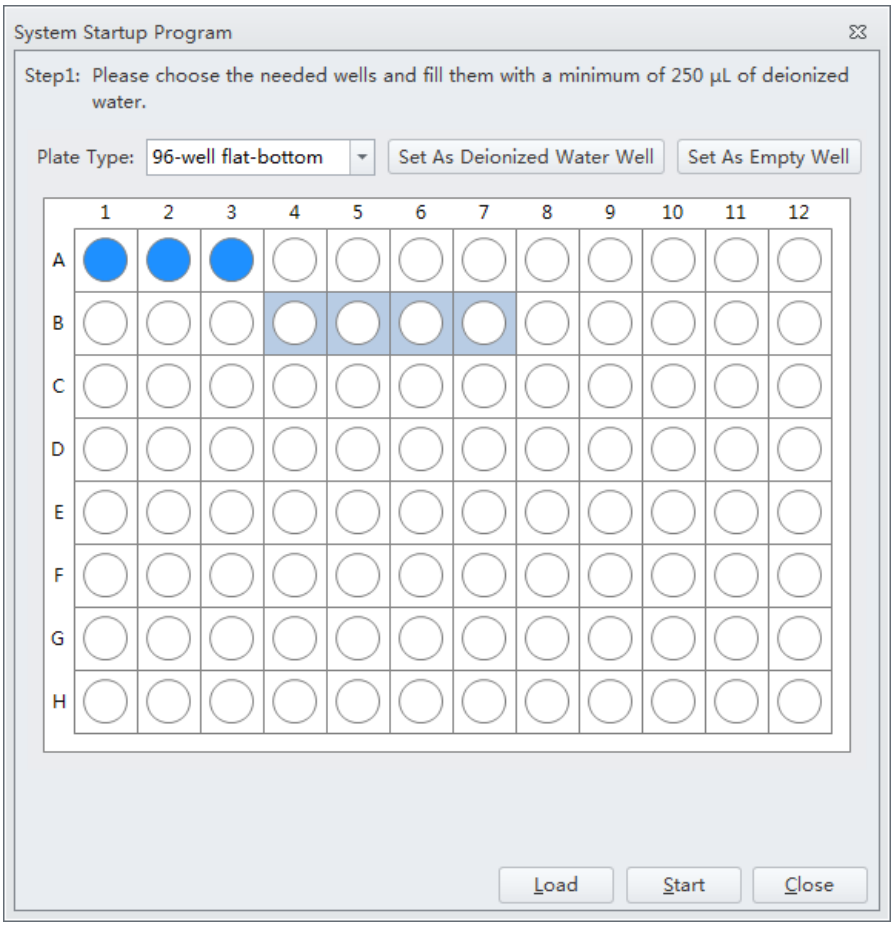


3 Select the desired plate type from the Plate Type drop-down menu.



NOTE The available plate types included in the drop-down menu depend on the settings selected in the Plate Library. To activate a plate type, refer to Plate Type Library in CHAPTER 2, Using the CytExpert Software.

4 Follow the on screen software prompts and select the desired wells and select **Set As Deionized Water Well.**



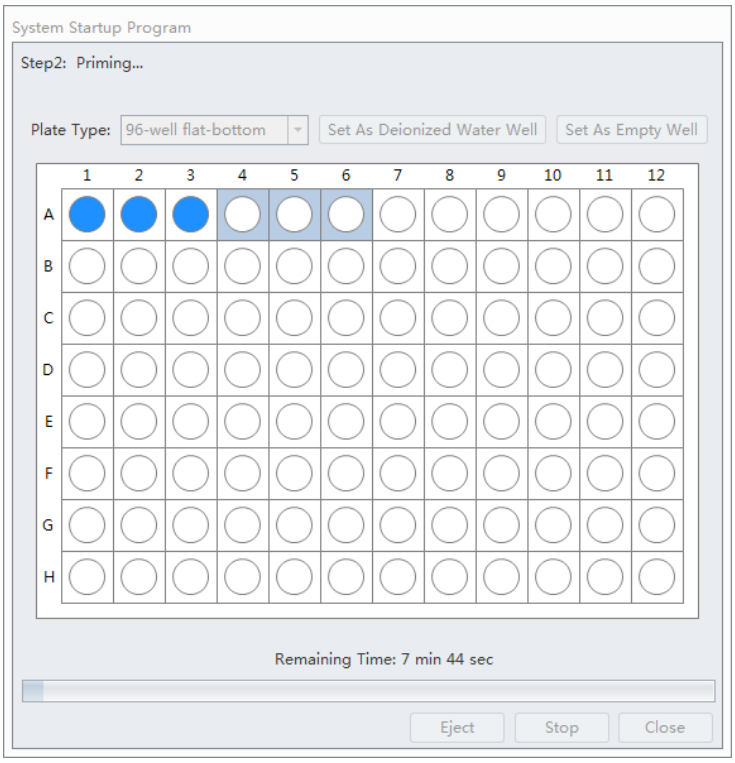
NOTE To deselect water wells, select the desired well and select **Set As Empty Well**.

NOTE Prepare two to six sample wells with deionized water.

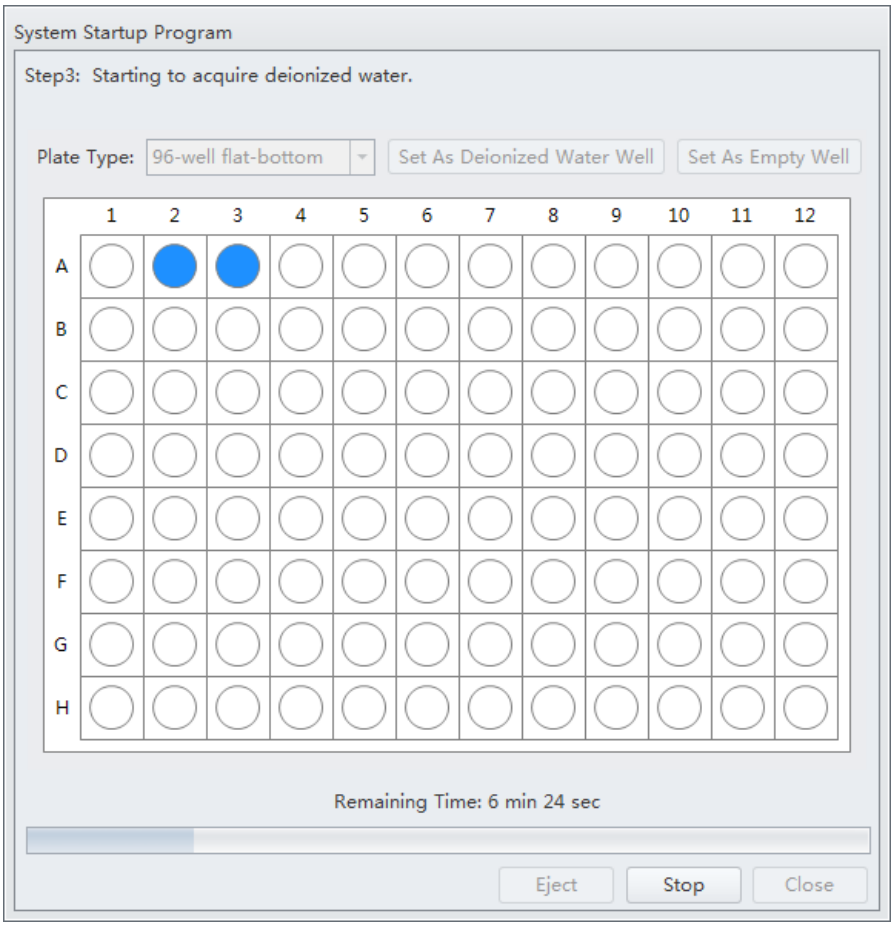
5 Select **Load** to load the plate.

6 Select **Start** to start the program. The message *Please confirm that the correct plate is placed properly and press OK* appears. Select **OK.**

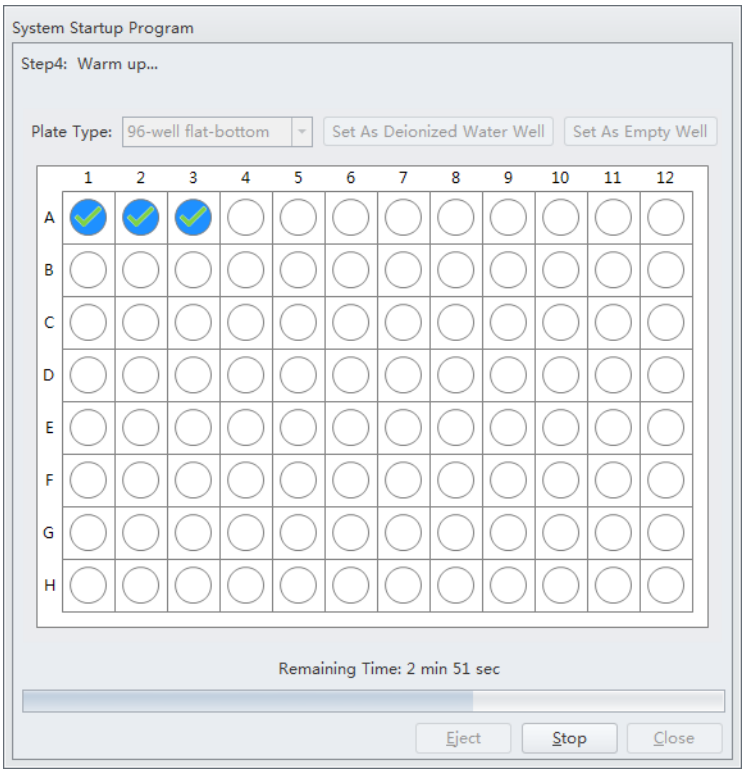
Wait for the system to initialize. The instrument begins prime. This process takes about 4 minutes.



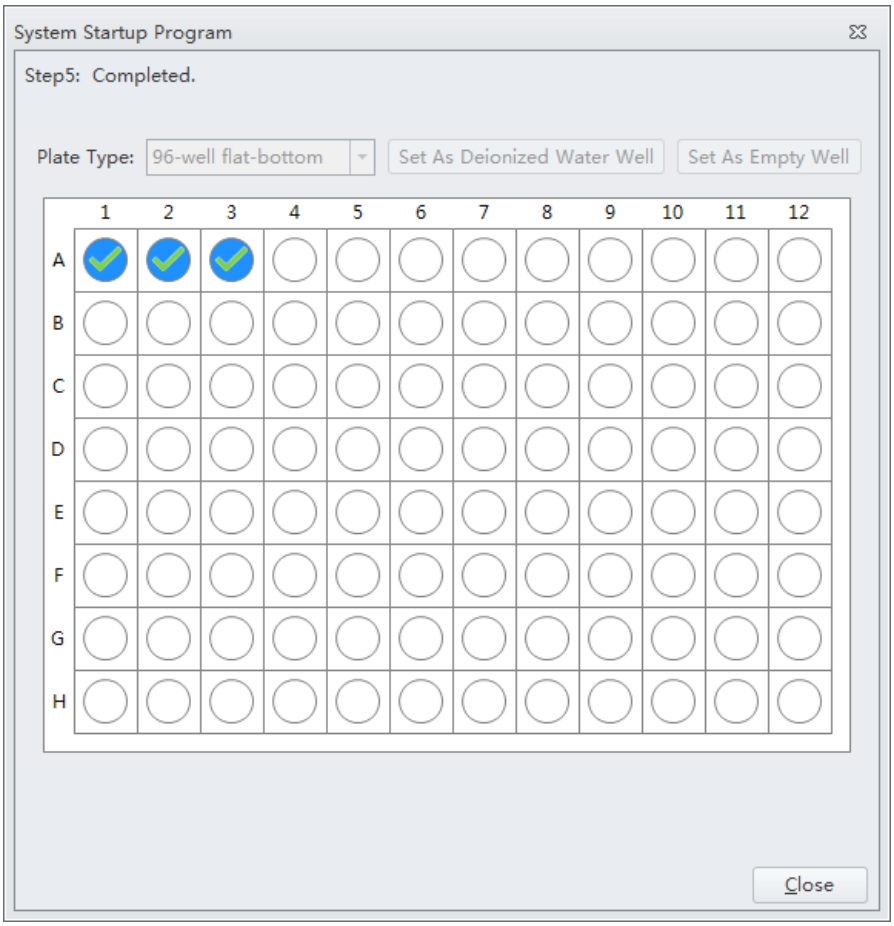
After a self check, the system initializes again. The sample is loaded automatically. This process takes about 1 minute per well.



When the system finishes acquiring the selected sample well, it uses the remaining time to warm up.



7 When warm up is complete the plate loader ejects the plate holder stage. Select **Close** to quit the startup program. The system is now initialized.

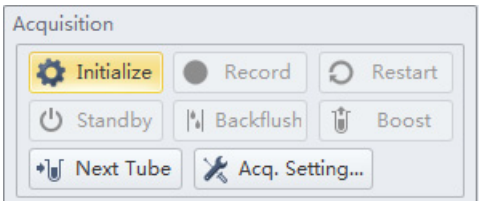


Selecting Experiments from the Start Page

Refer to Start Page in CHAPTER 2, Using the CytExpert Software.

Initializing the Instrument

1 Select **Initialize** in the Data Acquisition Control screen or select **Initialize** in the Cytometer Menu to initialize the instrument.



NOTE The system prompts you to initialize if the instrument remains in standby for over 24 hours.

NOTE If the instrument is in Semi-Automatic Injection mode during the initialization process, the sample tube holder automatically shifts into the sample loading position (see Figure 1.13).

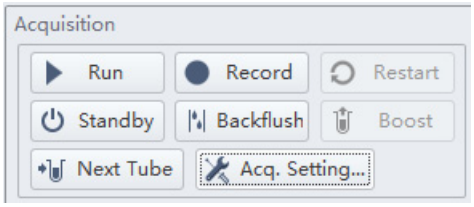
2 Wait for the beep indicating that the instrument properly initialized.

NOTE In the initialized state, the enabled lasers power on to achieve operating status, and the sheath fluid flows. Refer to Laser Settings in CHAPTER 5, Data Acquisition and Sample Analysis.

- If you need to execute a task with the Fluid Containers, do so with the instrument in standby state.
- If the instrument remains idle for 10 minutes, the Cytometer automatically enters the standby state.

NOTE After approximately 30 seconds, there should be a continuous flow of waste liquid from the Cytometer to the waste container.

3 Proceed to the subsequent operations or select **Standby** to put the instrument in standby state.



CHAPTER 4

Instrument Quality Control and Standardization

Overview

This chapter provides information on performing daily quality control (QC) on the CytoFLEX and CytoFLEX LX flow cytometer 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 or any other reference material that is relevant for your application(s) may be used as the standardization sample(s).

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.

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, Data Acquisition and Sample Analysis.
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. Notifies you if laser delay is >2 µs from the previous setting. The software automatically changes the laser delay setting.

   OR

   Notifies you if laser delay is >5 µs 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.

NOTE Beckman Coulter recommends performing QC on a daily basis.

NOTE QC can only be run in channels with the standard detector sets including, laser and band-pass. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Data Acquisition and Sample Analysis to verify that the default factory detector configuration is selected before running QC.

NOTE CytExpert QC includes auto daily QC routine with Levey-Jennings (LJ) charts tracking and logging.

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

QC Workflow:

Prepare the QC sample	→	Enter the QC experiment	→	Select QC target values	→	Acquire data	→	Confirm results

Standardization Workflow:

Prepare the standardization sample	→	Enter the standardization experiment	→	Select target values	→	Acquire data	→	Import standardization settings into associated experiments

This chapter contains information on:

- Preparing the QC Sample
- Preparing the QC Sample [With Plate Loader]
- Importing Lot-Specific Target Values
- Collecting QC Data
- Collecting QC Data [With Plate Loader]
- Confirming Results
- Standardization

Preparing the QC Sample

Required Materials

The following materials are required to complete the QC process:

- CytoFLEX Daily QC Fluorospheres
- CytoFLEX Sheath Fluid or another nonionic sheath fluid
- Sample tubes (12 x 75 mm).
- Vortexer
- CytoFLEX Daily IR QC Fluorospheres (for systems configured with an IR laser)

Preparation Process CytoFLEX Daily QC Fluorospheres

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 until a uniform mixture is achieved.
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 stored and sealed in a dark location at 2-8 °C for up to 5 days.

Preparation Process CytoFLEX Daily IR QC Fluorospheres

1. Take one sample tube and label it as the QC/IR sample tube.
2. Mix the CytoFLEX Daily IR QC Fluorospheres by inversion.

3 Add ten drops of CytoFLEX Daily IR QC Fluorospheres to the sample tube.

4 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 IR QC Fluorospheres should immediately be sealed and sealed in a dark location at 2-8 °C for up to 5 days.

Preparing the QC Sample [With Plate Loader]

Required Materials

The following materials are required to complete the QC process:

- CytoFLEX Daily QC Fluorospheres
- CytoFLEX Daily IR QC Fluorospheres (for systems configured with an IR laser)
- CytoFLEX Sheath Fluid or another nonionic sheath fluid
- Standard 96-well plate
  - 96-well flat-bottom
  - 96-well V-bottom
  - 96-well U-bottom
- 96-well deep well plate
  - 96-well V-bottom
  - 96-well U-bottom
- Vortexer

Preparation Process CytoFLEX Daily QC Fluorospheres

1 Take one 96-well plate and label one well as the QC sample well.

2 Use the vortexer or shake vigorously to thoroughly mix the bottle of CytoFLEX Daily QC Fluorospheres.

IMPORTANT Do not overfill the sample well.

3 Add one drop of CytoFLEX Daily QC Fluorospheres to the sample well.

4 Add approximately 200 µL of deionized water to the sample well.

5 Place the well plate in a dark location at 2-8 °C until ready to load the well plate into the instrument for QC.

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

Preparation Process CytoFLEX Daily IR QC Fluorospheres

1 Take one 96-well plate and label one well as the QC/IR sample well.

2 Mix the CytoFLEX Daily IR QC Fluorospheres by inversion.

IMPORTANT Do not overfill the sample well.

3 Add 3-4 drops of CytoFLEX Daily IR QC Fluorospheres to the sample well.

4 Place the well plate in a dark location at 2-8 °C until ready to load the well plate into the instrument for QC.

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

Importing Lot-Specific Target Values

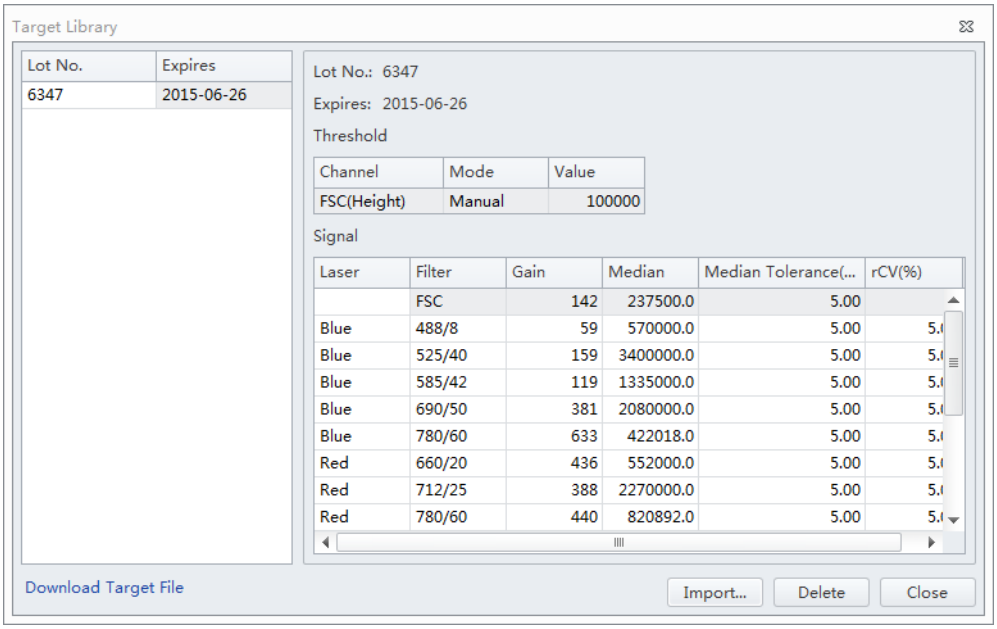
Import lot-specific target values for each new lot of CytoFLEX QC Fluorospheres and CytoFLEX Daily IR QC Fluorospheres.

⚠ CAUTION

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

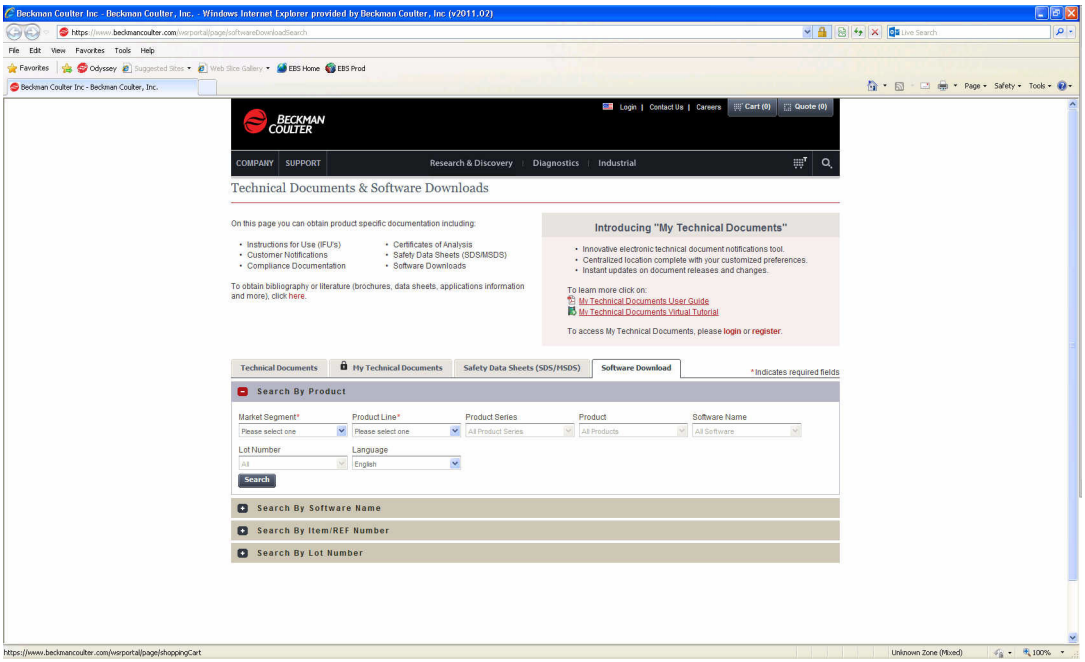
1 Open the CytExpert QC screen.

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 Technical Documents and Software Downloads page appears.



NOTE If your CytoFLEX 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 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 Series drop-down menu.

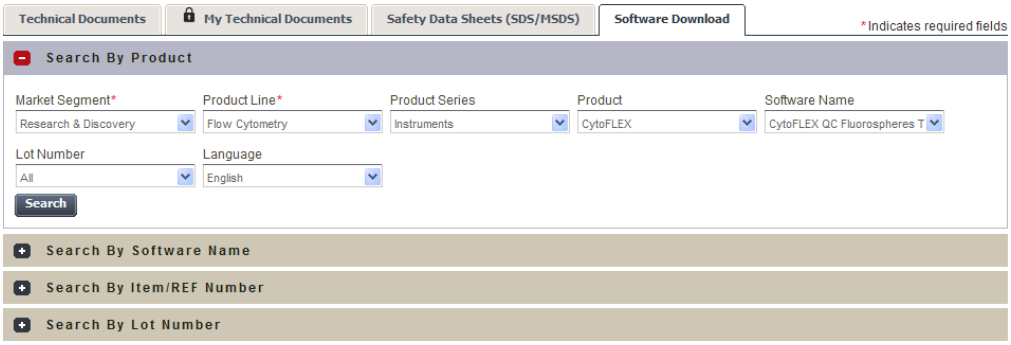
d. Select **CytoFLEX** from the Product drop-down menu.

e. Select **CytoFLEX QC Fluorospheres Target** or **CytoFLEX IR QC Fluorospheres Target** from the Software Name drop-down menu.

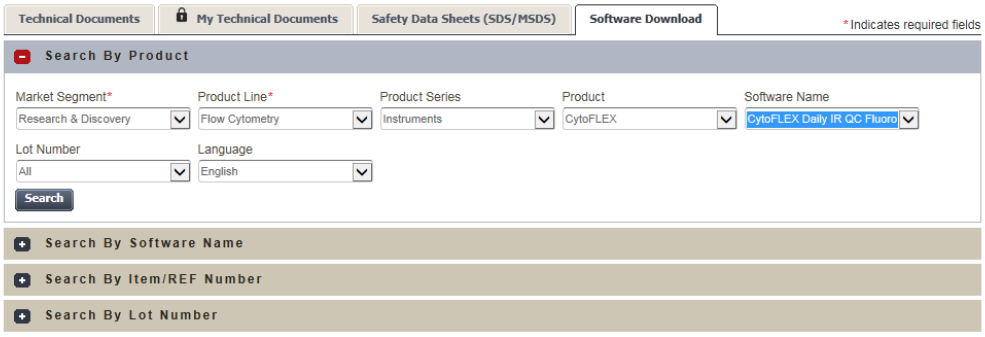
f. Select **All** from the Lot Number drop-down menu.

g. Select **English** from the Language drop-down menu.

[CytoFLEX QC Fluorospheres Target]



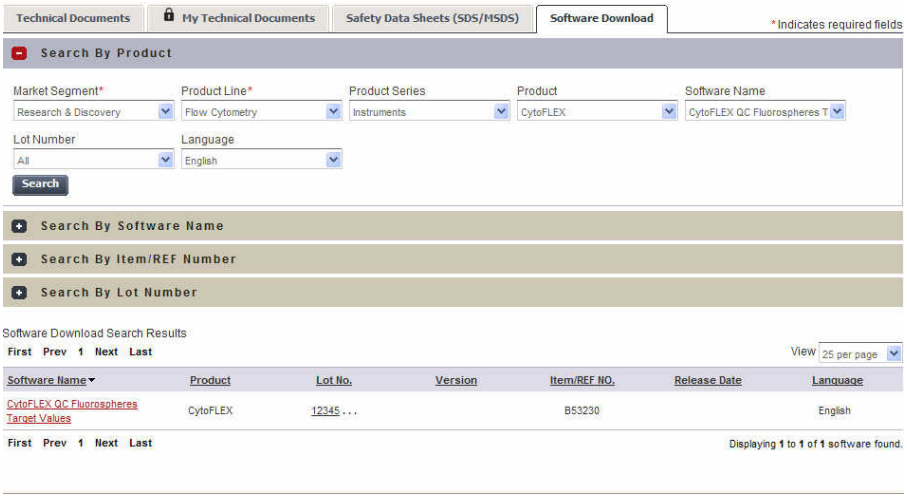
[CytoFLEX IR QC Fluorospheres Target]



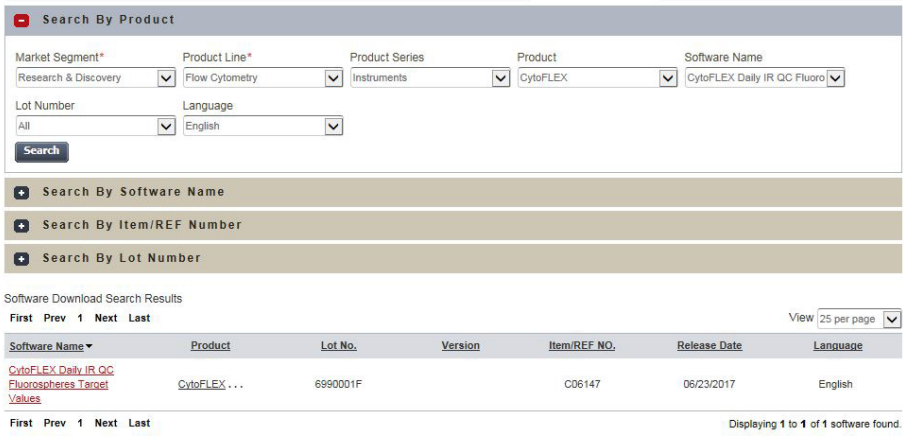
5 Select **Search**.

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

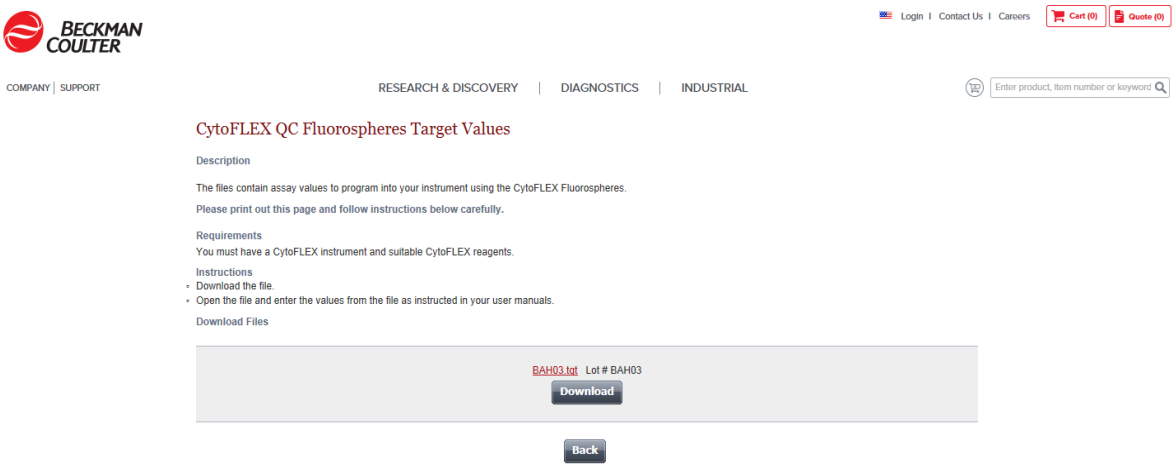
[CytoFLEX QC Fluorospheres Target]



[CytoFLEX IR QC Fluorospheres Target]

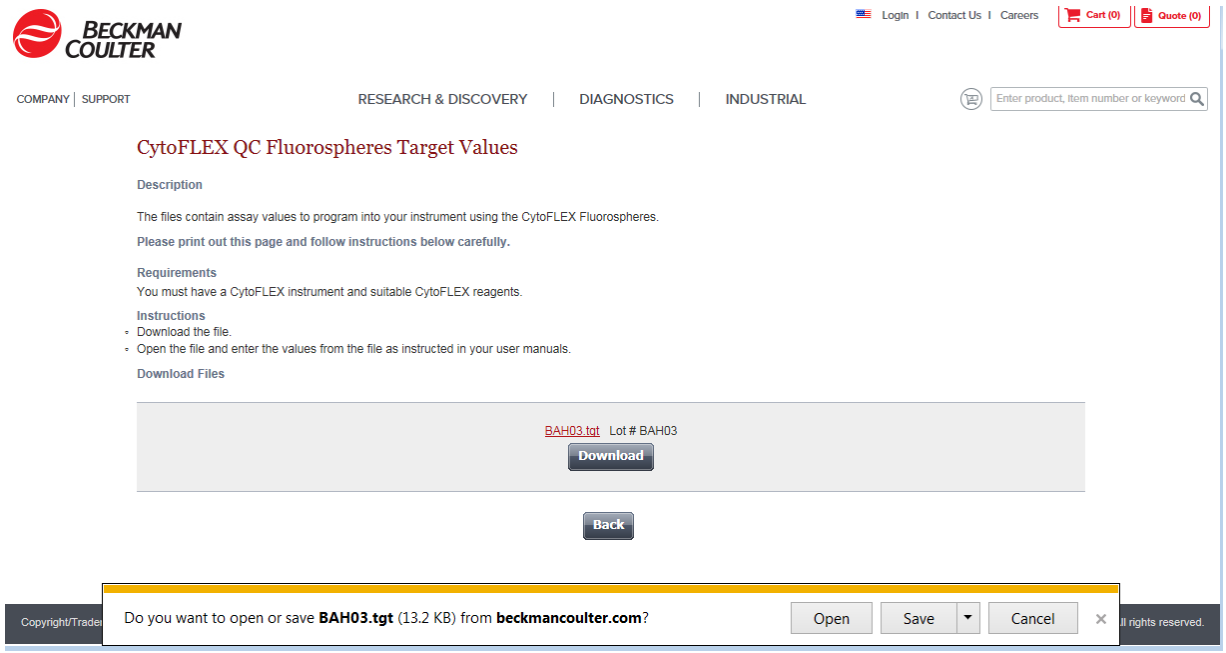


7 Select **CytoFLEX QC Fluorospheres Target Values** under the Software Name column. The CytoFLEX QC Fluorospheres Target Values page appears.



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

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



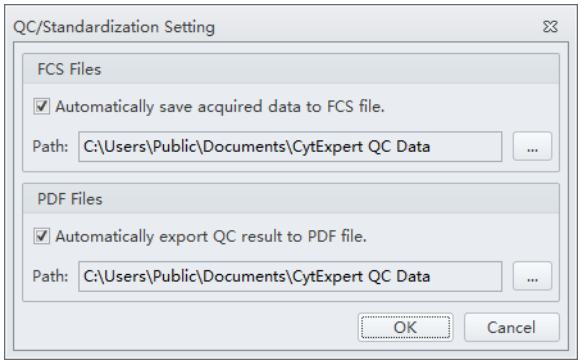
10 Select **Import** from the Target Library window in the CytExpert software.

11 Navigate to the file saved in step 9 and select **Open**.

12 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 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 Cytometer USB is securely connected to the Workstation and restart the Workstation.

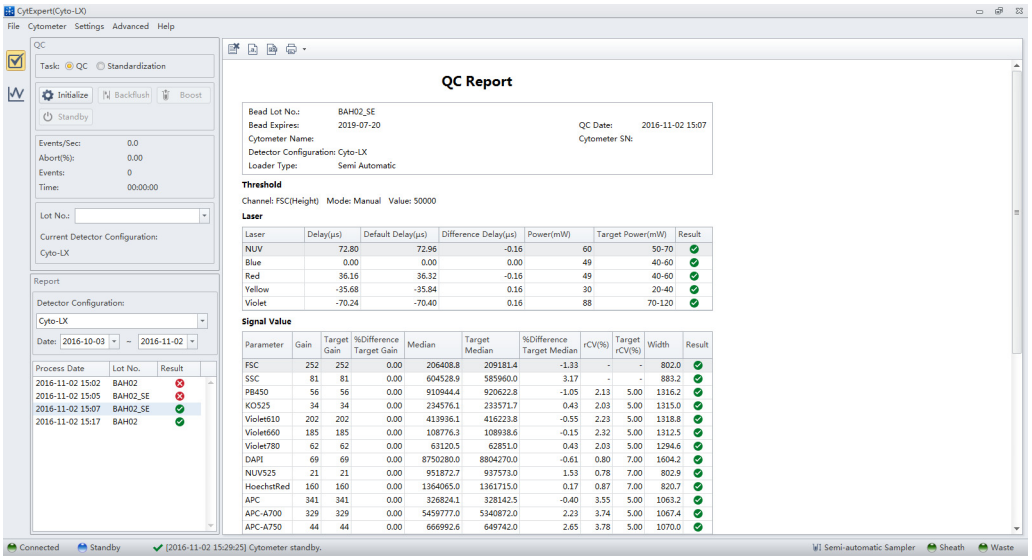
2 Verify the detector configuration. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Data Acquisition and Sample Analysis.

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, Data Acquisition and Sample Analysis.

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

[CytoFLEX LX Shown]



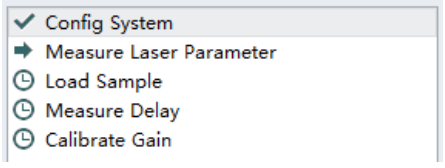
Ensure that the QC bead lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, refer to Importing Lot-Specific Target Values, then select the proper lot number.

5 Select **Initialize**.

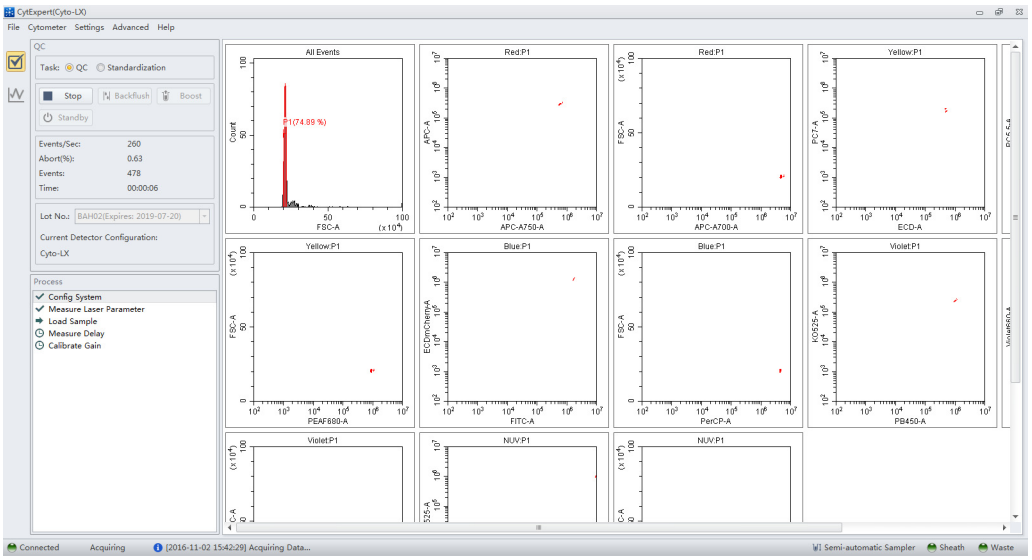
6 Insert the prepared QC sample tube (see Preparation Process CytoFLEX Daily QC Fluorospheres) into the tube holder.

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, and coefficient of variation.



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

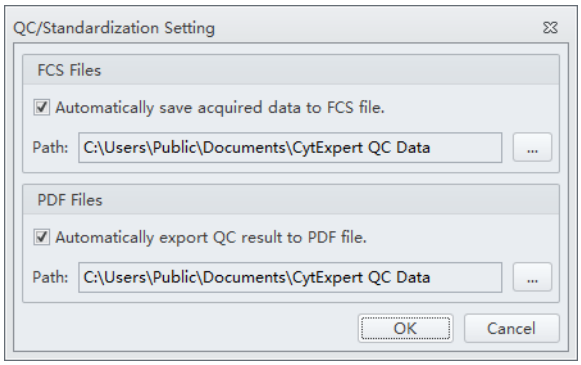


8 If the sampling rate is too low, the Cytometer stops the QC run and displays a prompt alerting you that the QC run failed to reach the required event flow rate. This is not considered a QC failure. If this situation occurs, increase the sample concentration by adding one drop of CytoFLEX Daily QC Fluorospheres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

9 Run Daily Clean to remove any residual fluorosphere particles. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures.

Collecting QC Data [With Plate Loader]

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 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 Cytometer 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, Data Acquisition and Sample Analysis.

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 QC 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, Data Acquisition and Sample Analysis.

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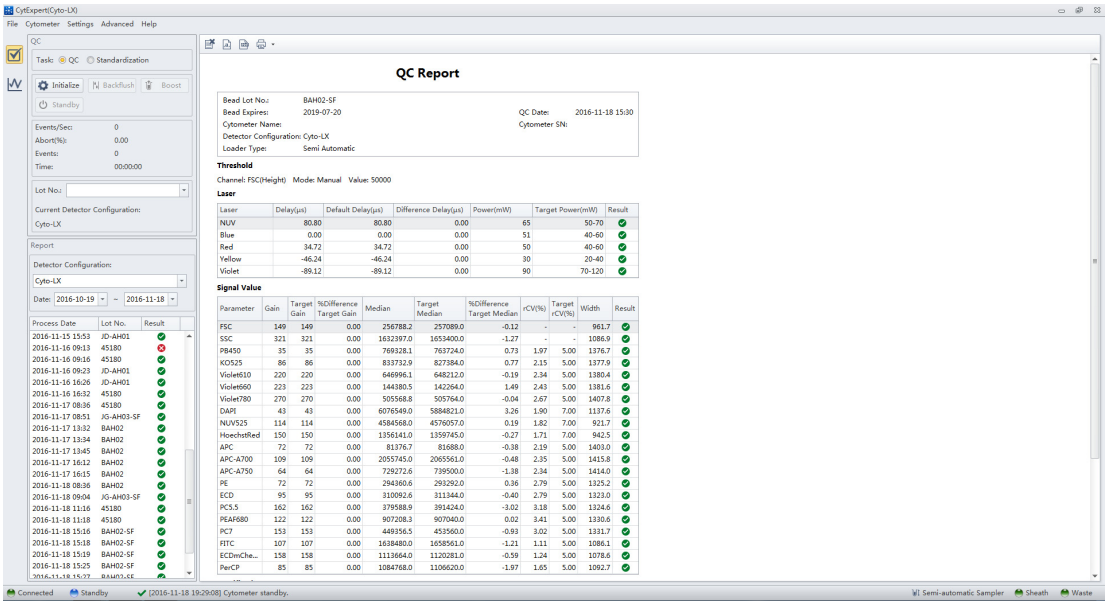
[CytoFLEX LX Shown]



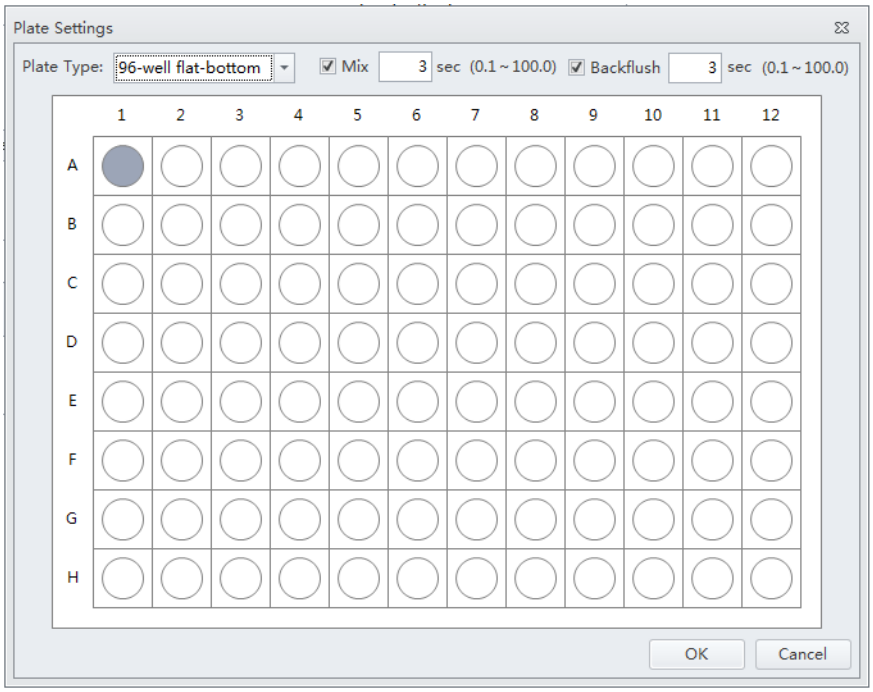
Ensure that the QC bead lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, refer to Importing Lot-Specific Target Values in CHAPTER 4, Instrument Quality Control and Standardization, then select the proper lot number.

5 Select **Initialize**.

6 Select **Eject**.

7 Insert the prepared QC well plate (see Preparation Process CytoFLEX Daily QC Fluorospheres) into the plate holder.

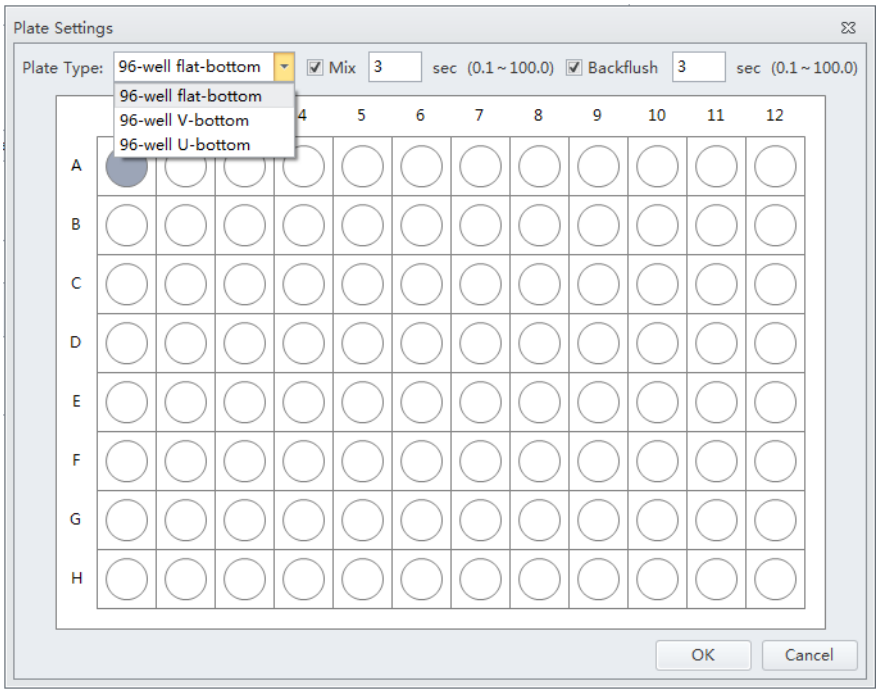
8 Select Plate Settings... . The Plate Settings window appears.



IMPORTANT Ensure the well position on the plate matches the well position selected in the software.

9 Select the appropriate QC well.

10 Select the desired plate type from the Plate Type dropdown menu.



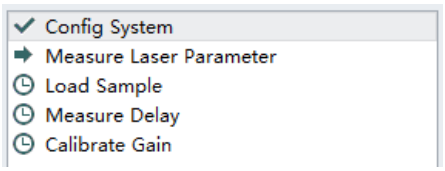
NOTE The available plate types included in the dropdown menu depend on the settings selected in the Plate Library. Refer to Plate Type Library in CHAPTER 2, Using the CytExpert Software.

11 Select the Mix and Backflush settings in the top of the Plate Settings window.

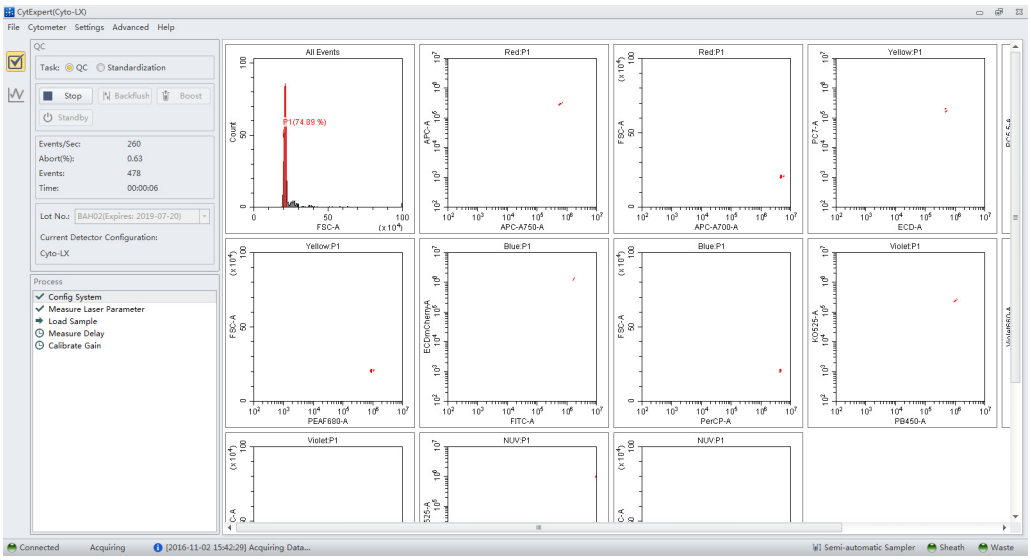
12 Select **OK**.

13 Select **Start** to load the sample and begin to run the QC procedure. The message *Please confirm that the correct plate is placed properly and press OK* appears. Select **OK**.

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



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



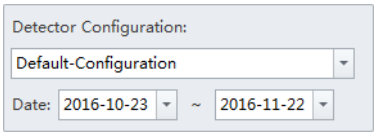
14 If the sampling rate is too low, the Cytometer stops the QC run and displays a prompt that the QC run fails to reach the required event flow rate. This is not considered a QC failure. If this situation occurs, increase the sample concentration by adding one drop of CytoFLEX Daily QC Fluorospheres to the sample tube and then perform the experiment.

15 Run Daily Clean to remove any residual fluorosphere particles. Refer to Daily Clean [With Plate Loader] in CHAPTER 10, Cleaning Procedures.

Confirming Results

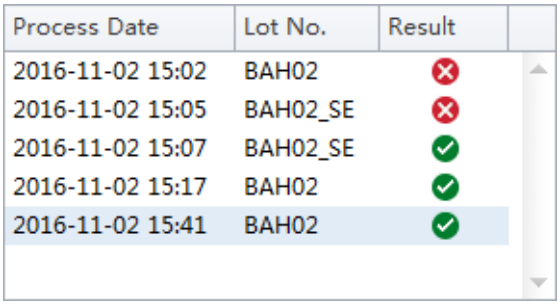
Select **Start QC/Standardization** in the QC/Standardization menu to return to the QC Setting screen at any time to review completed experiment results.

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.



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.



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:

- The gain differences must be ≤20% from the target gain.
- The median fluorescence intensity (MFI) differences must be ≤5% from the target MFI.
- The rCV must be within the range.

NOTE The CytoFLEX Daily QC Fluorospheres / Daily IR QC Fluorospheres (808 Laser) rCV must meet the following criteria to pass:

— The rCV for channels of the 405 nm, 488 nm, 561 nm, 638 nm lasers must be ≤5%.

— The rCV for channels of the 355 nm, 375 nm, 808 nm lasers must be ≤7%.

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.:	A555		
Bead Expires:	2015-09-30	QC Date:	2015-01-20 10:23
Cytometer Name:	CytoFLEX	Cytometer SN:	AR38012
Detector Configuration:	Default-Configuration		
Loader Type:	Semi Automatic		

Threshold

Channel: FSC(Height) Mode: Manual Value: 50000

Laser

Laser	Delay(μs)	Default Delay(μs)	Difference Delay(μs)	Power(mW)	Target Power(mW)	Result
Blue laser	0.00	0.00	0.00	53	40-60	✓
Red laser	-37.12	-39.36	2.24	53	40-60	✓

Signal Value

Parameter	Gain	Target Gain	%Difference Target Gain	Median	Target Median	%Difference Target Median	rCV(%)	Target rCV(%)	Width	Result
FSC	20	107	-81.31	242486.0	242482.7	0.00	-	-	1117.1	✗
SSC	14	96	-85.42	608237.2	601799.5	1.07	-	-	1187.8	✗
FITC	19	426	-95.54	3279233.0	3313982.0	-1.05	2.27	5.00	1188.6	✗
PE	26	71	-63.38	1117258.0	1145402.0	-2.46	2.22	5.00	1194.0	✗
APC	3000	472	535.59	29386.1	483724.0	-93.93	11.13	5.00	1700.8	✗
APC-A750	3000	495	506.06	20604.3	689351.1	-97.01	17.46	5.00	1433.5	✗

Specifications

Delay: -5.00μs ≤ Difference Delay ≤ 5.00μs.
Gain: -20.00% ≤ %Difference Target Gain ≤ 20.00%.
Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.
rCV: rCV(%) ≤ Target rCV(%).

Result

QC Failed.

Comment

The difference between FSC gain and target is more than 20%.
The difference between SSC gain and target is more than 20%.
The difference between FITC gain and target is more than 20%.
The difference between PE gain and target is more than 20%.
APC gain calibration was failed, median value is out of the target value range.
APC-A750 gain calibration was failed, median value is out of the target value range.

If QC fails, follow the procedure below:

a. Verify whether the beads used were within their shelf life and stored in accordance with the appropriate instruction manual.

b. Verify whether the allocated sample tube was prepared as required and correctly positioned.

c. Run Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures, and retest.

d. Run Daily Clean in CHAPTER 10, Cleaning Procedures, and retest.

e. Run Deep Clean Procedure in CHAPTER 10, Cleaning Procedures, and retest.

f. Repeat Steps c-d.

NOTE If QC fails two times in a row on the same day after repeating Steps a-f, contact us.

3 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.

4 Select **Close QC/Standardization** in the File menu to exit the QC screen.

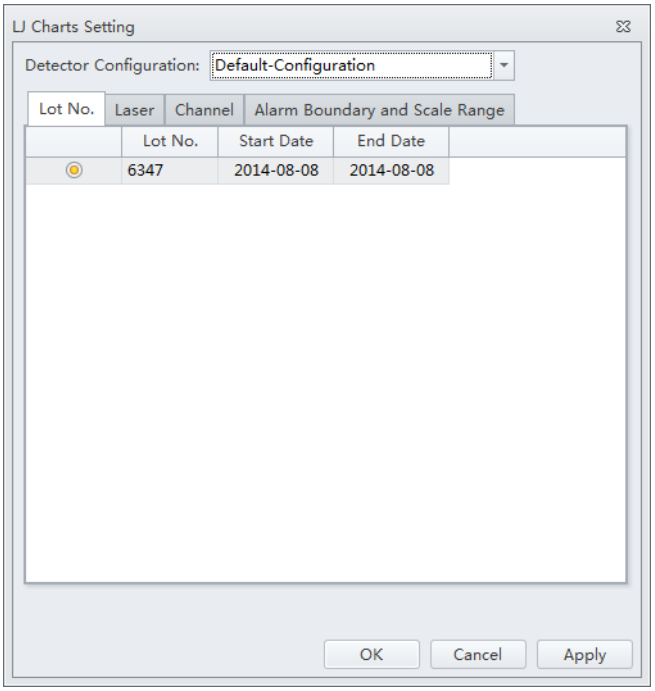
Creating Levey-Jennings Charts

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

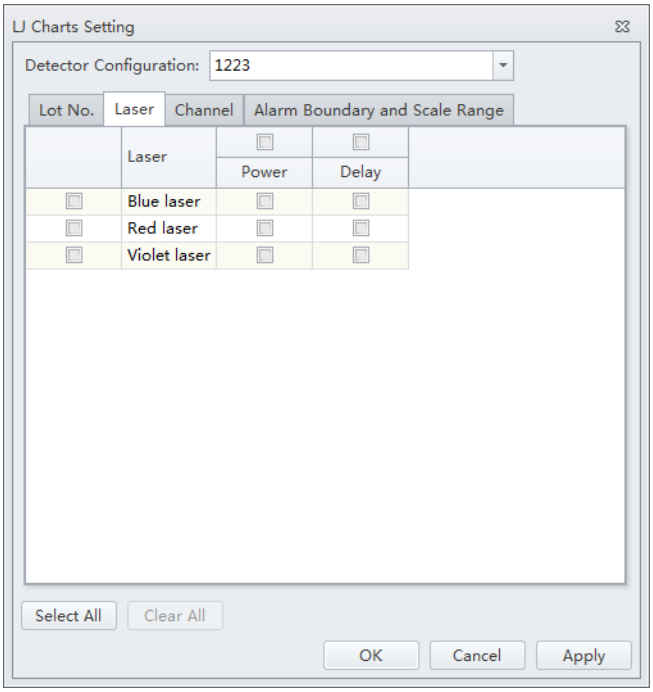
2 Select LJ chart on the left side of the screen.

IMPORTANT When there are multiple lots, select which lot to create the LJ charts from.

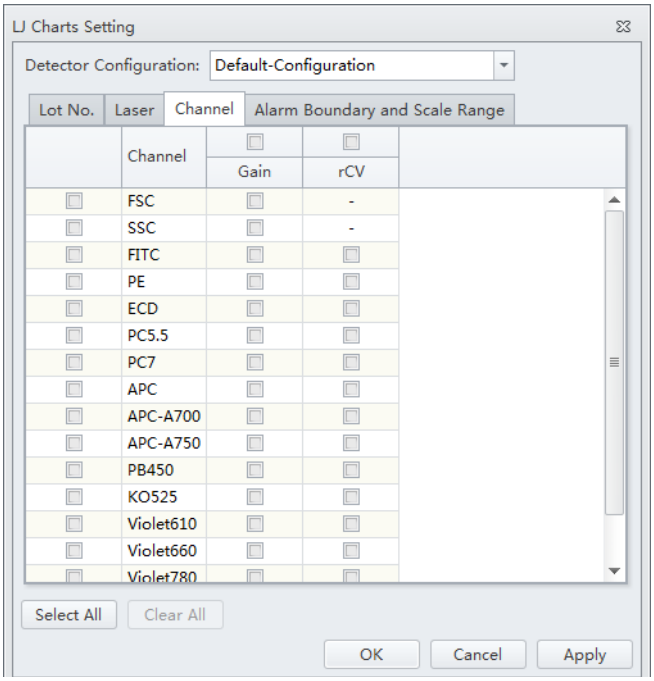
3 Select LJ Chart Settings on the top of the LJ Chart screen. The LJ Chart Settings screen appears.



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



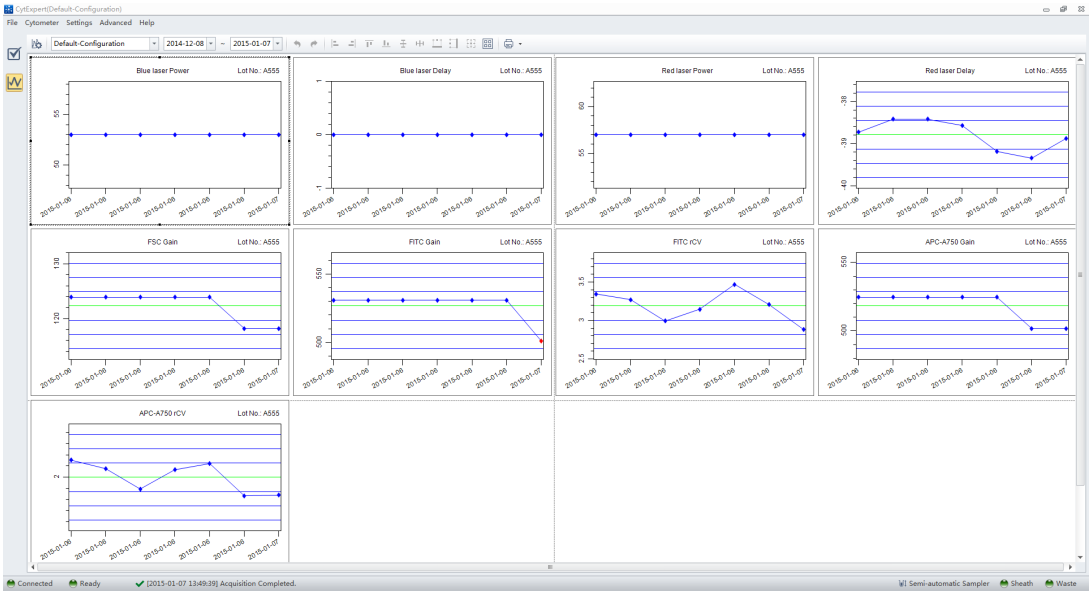
5 Select the **Channels** tab, and select each channel checkbox as needed.



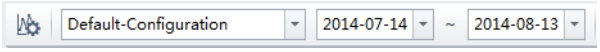
6 Select **Apply**.

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.

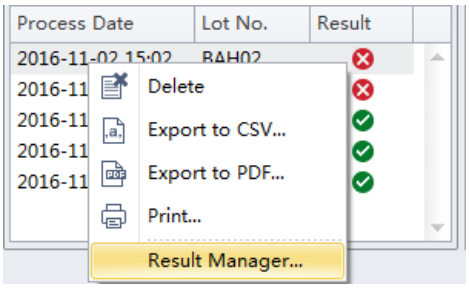


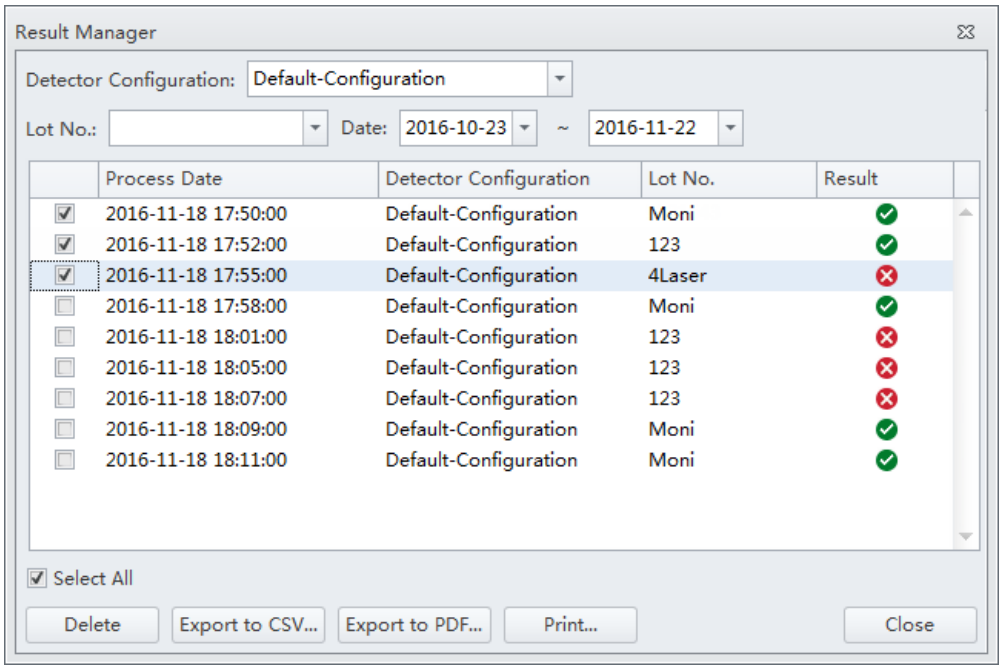
9 Select **Close QC/Standardization** in the File menu to exit the QC screen.

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.



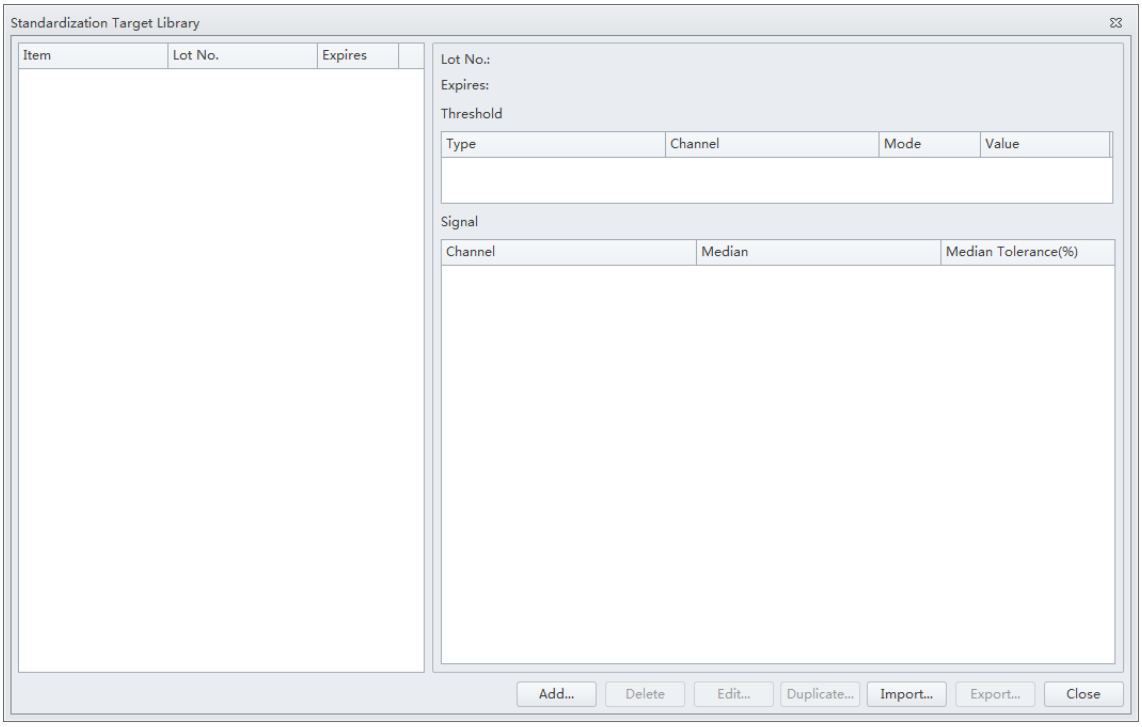


Standardization

Use Beckman Coulter CytoFLEX Daily QC fluorospheres or any other reference material that is relevant for your application. Ensure that either the Beckman Coulter CytoFLEX Daily QC fluorospheres or other reference material has optimized settings appropriate for your experiment.

Adding, Editing, Importing, and Deleting Standardization Laser Target Values in the QC Experiment

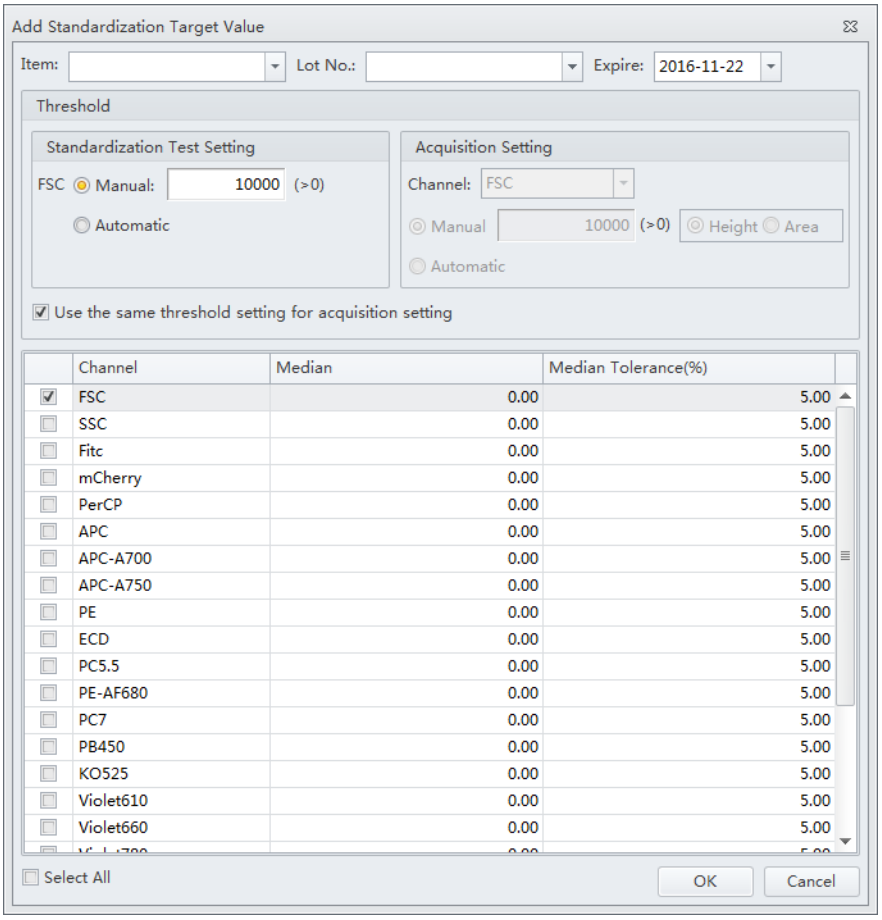
Select **Standardization Target Library...** from the Settings menu. The standardization Target Library window appears.



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

Adding a New Standardization Item

1 Select Add... . The Add Standardization Target Value window appears.



NOTE Select Duplicate... to create a duplicate copy of an existing standardization item.

NOTE Unchecking the *Use the same threshold setting for acquisition setting* checkbox allows you to specify custom threshold settings and when to save the test item into the Acquisition Setting Catalog.

2 Enter the Item, Lot No., and Expire date from the drop downs 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.

3 Choose either Manual or Automatic threshold from the Standardization Test Setting or Acquisition Setting section of the screen.

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

4 Set the channels, median, and median tolerance values.

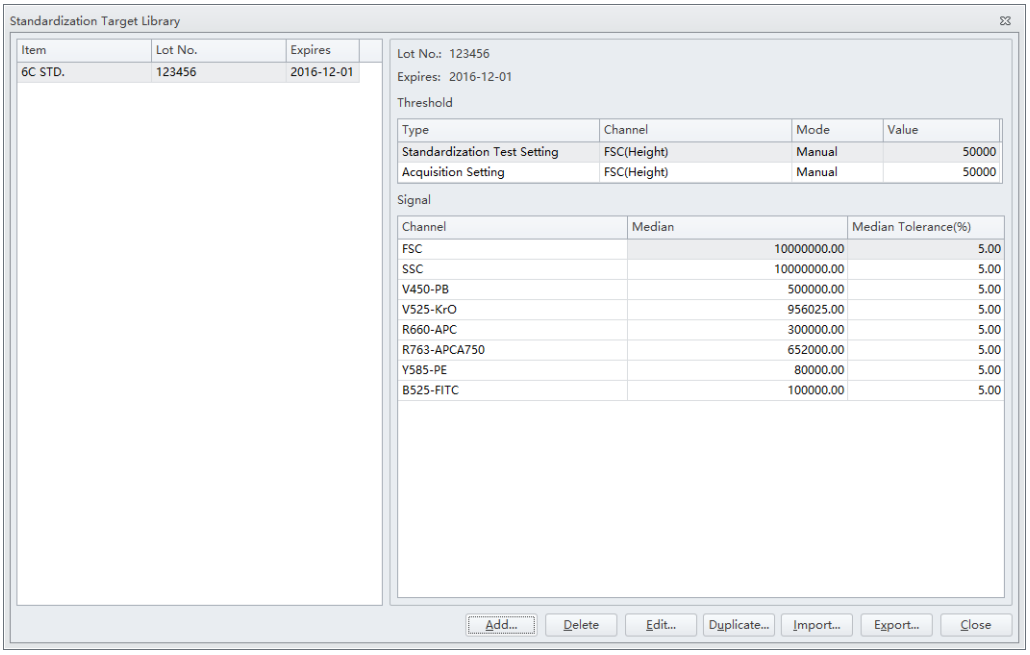
NOTE The contents of channel, laser and filter column come from current detector configuration setting.

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

NOTE FSC is a required channel.

5 Select **OK** to save the target value.

The saved results display in the Standardization Target Library window.



6 Select Close 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 Edit....

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 Export... and save the file.

4 Select Close to exit the Standardization Target Library window.

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.

3 Select Close to exit the Standardization Target Library window.

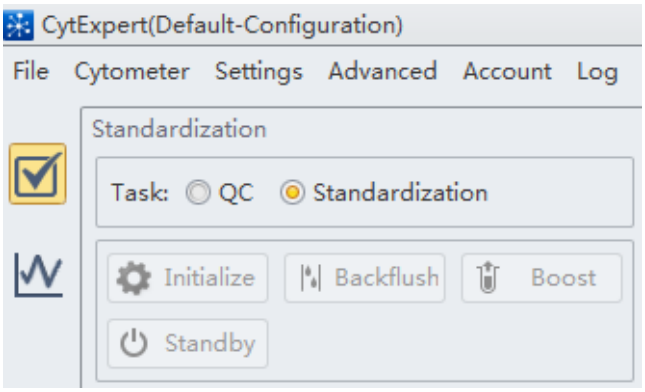
Deleting Standardization Items

Select an item from the Item column on the Standardization Target Library window and select Delete. Select Close to exit the Standardization Target Library window.

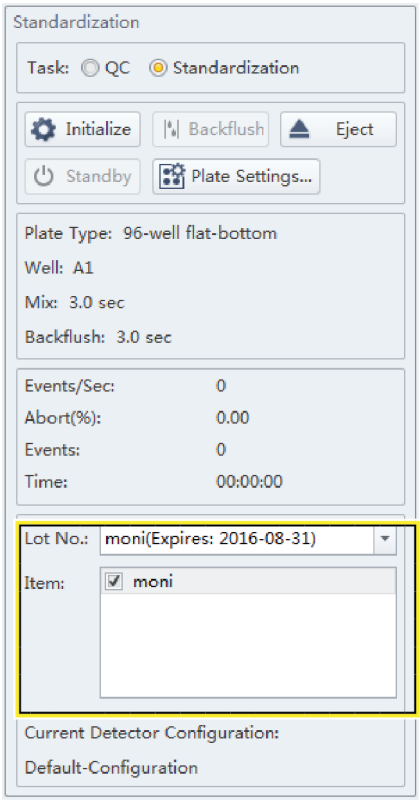
Applying the Standardization in QC

1 Open the CytExpert QC screen.

2 Select the **Standardization** radio button.

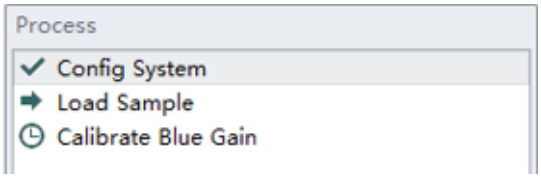


3 Select the Lot No. and the Items to be applied.

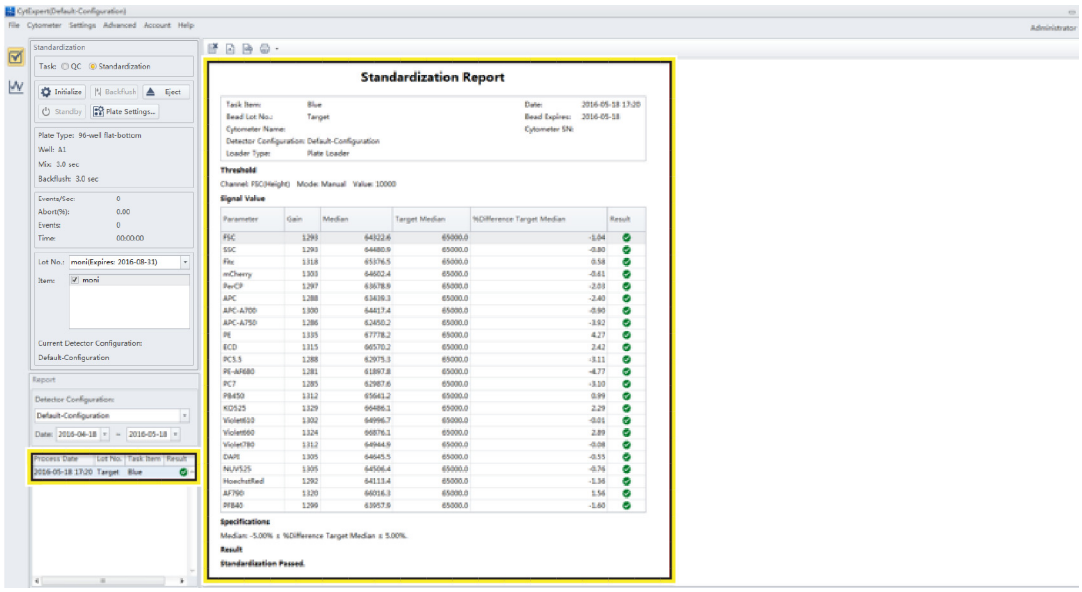


4 Select Start.

The Process section of the screen displays the process details.

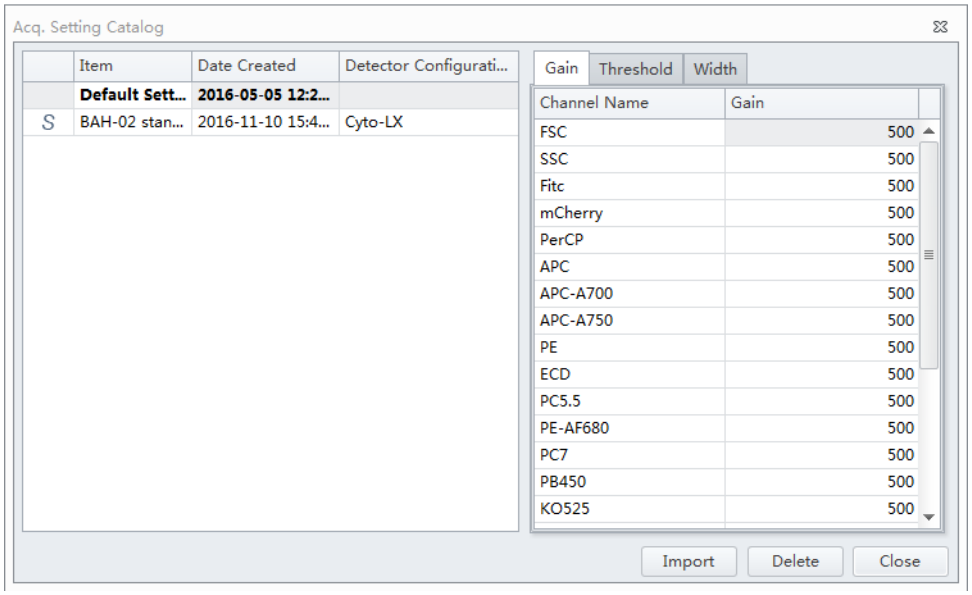


Once the process is complete, the Standardization Report displays.



5 Verify the gain settings.

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



NOTE S designates test items from Standardization.

b. Select the desired Test Item, from the list in the Gain, Threshold, and Width tabs.

CHAPTER 5

Data Acquisition and Sample Analysis

Overview

This chapter contains information on how to use your CytoFLEX and CytoFLEX LX flow cytometer, including data acquisition, analyzing and exporting results, and compensation procedures that will be executed manually during the process.

Workflow:

Create experiment	→	Load sample	→	Adjust instrument settings and gates	→	Record Data	→	Analyze and export data	→	Save experiment

This chapter contains information on:

- Creating an Experiment
- Creating an Experiment [With Plate Loader]
- Load Sample and Record Data
- Configuring Acquisition Settings
- Analyzing and Exporting Data
- Saving the Experiment

Creating an Experiment

⚠ CAUTION

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 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, Data Acquisition and Sample Analysis.

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.

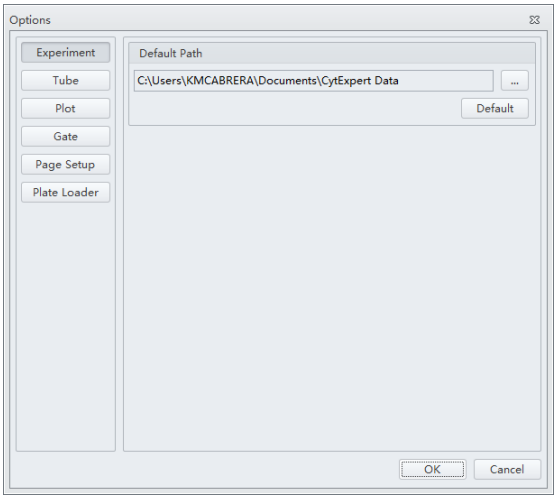
- Create a new experiment from a template:
  - Select **New Experiment from Template** on the Start page. Select **Browse** next to New Experiment and specify the file path for the new experiment, then select **Browse** next to Template and specify the file path to the existing template.
    Or
  - Select **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:
  - **If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option:** Select **Open Experiment** on the Start page, specify the file path and save the experiment.
    If you are using the CytExpert Electronic Record Management Software Option: Select **Open Experiment** on the Start page, specify the experiment file and open the experiment.
    Or
  - **If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option:** Select **Open Experiment** in the File menu, specify the path and save the experiment.
    If you are using the CytExpert Electronic Record Management Software Option: Select **Open Experiment** in the File menu, specify the experiment file and open the experiment.
    Or
  - **If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option:** 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.
    If you are using the CytExpert Electronic Record Management Software Option: Select the Experiment tab on the Start page and select the experiment from the list of recently opened experiments. Specify the experiment file and open the experiment.

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

NOTE **If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option:** If you need to change the default save path, select **Options** in the Settings menu and modify the Default Path displayed to the right of the Experiment tab. Then select **OK**.

If you are using the CytExpert Electronic Record Management Software Option: Select **File > Experiment Explorer** to modify any Experiment Directory sub folders for the Experiment. Refer to Experiment Directory Management in APPENDIX B, CytExpert Electronic Record Management.



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

Creating an Experiment [With Plate Loader]

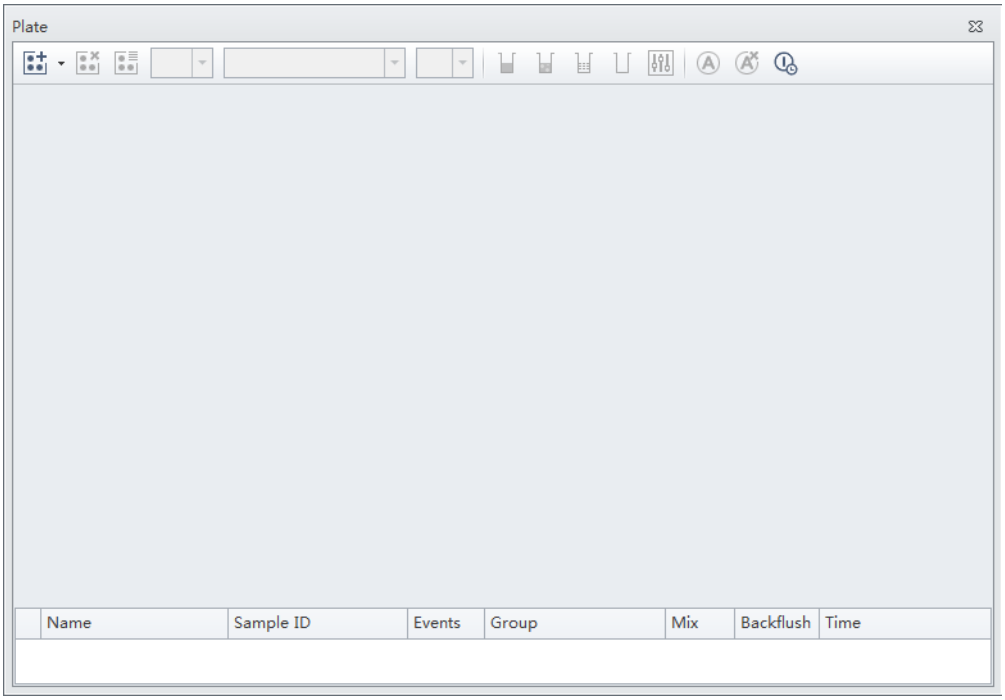
⚠ CAUTION

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

1 Create an experiment. Refer to Creating an Experiment in CHAPTER 5, Data Acquisition and Sample Analysis

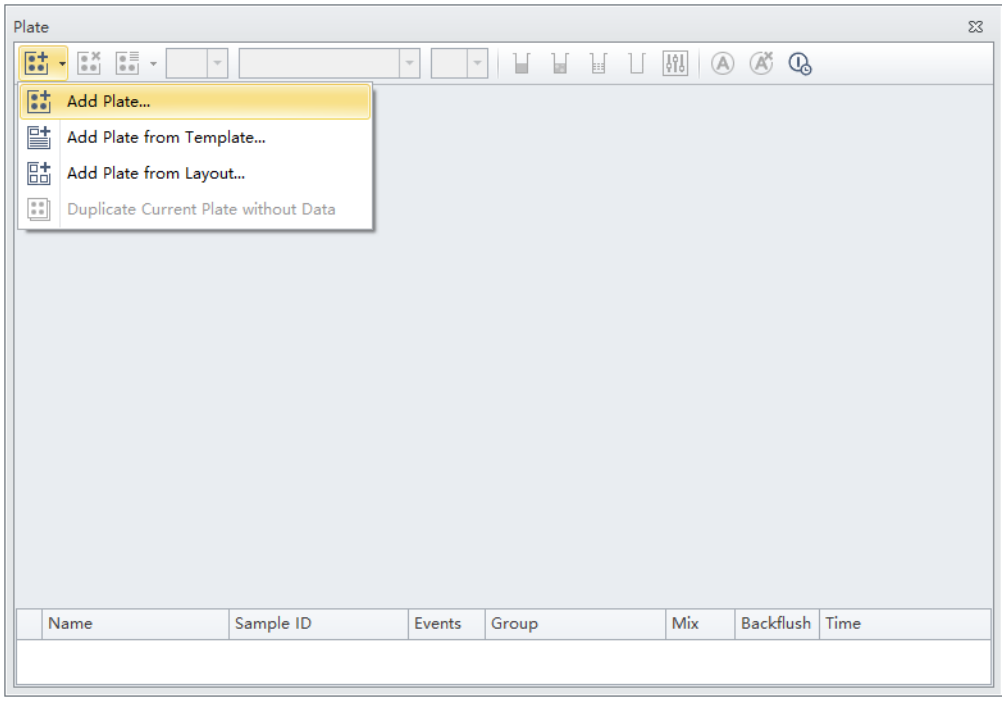
2 Select  . The Plate window appears.

[CytoFLEX LX Shown]

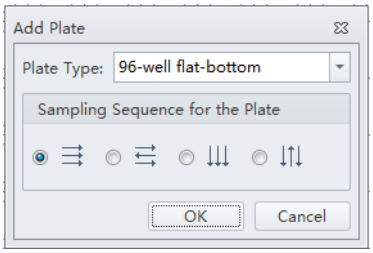


3 Select the Add Plate dropdown and select **Add Plate**.

[CytoFLEX LX Shown]



The Add Plate window appears.



NOTE Select **Add Plate from Template** to add a plate template with preset plate settings.

NOTE Select **Duplicate Current Plate without Data** to create a copy of the selected plate without data.

NOTE Select **Add Plate from Layout** to create a plate from a preset .csv file. The CSV file can be generated by selecting , or self-defined by other tools like Excel or Notepad.

When you create a CSV file, make sure to follow the example:

1. In the first row, enter the following column titles WellLabel, TubeName, SampleID, Group in sequence as shown in Figure 5.1. The table header cannot be customized.
2. In the WellLabel column, a maximum of 96 wells can be defined, beginning with A1-A12, B1-B12, and so on.

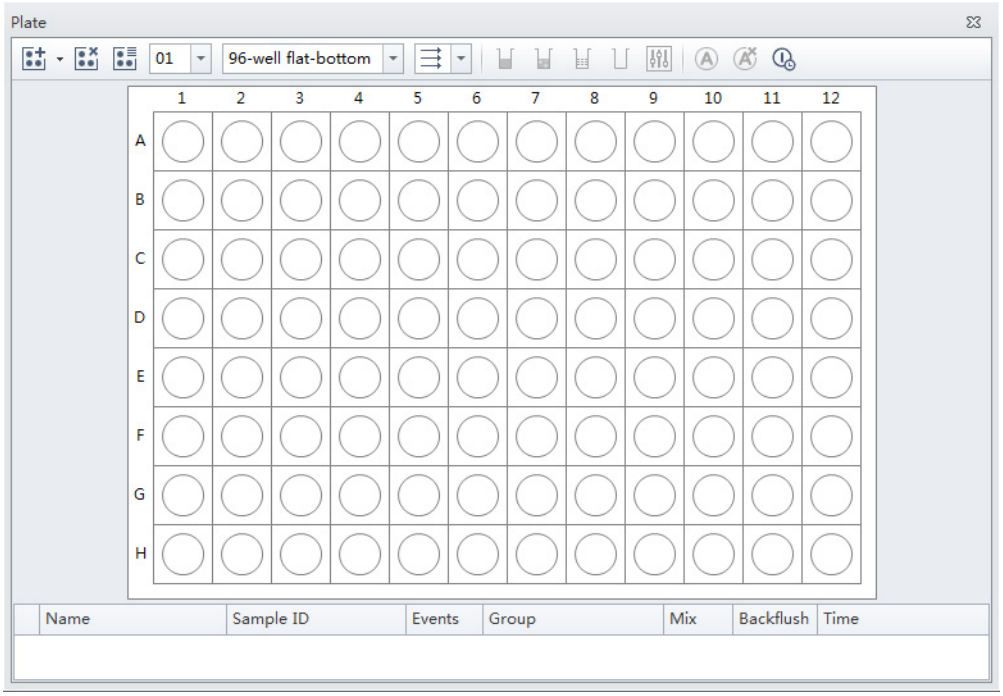
Figure 5.1 CSV template

	A	B	C	D
1	WellLabel	TubeName	SampleID	Group
2	A1			
3	A2			
4	A3			
5	A4			
6	A5			
7	A6			
8	A7			
9	A8			
10	A9			
11	A10			
12	A11			
13	A12			
14	B1			
15	B2			
16	B3			
17	B4			

4 Select the Plate type from the dropdown.

5 Select the direction of the workflow from the sampling sequence from the plate section of the window. The Plate window appears.

[CytoFLEX LX Shown]



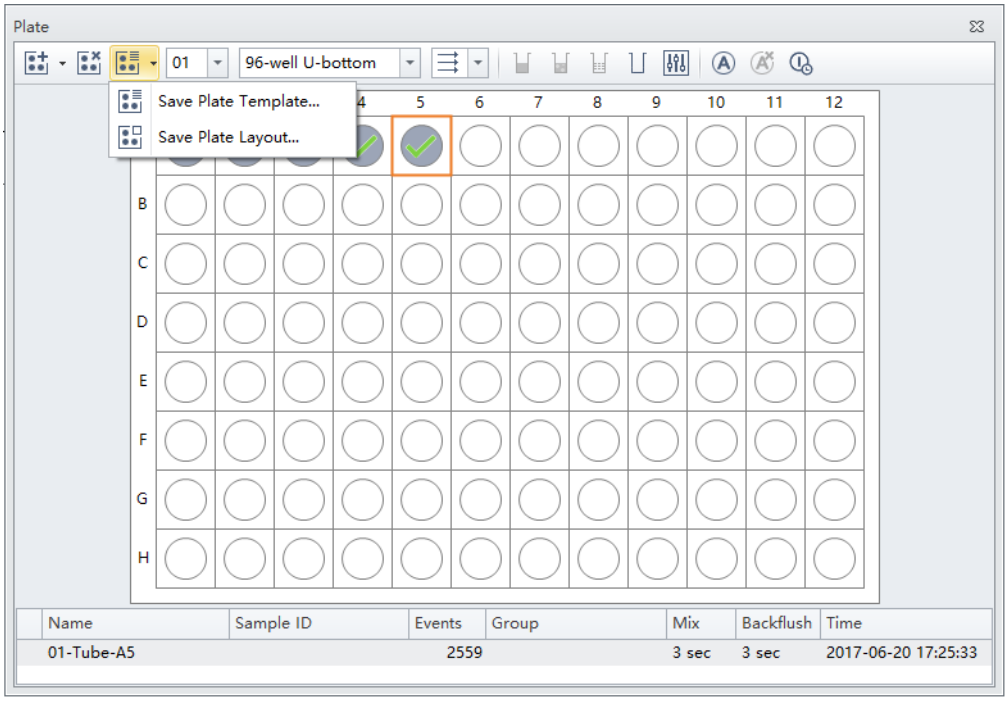
6 If you need to automatically turn off the cytometer after auto acquisition is complete **[CytoFLEX LX Only]**:

a. Ensure you set the last sample wells with the appropriate number of cleaning agent wells and deionized water wells.

b. Select .

NOTE A gray background ( ) indicates autoshutdown is disabled. A yellow background ( ) indicates autoshutdown is enabled.

NOTE Select **Save Plate Template** to save the acquisition settings as a template in SCTM format. Select **Save Plate Layout** to save the name, sample ID, or metadata as a layout in CSV format.

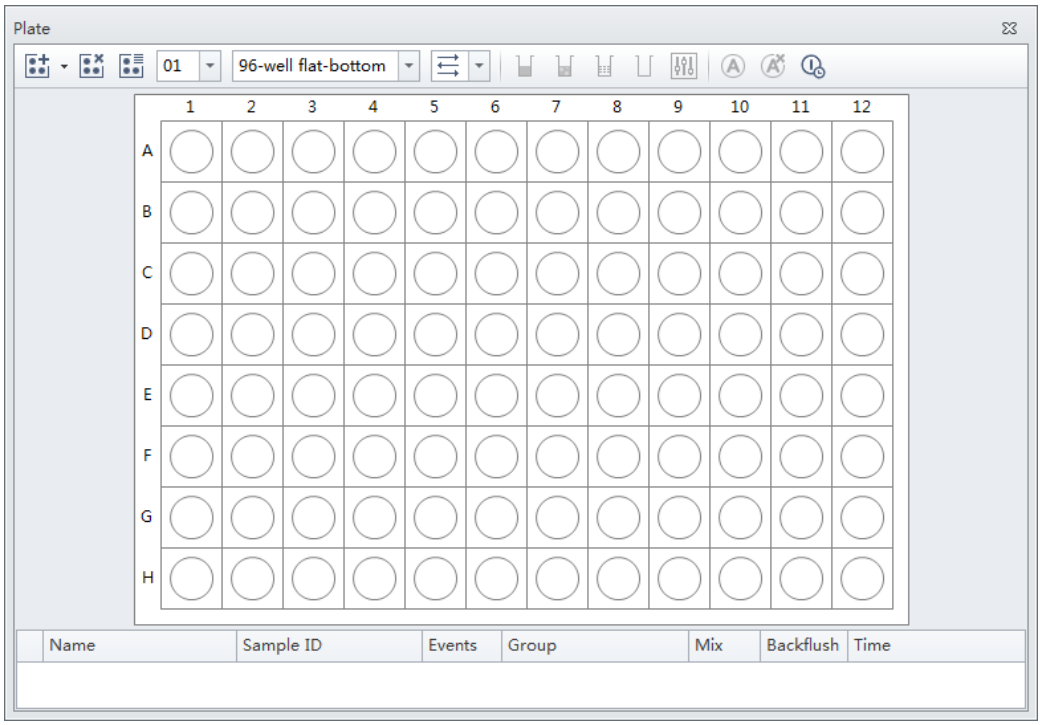


7 Select **OK**.

Setting Sample Wells

Once the plate protocol is created, the plate window appears. Refer to Figure 5.2.

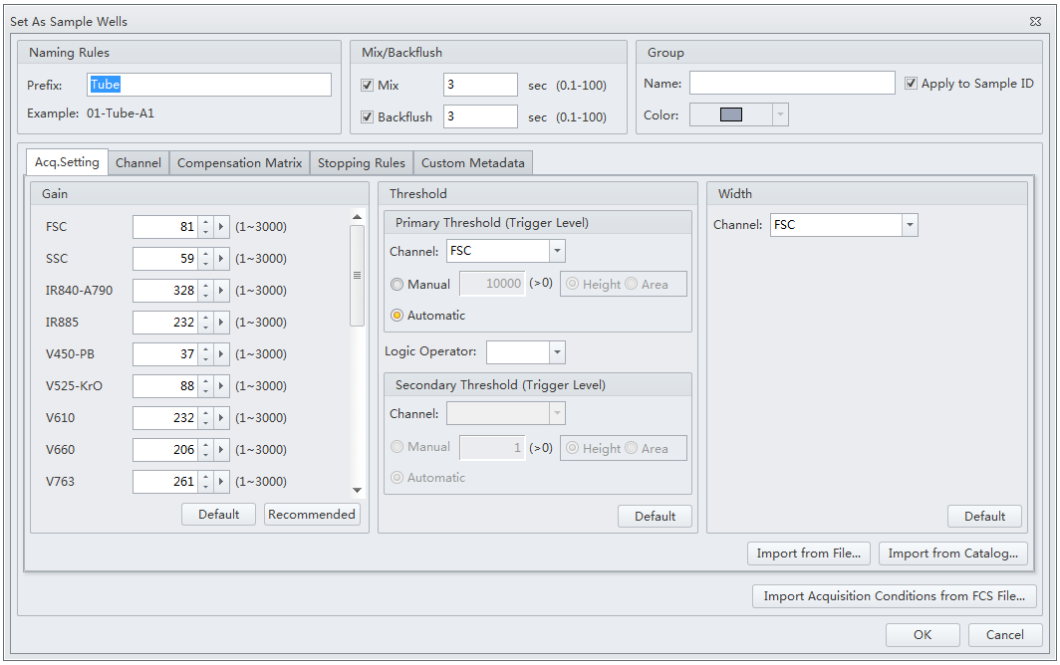
Figure 5.2 Plate Window [CytoFLEX LX Shown]



	Empty well
	The well with color is set as sample well, but is not set for Auto Record
	The well is set as sample well, and is ready for Auto Record. The number labeled at the right bottom shows the order of auto record.
	Cleaning Agent well
	Deionized water well
	A blue check mark means the data is acquired, not recorded.
	A green check mark means the data is recorded.
	A red cross mark means the data is recorded by Auto Record mode. But the acquisition is terminated abnormally. For example, the well is skipped or the acquisition is manually stopped.

1 Left-click and drag your mouse to highlight the desired wells or hold the Control key and select each desired well.

2 Select  or right-click the selected wells and select **Set As Sample Wells**. The Set As Sample Wells window appears.



NOTE Select  or right-click and select **Set as Empty Wells** to reset selected wells as empty.

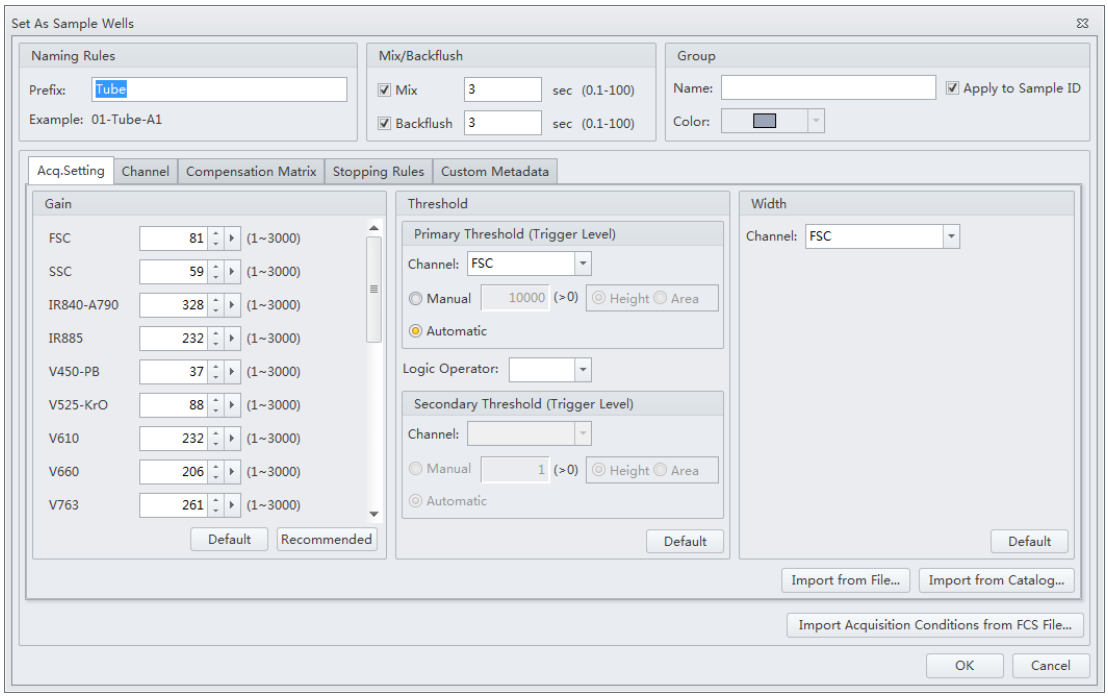
3 Enter the name in the Prefix box in the Naming Rules section of the window.

4 Select the desired Mix and Backflush duration from the Mix/Backflush section of the window.

5 Enter the Group Name in the Name box in the Group section of the window.

6 Select the sample well color using the color dropdown under the Group section of the window.

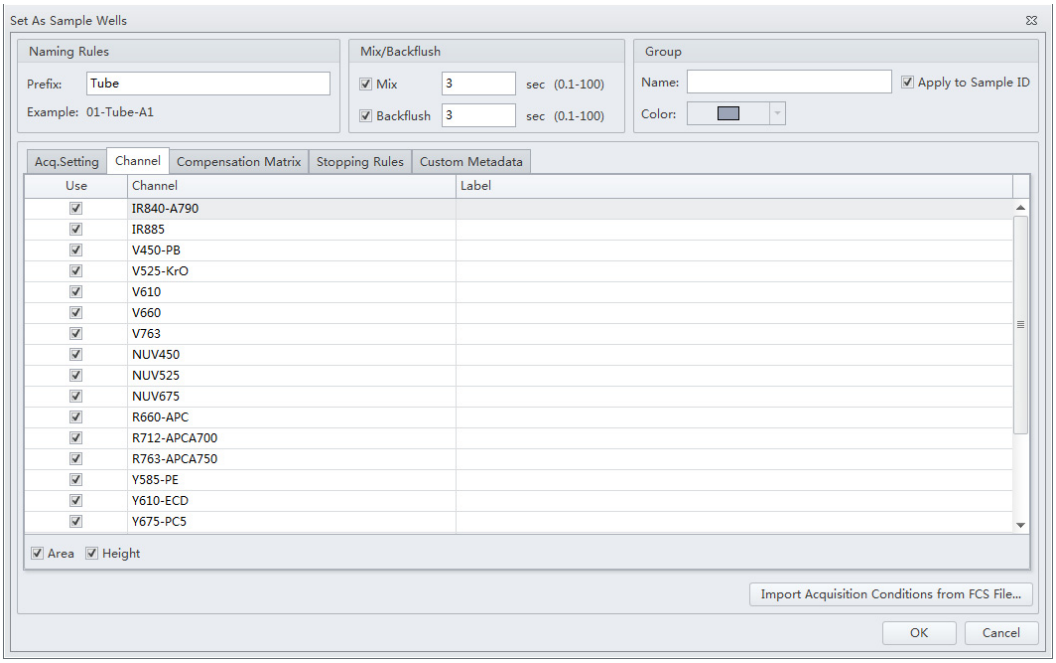
7 Select the desired acquisition settings under the Acq. Setting tab.



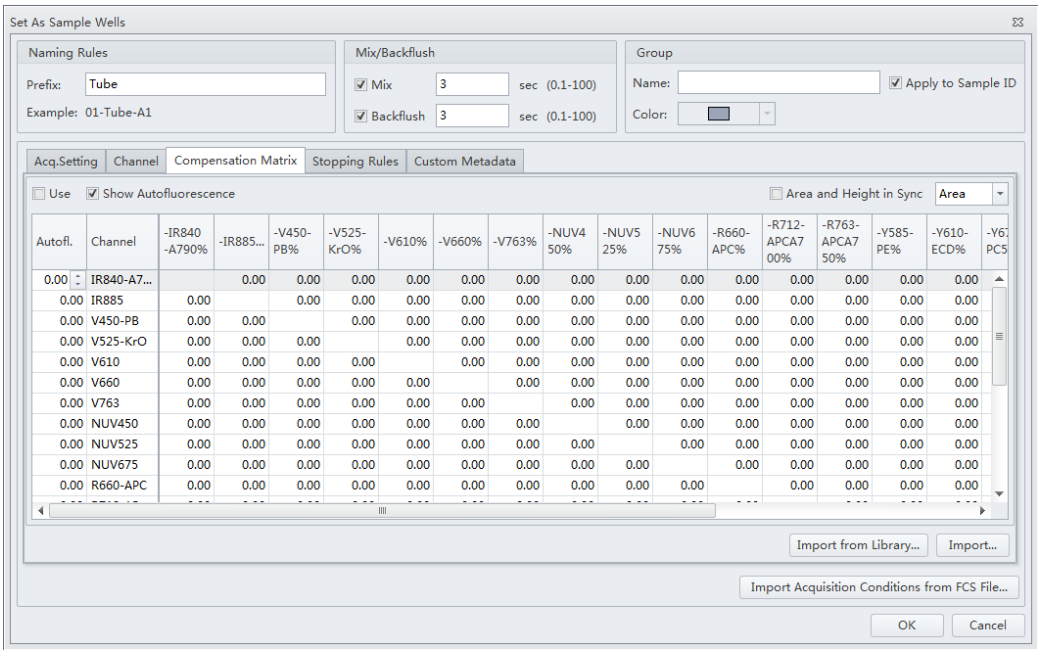
NOTE Select **Import from File** to import the settings from a FCS file.

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

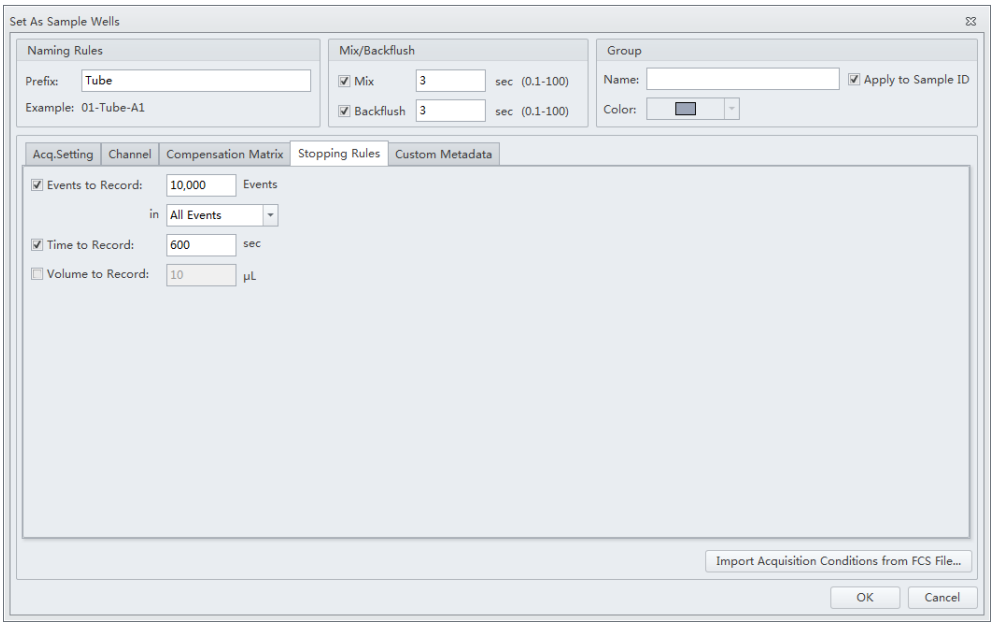
8 Select the channels and create label names under the Channel tab.



9 Set compensation under the Compensation Matrix tab. Refer to CHAPTER 6, Compensation for detailed instructions on setting compensation.

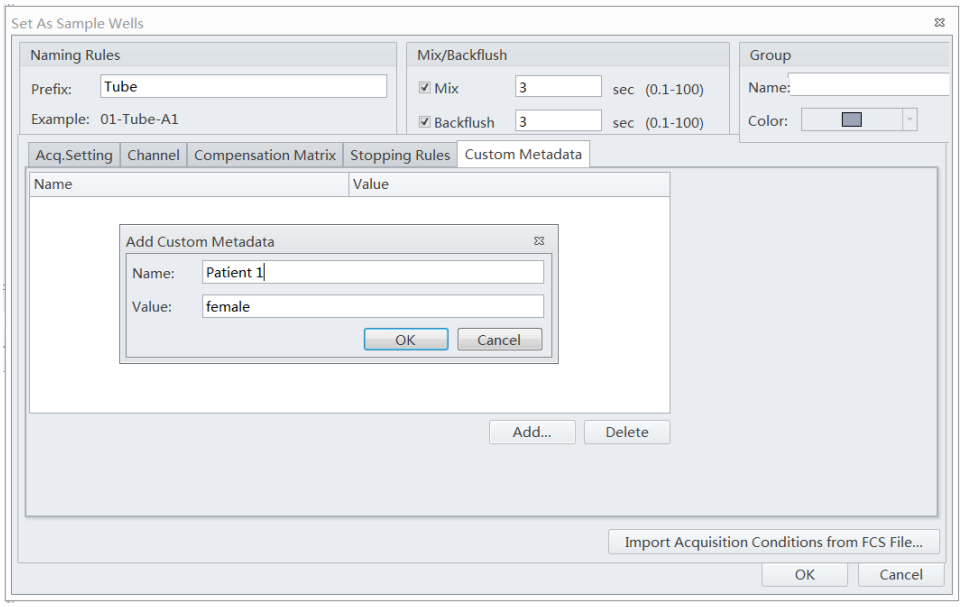


10 Select Events to record, time to record, or volume to record under the Stopping Rules tab.



NOTE Beckman Coulter recommends setting an acquisition time limit to stop the acquisition if the event limit cannot be reached.

11 Create the desired name and value under the Custom Metadata tab.

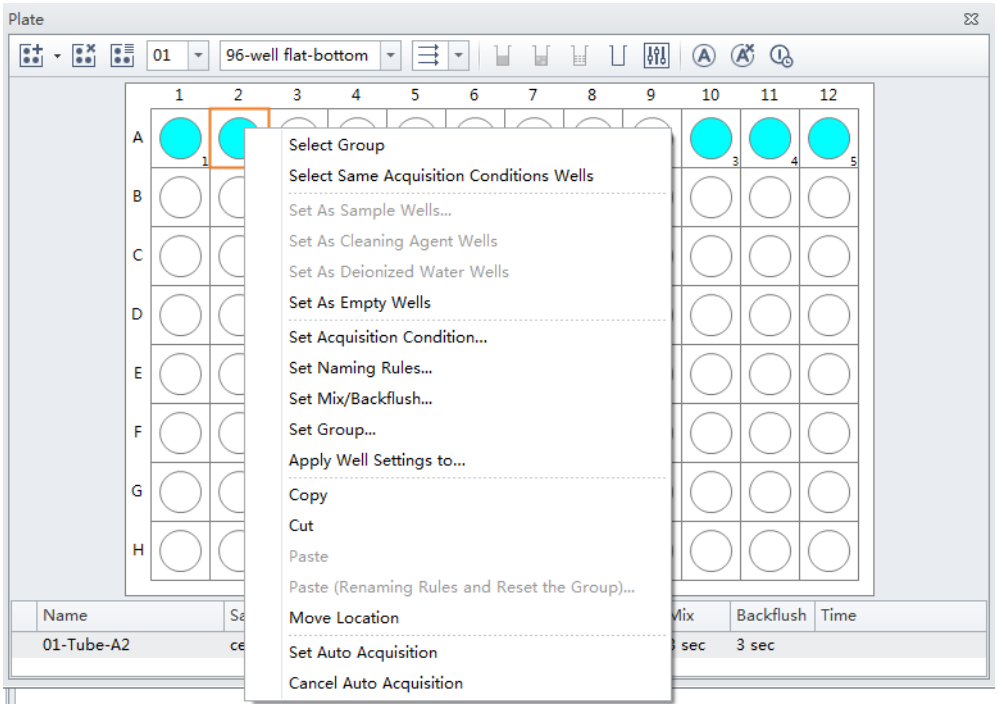


12 Select **OK**.

Modifying Well Settings

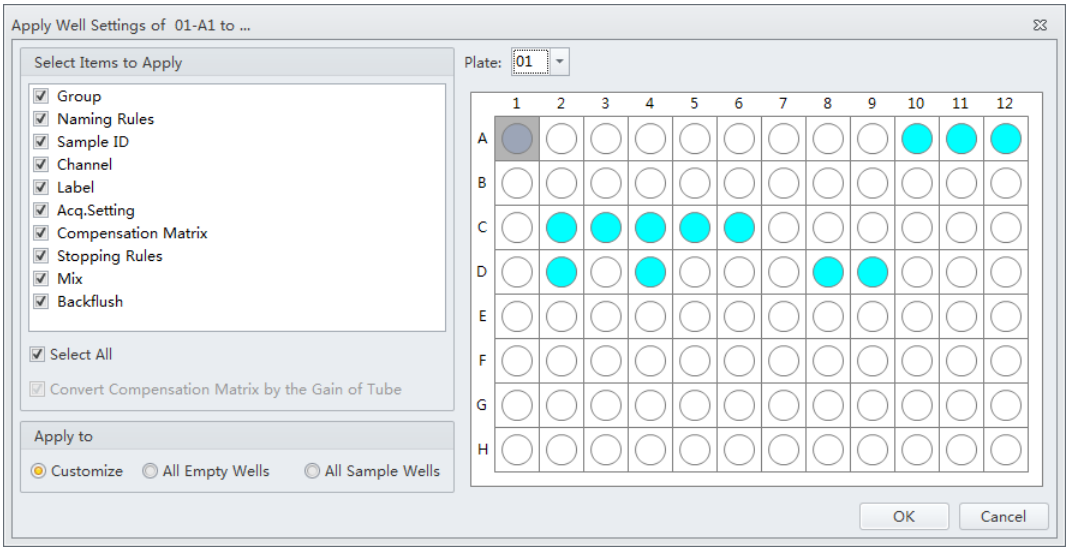
If any well settings require modification, right-click the sample well and select the setting to change.

[CytoFLEX LX Shown]



Applying Existing Well Settings to Additional Wells

1 Right-click the well with the desired settings and select **Apply Well Settings to.** The Apply Well Settings of window appears.



NOTE The *Convert Compensation Matrix by the Gain of Tube* checkbox is only available when Acq. Setting is deselected. Selecting the *Convert Compensation Matrix by the Gain of Tube* checkbox converts the compensation matrix automatically by the gain of the well selected.

2 Select the wells to apply the well settings to.

NOTE Select the **Customize** option to select individual wells to apply the settings to. Select the **All Empty Wells** option to apply the settings to all remaining empty wells. Select the **All Sample Wells** option to apply the settings to all existing colored sample wells. You cannot apply settings to wells that already contain data.

3 Checkmark which settings to apply from the Select Items to Apply section of the window.

NOTE

- If Group and Name Rule are selected, the settings can be applied to any wells, and empty wells will be set as sample wells after applied.
- If Group and Name Rule are not selected, the settings can only be applied to sample wells.

Copying, Cutting, and Pasting Wells

1 Left-click and drag your mouse to highlight the desired wells or hold the Control key and select each desired well.

2 Right-click and select **Copy** or **Cut**.

NOTE When **Copy** or **Cut** is selected, the well displays as follows: 

3 Right-click an empty well and select **Paste**.

NOTE The same number of wells will paste in the same orientation the wells were selected.

Moving the Location of a Well

1 Right-click the desired well to move and select **Move location**.

NOTE When **Move location** is selected, the well displays as follows: 

2 Select the new well location. The well will automatically move to the new selected location.

Running Samples

IMPORTANT Ensure the sample plate is loaded properly before acquiring the samples.

1 To run a single well:

a. Select the well in the Plate window.

b. Select **Run** to prompt the system to begin sample aspiration.

NOTE Acquisition settings can be adjusted during acquisition.

c. Select **Record** to save the data.

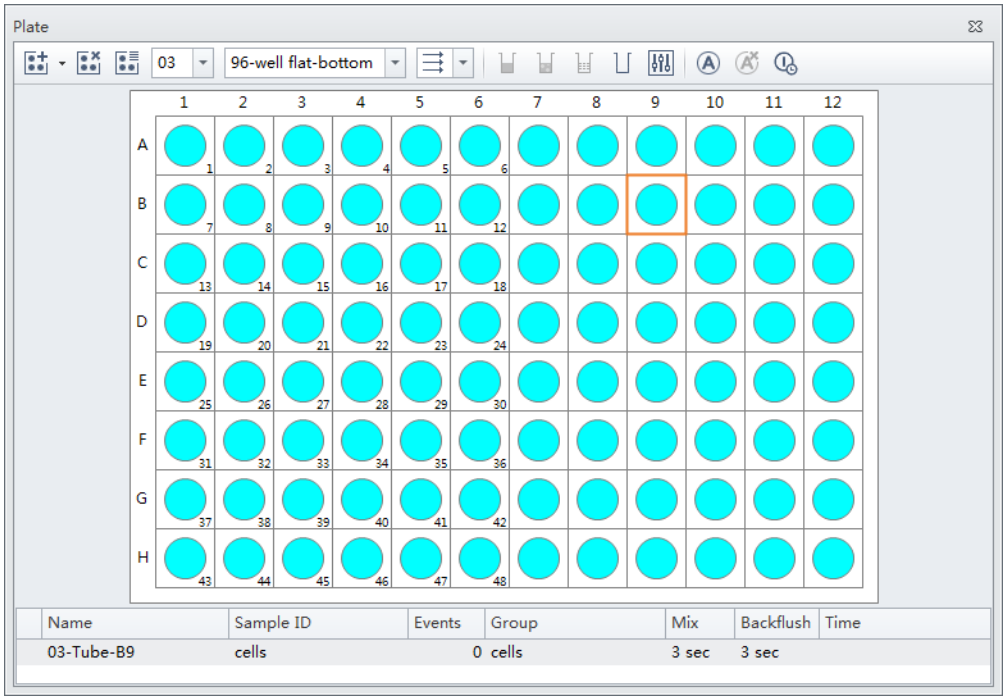
d. Select **Eject** to prompt the plate loader to eject.

To run a set of wells:

a. Select the desired wells.

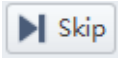
b. Select  or right-click and select **Auto Record** to set the selected wells for auto record. Number labels appear in the bottom right corner of each well set for auto record. Sample acquisition occurs in the order indicated by the numbers.

[CytoFLEX LX Shown]



NOTE Select  to remove the auto record setting from the selected wells.

2 Select  to begin sample acquisition.

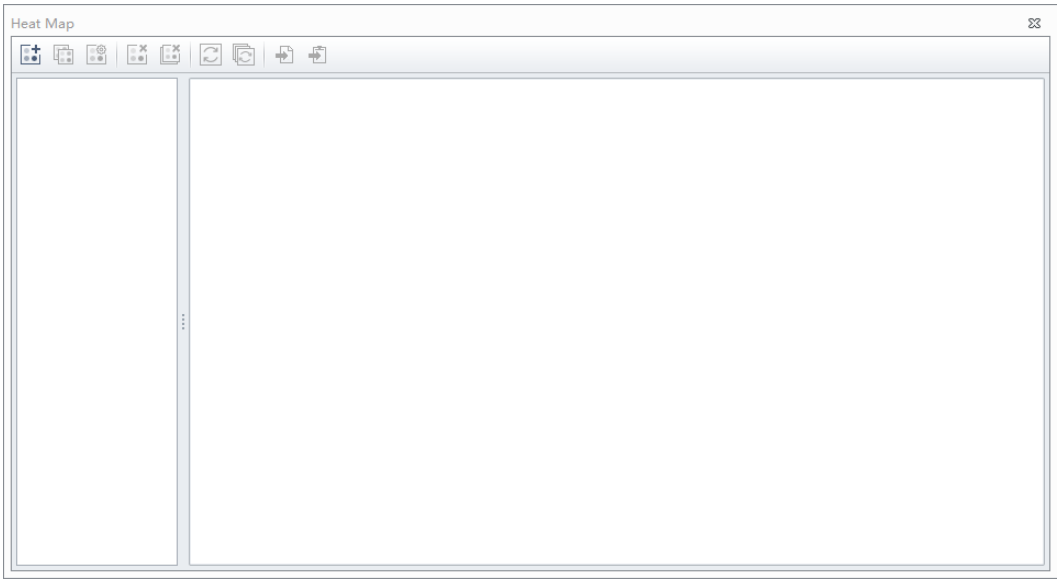
NOTE Select  to skip the current well and move to the next well for acquisition.

NOTE Select  to pause acquisition. The software completes the current sample well acquisition and pauses when the current well is complete. The plate can be loaded and unloaded while the sample acquisition is paused.

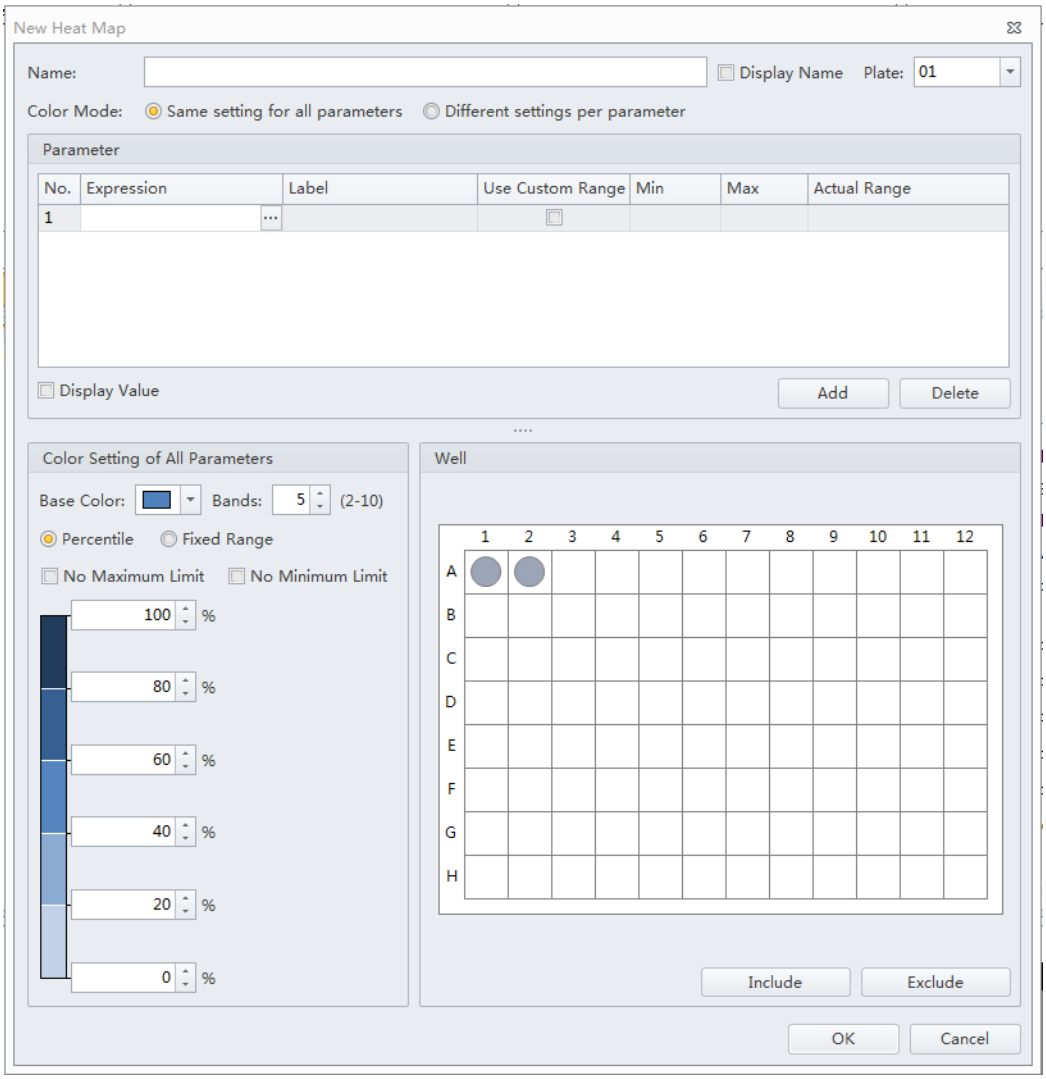
NOTE Select  to continue the acquisition.

Creating a Heat Map

1 Select  from the Tube management controls (refer toTest Tubes in CHAPTER 2, Using the CytExpert Software CHAPTER 5, Data Acquisition and Sample Analysis). The Heat Map window appears.



2 Select  from the Heat Map window. The New Heat Map window appears.



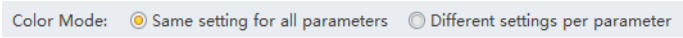
NOTE You must have at least one plate that contains data in at least two wells to create a new heat map.

NOTE Select **Display Value** to display the value within the heat map well on the Heat Map window. Display Value is only selectable when using a single parameter.

3 Enter the heat map name, select the Display Name checkbox if you want the name to display in the heat map view, and select the heat map data set from the Plate drop down menu.

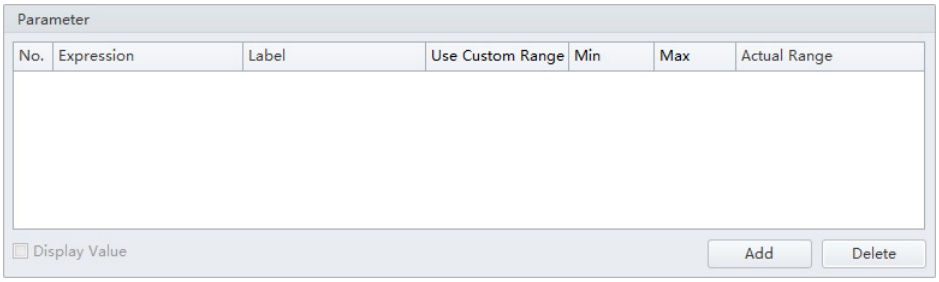
NOTE The plate drop down menu only displays plates that contain data.

4 Select the desired color mode.



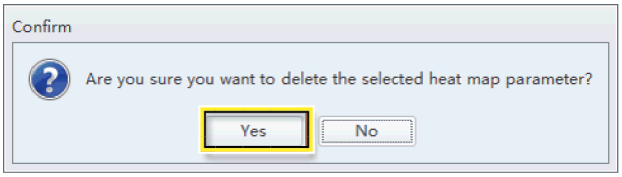
NOTE Select **Same setting for all parameters** to use the same color for all parameters.
Select **Different setting per parameters** to use different colors for different parameters.

5 Select Add to add a parameter item to the Parameter section of the New Heat Map window.



NOTE A maximum of 6 parameters can be added to a single heat map.

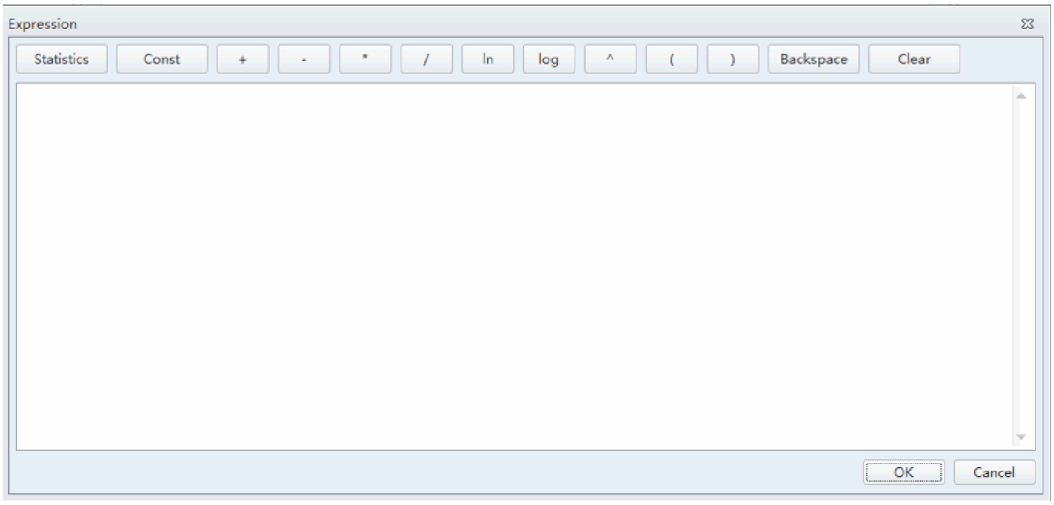
NOTE Select a parameter then select Delete to delete a parameter. Select **Yes** when the following message appears.



6 Change the Parameter elements in the Parameter section of the New Heat Map window.

a. Change the Label name if needed.

b. Select ... from the Statistic Expression section of the Parameter list. The Expression window appears.

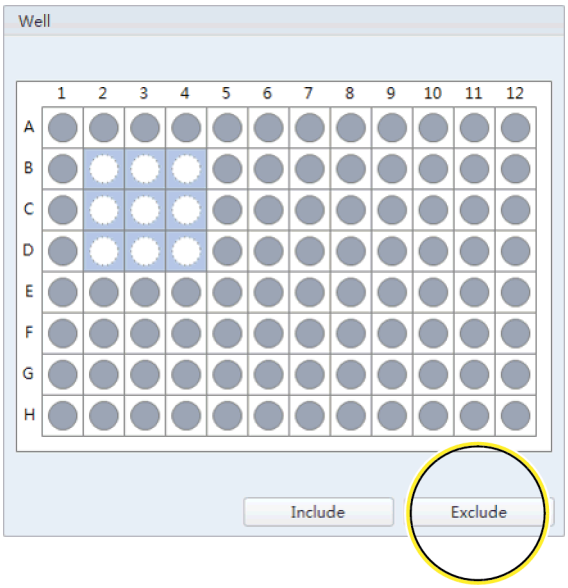


c. Enter the desired expression for the selected parameter then select **OK**. The Actual Range displays in the Parameter section of the Heat Map window.

d. Select the **Use Custom Ranges** checkbox if needed and enter the Min and Max ranges.

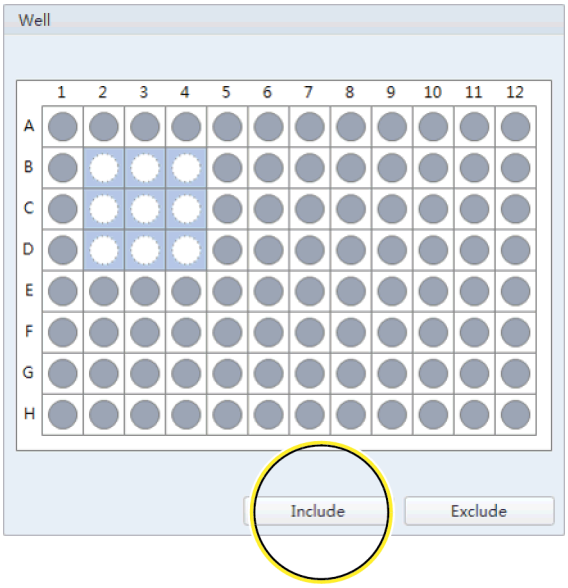
NOTE The Actual Range displays when the statistics parameter can be calculated.

7 Select any wells that should be excluded from the heat map in the Wells section of the New Heat Map window then select Exclude.



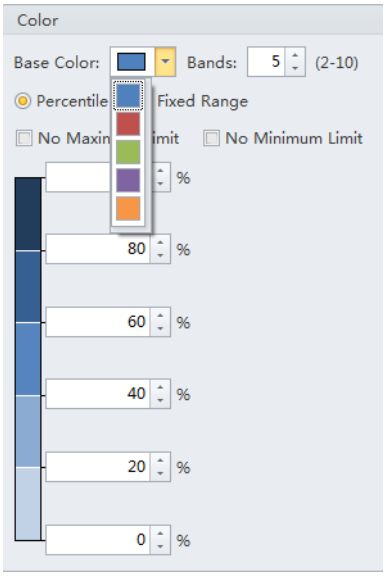
NOTE  designates that a well is included. All wells are included by default.

NOTE  designates that a well is excluded. Select any wells that should be included and select Include.

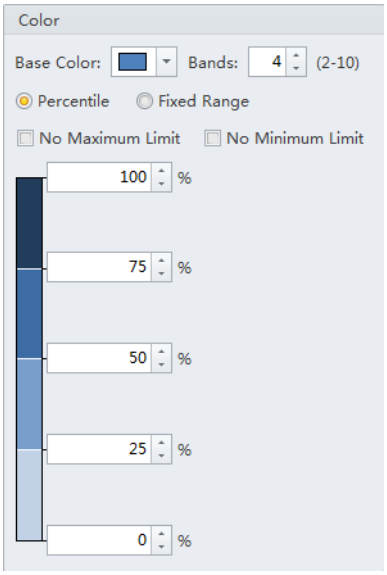


8 Edit the color elements in the Color section of the New Heat Map window.

a. Select a color from the base color drop down menu.



b. Select the number of color bands desired from the Bands drop down menu. The window refreshes to display the appropriate number of bands.



NOTE You can choose between 2 and 10 color bands.

c. Select **Percentile** to assign colors based on a percentage range.

NOTE If Use **Custom Range** is selected, the percentile is calculated according to “Min” and “Max”. If **Use Custom Range** is not selected, the percentile is calculated according to “Actual Range”.

Or

Select **Fixed Range** to assign colors based on a fixed range specified by the user. The heat map is created directly based on the result of the expression. The color of the heat map displays according to the legend range.

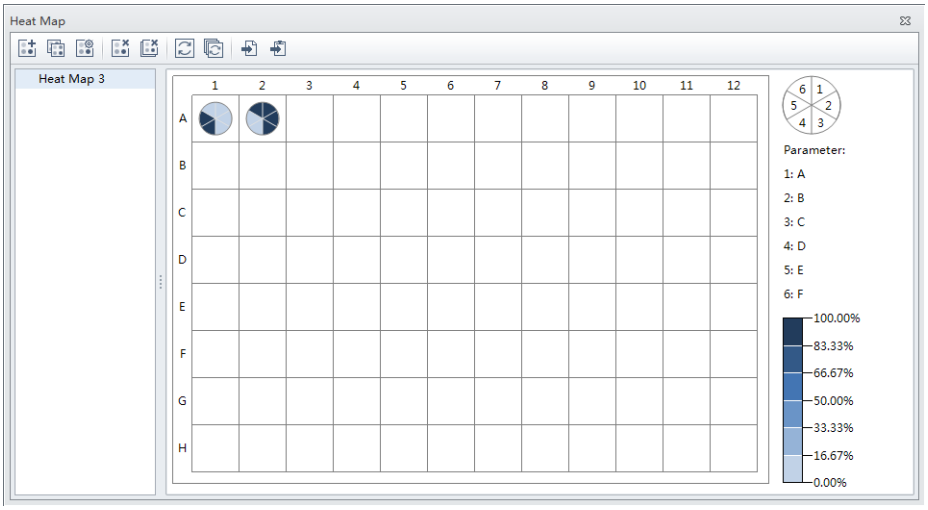
NOTE Fixed Range can only be used with one parameter.

NOTE If **Different settings per parameter** is selected, repeat Steps a-c to assign the color setting to each parameter.

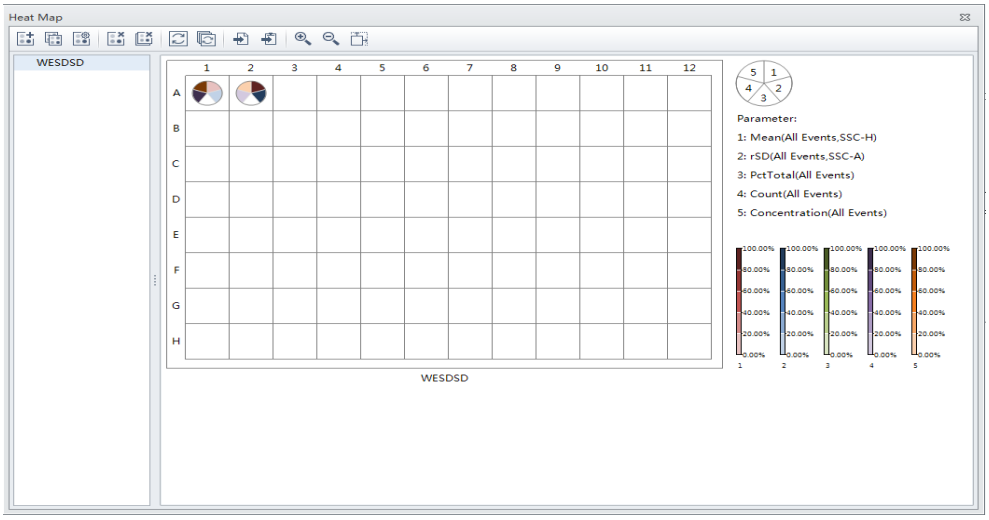
---

9 Select **OK**. The New Heat Map window closes to display the Heat Map window.

Heat Map with 6 Parameters [Same setting for all parameters]

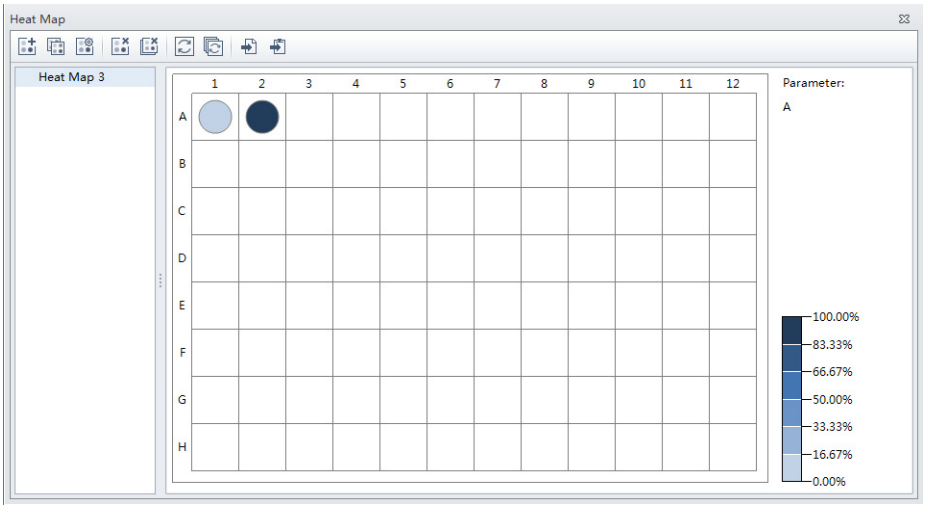


Heat Map with 6 Parameters [Different settings per parameter]



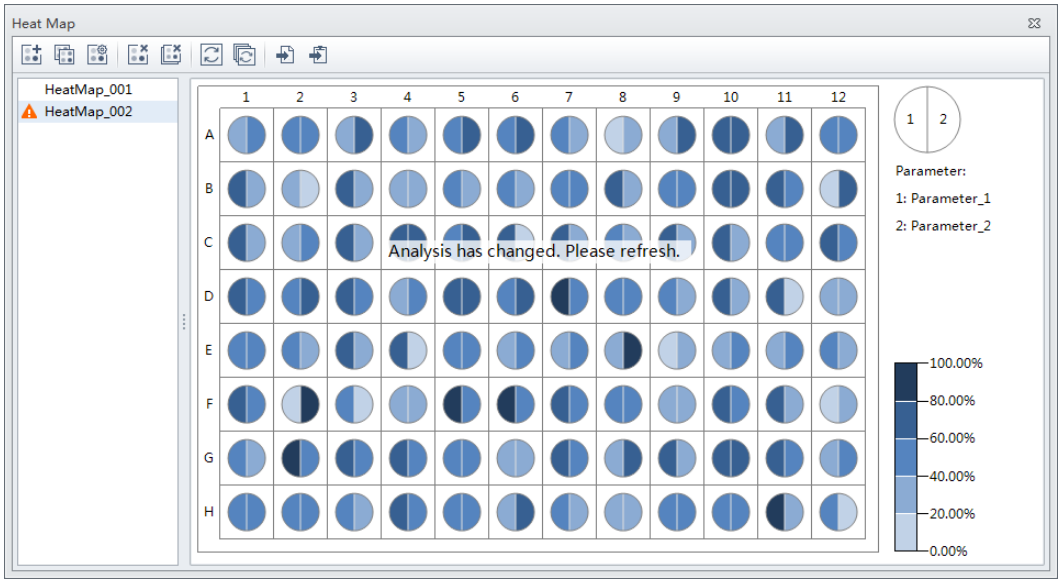
NOTE When viewing more than one parameter, the parameter location relative to the pie chart is visible in the top, right corner of the Heat Map screen.

Heat Map with 1 Parameter



Refreshing a Heat Map

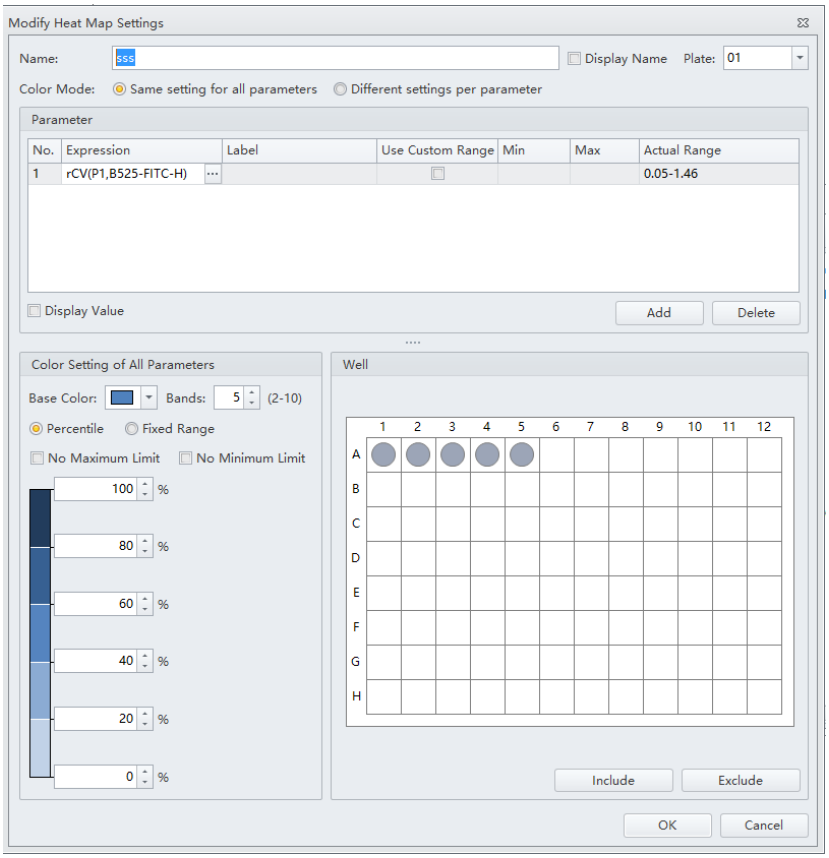
When data displayed in a heat map is no longer current, the  symbol appears next to the heat map name and the message *Analysis changed. Please refresh.* appears on the Heat Map window.



Select  from the heat map toolbar to refresh the analysis.

Modifying Existing Heat Map Settings

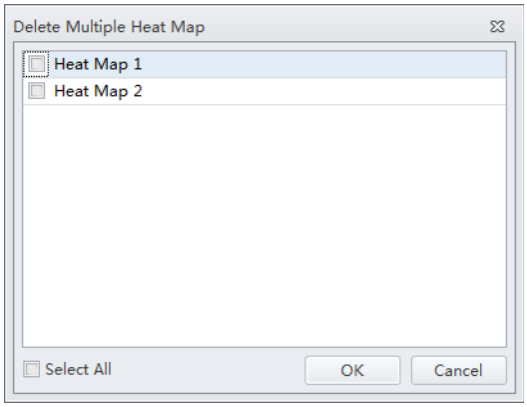
Select  from the heat map toolbar to modify existing heat map settings. The Modify Heat Map Settings window appears.



Deleting an Existing Heat Map

To delete a single heat map, select the heat map to be deleted from the list of heat maps in the Heat Map window then select  from the heat map tool-bar.

To delete multiple heat maps, select  from the heat map toolbar. The Delete Multiple Heat Maps window appears.



Select the heat maps to be deleted then select **OK**.

NOTE The Select All checkbox allows you to delete all of the heat maps listed.

Exporting a Heat Map

Heat maps can be exported as a graphics file (.bmp or .emf) or to a clipboard (.bmp).

To export a heat map as a graphics file, select  from the heat map toolbar.

To export a heat map to a clipboard, select  from the heat map toolbar.

Load Sample and Record Data

Before Running Samples

⚠ CAUTION

Risk of erroneous results if the Cytometer has been idle for an extended period of time. Perform a prime if the system has been idle for an extended period of time (see Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures.)

1. Run the Daily Startup procedure.
2. Run the Instrument Quality Control and Standardization procedure.
3. Create an experiment. Refer to Creating an Experiment.
4. Verify mixer settings. Refer to Changing Sample Mixing and Backflush Settings in CHAPTER 11, Replacement/Adjustment Procedures.
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, Data Acquisition and Sample Analysis.

Verifying, Selecting, Editing, and Creating Detector Configuration

⚠ CAUTION

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.

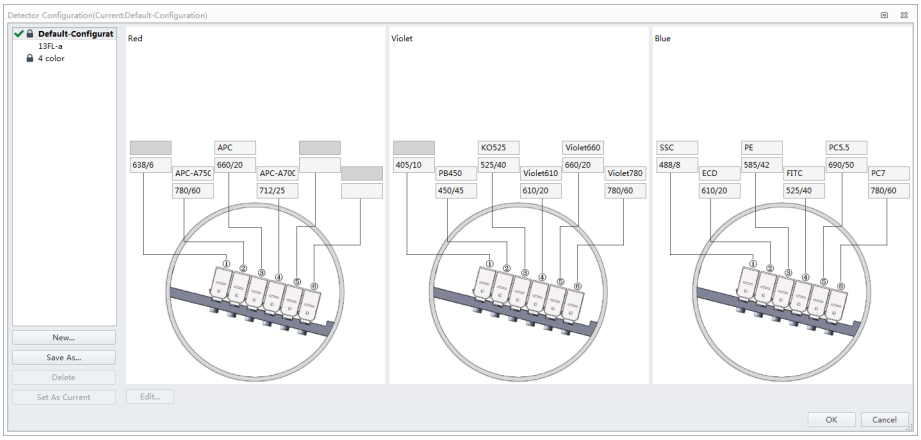
1 Select **Detector Configuration** in the Cytometer menu to verify the correct detector configuration is selected. To change the configuration:

a. Select the desired configuration.

b. Select **Set as Current**.

A green checkmark appears in front of the selected configuration.

[CytoFLEX Shown]



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.

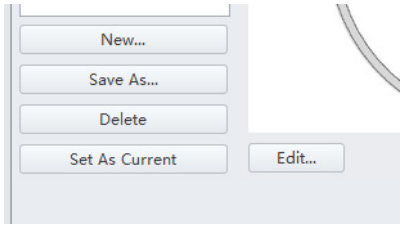
2 Select **OK** to close the Detector Configuration screen.

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.

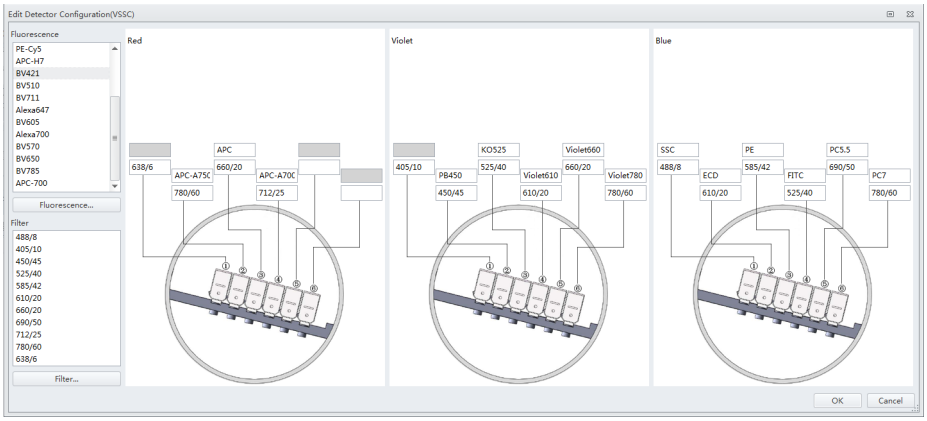
4 If a saved configuration requires changes, edit that configuration.

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.

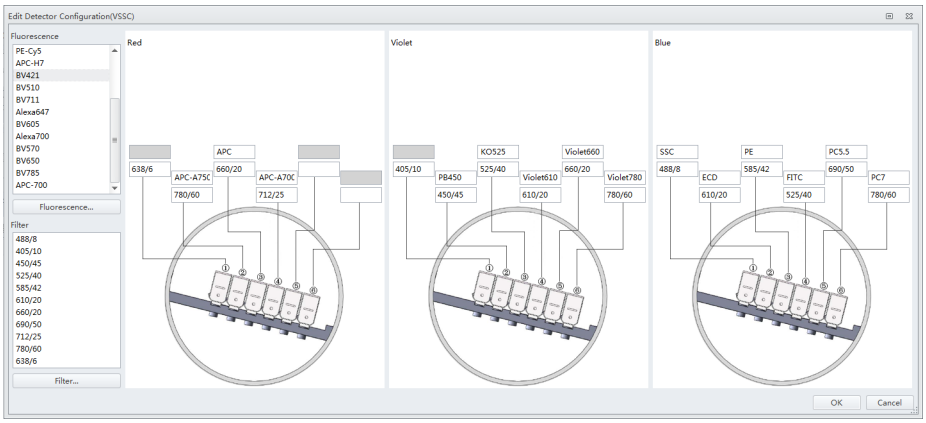
a. Select **Detector Configuration...** in the Cytometer menu.

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.

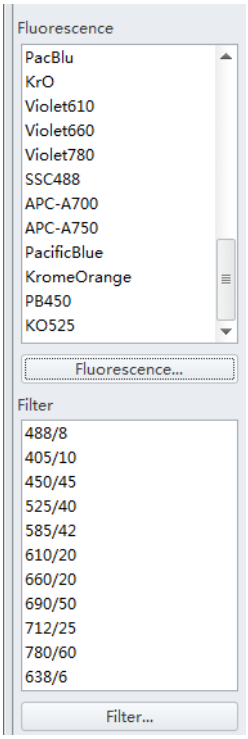


e. Customize the new configuration. Channels with a white background can be edited. Drag the names of the appropriate fluorescence channels and optical filters on the left to the correct channels.

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**.

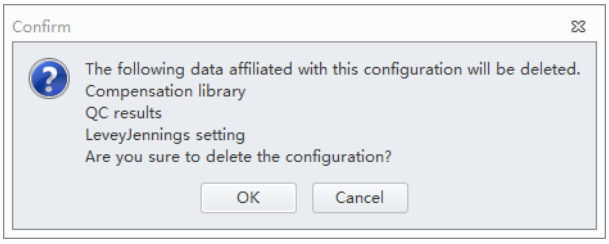
8 Select the appropriate configuration.

9 Verify that the correct optical filters are installed in the Cytometer 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**.



Setting Up Violet Side Scatter (VSSC) Channel

For microparticles, 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.

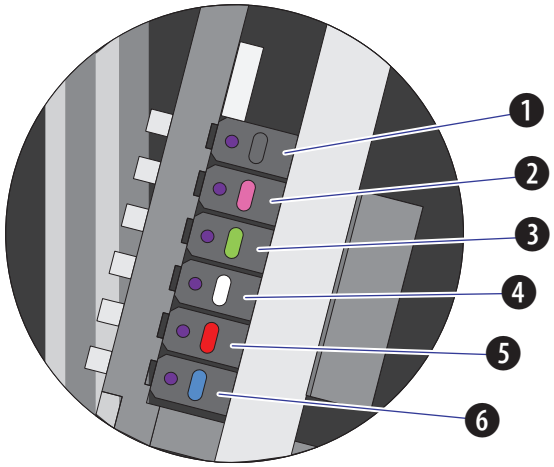
NOTE Since the total available channel numbers remain the same when the VSSC channel is used, the number of fluorescent channels in the violet WDM is reduced by 1 channel.

⚠ 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 (see 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.

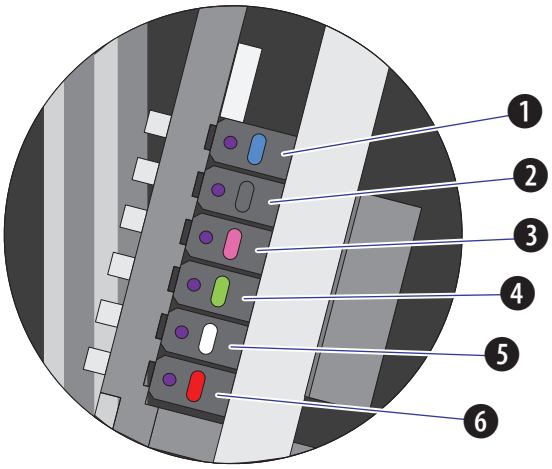


1. Position 1
2. Position 2
3. Position 3
4. Position 4
5. Position 5
6. Position 6

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.

2 Place the third filter in position 1, the 405-nm filter in position 2, and the 450-nm filter in position 3.

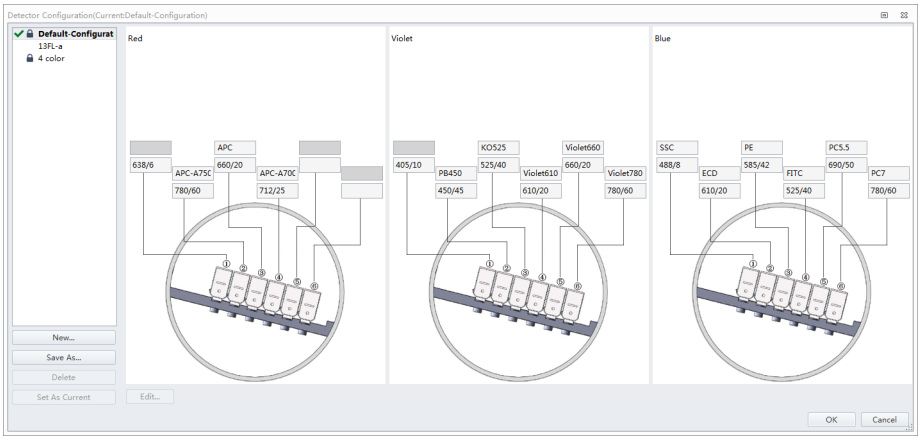
NOTE For the Violet WDM, Beckman Coulter recommends placing the filters in sequential order from the shortest wavelength to the longest wavelength in positions 2 to 6. Position 1 will always contain the unused filter.



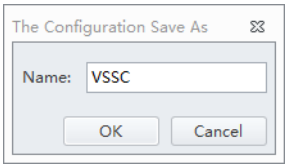
1. Position 1
2. Position 2
3. Position 3
4. Position 4
5. Position 5
6. Position 6

3 Start the CytExpert software. Refer to Logging Into the Software in CHAPTER 3, Daily Startup.

4 Select **Detector Configuration** from the Cytometer menu. The Detector Configuration window appears.

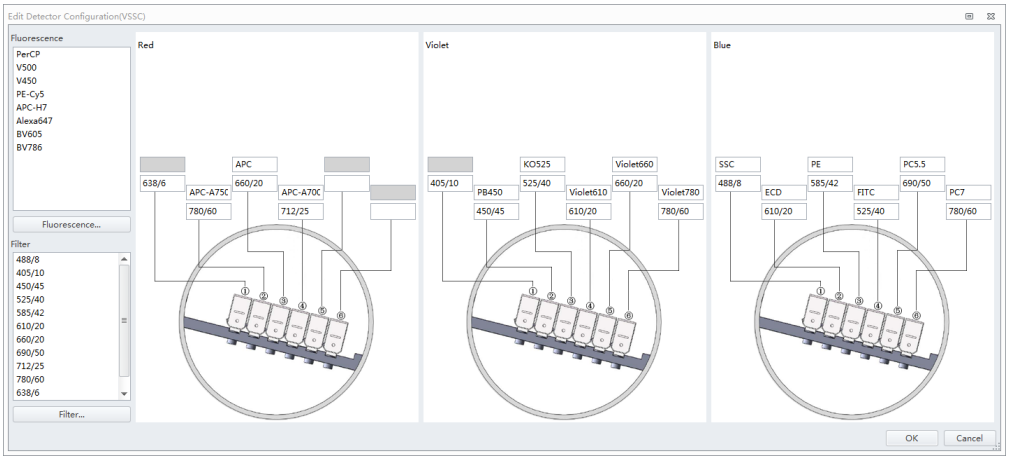


5 Select the Default Configuration and select **Save As**. The Configuration Save As window appears.



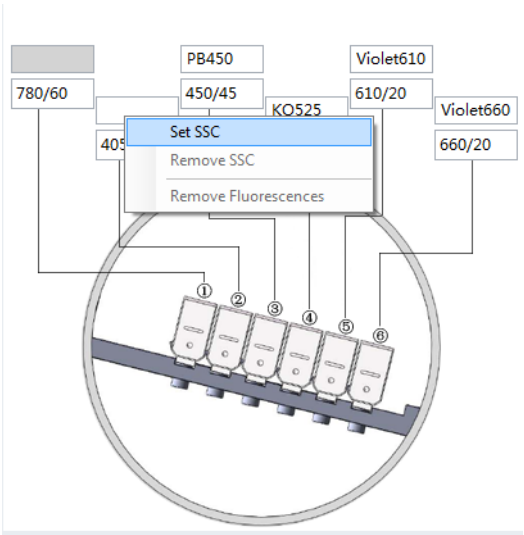
6 Name the new configuration VSSC and select **OK**.

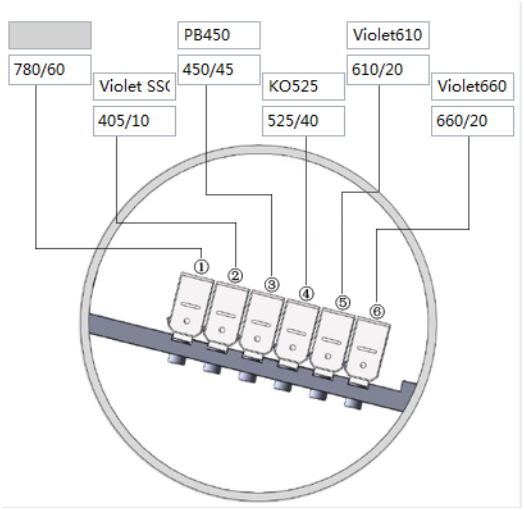
7 Select the VSSC configuration and select **Edit**. The Edit Detector Configuration window appears.



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.

Sampling and Collecting Data

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 toImporting and Exporting Compensation in CHAPTER 6, Compensation.

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.

3 Mix the sample tube intended for testing.

4 Ensure that the sample tube holder is in the sample loading position (see Figure 1.13). If the sample tube holder is not in the sample loading position, select **Initialize**.

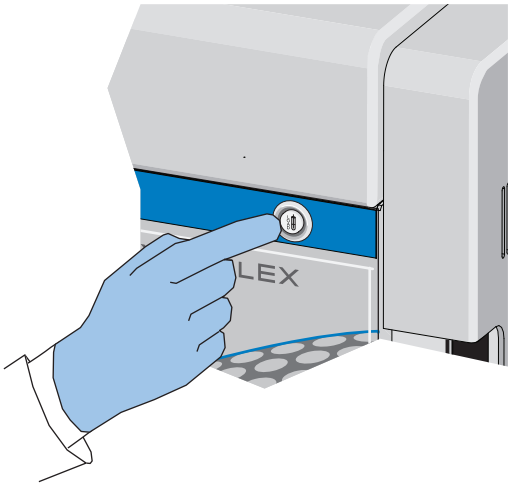
⚠ WARNING

Risk of biohazardous contamination. When using 1.5-mL and/or 2-mL sample tubes, always cut the cap off and do not exceed 300-µL sample volume. Running samples with a cap attached to the sample tube or with volumes exceeding 300-µL can result in sample splashing.

5 Place the sample tube in the sample tube holder.

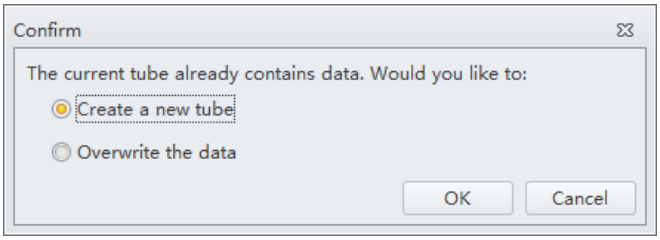
6 Select the desired acquisition parameters (Events/Time to Record/Volume to Record and Sample Flow Rate) on the left side of the screen.

NOTE You can also push the load button on the front of the instrument to automatically start the run and record the data.



7 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:



- **Create new tube.** Saves the current tube and creates an additional tube.
- **Overwrite the data.** Overwrites the current tube data with new data.

8 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.

9 Adjust the gain settings. Refer to Adjusting the Gain.

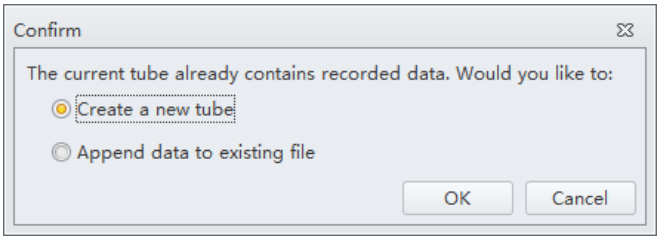
10 Adjust the threshold settings. Refer to Adjusting the Threshold.

11 Adjust the Acquisition conditions. Refer to Setting Collection Conditions.

12 Select **Record** to save the data.

Wait for the saving process to finish. The sample tube holder returns to the sample loading position (see Figure 1.13).

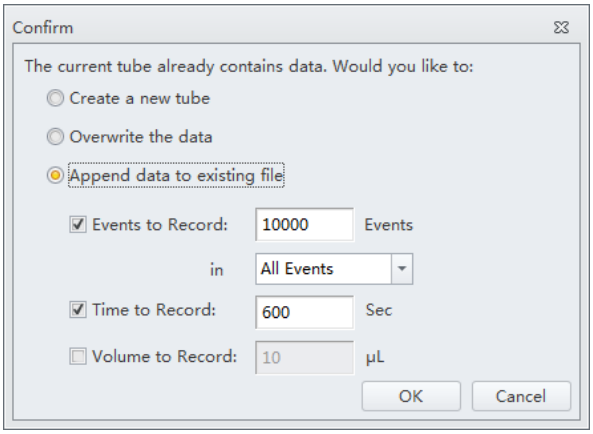
NOTE When you select a tube that contains recorded data, as indicated by the green tube in the test tube section of the screen, the following message appears:



- **Create new tube.** Creates a new tube in the test tube section of the screen for the data.

- **Append data to existing file.** Adds new data to the existing data.

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.

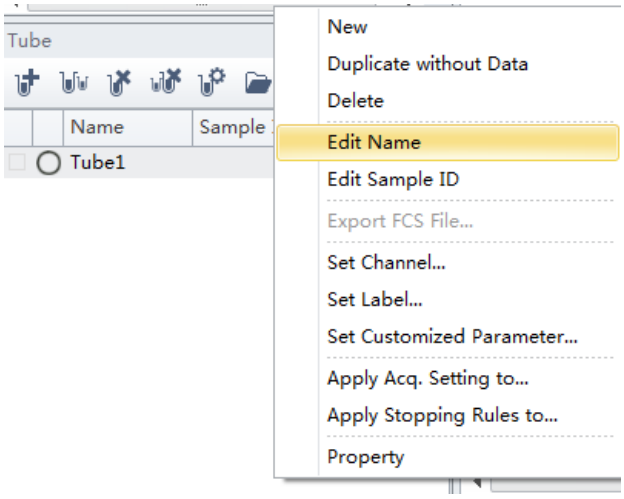
13 Repeat steps 1-12 until all sample tube data required for testing has been collected.

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 in CHAPTER 10, Cleaning Procedures to flush out the sample probe. If you are still unable to clear the sample probe, contact us.

Configuring Acquisition Settings

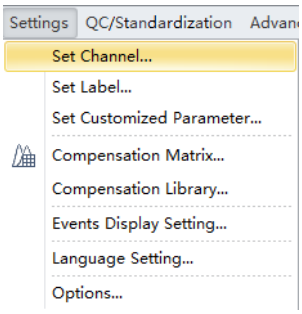
Changing the Tube Name

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.



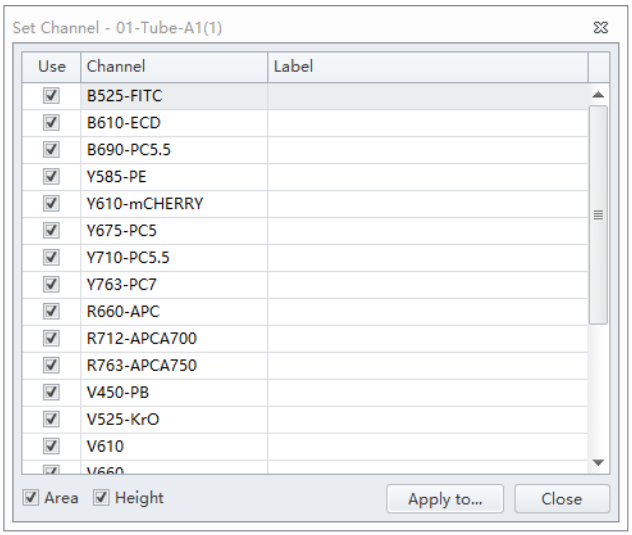
Setting the Channel and Label

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



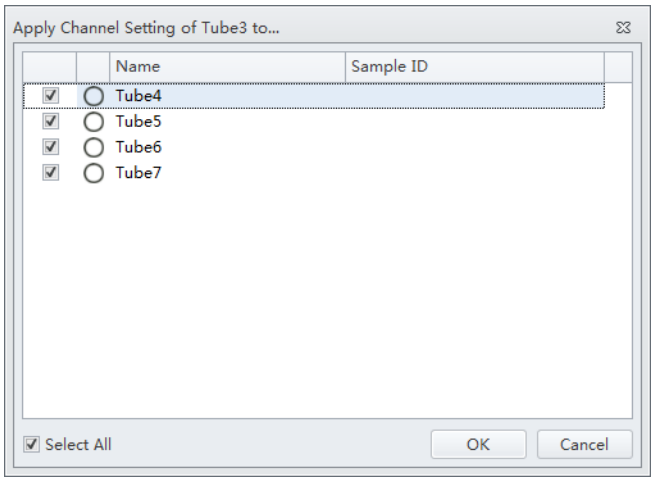
2 In the Set Channel window, modify which channels are used and how they are displayed.

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.



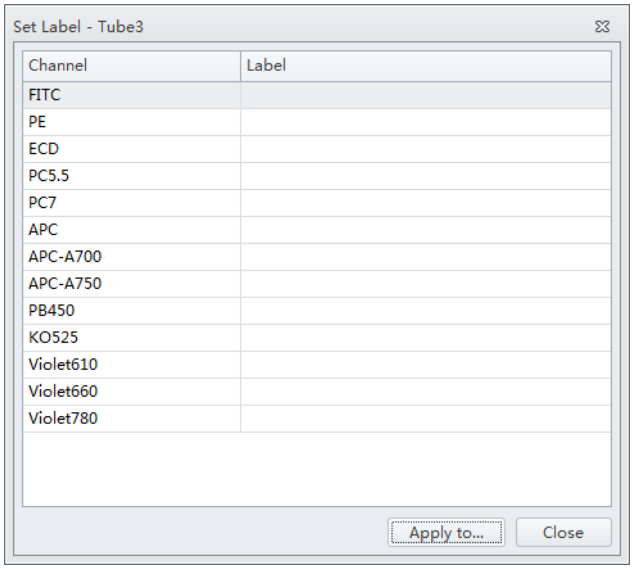
NOTE You can select which signal type to use Height or Area.

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

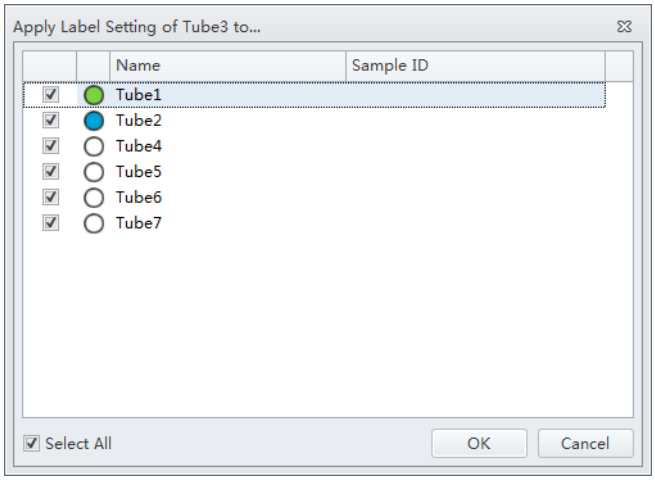


c. Select the tubes to apply the channel settings to and select **OK**.

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.

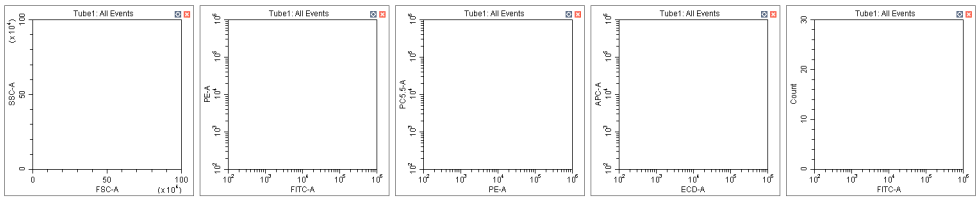
IMPORTANT The maximum number of gates allowed in an experiment is 200.

---

1 Use the plotting controls (see Figure 2.1) in the plot area to create plots and gates and to generate graphs as shown.

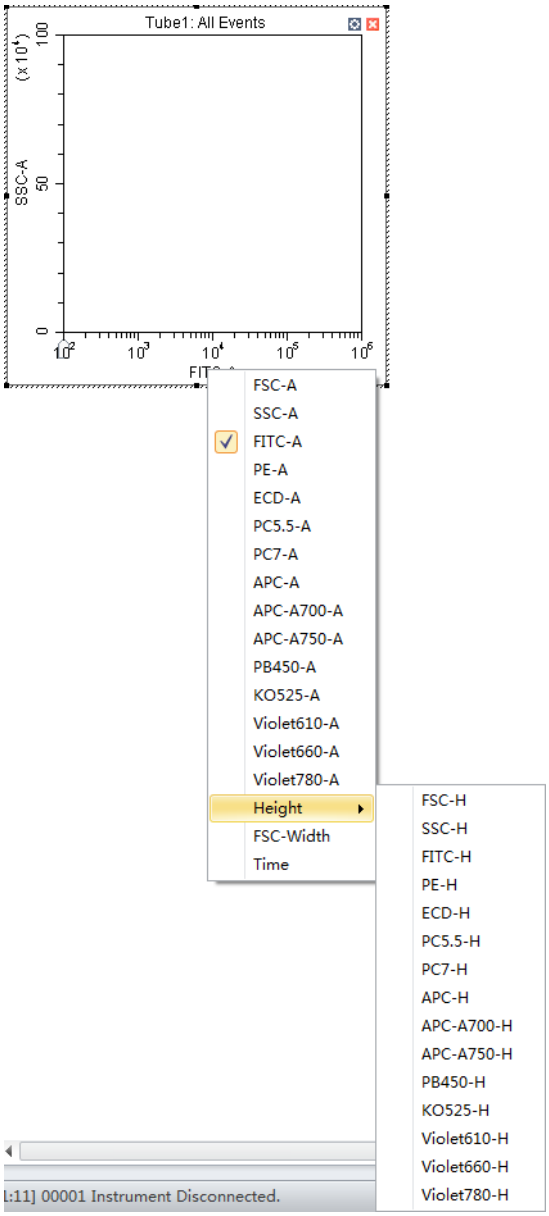
Use the  icons to generate histograms, dot plots, density plots, pseudo color plots, and contour plot.

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.

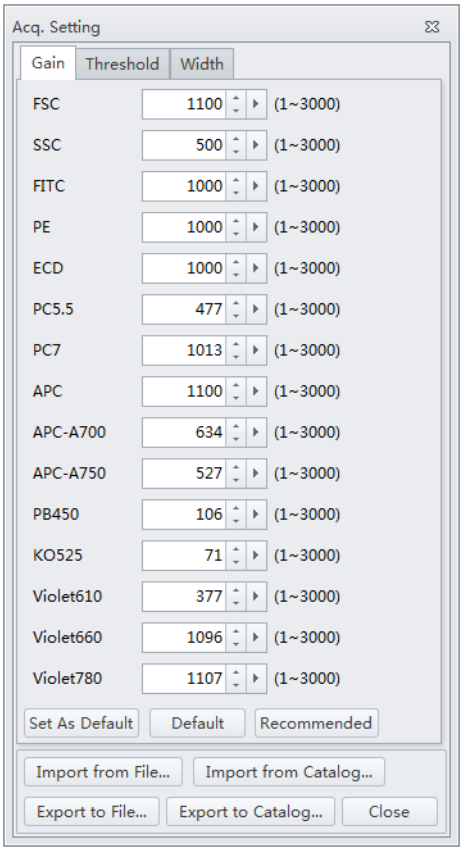
b. Select an axis name to change which channel is displayed. An “A” after the channel name indicates signal pulse area, while an “H” indicates height. The default setting is "A".



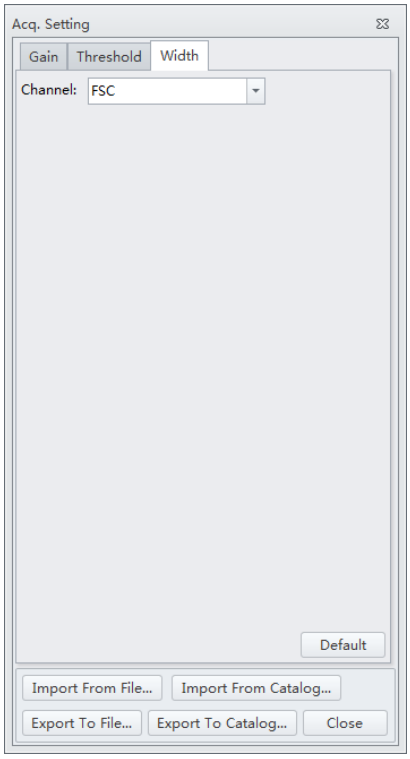
NOTE To modify the default settings, select **Options** in the Settings menu. The Options window appears. Select **Plot** on the left side of the Options window. Under the Signal section of the window, change the Main Channel default by selecting the **Height** or **Area**.

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 Acq. Setting... to open the Acq. Setting window.

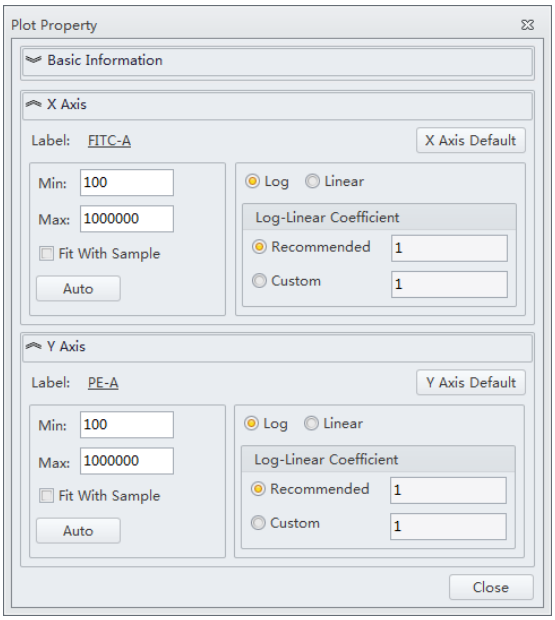


d. Select the **Width** tab, and select a channel with the required signal width.



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**.

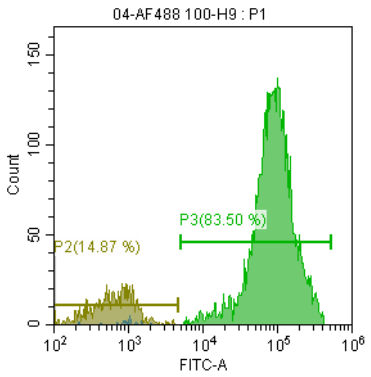
Or

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.

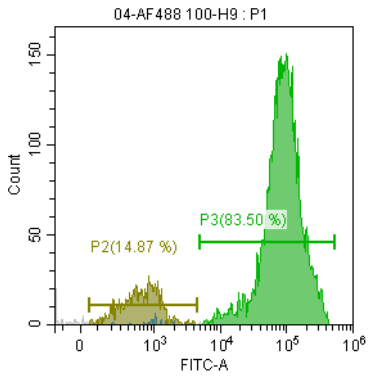
NOTE The log-linear slider is also available during data acquisition.

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.

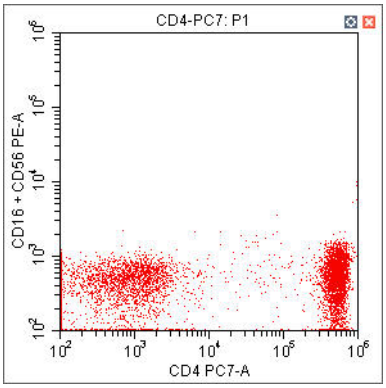
Histogram with logarithmic X-axis



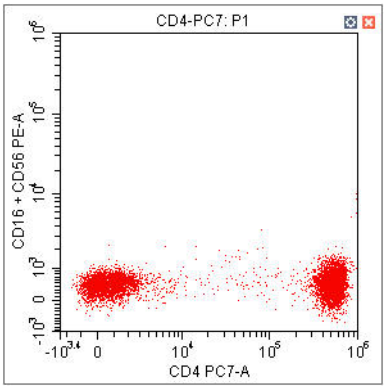
Histogram with log-linear X-axis



Dot plot with logarithmic X-axis



Dot plot with log-linear X-axis

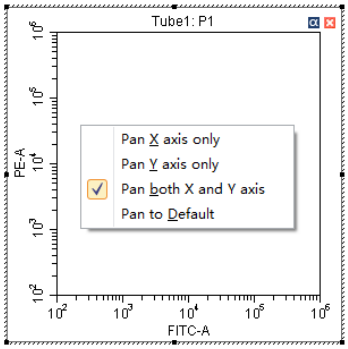


f. You can adjust axis ranges using the pan axis display controls located at the top of the screen.

- 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.

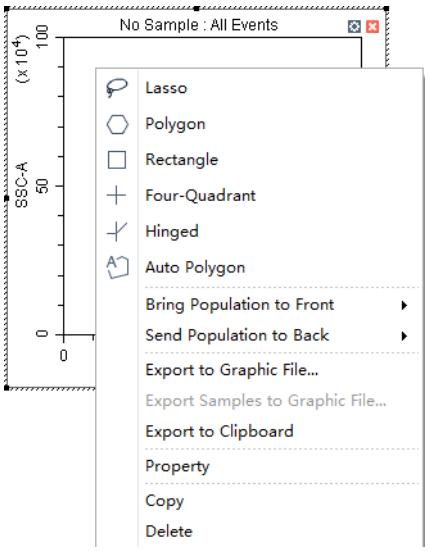
- Double-click the border area of the plot to open the Plot Property window, or right-click the plot, then select **Property** to open the same Plot Property window.
- In the Plot Property window, manually enter the minimum and maximum display values for the X- and Y-axes. You can also select **Fit With 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.

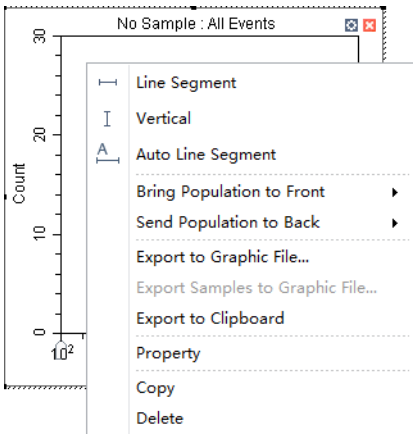
  NOTE Select **Fit With Sample** to identify the signal's lower limit, adjusting automatically as warranted. Selecting this item is recommended whenever the signal appears to be relatively low.

  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.





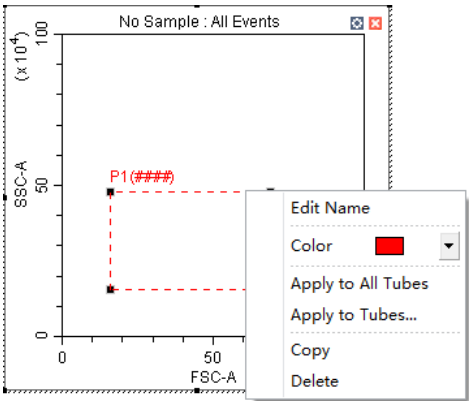
NOTE To add a vertex to a polygon gate:

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.

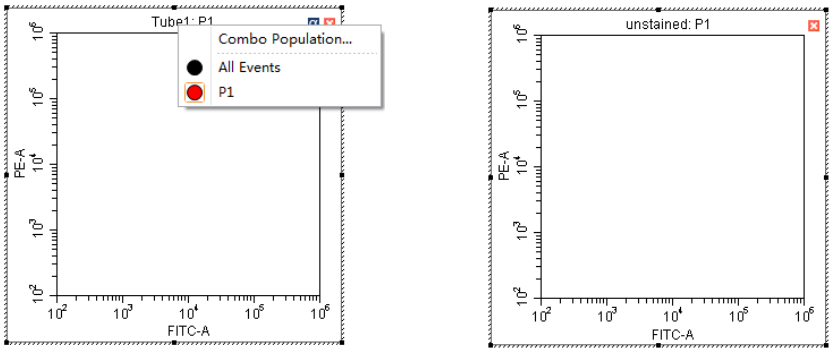
The position of the same gate in different sample tubes may vary. To change the position of a gate and apply the change to all sample tubes accordingly, you can right-click the gate and select **Apply to All Tubes**.

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

Figure 5.3 shows all gates defined in the example experiment below. Note that the only gate option in plot 1 of Figure 5.4 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 5.3 All Gates - Example Experiment

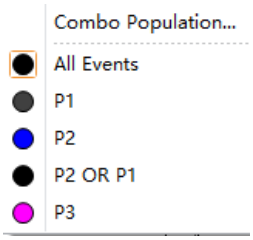
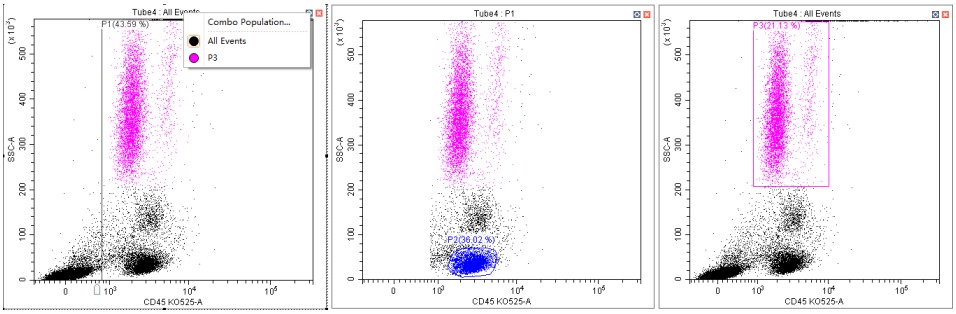
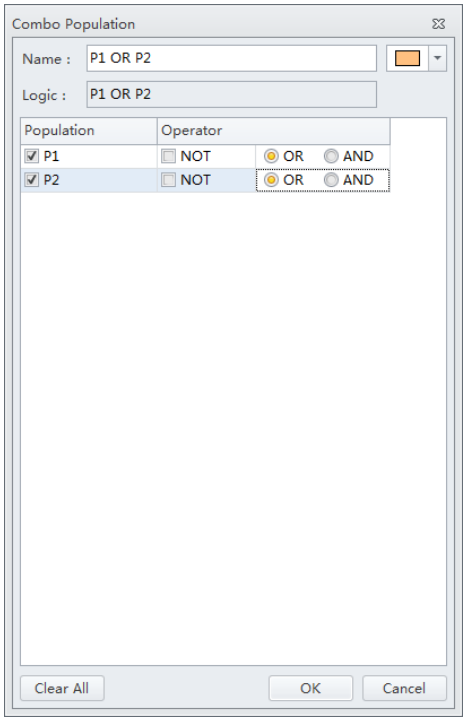


Figure 5.4 Circular Gating Logic - Example Experiment



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.

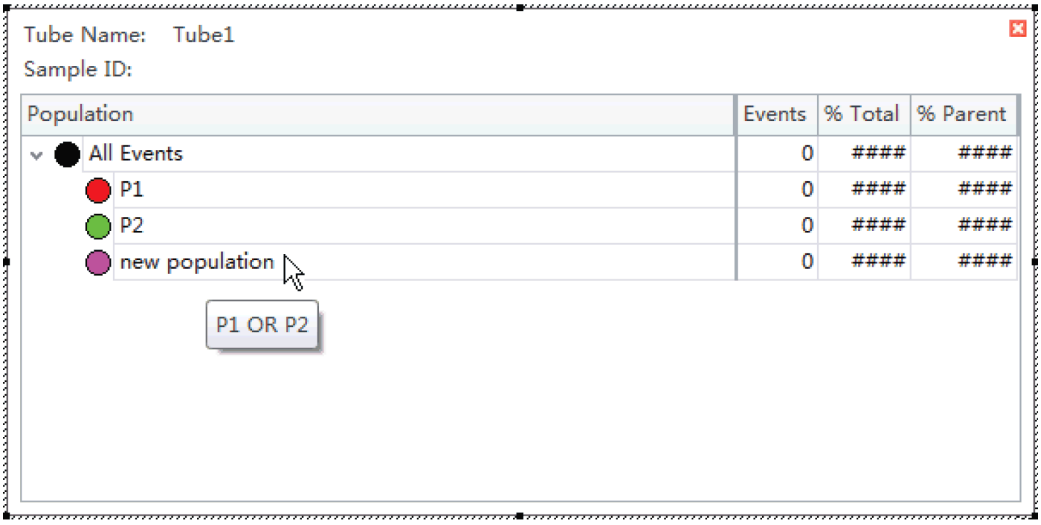


- “And” indicates that all selections must be satisfied. For example, “P1 and P2” means that the data for the newly added gate represent the intersection of P1 and P2.
- “Or” indicates that only one of the selections need be satisfied. For example, “P1 or P2” means that the data for the newly added gate 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.

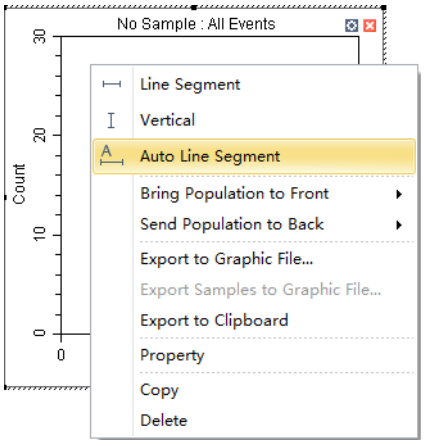
The Population Hierarchy function allows you to view how gates rank in relation to one another. To change the display color, double-click the default color and select the desired color from the drop-down color palette. To change the name of each gate, double-click the name of the desired gate. By hovering your mouse pointer over a combo population whose display name has just been changed, you can view its corresponding Boolean logical operation.



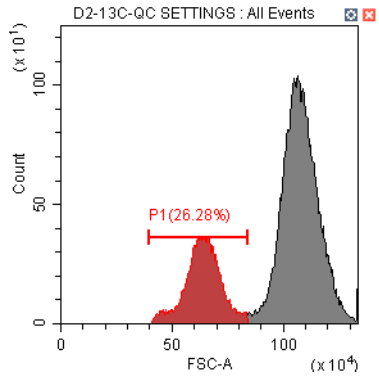
Creating and Adjusting Auto Gates

There are two types of autogates available in the CytExpert software: auto line segment and auto polygon.

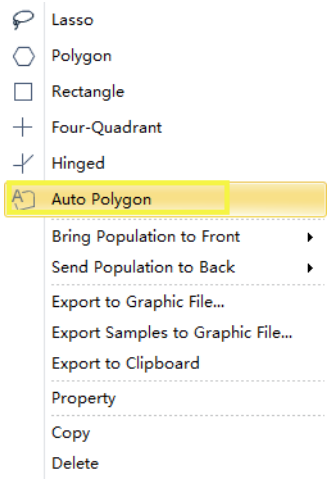
To create an auto line segment gate, select  from the toolbar or right-click on the histogram and select **Auto Line Segment** from the drop down menu.



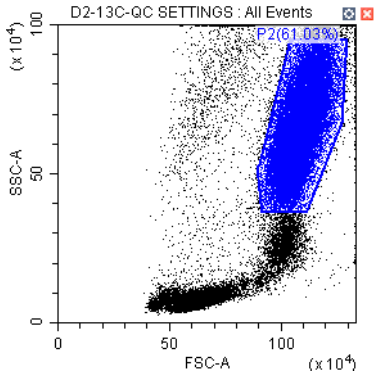
Select the population you want to gate in the histogram to automatically gate that population.



To create an auto polygon gate, select from the toolbar 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

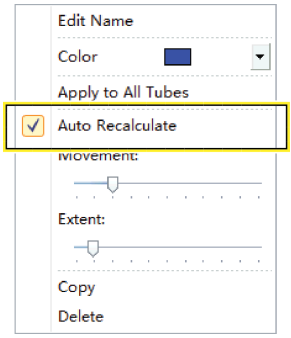
When auto recalculate is turned on, all autogates will recalculate when:

- 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.

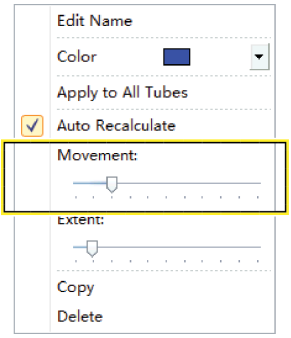
Right-click an autogate and select **Auto Recalculate** from the auto gate menu to toggle auto recalculate on and off.



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 5.5 for an example of the default movement setting. Refer to Figure 5.6 for an example of the maximum movement setting.

Figure 5.5 Movement - Default Setting

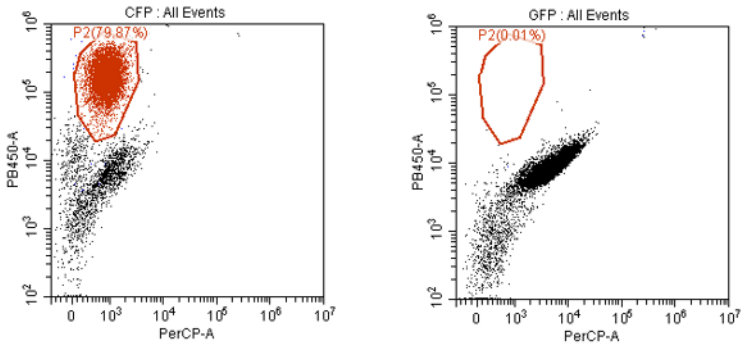
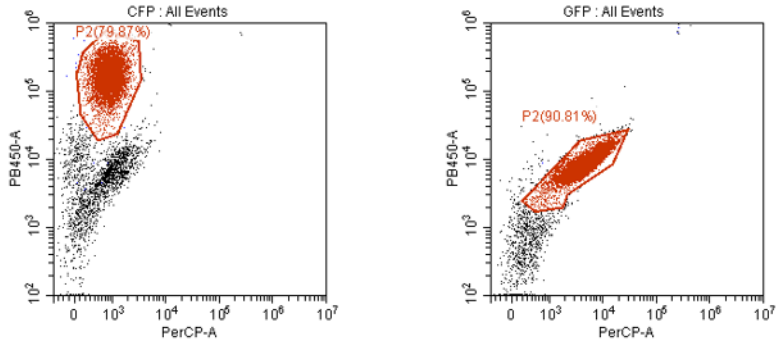
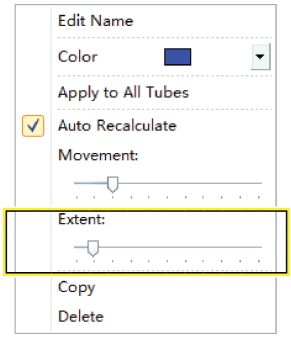


Figure 5.6 Movement - Max Setting



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 5.7 for an example of the default extent setting. Refer to Figure 5.8 for an example of the maximum extent setting.

Figure 5.7 Extent - Default Setting

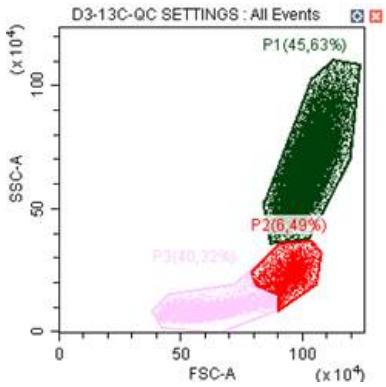
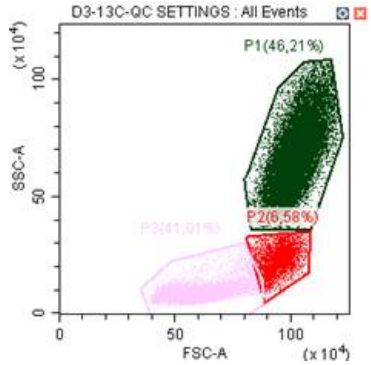


Figure 5.8 Extent - Maximum Setting

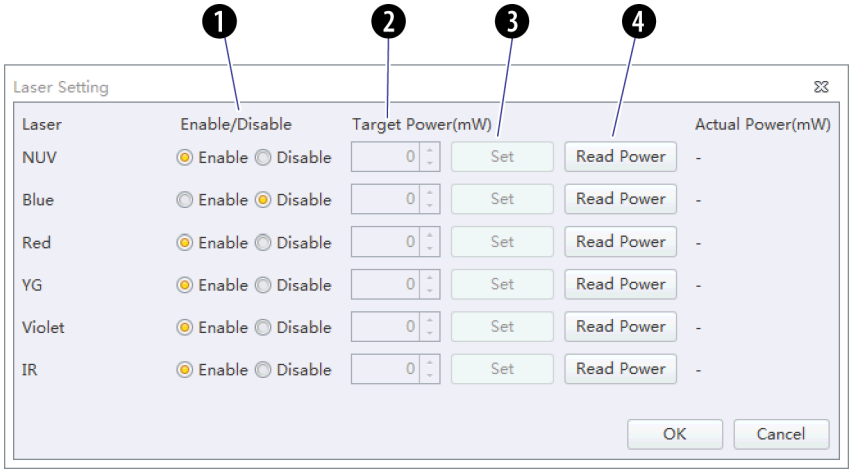


Laser Settings

To access the Laser Setting window, select **Advanced** > **Laser Setting.** The Laser Setting window appears. Refer to Figure 5.9.

NOTE The instrument must be in Standby mode to access the Laser Setting window.

Figure 5.9 Laser Setting Window [CytoFLEX LX shown]



1. **Enable/Disable:** Enables or disables the laser.
2. **Target Power (mW):** Used to change the laser target power.

   NOTE Laser target power can only be adjusted on the CytoFLEX LX system. The power detector has ±1 mW tolerance. Refer to Table 5.1 for the target power ranges allowed in the Laser Setting screen.

   NOTE Refer to the target powers listed for each laser in the QC reports area of the QC Report Screen (refer to Figure 2.2) for range limits.

3. Set : Sets the laser target power setting.

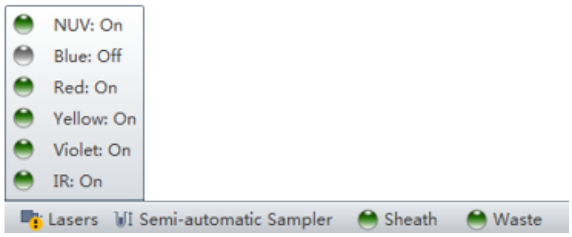
IMPORTANT The Actual Power readings are +/- 1 mW.

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. Refer to Table 5.1 for a list of laser ranges.

Table 5.1 Target Power Ranges in the Laser Setting Screen

Laser	Min Power (mW)	Max Power (mW)
355	19	21
375	51	69
405	71	119
488	41	59
561	21	39
638	41	59
808	51	69

Select the **Enable** or **Disable** radio button next to each laser on the Laser Setting window to enable or disable lasers. The laser status for each laser displays in the software status bar. Hover your mouse over Lasers to display details for each laser.



NOTE Lasers can only be enabled and disabled when the system is in standby mode.

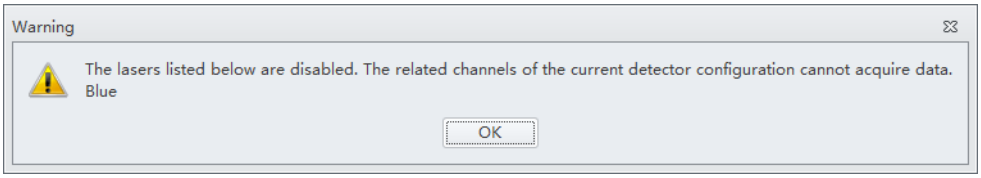
Setting Laser Target Power Settings [CytoFLEX LX Only]

IMPORTANT When you change the laser target power, it will impact all of your results including QC and standardization.

1 To access the Laser Setting window, select **Advanced** > **Laser Setting**. The Laser Setting window appears.

2 Ensure that all desired lasers are enabled.

NOTE If a laser is disabled on an experiment that requires that laser, the following message appears:



3 Select **Read Power** to view the real-time laser power reading.

The laser power displays under the **Actual Power (mW)** column located in the far right section of the Laser Setting window.

4 Adjust the target power for each laser as needed.

5 Select **Set** to set the device power.

6 Standardize your laser target values in the QC Experiment. Refer to Standardization in CHAPTER 4, Instrument Quality Control and Standardization.

NOTE Disabled lasers are marked *Laser XXX is disabled* in the QC screen and do not provide laser power values.

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 Acq. Setting... on the left side of the screen. The Acq. Setting window appears.

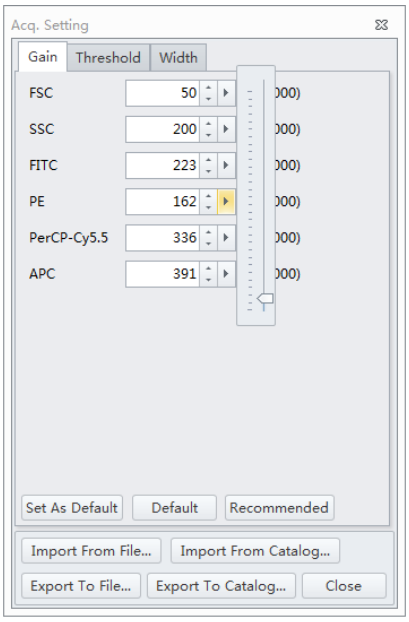
2 Select the **Gain** tab in the Acq. Setting window.

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 **Recommended** to use the instrument's QC settings.

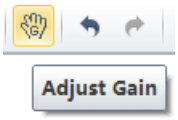
NOTE In cases where you do not specify your own default parameters, the recommended settings and default settings are identical.

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.

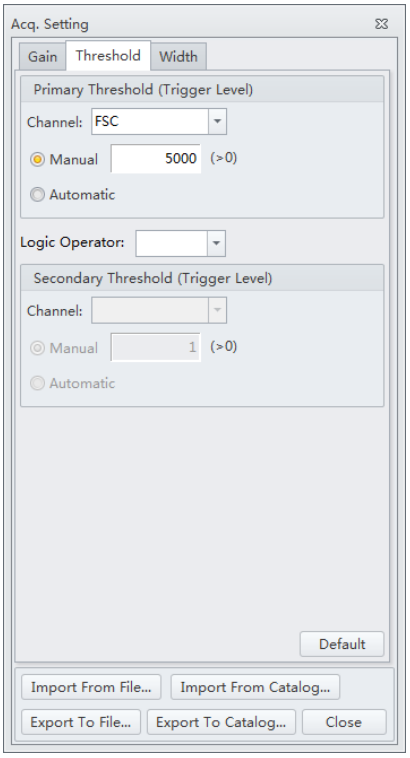
NOTE Threshold can be defined for any of the fluorescence channels.

---

2 Select Acq. Setting... 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**.

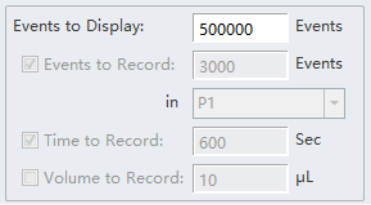
Setting Collection Conditions

1 Check mark the conditions required to set the necessary stop count events on the left side of the Acquisition screen.

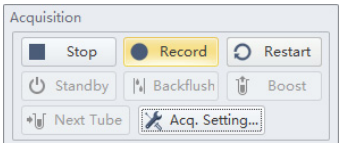
Three stop count collection conditions are available for sample recording:

- **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.
- **Volume to Record**. Used to set the collection volume in µL.

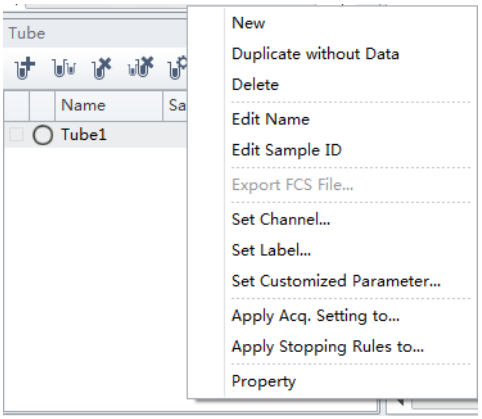
For example, if the event to record is set to record 1,000 P1 events, the software automatically stops recording when P1 events reach 1,000 events. However, the software saves all data acquired, including events outside of P1, when 1,000 P1 events is reached. You can also specify the time to store if necessary. When multiple acquisition conditions are established, any one of these conditions stops the collection process.



2 Select **Record** and wait for the software to complete collecting the data, at which time the sample tube holder returns to the sample loading position (see Figure 1.13).

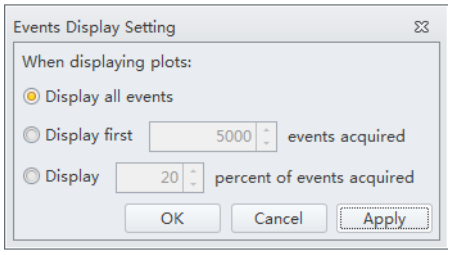


3 If you made changes to the data acquisition conditions and need to apply these changes to an established sample tube, right-click the sample tube and select **Apply Acq. Settings To**, to apply the conditions accordingly.



Setting Plot Display Conditions

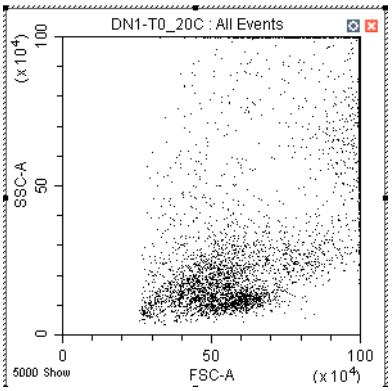
Select **Events Display Setting** in the Settings menu. The Events Display Setting window appears.



Three display options are available:

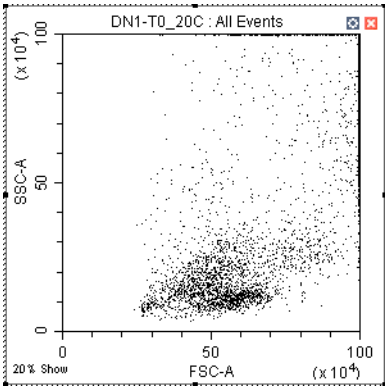
- **Display all events.** Used to view all events on the plot.
- **Display first XXXX events.** Used to set the set number of events to display.

  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.

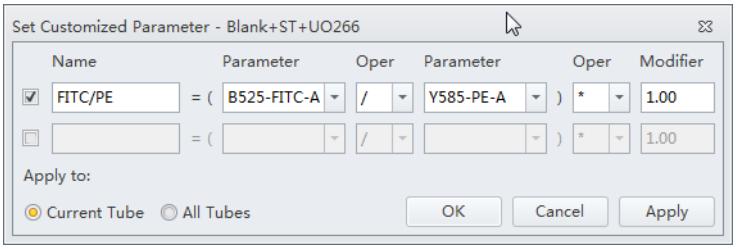
  NOTE The selected percentage of events displays in the bottom, left corner of the plot. For example if you choose to show 20 percent of events acquired, the bottom, left corner of the plot displays *20% Show*.



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.

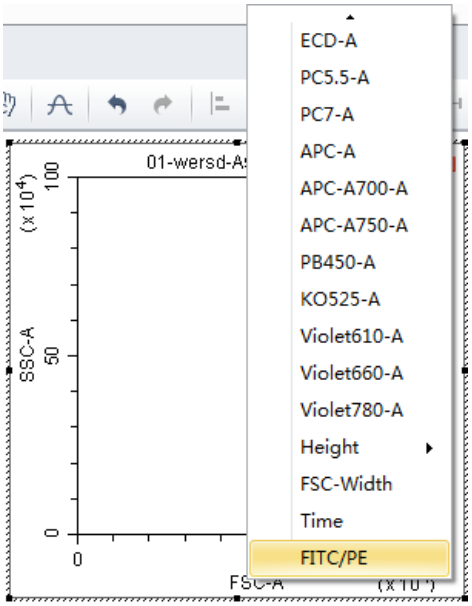


2 Enter a name for the parameter in the Name section.

3 Select the parameters for calculation in the Parameter dropdowns.

4 Select the equation operations from the Open dropdown menu.

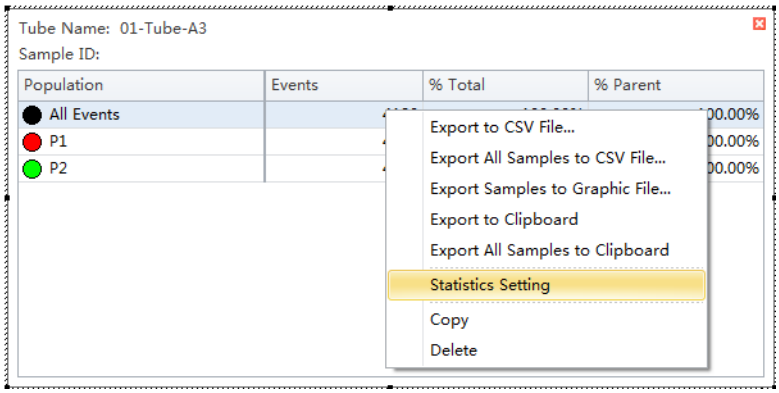
The new parameter name is displayed in the list of parameters and statistic items.

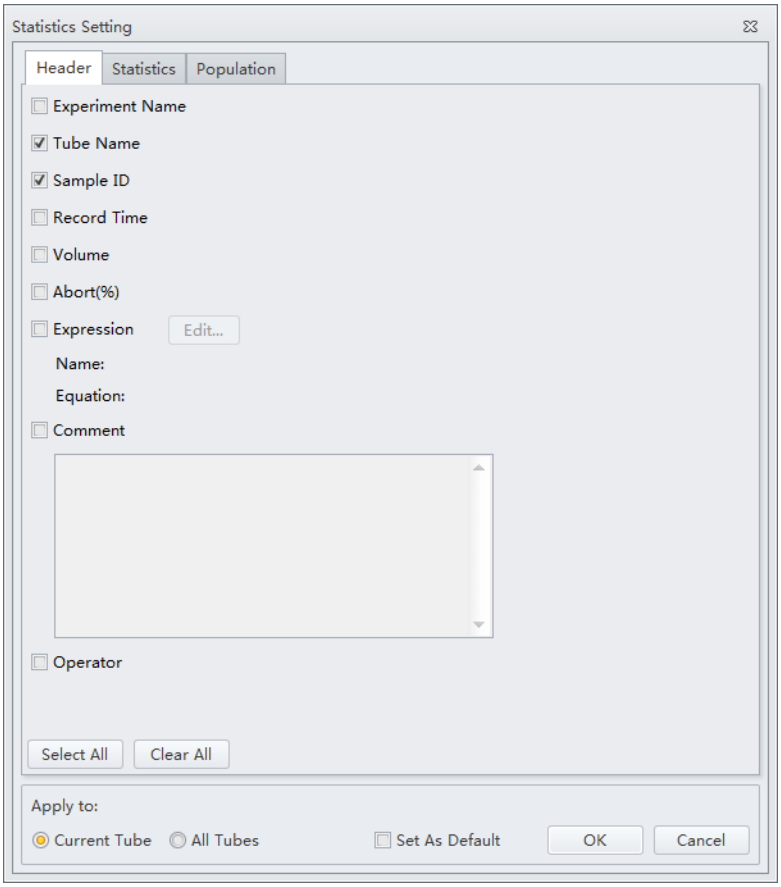


Setting Custom Statistics

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.

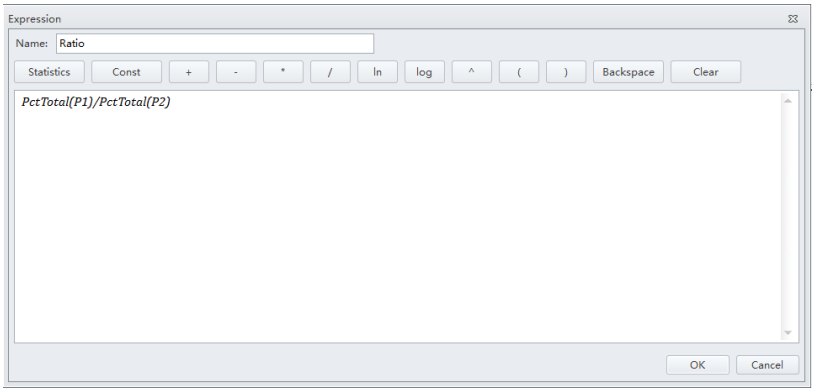




2 Select **Expression**.

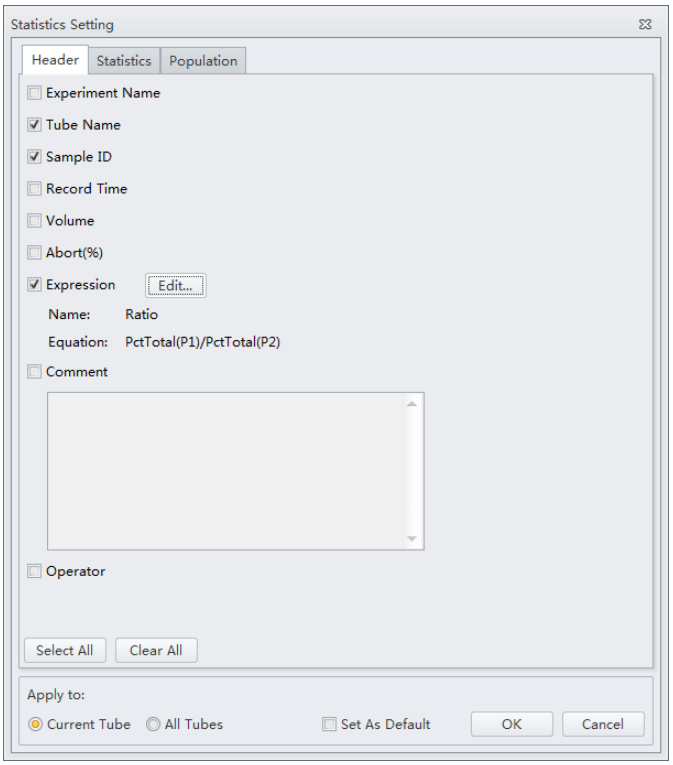
3 Select **Edit.** The Expression window appears.

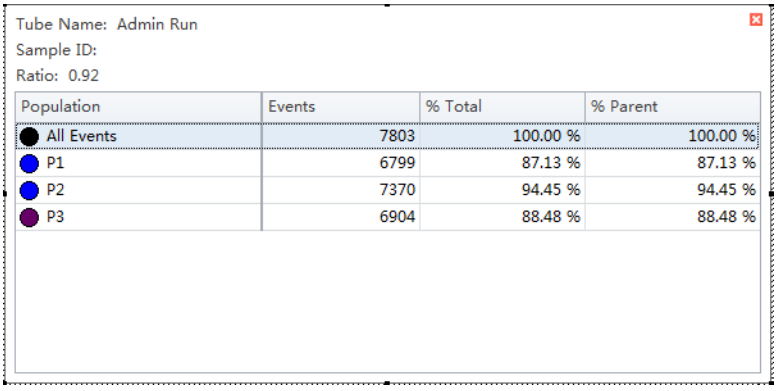
4 Enter the expression name in the Name section and enter the expression using the equation buttons.



5 Select **OK**.

NOTE The equation populates in the Statistics Setting window under the Expression selection.

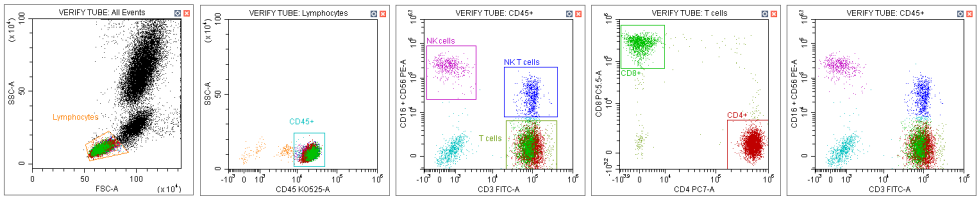




Analyzing and Exporting Data

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.



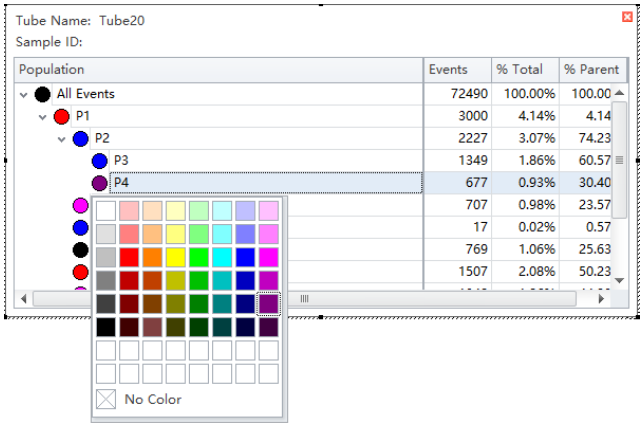
NOTE Changing a gate's position does not affect the positions of other gates already established on a given sample tube. Each test tube individually records the positions of its associated gates. If you need to make a change that concerns all the tubes, you must select the gate, then right-click the correctly positioned gate and select **Apply to All Tubes**.

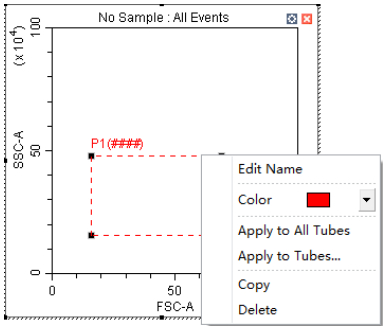
3 Select  . The Gate Hierarchy screen appears.

4 Check the relationship between the parent and daughter gates in the Gate Hierarchy window.

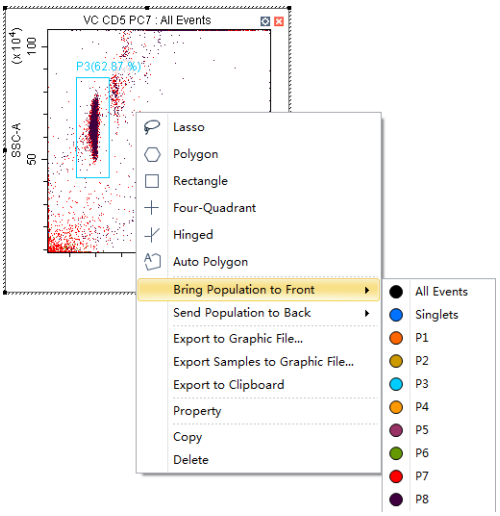
NOTE Newly added gates become subsets of populations displayed in plots with existing gates. Name and display color can be modified. Right-click directly on a gate plot to change the name and color.

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.



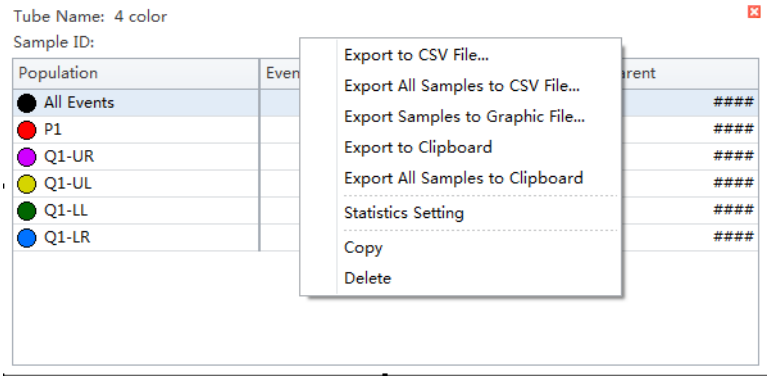


5 Right-click the plot and select **Bring population to front** to make the display color of the specified gate appear in front of all other colors, or select **Send population to back** to hide the display color of the specified gate behind all other colors.

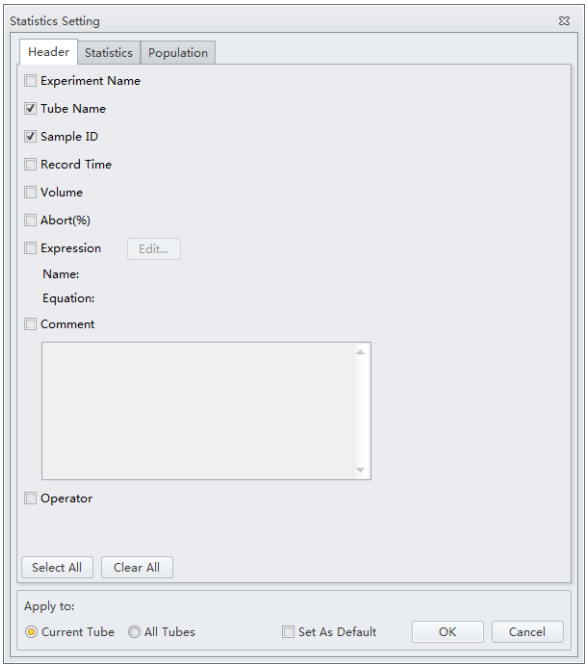


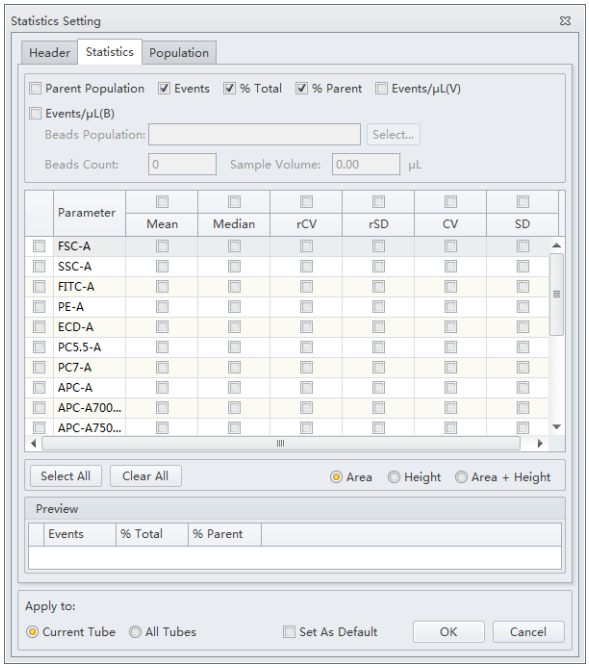
6 Select  in the plot area to generate a statistical table.

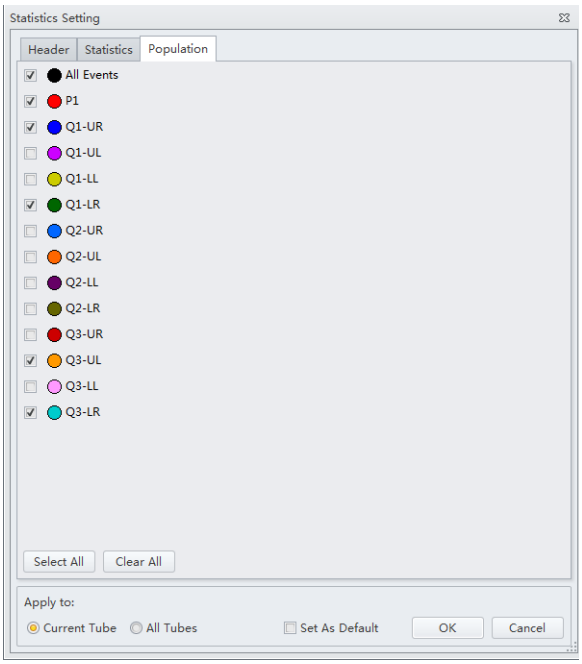
7 Right-click the table and select **Statistics Setting** to modify the settings of the statistics display parameters. The Statistics Setting window appears.



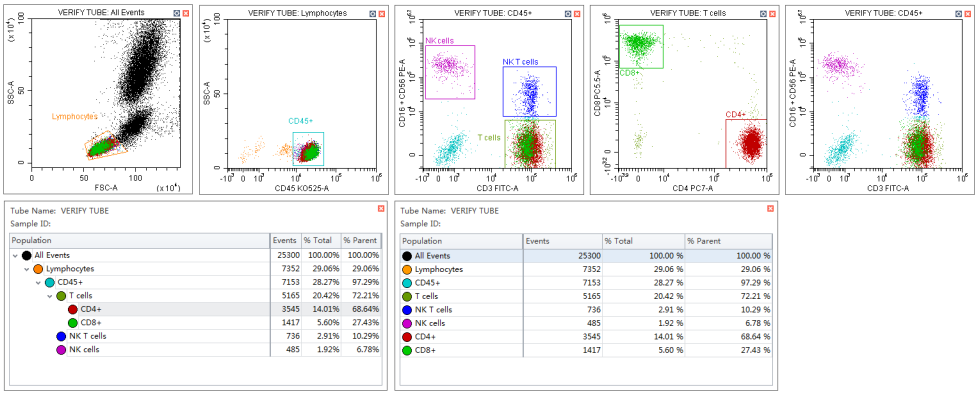
The Statistics Setting window allows you to change the display of the header, statistical elements and cell populations included.



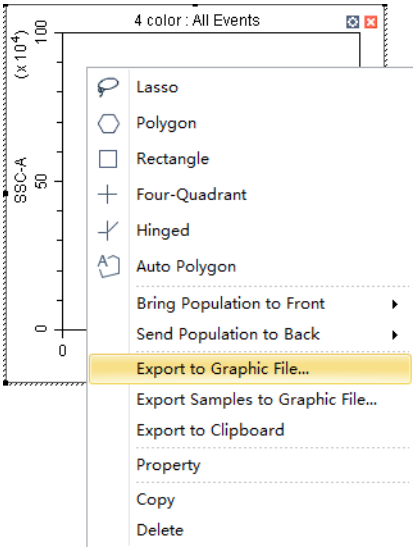




The final generated plots appear as below.



8 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.



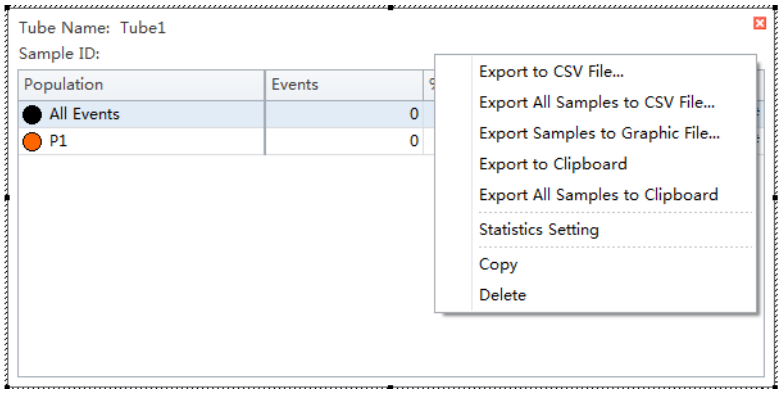
- **Export to Clipboard** copies the plot to the clipboard, allowing you to paste it directly into documents in common file formats.

  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.

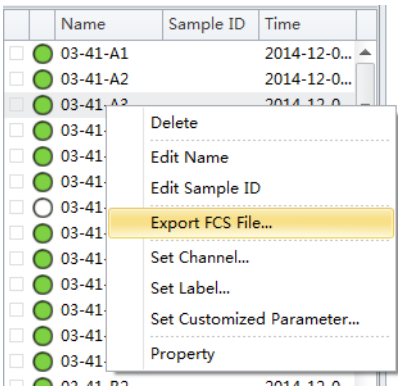
10 Export the FCS file if necessary. Refer to Exporting FCS Files.

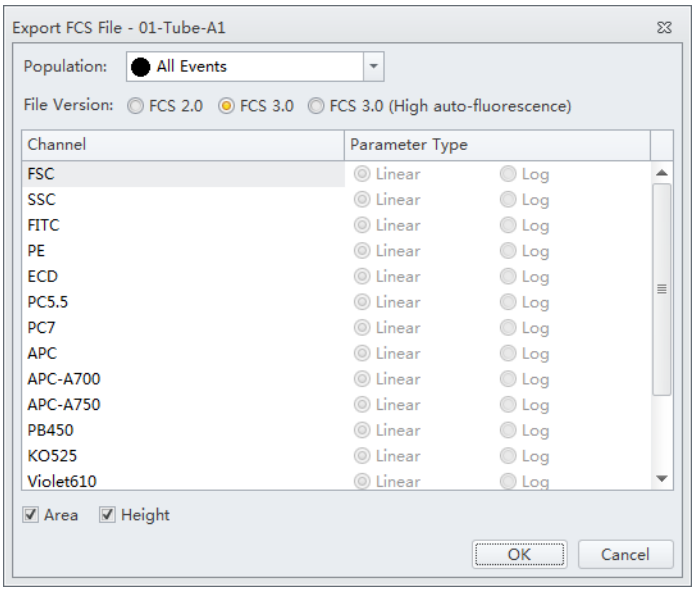
NOTE Ensure that any storage devices used with the instrument are free from viruses. To guard against data loss, Beckman Coulter recommends backing up data on a frequent and regular basis. Beckman Coulter is not liable for any loss of data resulting from computer viruses or damage to hardware.

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.



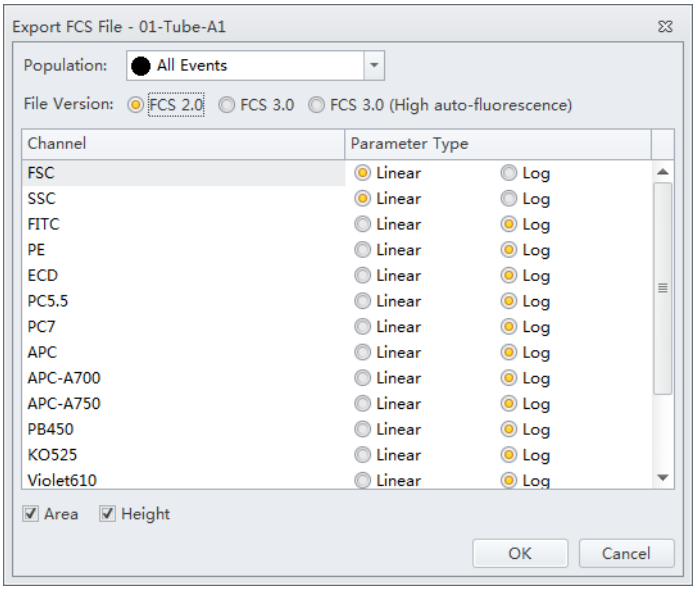


2 Select the population from the Population dropdown menu.

3 Select either **Area** or **Height**.

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.



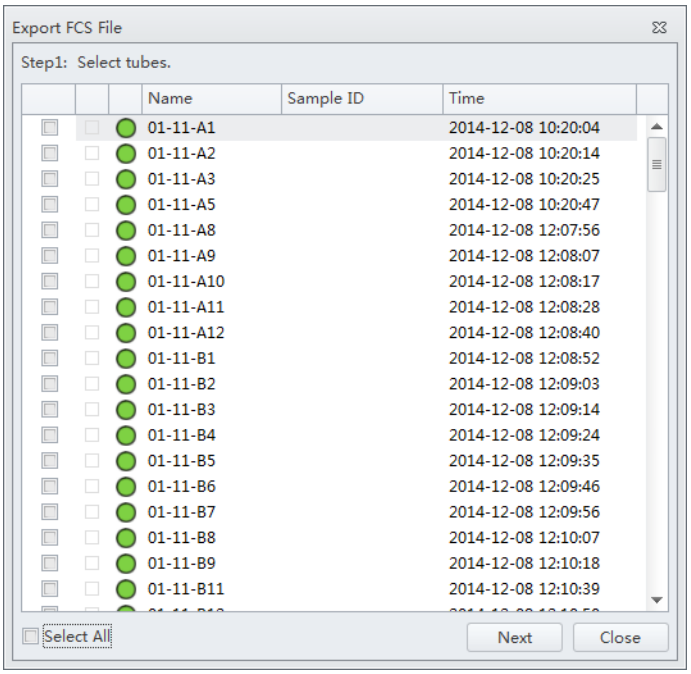
NOTE The default CytExpert FCS file contains high auto-fluorescence vector values that may not be recognized by third party software. Therefore, the data displays differently in third part software packages than in CytExpert. Auto-fluorescence values are added for the FCS 3.0 (High auto-fluorescence) 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 the path to save the FCS file to from the Path section of the window.

6 Select **Next** to export the file.

Exporting Multiple FCS Files

1 Select **Export FCS File** from the File menu. The Export FCS File window appears.

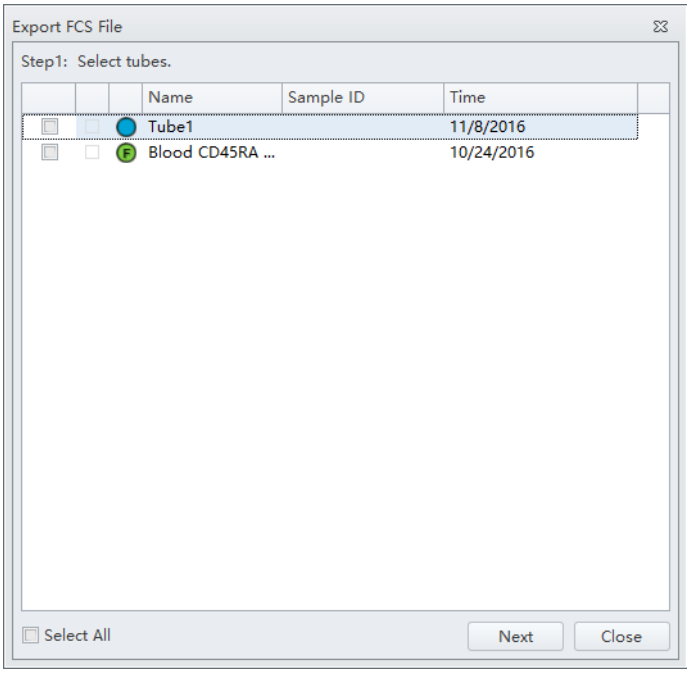


2 Select the tubes to export.

3 Repeat Steps 2-6 from Exporting Single Tube Files.

Exporting Plots or the Statistics Table of Multiple Tubes as Picture Files

1 Select **File > Export FCS File.** The Export Tubes to Files window appears.



2 Select the tubes to export.

3 Select the path.

4 Select **OK**.

NOTE The plots of the selected tubes save as .bmp file.

Importing and Exporting Instrument Settings

The CytExpert 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 Acq. Setting... to edit gain, threshold, and width. These can be imported from an experiment file or from a catalog of instrument settings.

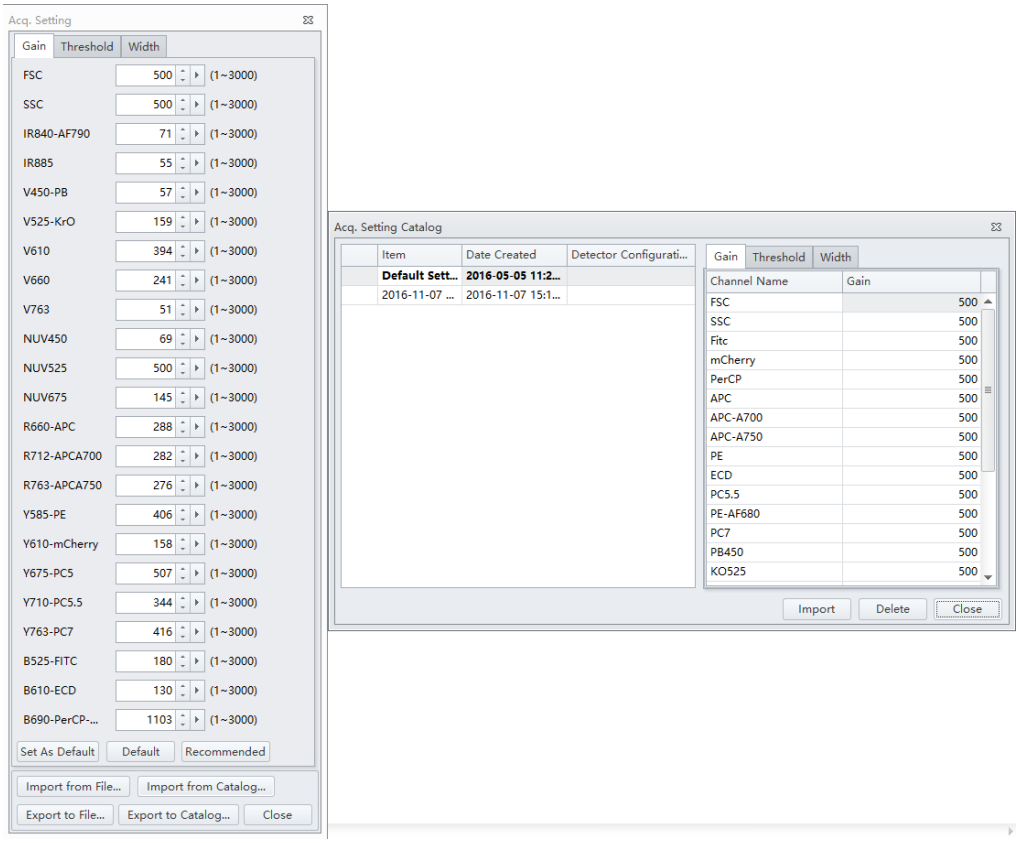
Importing Instrument Settings

1 Select the desired sample tube to import. Then select Acq. Setting....

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.

[CytoFLEX LX Shown]



3 Select **Close**.

Exporting Instrument Settings

1 Select the desired sample tube to export. Then select Acq. Setting....

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**.

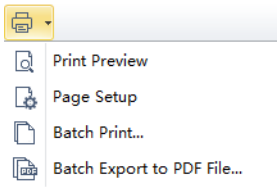
Importing and Exporting Compensation Settings

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 CHAPTER 5, Importing and Exporting Compensation Settings in CHAPTER 6, Compensation.

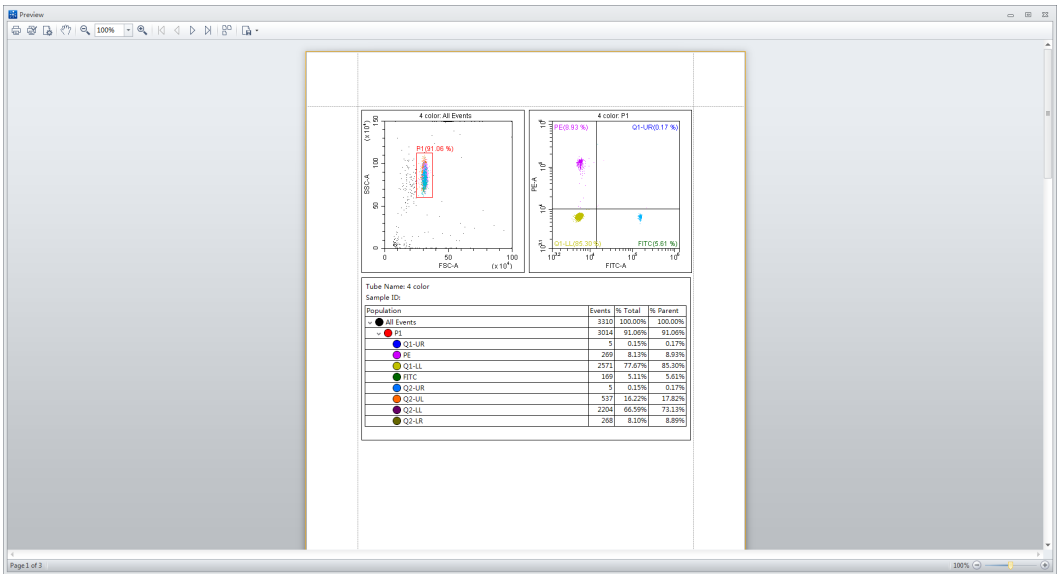
Printing Graphics

CytExpert offers printing functionality for the plots and tables that appear in the plot area. The software also lets you save these images by converting them into .jpg or .pdf files.

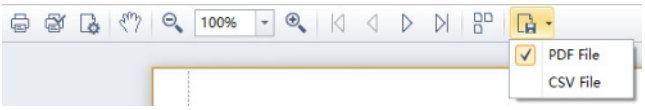
Select  in the printer control area to print directly. Or, select the print drop-down arrow for the following options:



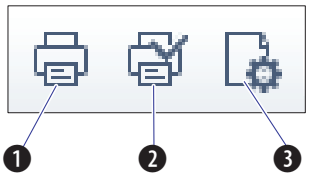
-  **Print Preview.** Used to access the Preview screen.



  — Select  to select the required format of the file to be exported and to save the file in that format.

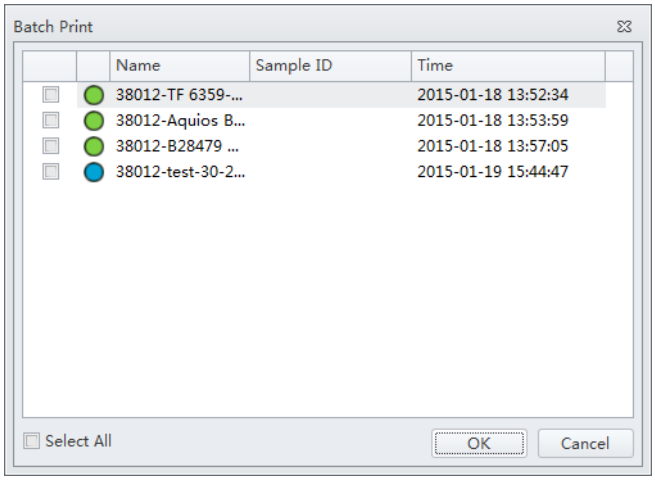


  — Print preview also lets you choose between printing directly **(1)**, modifying the printer configuration **(2)**, or adjusting the page settings **(3)**.



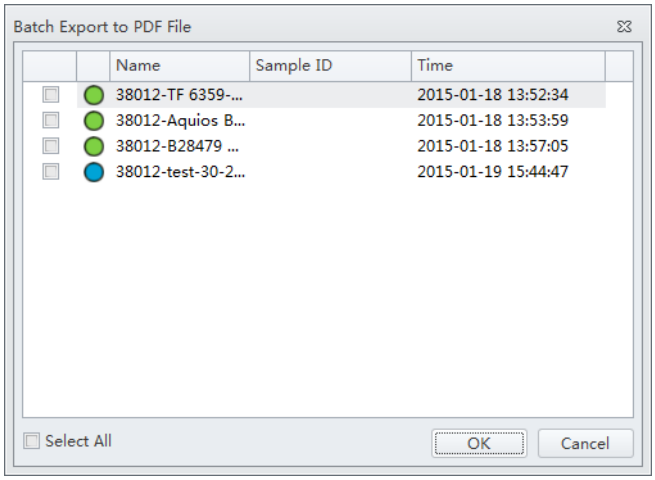
-  **Page Setup.** Used to adjust the page settings.

- **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 **Standby** to return the instrument to the standby state.
- Select **File > Close** Experiment to clear 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, Daily Shutdown.

CHAPTER 6

Compensation

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

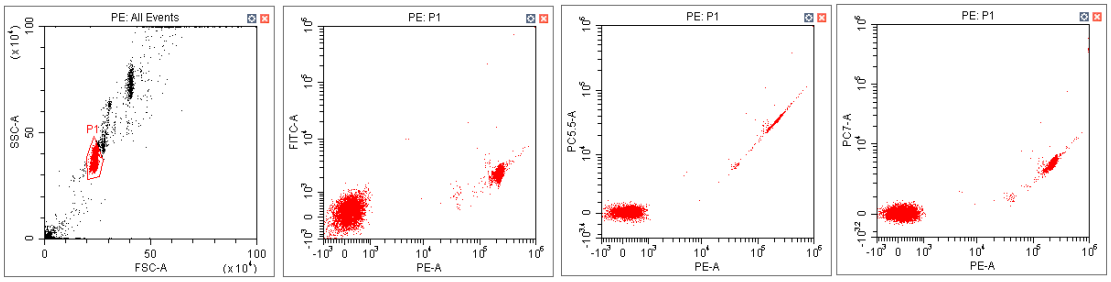
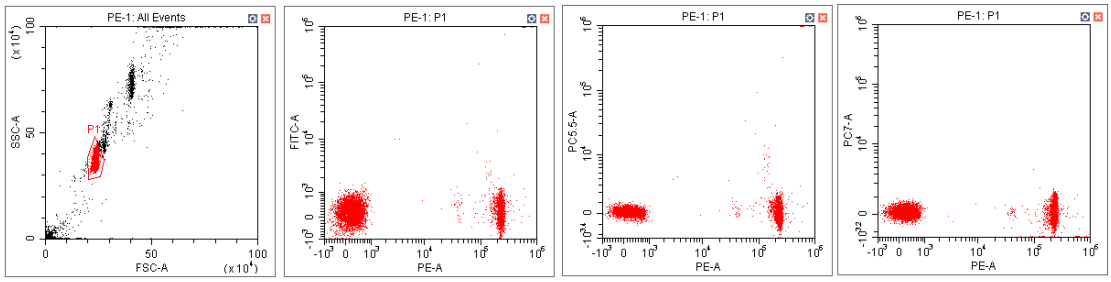


Figure 6.2 After Compensation



NOTE CytExpert compensation allows full matrix compensation, manual, and automatic.

CytExpert 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

Before creating a compensation experiment, you must verify the instrument's detector configuration settings (see Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Data Acquisition and Sample Analysis).

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 ".xitc" suffix.

2 Navigate to the desired file path and select **Save**. The Compensation Setup window appears.

⚠ 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.

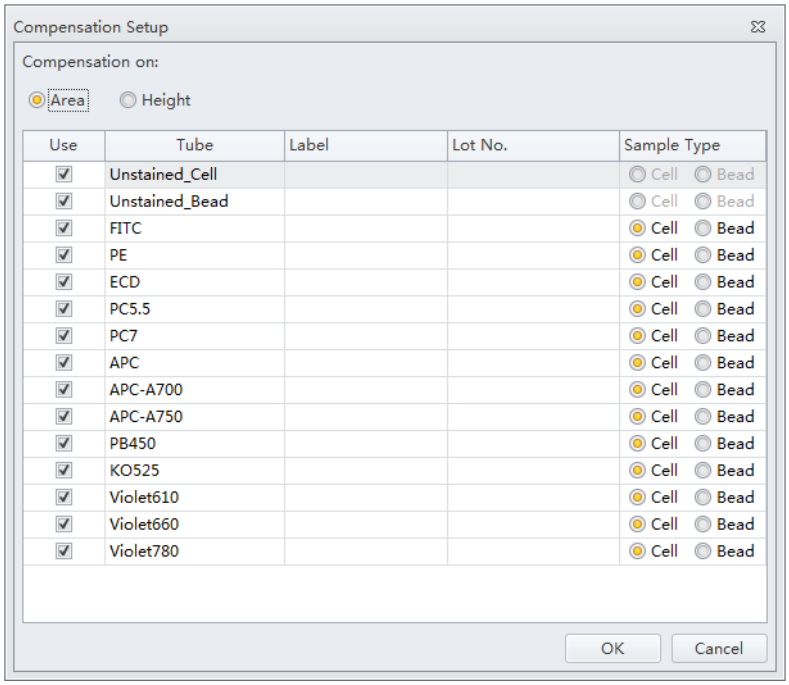
It is important to specify the appropriate sample type. Otherwise, the background information could be incorrectly calculated and lead to erroneous compensation results.

3 Select the channel requiring compensation calculation and the sample type.

If a negative population is not present in each single color tube, then an unstained control tube is recommended.

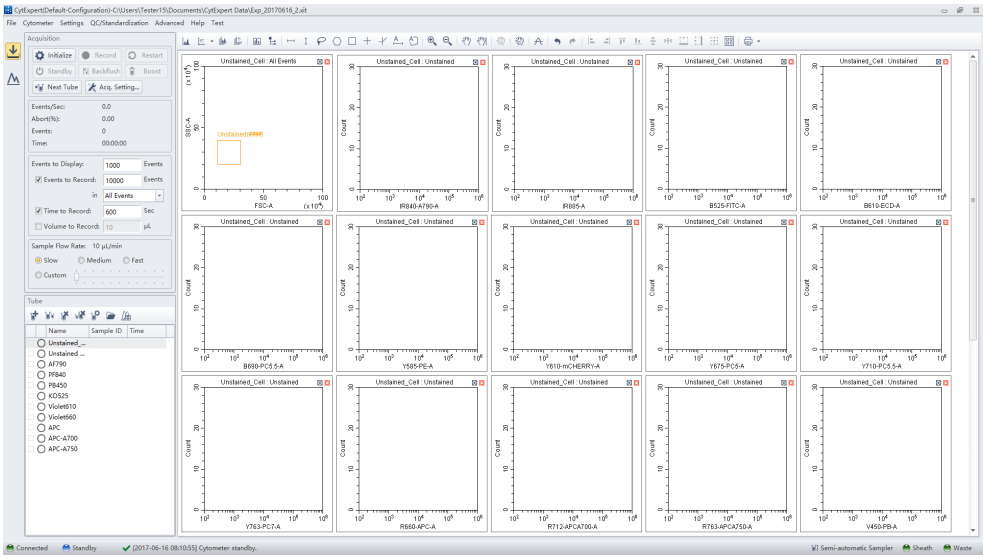
NOTE The default selection is **Area**. The unstained negative control tube can be selected if needed.

NOTE Label and lot number information can be retained in the Compensation Library to facilitate future compensation calculations.



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

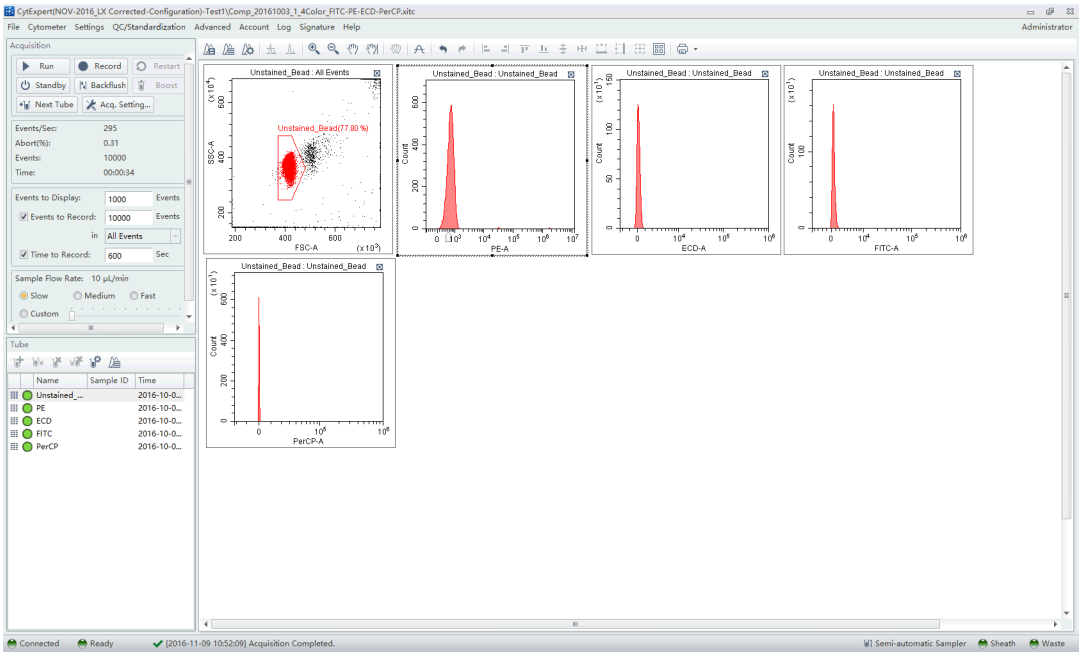
Defining the Negative Population Using Unstained Samples

1 Confirm that the instrument has been initialized. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup.

⚠ 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, Data Acquisition and Sample Analysis. 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 (see Creating Plots and Gates in CHAPTER 5, Data Acquisition and Sample Analysis).

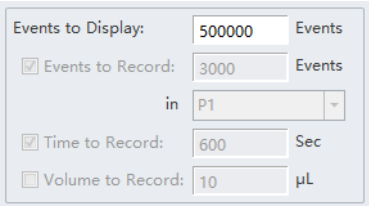


3 Place the negative control tube in the sample tube holder.

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.



7 Select **Record** to save the data.

Running the Single Positive Control Samples

1 Place the single positive tube in sample loading position (see Figure 1.13).

2 Select the appropriate, corresponding tube.

3 Select **Run** to load the sample.

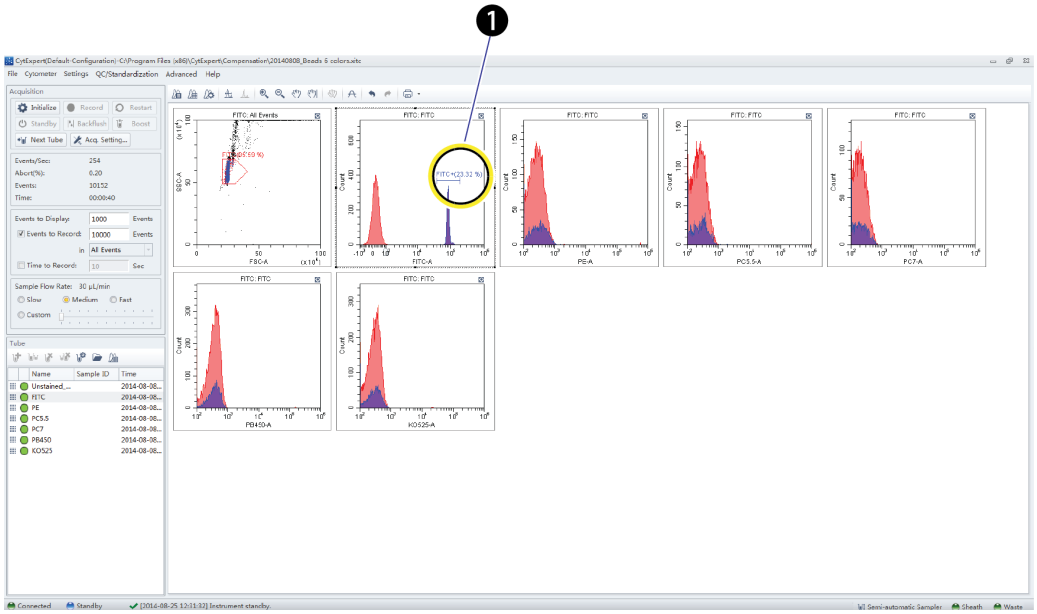
⚠ CAUTION

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 ratio 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. If necessary, move the positive gate 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.

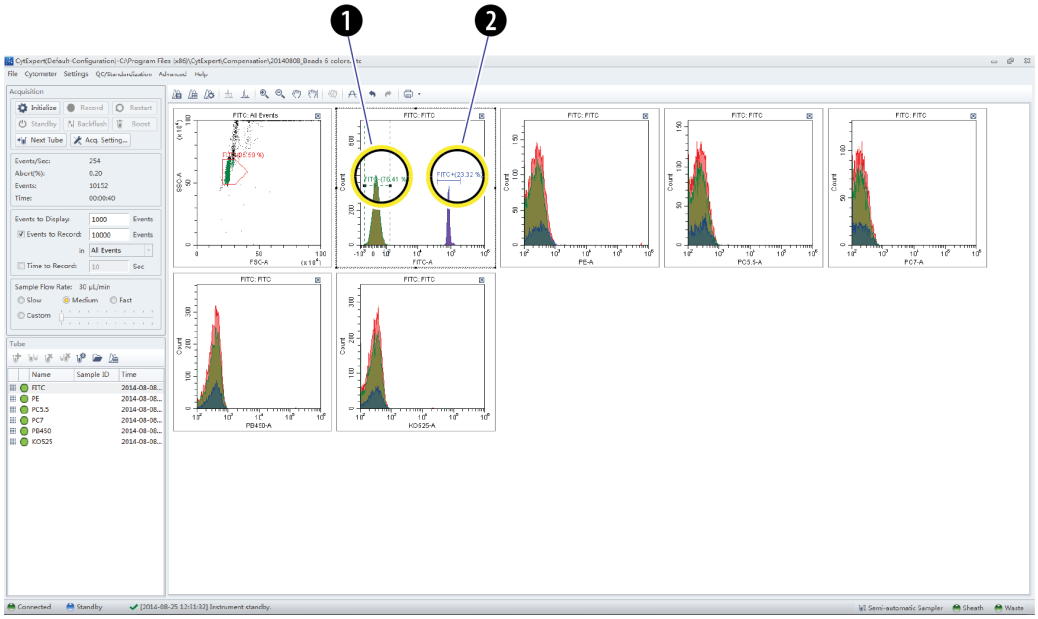
Figure 6.3 Positive Population Selected from the Single-Stained Sample



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

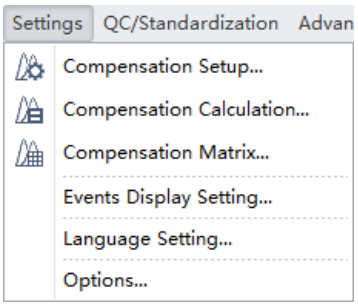
Risk of erroneous results. While the software automatically adjusts the compensation calculation according to gain, excessive manual adjustment of the fluorescence gain could lead to inaccurate results.

7 If necessary, adjust gain while acquiring data from single positive sample tubes. Refer to Adjusting the Gain in CHAPTER 5, Data Acquisition and Sample Analysis.

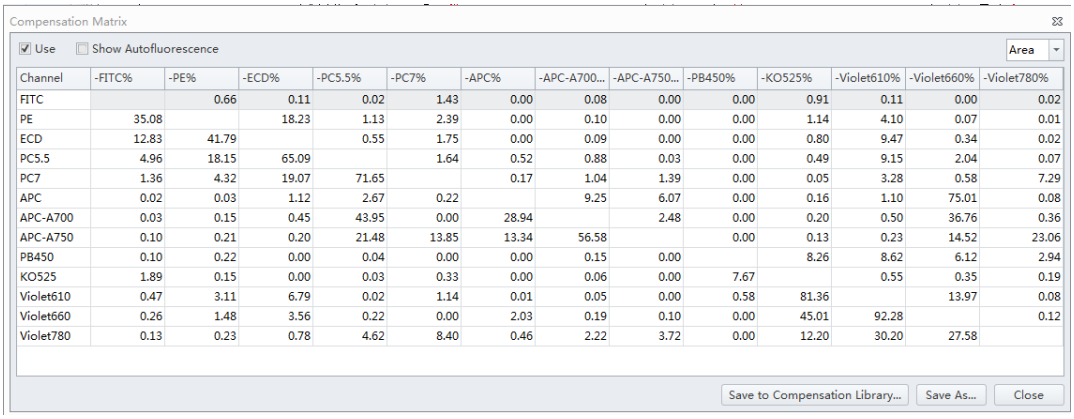
Calculating Compensation Values

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%
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:

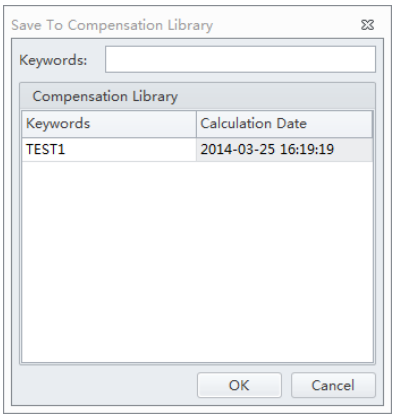
- The *Use* checkbox applies the compensation to the selected sample.
- The *Show Autofluorescence* checkbox displays the vectors for the autofluorescence.

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 a Compensation Experiment [With Plate Loader]

Before creating a compensation experiment, you must verify the instrument's detector configuration settings (see Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Data Acquisition and Sample Analysis).

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 ".xitc" suffix.

2 Navigate to the desired file path and select **Save**. The Compensation Setup window appears.

3 Select the plate type and sampling sequence located in the top, right of the Compensation Setup window.

⚠ 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, then each single marker tube must have a negative population.

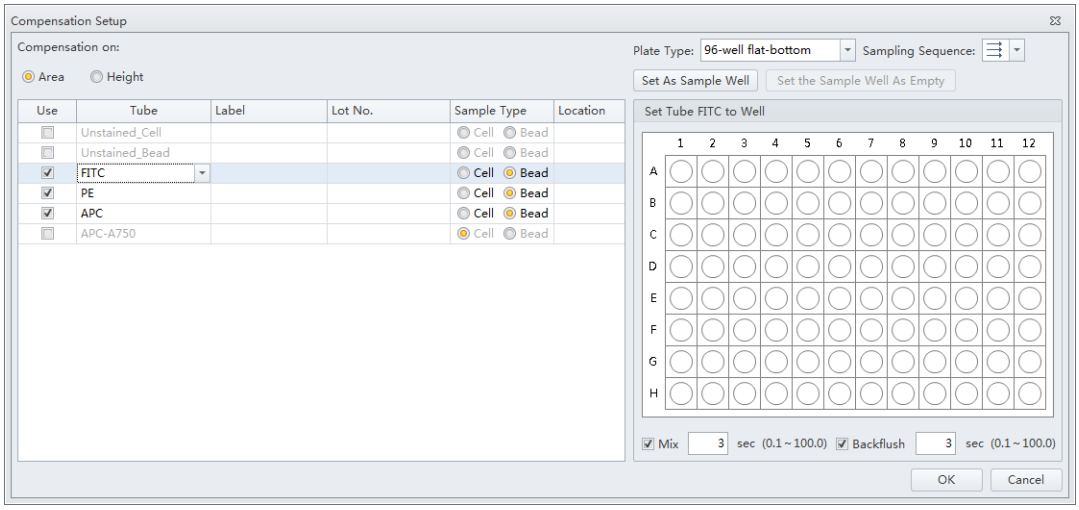
It is important to specify the appropriate sample type. Otherwise, the background information could be incorrectly calculated and lead to erroneous compensation results.

4 Select the channel requiring compensation calculation, and the sample type.

If a negative population is not present in each single color well, then an unstained control well is recommended.

NOTE The default selection is **Area**. The unstained negative control well can be selected if needed.

NOTE Label and lot number information can be retained in the Compensation Library to facilitate future compensation calculations.



5 Select the Mix and Backflush settings in the bottom, right of the Compensation Setup window.

6 Assign the well locations.

a. Select the fluorochrome.

b. Select the desired sample well location for the fluorochrome.

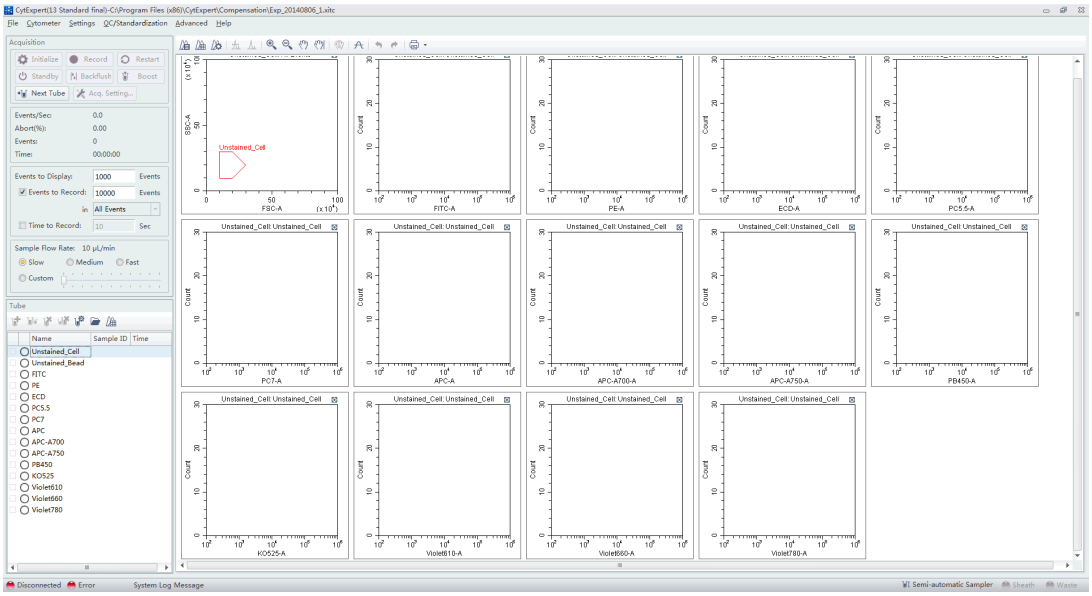
c. Select **Set As Sample Well**.

NOTE The well location populates in the location column.

d. Repeat Steps a-c for each fluorochrome.

7 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.

NOTE If the plate settings require modification, select  . The Compensation Setup window appears.

8 Before acquiring data, ensure the plate has been loaded properly. Data can be acquired as a single well or as a set of wells. Refer to Running Samples in CHAPTER 5, Data Acquisition and Sample Analysis.

Preparing the Compensation Sample

To perform a compensation experiment, prepare:

- A single positive control well for each color
- A negative control well (optional)

NOTE A negative control well is required if a single positive control well does not contain a negative population.

For the negative control sample and single positive control sample, you can use blood, cell lines, 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

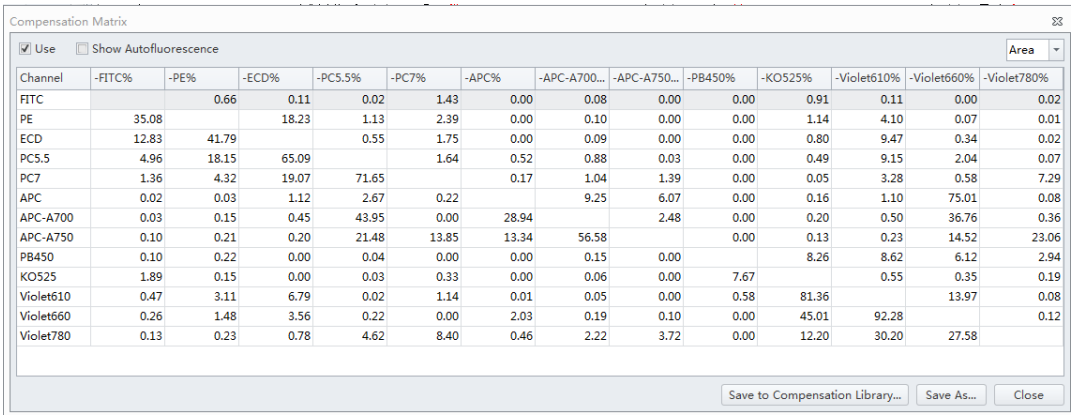
Refer to Defining the Negative Population Using Unstained Samples and Running the Single Positive Control Samples in CHAPTER 6, Compensation.

Calculating Compensation Values

1 Check all acquired sample tubes and confirm that the gating is appropriate.

2 Select or select **Compensation Calculation** in the Settings menu to calculate the compensation values.

The Compensation Matrix window appears, displaying the calculated compensation values.



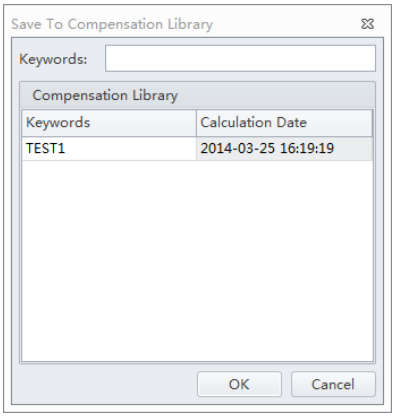
Channel	-FITC%	-PE%	-ECD%	-PC5.5%	-PC7%	-APC%	-APC-A700...	-APC-A750...	-PB450%	-KO525%	-Violet610%	-Violet660%	-Violet780%
FITC		0.66	0.11	0.02	1.43	0.00	0.08	0.00	0.00	0.91	0.11	0.00	0.02
PE	35.08		18.23	1.13	2.39	0.00	0.10	0.00	0.00	1.14	4.10	0.07	0.01
ECD	12.83	41.79		0.55	1.75	0.00	0.09	0.00	0.00	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	

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**.

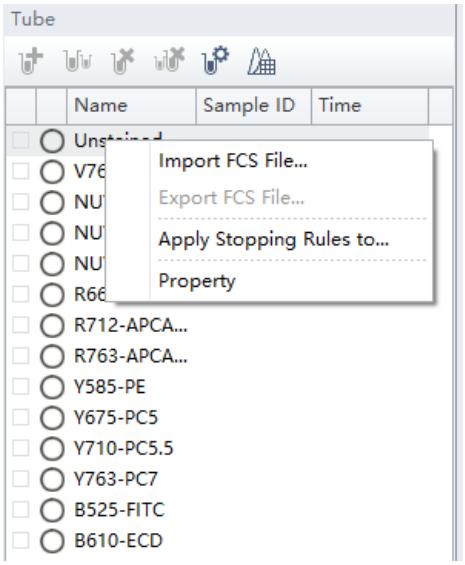
Creating the Compensation Matrix from Previously Acquired Data

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.

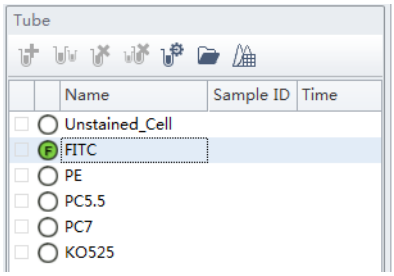
1 Select **New Compensation** from the File menu or the start page.

2 To create a compensation experiment, select the required channels. Refer to Setting the Channel and Label in CHAPTER 5, Data Acquisition and Sample Analysis.

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.



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 samples.

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.

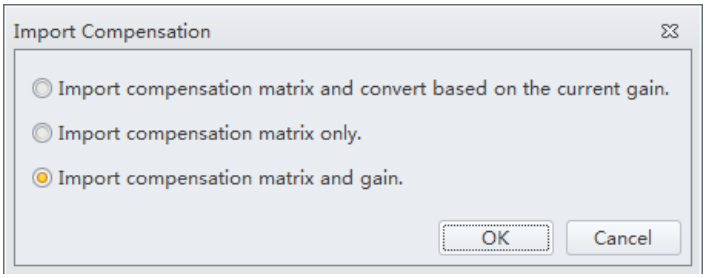
3 Select **Import** and locate the path where compensation matrix files are saved. Select the corresponding compensation matrix file (.comp) to import the compensation values.

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.
- 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 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**.

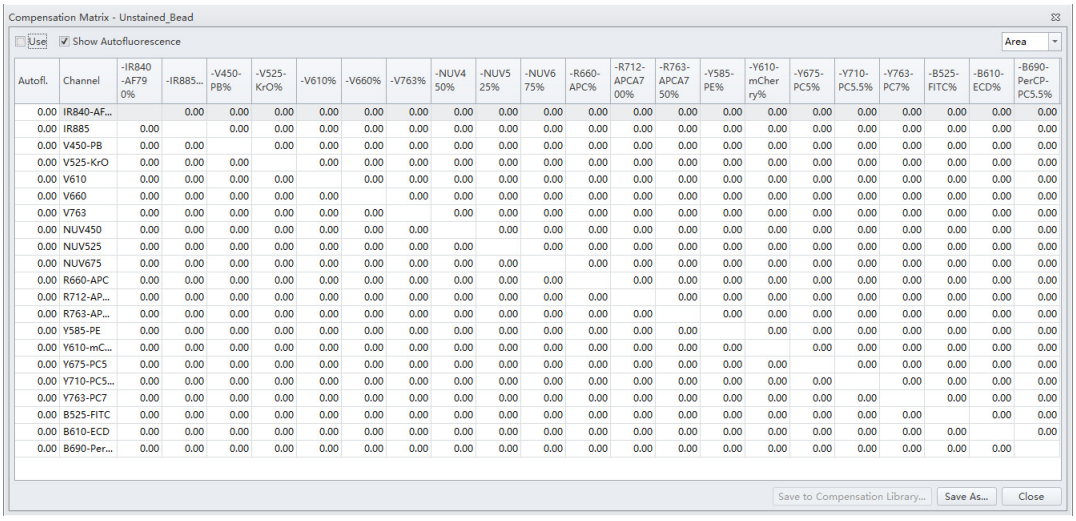
Importing Compensation Settings from the Compensation Library

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.

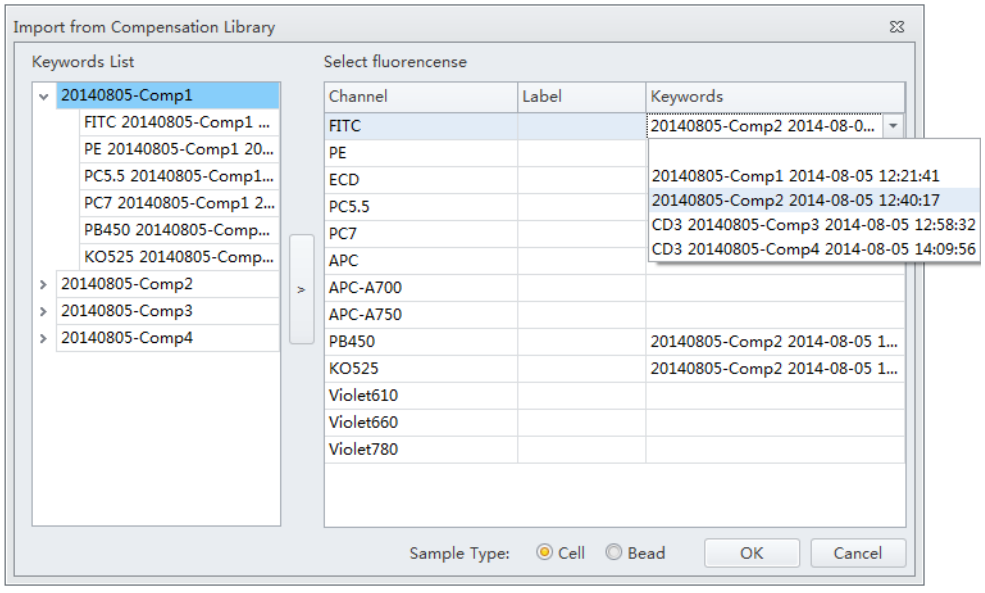
NOTE Files available in the compensation library are configuration-specific. The compensation library only displays the files created under the current default configuration.

1 Select **Import From Compensation Library** to select which compensation values to import from the compensation library.

[CytoFLEX LX Shown]



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.

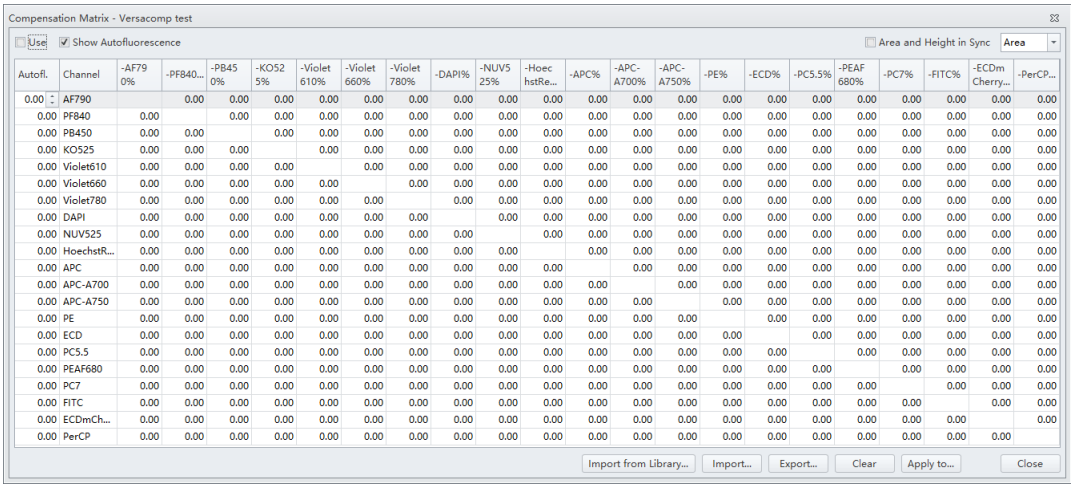
Exporting Compensation Settings

1 Select the desired sample tube to export.

2 Select **Compensation Matrix** in the Setting menu.

3 Select **Export** to specify a path and filename for the compensation file you are saving.

[CytoFLEX LX Shown]



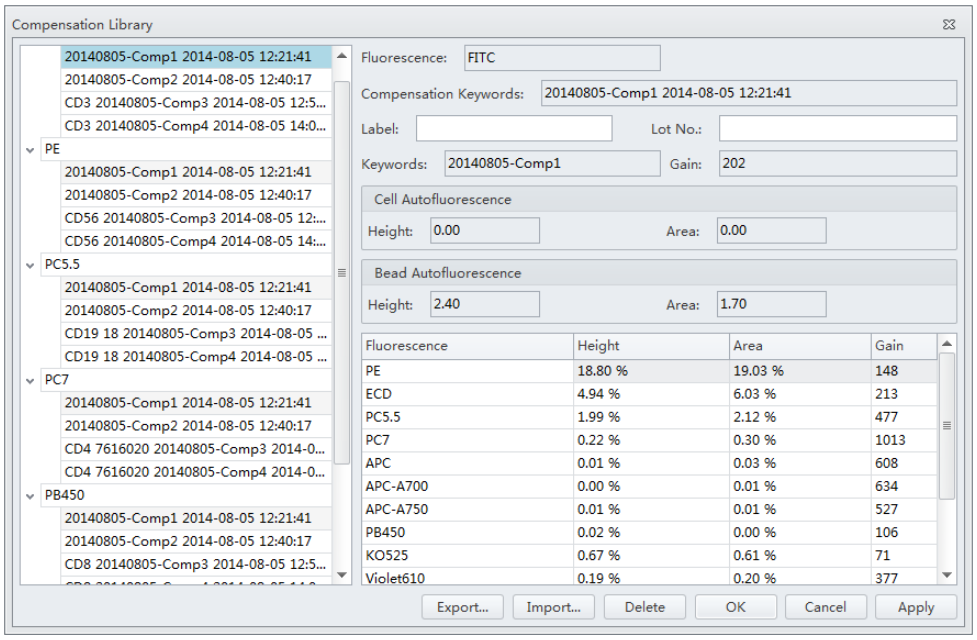
4 Select **Save**.

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.



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

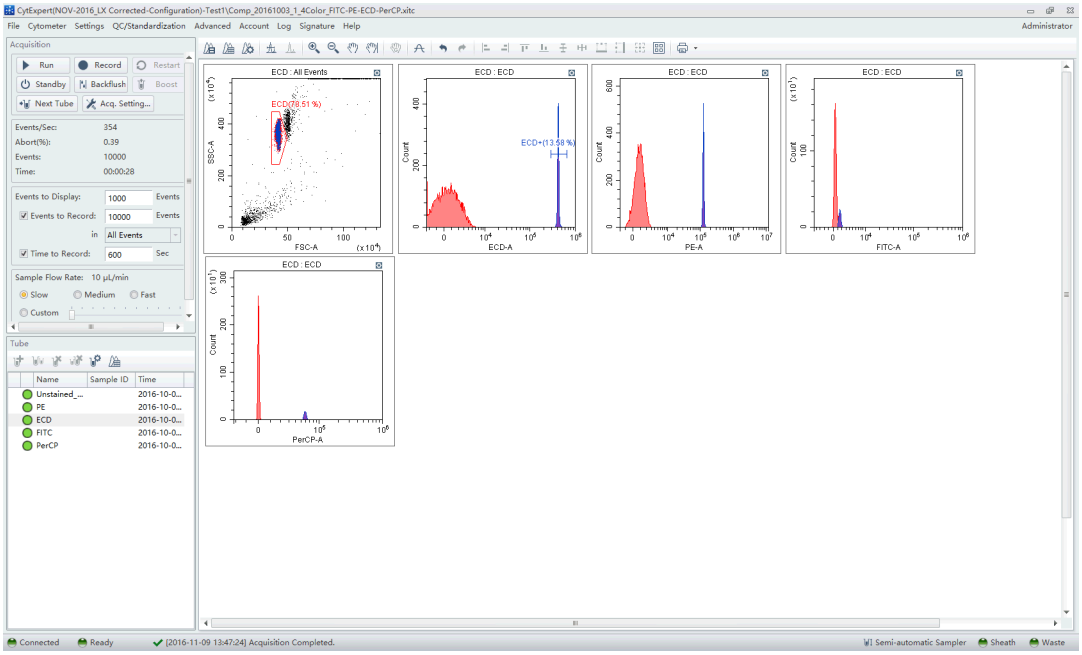
Channels requiring compensation calculations that have not been previously acquired can be added to the compensation experiment by acquiring the necessary positive tubes.

1 In the compensation experiment, select  in the compensation controls, or select **Compensation Setup** in the Compensation 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.

CHAPTER 7

Data Review

Overview

This chapter discusses how to use the Analysis screen to analyze data. Data can be analyzed using any computer equipped with the CytExpert 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

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 software.

Select **Open Experiment** from the Start page or select **Open Experiment** in the File menu to open the copied experiment. Then, select **Save As**.

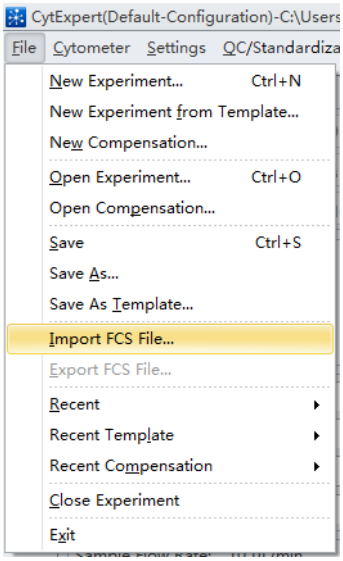
NOTE The .xit and data folder must be stored in the same path.

Importing Previously Acquired Data

The CytExpert software can import and analyze compatible FCS data files acquired by other CytoFLEX flow cytometers.

1 Create a new experiment or open a saved experiment. Refer to Creating an Experiment in CHAPTER 5, Data Acquisition and Sample Analysis.

2 In the new or opened experiment, select **Import FCS File** in the File menu to import the data files.



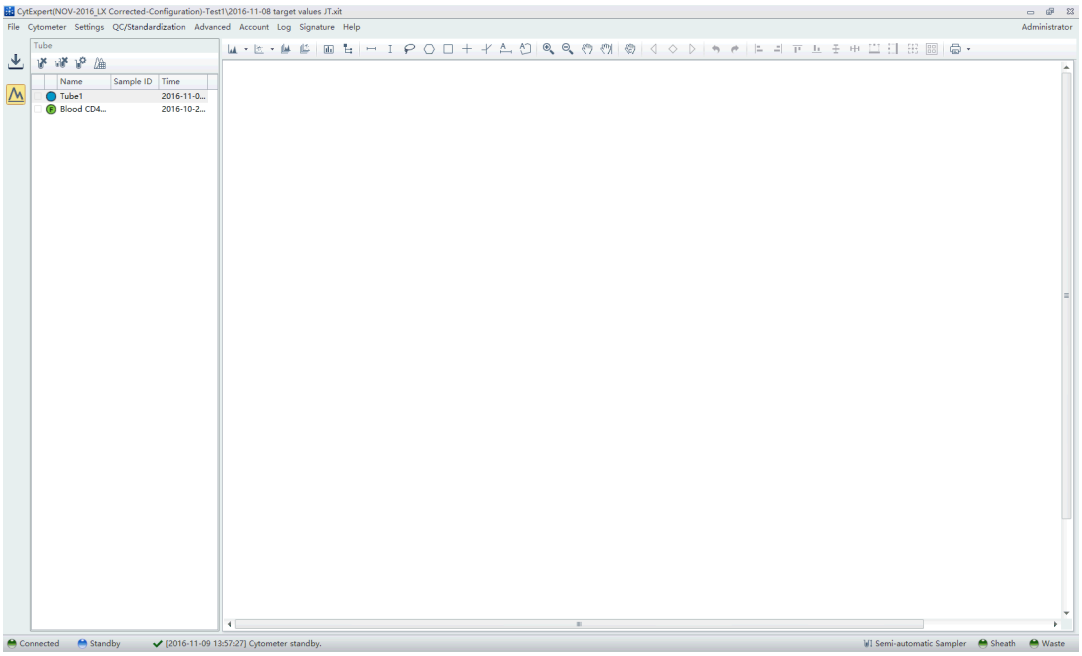
Imported data files appear in the Tube screen.

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.

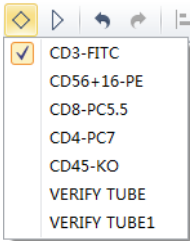
f. Right-click the plot area and select **Paste** from the drop-down menu or press Ctrl+V to paste the plot.

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 toolbar at the top of the page (see Figure 2.1) to change the data displayed in a plot.



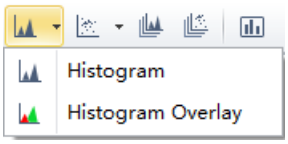
a. Select the plot requiring a change to the data displayed. By pressing and holding the Ctrl key while selecting plots, you can select several plots at one time.

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

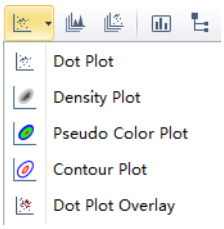
The CytExpert software supports histogram and dot plot data overlay functionality, allowing you to combine data from differing sources onto the same histogram or dot plot.

1 Select **Histogram Overlay** under the histogram icon drop-down list to create a new multi-data histogram.



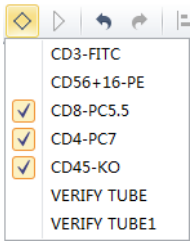
Or

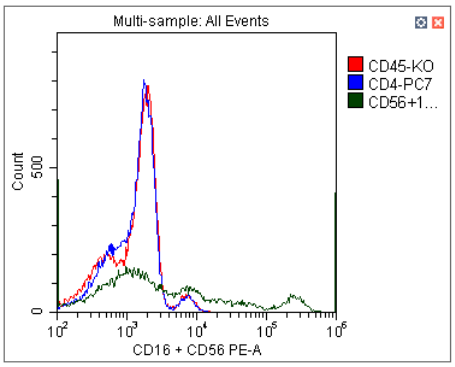
Select **Dot Plot Overlay** under the dot plot icon drop-down list to create a new dot plot overlay.



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, Data Acquisition and Sample Analysis.

Calculating Sample Volume and Concentration

The CytoFLEX flow cytometer supports the calculation of the sample concentration based on the volume consumed and/or based on the known concentration of reference beads.

NOTE If necessary, calibrate the sample uptake rate (see Calibrating the Sample Flow Rate in CHAPTER 11, Replacement/Adjustment Procedures) prior to collecting data for volumetric analysis:

- Select the *cells/µL(V)* checkbox to calculate concentration directly.

  NOTE The direct calculation of concentration can be affected by several conditions such as, the sample's viscosity and sample mixing. Uncalibrated sample volume uptake rates may lead to erroneous results.

- If using reference beads to calculate the concentration, select the *cells/µL(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. The software automatically calculates the original sample concentration based on the input values. (You can also enter the reference bead concentration directly in the beads count field and set the sample volume as 1.)

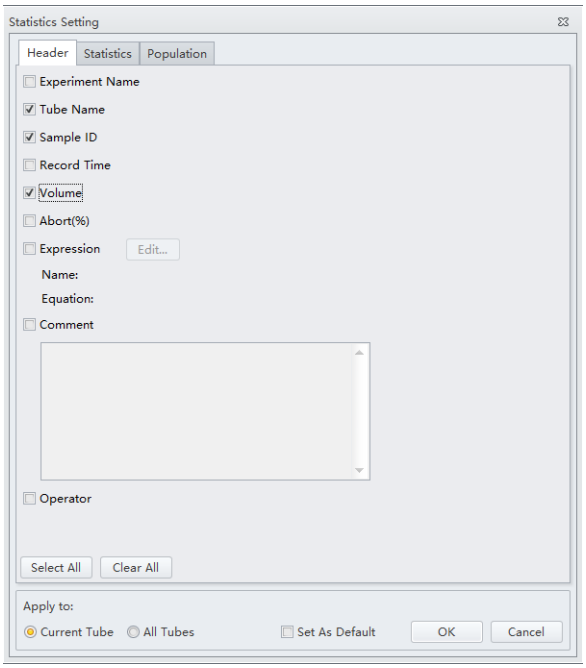
To obtain accurate calculations, throughout the data acquisition process, ensure that:

- 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. When the detection rate does not exceed the stated event rate, running at medium to high acquisition speeds are considered more accurate.
- A constant sampling rate is maintained when recording data.
- You acquire at least 10 µL of sampling volume.

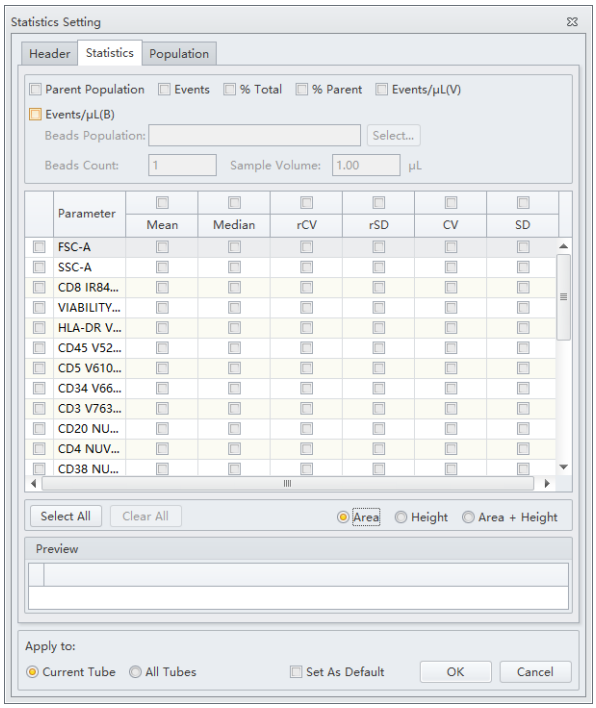
In the Statistics Setting screen, select **Volume** and the concentration item to see the corresponding information in the statistics table.

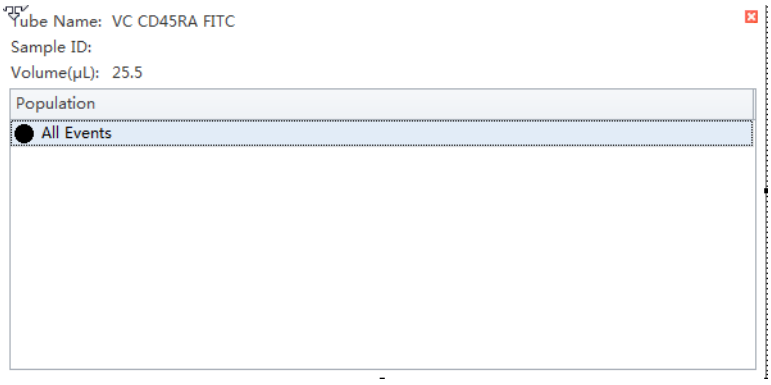
NOTE While collecting samples, instantaneous data calculation can appear inaccurate. Regard the calculation as accurate only after data acquisition has been completed.

[CytoFLEX LX Shown]



[CytoFLEX LX Shown]





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 CHAPTER 5, Data Acquisition and Sample Analysis.

CHAPTER 8

Daily Shutdown

Overview

This chapter provides procedures for shutting down the CytoFLEX instrument.

Workflow:

Prepare the cleaning solution	→	Clean the instrument	→	Turn the instrument off

This chapter contains information on:

- Preparing the Cleaning Solution
- Shutting Down the Instrument
- Auto Shutdown [CytoFLEX LX Only]

Preparing the Cleaning Solution

Required materials

Materials to prepare:

- 12 x 75 mm sample loading tube
- FlowClean
- Deionized water
- Bleach

Set aside 2 mL of FlowClean in one sample tube and 3 mL of the deionized water in a separate sample tube.

Shutting Down the Instrument

1 Run Daily Clean to clean the sample line. Refer to Daily Clean or Daily Clean [With Plate Loader] in CHAPTER 10, Cleaning Procedures.

2 If necessary, empty all waste liquid from the waste container. Refer to Emptying the 4 L Waste Container [CytoFLEX] in CHAPTER 11, Replacement/Adjustment Procedures.

3 Remove the sample tube from the instrument and store according to your laboratory procedures.

4 Select **Standby**.

5 Exit the software.

6 **Optional**: Turn the computer off.

7 **Optional**: Turn the Cytometer's main power switch off.

8 If there are any spills, clean the sample station. Refer to Cleaning the Sample Station in CHAPTER 10, Cleaning Procedures.

Auto Shutdown [CytoFLEX LX Only]

You can set up the system to automatically Shutdown the Cytometer.

To schedule an auto shutdown after acquisition refer to Step 6 of Creating an Experiment [With Plate Loader] in CHAPTER 5, Data Acquisition and Sample Analysis.

To schedule an auto shutdown during Daily Clean refer to Step 3 of Daily Clean [With Plate Loader] in CHAPTER 10, Cleaning Procedures.

CHAPTER 9

Troubleshooting

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:

- Precautions/Hazards
- Hazard Labels and Locations
- RoHS Notice
- Disposal Precaution
- Troubleshooting Table
- Backup and Restore

Precautions/Hazards

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 Cytometer 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 Series flow cytometer can contain up to 6 solid-state diode lasers that are capable of producing laser light at the following levels:

- 355-nm, 20-mW solid-state diode laser
- 375-nm, 60-mW solid-state diode laser
- 405-nm, 80-mW solid-state diode laser
- 488-nm, 50-mW solid-state diode laser
- 561-nm, 30-mW solid-state diode laser
- 638-nm, 50-mW solid-state diode laser
- 808-nm, 60-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.

⚠ WARNING

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.

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, it is important to:

- Limit access to the Cytometer to trained and experienced personnel.
- Never attempt to remove a shield housing a laser.
- Never remove a warning label.
- Contact us if a label is missing or unclear.

Laser Warning Labels

⚠ WARNING

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:

See Figure 9.1 and Figure 9.2 for the Laser Warning Label on the Cytometer optical bench.

See Figure 9.3 and Figure 9.4 for the Laser Warning Label on the optical bench (located Inside the Cytometer).

See Figure 9.6 and Figure 9.7 for the Laser Warning Labels on the Cytometer 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. See Figure 9.1.

Figure 9.1 Laser Warning Label on the Laser Optical Bench [**CytoFLEX**]

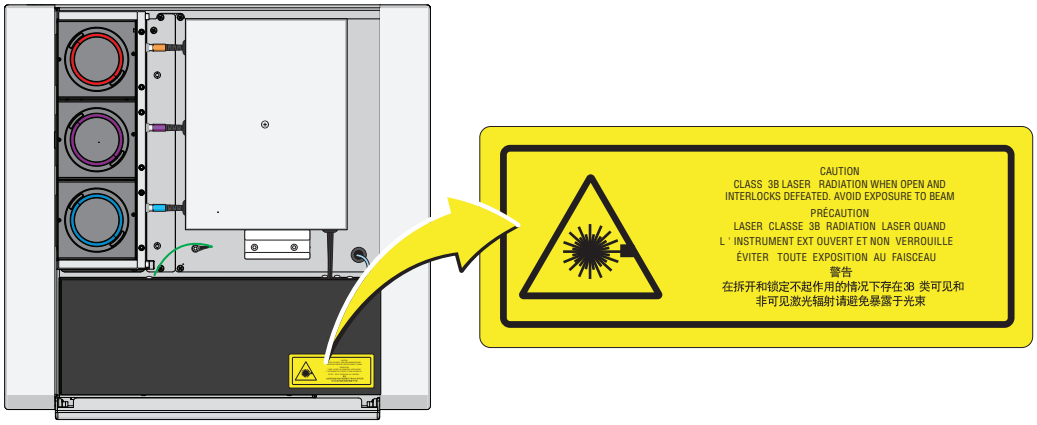


Figure 9.2 Laser Warning Label on the Laser Optical Bench [**CytoFLEX LX**]

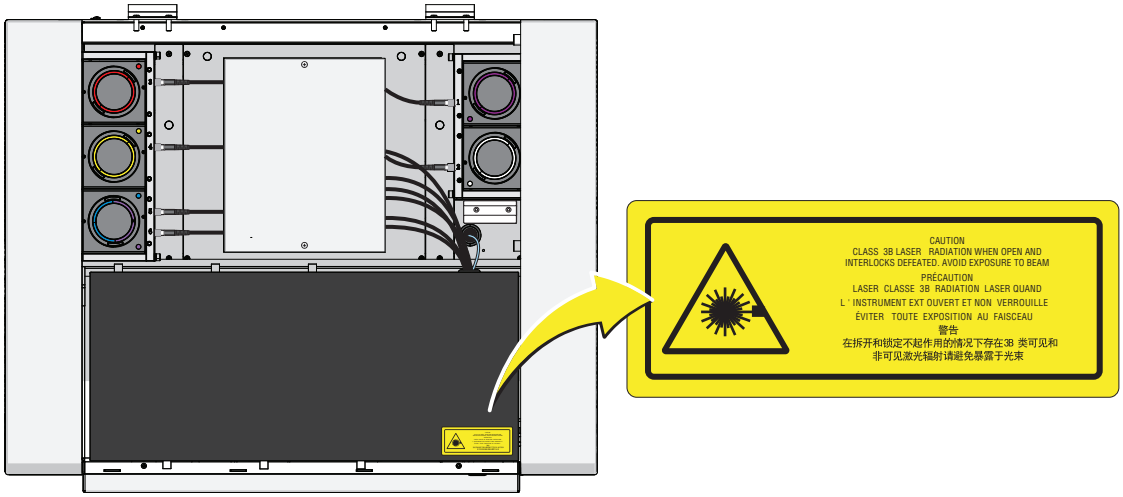


Figure 9.3 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [**CytoFLEX**]

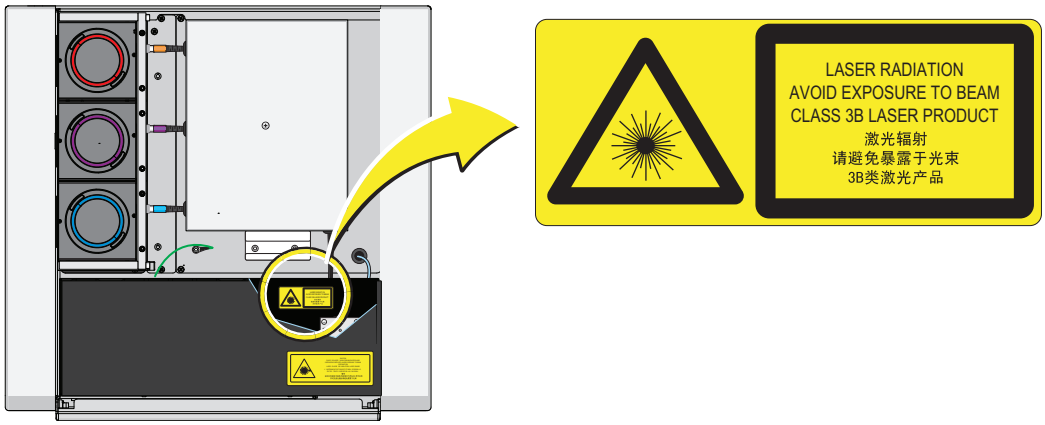


Figure 9.4 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [**CytoFLEX LX**]

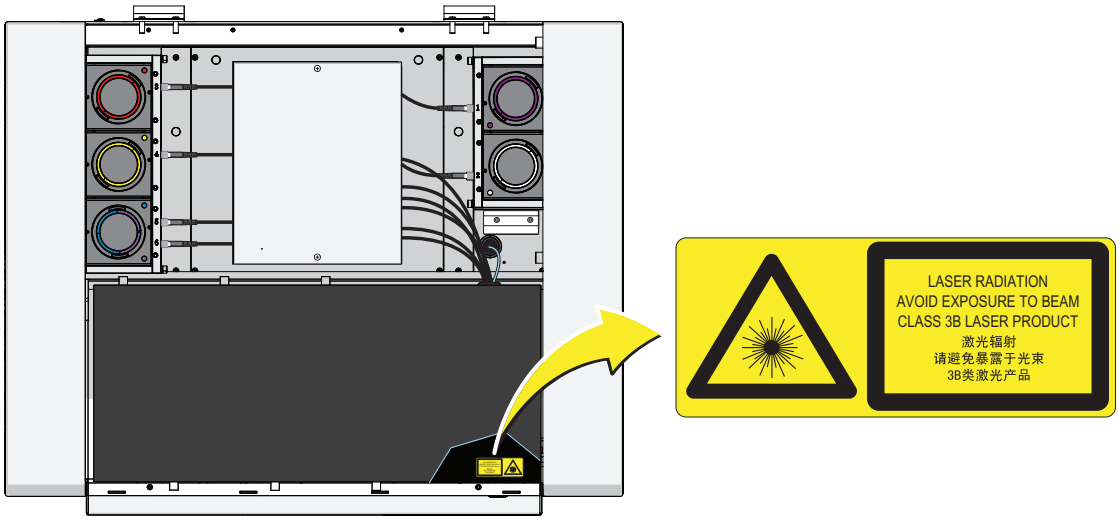


Figure 9.5 Laser Warning Label on the 355-nm Laser [**CytoFLEX LX**]

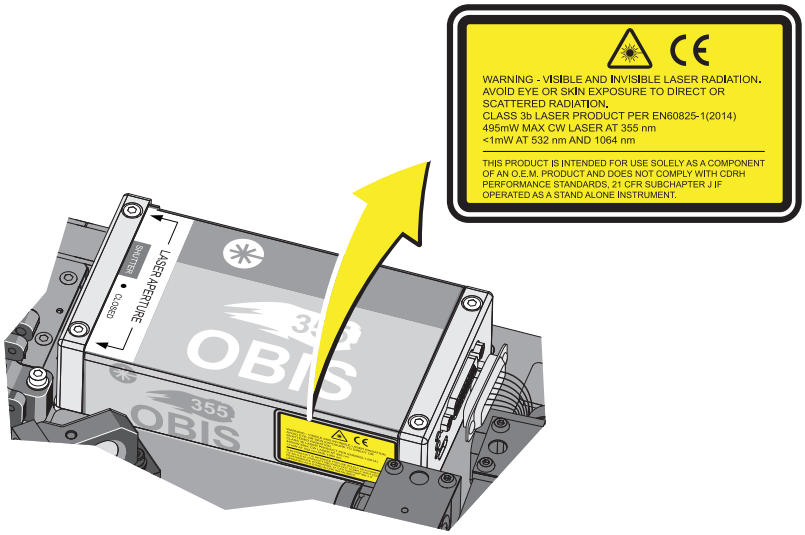


Figure 9.6 Laser Warning Labels on the Cytometer Back Cover [**CytoFLEX**]

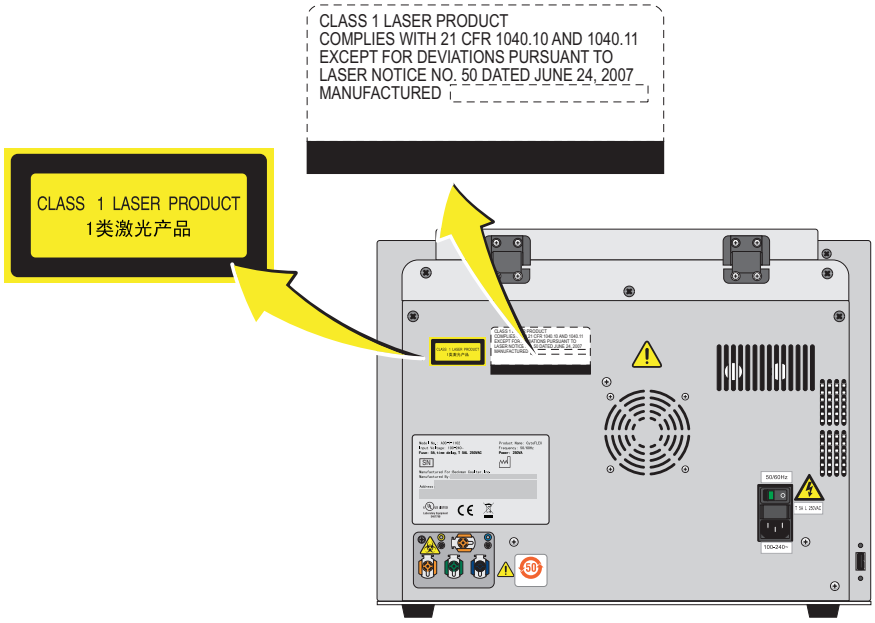
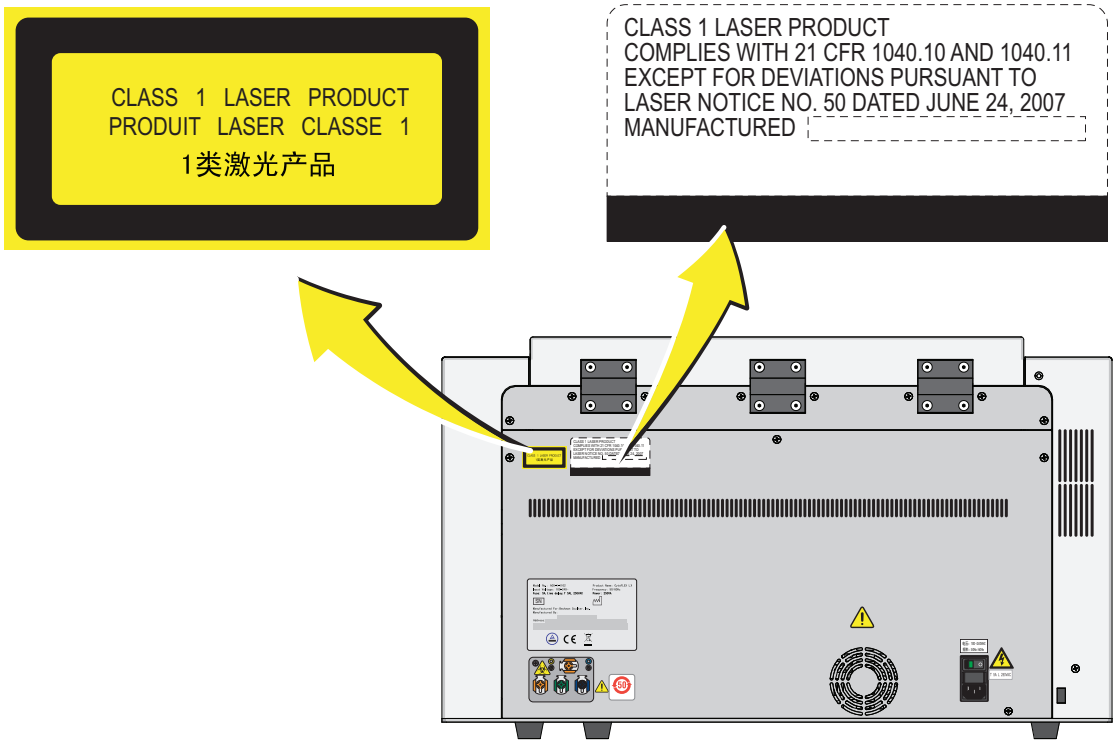


Figure 9.7 Laser Warning Labels on the Cytometer Back Cover [**CytoFLEX LX**]



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 9.8 Biohazard Label on the 4 L Fluid Containers

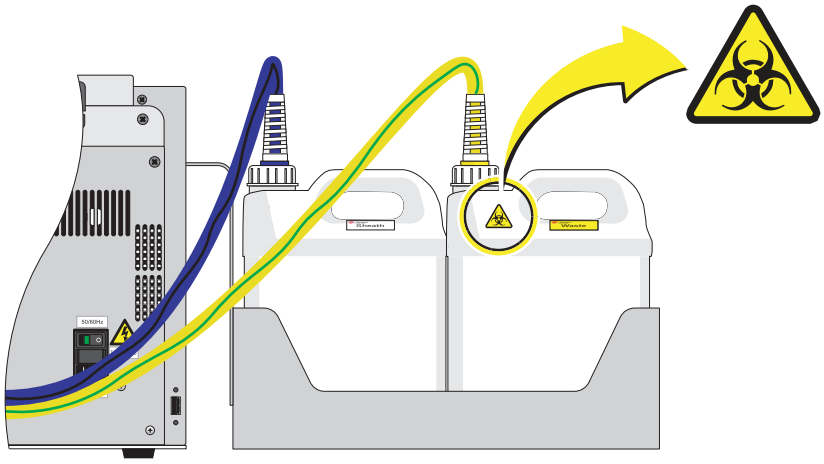


Figure 9.9 Biohazard Label on the 10 L Fluid Cubitainers

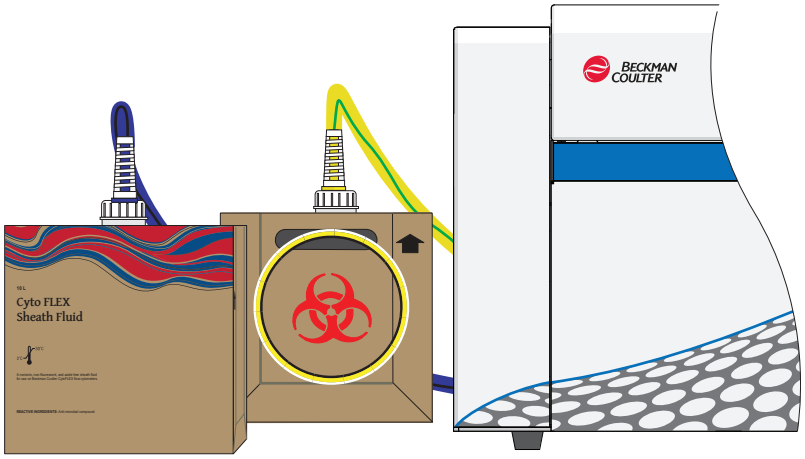
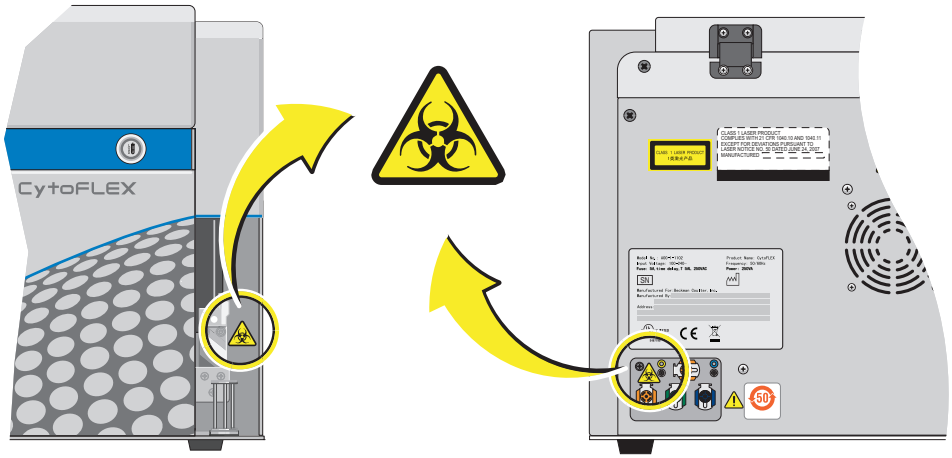
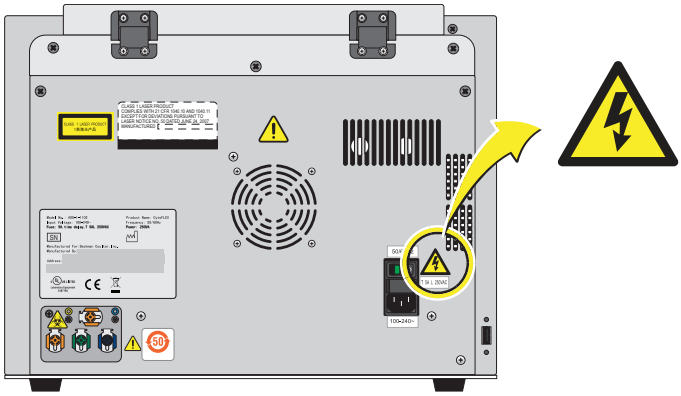


Figure 9.10 Biohazard Label Located in the Sample Station and on the Back of the Cytometer [CytoFLEX Shown]



Electrical Shock Hazard Label and Location

Figure 9.11 Electrical Shock Hazard Label by the Power Switch [CytoFLEX Shown]



Caution Labels and Location

Figure 9.12 Caution Labels [CytoFLEX Shown]

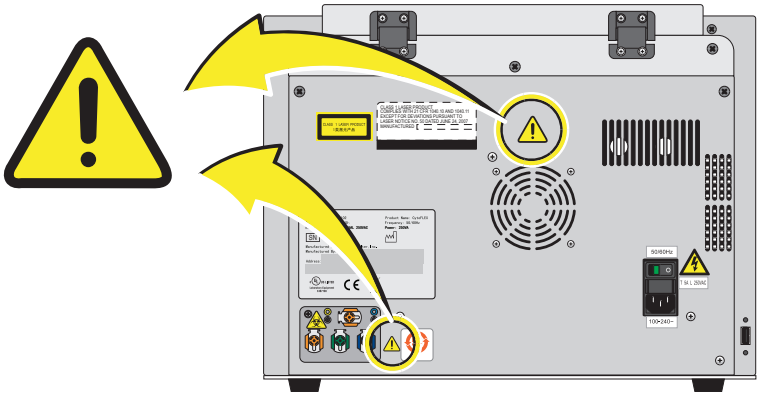
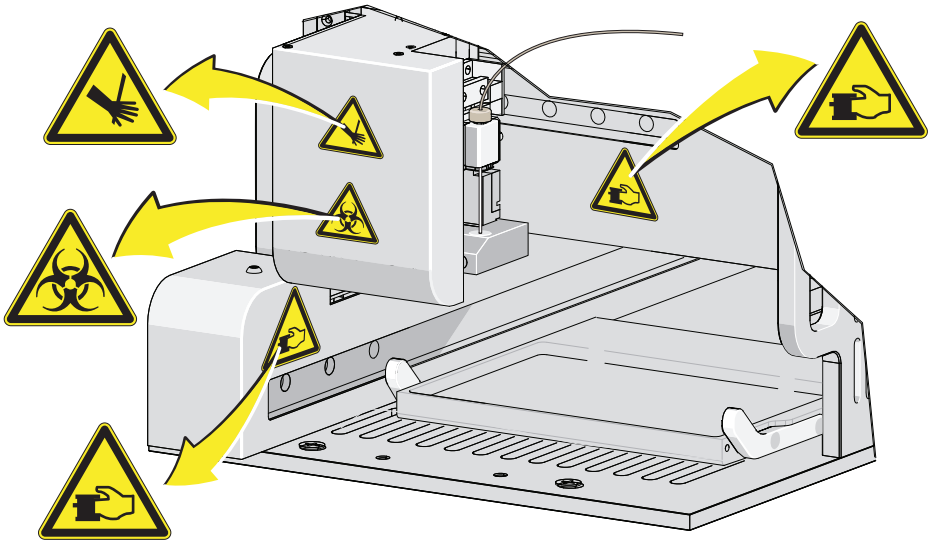


Plate Loader Hazard Labels and Location

Figure 9.13 Plate Loader Hazard Labels



Disposal Of Electrical Instrumentation

It is very important that customers understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

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:

- that the device was put on the European Market after August 13, 2005 and
- that the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

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.

RoHS Notice

These labels and materials declaration table (the Table of Hazardous Substance's Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/T11364-2006 "Marking for Control of Pollution Caused by Electronic Information Products" requirements.

RoHS Caution Label

This label indicates that this 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 recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

RoHS Environmental Label

This label indicates that the electronic information product does not contain any toxic or hazardous substances. The center "e" indicates the product is environmentally safe and does not have an Environmentally Friendly Use Period (EFUP) date. Therefore, it can safely be used indefinitely. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

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 lists problems that you could encounter while running the CytoFLEX flow cytometer, 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

Problem	Probable Cause	Corrective Action
The Cytometer cannot be turned on.	• The instrument is turned off in the Cytometer menu. [CytoFLEX LX] • The power switch is in the off position and the Turn On selection will not function in the Cytometer menu. [CytoFLEX LX] • The power cable is not securely connected. • The fuse is blown.	1. Ensure the power switch is in the on position on the back of the Cytometer. [CytoFLEX LX] 2. Select **Turn On** in the Cytometer menu. [CytoFLEX LX] 3. Ensure that the power cable is securely connected to the back of the Cytometer. 4. Replace the fuse. Refer to CHAPTER 11, Replacing the Fuse in CHAPTER 11, Replacement/Adjustment Procedures. 5. If the problem persists, contact us.
The Workstation cannot be turned on.	• The power cable is not securely connected. • The Workstation was restarted too fast.	1. Ensure that the power cable is securely connected to the back of the Cytometer. 2. Unplug the power cable. Wait 10 seconds, then plug the power cable back in. Then, restart the computer. 3. If the problem persists, contact us.
The connection indicator light in the lower left corner of the software screen is red and displays *Disconnected* and *Error*.	• Data connection error • The Cytometer is not turned on. • The Cytometer's power cable is disconnected.	1. Ensure that the USB data cable is securely connected to the back of the Cytometer and the back of the Workstation. Refer to Figure 1.23. 2. Restart the software. Restart the Workstation.Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. 3. Turn on the Cytometer using the power switch on the back of the instrument. 4. Verify that the power cable is securely connected to the back of the Cytometer. 5. If the problem persists, contact us.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
The alarm does not sound when the waste container is full or the sheath fluid container is low and the software status display is red.	• The alarm is not working. • Instrument data connection error. • The sheath fluid/waste harness float is restricted. • The sheath fluid/waste harnesses have been secured on the wrong container.	**1.** Ensure the sheath fluid/waste harnesses are secured to the correct container. **2.** Ensure that the USB data cable is securely connected to the back of the Cytometer and the back of the Workstation. Refer to Figure 1.23. **3.** Restart the Workstation. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. **4.** Restart the software. **⚠ 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.** **5.** Verify that the float of the sensor in the sheath fluid/waste container moves freely. **6.** Replace the sheath fluid/waste harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. **7.** If the problem persists, contact us.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
The fluid status information displays red for *Sheath* and/or *Waste* even though the sheath fluid container is full and the waste container is empty.	• Instrument data connection error. • The sensor connection is not working properly. • The sensor does not work properly. • The sheath fluid/waste harnesses have been secured on the wrong container.	**1.** Ensure the sheath fluid/waste harnesses are secured to the correct container. **2.** Ensure that the USB data cable is securely connected to the back of the Cytometer and the back of the Workstation. Refer to Figure 1.23. **3.** Restart the software. **4.** Ensure the sheath fluid harness and/or the waste harness are properly connected. **5.** Verify that the float of the sensor in the sheath fluid container and/or waste container moves freely. **6.** Replace the sheath fluid harness and/or the waste harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/ Adjustment Procedures. **7.** If the problem persists, contact us.
The sample tube holder cannot move up and down automatically.	The setting is incorrect.	**1.** Ensure that the sample injection mode in the software is in Semi-Automatic Injection mode. Refer to Selecting the Proper Sample Injection Mode in CHAPTER 3, Daily Startup. **2.** If the problem persists, contact us.
The sample flow rate is unstable.	• The sample probe is clogged. • The sample contains aggregates or clumps. • There are air bubbles in the flow cell. • The sample peristaltic pump tubing is aged. • The sample peristaltic pump tubing is not properly connected.	**1.** Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. **2.** Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures. **3.** Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures. **4.** Filter the sample using an appropriately sized mesh aperture filter. **5.** Ensure that the sample tubing is properly connected. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. **6.** Replace the sample probe and sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. **7.** If the problem persists, contact us.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
The sampling flow rate is too fast.	• The threshold setting is too low. • The sample concentration is too high. • There are too many sample fragments. • The sheath fluid filter is clogged. • The sample flow rate requires calibration.	**1.** Use the manual threshold setting to increase the threshold. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. **2.** Calibrate the sample flow rate. Refer to Calibrating the Sample Flow Rate or Calibrating the Sample Flow Rate [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures. **3.** Dilute the sample and adjust the concentration to approximately 10^6/mL. **4.** Filter the sample using an appropriately sized mesh aperture filter. **5.** Restain the sample. **6.** Replace the Sheath Fluid Filter. Refer to Replacing the Sheath Fluid Filter in CHAPTER 11, Replacement/Adjustment Procedures. **7.** If the problem persists, contact us.
Laser power is low.	Communication error	**1.** Reinitialize. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. **2.** Restart the Cytometer. **3.** If the problem persists, contact us.
Populations are drifting.	• Air bubbles are in the flow cell. • Air bubbles are in the system. • The sheath fluid harness float is restricted.	**1.** Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. **2.** Ensure that the sheath fluid harness and/ or waste harness is not kinked. **3.** Ensure that the sheath fluid harness and/ or waste harness is securely connected. **4.** Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. **5.** Verify that the float of the sensor in the sheath fluid container moves freely. **6.** Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. **7.** If the problem persists, contact us.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
Population amplitude is decreasing and CV values are increasing.	• Air bubbles are in the flow cell. • The flow cell is dirty. • The sheath fluid harness float is restricted.	**1.** Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. **2.** Run the Deep Clean procedure. Refer to Deep Clean Procedure in CHAPTER 10, Cleaning Procedures. **3.** Verify that the float of the sensor in the sheath fluid container moves freely. **4.** Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. **5.** If the problem persists, contact us.
The laser delay values are out of range.	• Air bubbles are in the flow cell. • Air bubbles are in the system. • The sheath fluid harness float is restricted.	**1.** Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. **2.** Ensure that the sheath fluid harness and/ or waste harness is not kinked. **3.** Ensure that the sheath fluid harness and/ or waste harness is securely connected. **4.** Verify that the float of the sensor in the sheath fluid container moves freely. **5.** Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. **6.** If the problem persists, contact us.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
No data acquisition.	• The threshold setting is too high. • The gain setting is too low. • Sheath fluid flow is insufficient. • Laser power is insufficient. • The sample probe is clogged.	**1.** Decrease the threshold setting. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. **2.** Increase the gain setting. Refer to Adjusting the Gain in CHAPTER 5, Data Acquisition and Sample Analysis. **3.** Ensure that the sheath fluid harness and/or waste harness is not kinked. **4.** Ensure that the sheath fluid harness and/or waste harness is securely connected. **5.** Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. **6.** Reinitialize. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. **7.** Restart the Cytometer. **8.** Verify that your sample does not have excessive debris. If it does: **a.** Filter the sample using an appropriately sized mesh aperture filter. **b.** Restain the sample. **9.** Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures. **10.** Replace the sample probe and the sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. **11.** If the problem persists, contact us.
Data populations are normal on one laser, but too low on another laser.	The laser delay setting is incorrect.	**1.** Ensure that the laser delay is set correctly. Refer to Setting Laser Delay in CHAPTER 11, Replacement/Adjustment Procedures. **2.** If the problem persists, contact us

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
Data populations are not where they are expected.	• The detector configuration setting is incorrect. • The optical filter is not placed correctly. • QC was not completed. • Gain and threshold is not set correctly.	**1.** Ensure that the detector configuration is set correctly. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Data Acquisition and Sample Analysis. **2.** Ensure that the position of the optical filter in the WDM matches the detector configuration setting. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Data Acquisition and Sample Analysis. **3.** Ensure that the optical filter is installed correctly. Refer to Replacing the Optical Filter in CHAPTER 11, Replacement/ Adjustment Procedures. **4.** Follow the QC procedure. Refer to CHAPTER 4, Instrument Quality Control and Standardization. **5.** Review the gain and threshold settings. Refer to Adjusting the Gain and Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. **6.** Review the display ranges. Refer to Creating Plots and Gates in CHAPTER 5, Data Acquisition and Sample Analysis. **7.** If the problem persists, contact us.
No changes occurred after manually adjusting compensation settings.	Compensation was applied to the wrong channel.	Ensure that the adjustment is applied to the correct primary and secondary channels in the compensation matrix. Or Select  to modify compensation in the desired plot.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
The calculation of the automatic compensation experiment is incorrect.	• Erroneous data acquisition. • The gate is not set on the appropriate population. • The events of the acquired cells are too low. • The mean fluorescence of the positive cells is too weak.	**1.** Ensure that the corresponding negative control tube and the individual positive tube acquired are from the same sample type. **2.** Ensure that the single colors collected correspond to the correct tube name. **3.** Ensure that the gate in the FSC/SSC plot encloses the correct sample population. **4.** Ensure that the positive gate in each tube is correctly placed. **5.** Modify the events to record to ensure that enough events are collected for the data population. **6.** Select samples with a stronger positive signal as the positive control. Or Use dedicated compensation beads such as VersaComp Antibody Capture Beads
The sample is flowing, but no signal appears in the plot.	• The signal is outside of the display range. • The parent gate is not positioned properly and does not contain events. • The population color setting is too light. • The threshold is too high.	**1.** Use either  or  to modify the display range. Or Right-click the plot and select **Property**. The Plot Property window appears. Select **Fit with sample**. **2.** Ensure that the parent gate is gated correctly. **3.** Change the display color. **4.** Move the sample above the threshold using one of the following methods: • Decrease the threshold setting. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. • Increase the gain setting. Refer to Adjusting the Gain in CHAPTER 5, Data Acquisition and Sample Analysis

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
The concentration calculation is incorrect.	• The sample concentration is not within the specified range. • The sample settled. • The sample flow rate is too fast. • The sample volume analyzed is too low. • The cell population is not detected.	**1.** Ensure that the pipette used in sample processing is calibrated. **2.** Verify that the concentration of the sample is between $2x10^4$-10^7 events/mL. **3.** Vortex the sample before loading and verify that the sample is evenly mixed before loading the sample. **NOTE** An excessively long sample loading time leads to sample settlement. **4.** Ensure that the sample flow rate does not exceed 10,000 events/second. **5.** Adjust the threshold to remove sample debris. **6.** Ensure that the sample volume analyzed exceeds 10 µL. **7.** Ensure that the following are correct: • Gain. Refer to Adjusting the Gain in CHAPTER 5, Data Acquisition and Sample Analysis. • Threshold. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. • Compensation settings. Refer to CHAPTER 6, Compensation) **8.** Ensure that the gating and population hierarchy are correct.
The sample probe is too low.	The sample probe is not securely connected.	**1.** Ensure that the sample probe is securely connected to the sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. **2.** Ensure the sample pump cover is securely fastened. **3.** If the problem persists, contact us.
The wash station drips during backflush.	• The sample probe is not securely connected. • The wash station height adjustment is not correct.	**1.** Ensure that the sample probe is securely connected to the sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. **2.** Ensure the sample pump cover is securely fastened. **3.** If the problem persists,contact us.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
The mixer is not functioning.	• Sample mixing is disabled in the software. • The mixer motor is defective.	**1.** Ensure sample mixing is enabled in the software. Refer to Changing Sample Mixing and Backflush Settings in CHAPTER 11, Replacement/Adjustment Procedures. **2.** If the problem persists, contact us.
Instrument operations cannot be performed in the Acquisition screen.	• The instrument is in standby mode. • The software is frozen. • Data connection error.	**1.** Select **Initialize**. **2.** Ensure that the power switch to the Cytometer is turned on. **3.** Restart the software. **4.** Restart the Workstation. **5.** Ensure that the USB data cable is securely connected to the back of the Cytometer and the back of the Workstation. Refer to Figure 1.23. **6.** If the problem persists, contact us.
Software installation fails.	Multiple issues.	Contact us.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
QC aborted due to low event rate.	• The diluted CytoFLEX Daily QC Fluorospheres or CytoFLEX Daily IR QC Fluorospheres concentration is too low. • The sample probe is clogged. • The sample line is clogged.	**1.** Add 1 drop of CytoFLEX Daily QC Fluorospheres to the QC solution. Then, rerun QC. **2.** Reload the target value file. Refer to Importing Lot-Specific Target Values in CHAPTER 4, Instrument Quality Control and Standardization. Then, rerun QC. **3.** Prepare a new sample of the CytoFLEX Daily QC Fluorospheres. Then, rerun QC. **4.** Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures. Then, rerun QC. **5.** Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Then, rerun QC. **6.** Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures. Then, rerun QC. **7.** Replace the sample probe and sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. Then, rerun QC. **8.** If problem persists, contact us.

Table 9.1 Troubleshooting *(Continued)*

Problem	Probable Cause	Corrective Action
QC failed.	• The sheath fluid container and the waste container are not on the same level as the Cytometer. • The median fluorescence fails to meet the target specification. • The QC gain value does not meet the target gain specifications. • The laser delay settings are too high. • rCV fails specifications.	**1.** Ensure the sheath fluid container and the waster container are on the same level as the Cytometer. **2.** Rerun QC. Refer to CHAPTER 4, Instrument Quality Control and Standardization. **3.** Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Then, rerun QC. **4.** Prime the sheath fluid filter with sheath fluid as follows, then rerun QC. **a.** Remove the vent cap from the sheath fluid filter. **b.** Ensure that the instrument is in Standby. **c.** At the Workstation, select **Cytometer** > **Prime**. **IMPORTANT** If the vent cap is not reinstalled as soon as the sheath fluid approaches the vent port, the sheath fluid will overflow. **d.** Wait until the sheath fluid approaches the vent port in the sheath fluid filter, then immediately reinstall the vent cap to avoid overflow. **5.** Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures. Then, rerun QC. **6.** Run Deep Clean. Refer to Deep Clean Procedure in CHAPTER 10, Cleaning Procedures. Then, rerun QC. **7.** If the problem persists, contact us.

Table 9.2 Troubleshooting [With Plate Loader]

Problem	Probable Cause	Corrective Action
The sample probe comes in contact with the bottom of the well plate.	• Incorrect plate type selected. • The plate or plate holder is installed incorrectly. • The sample probe sampling position is not calibrated.	**1.** Verify that the correct plate type is selected in the Plate window. **2.** Ensure the plate and the plate holder are installed correctly. Refer to Replacing the Plate Holder [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures. **3.** Calibrate the sample probe sampling position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.
The dead volume of the plate wells are high.	• Incorrect plate type selected. • The sample probe sampling position is not calibrated.	**1.** Verify that the correct plate type is selected in the Plate window. **2.** Calibrate the sample probe sampling position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.
Mixing does not sufficiently suspend the sample particles.	• Incorrect plate type selected. • Incorrect sample mixing setting. • The sample probe sampling position is not calibrated.	**1.** Verify that the correct plate type is selected in the Plate window. **2.** Verify the sample mixing duration in the Plate window. **3.** Calibrate the sample probe sampling position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.
Gain is over 3,000 and default gain settings are needed.	Corrupt/incorrect configuration file.	Reload the configuration file. Refer to Installing the Instrument Configuration File in APPENDIX A, Instrument Installation.
The configuration file does not match the instrument.	Corrupt/incorrect configuration file.	**1.** Verify the correct configuration file is installed. **2.** Reload the configuration file. Refer to Installing the Instrument Configuration File in APPENDIX A, Instrument Installation.
The Administrative account is locked.	The Admin password was forgotten and the attempts exceed the limit.	**1.** Select **Forgot password**. **2.** Contact us
Plots are cut off when printing a PDF.	Plots need to be rearranged.	Rearrange the plots until the print preview screen shows all plots correctly.

Backup and Restore

IMPORTANT If you have the Electronic Record Management software option installed, ensure the following prior to attempting to restore data:

- The target system includes all of the disk volumes that each experiment directory is saved to.
- The user performing the restore has *Set Experiment Directory* permission in CytExpert.

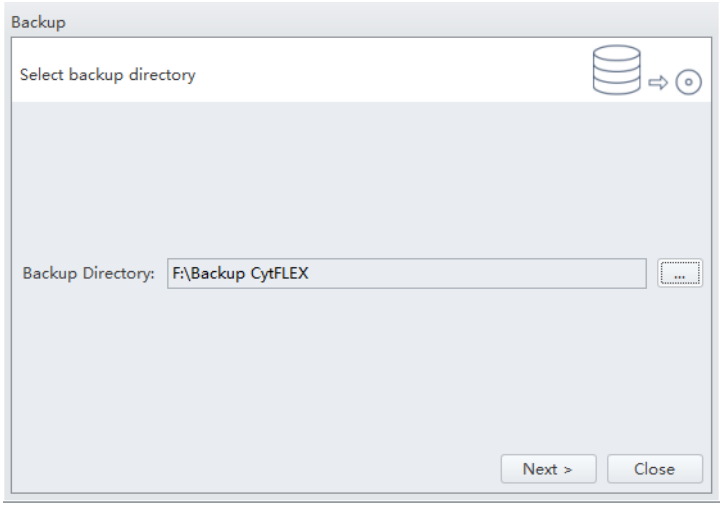
NOTE These procedures are only available if you have either the User Management or Electronic Record Management software option installed.

- **Electronic Record Management**: Both the database and experiment files can be backed up or restored.
- **User Management**: Only the database can be backed up or restored.

Backup

1 Launch CytExpert.

2 Select **Backup/Restore > Backup**. The Backup window appears.

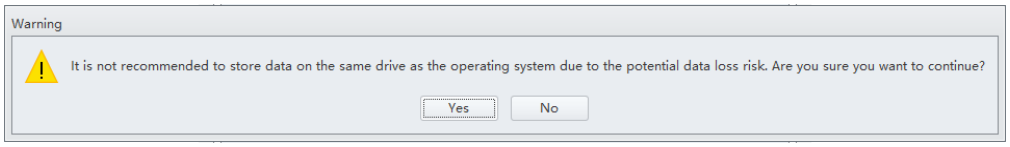


⚠ CAUTION

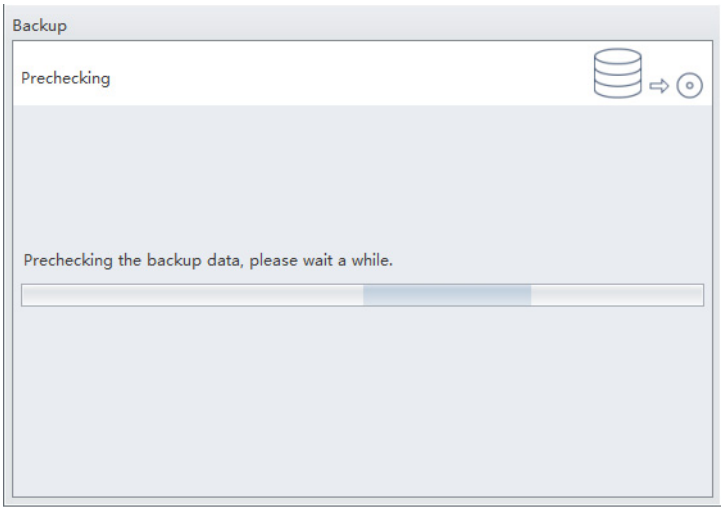
Risk of data loss. Beckman Coulter recommends storing data on a drive other than the operating system.

3 Select ... and browse to the desired backup directory to store the backup data.

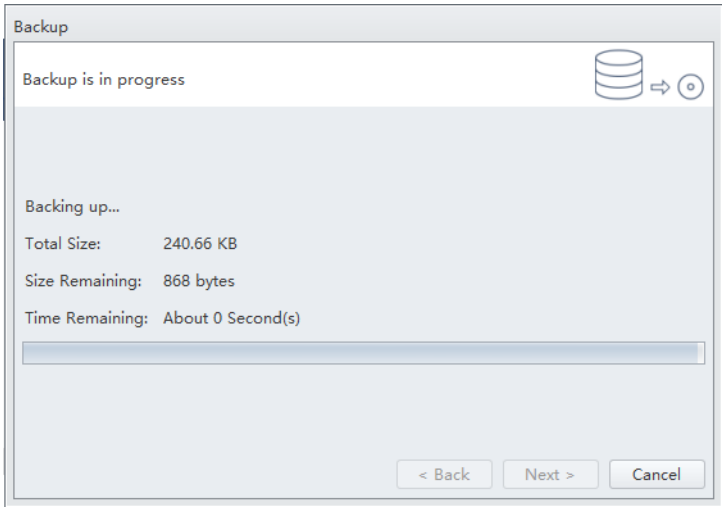
Selecting the same drive as the operation system prompts the following system warning:

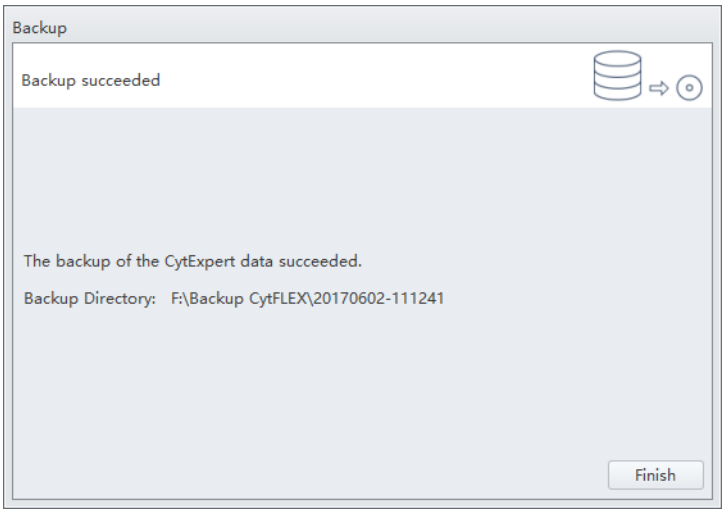


4 Select **Next**. The Prechecking window appears.



The backup starts automatically after the prechecking.

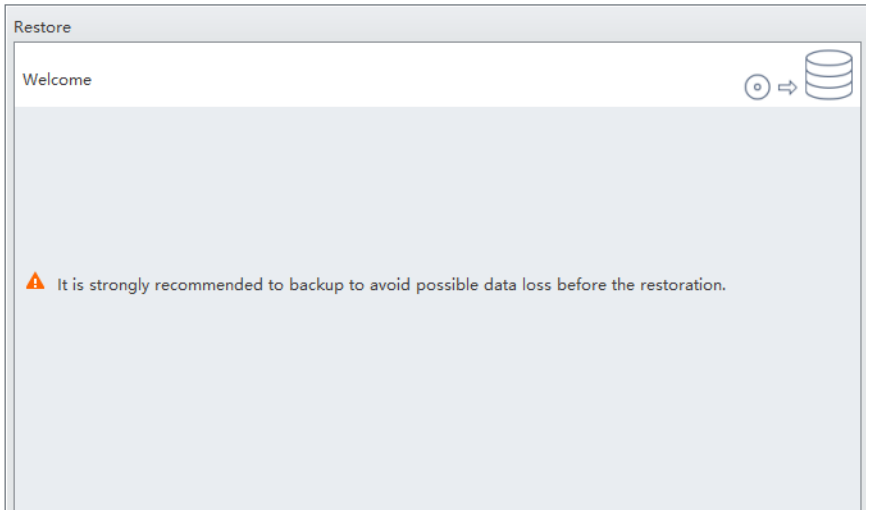




5 Select **Finish**.

Restore

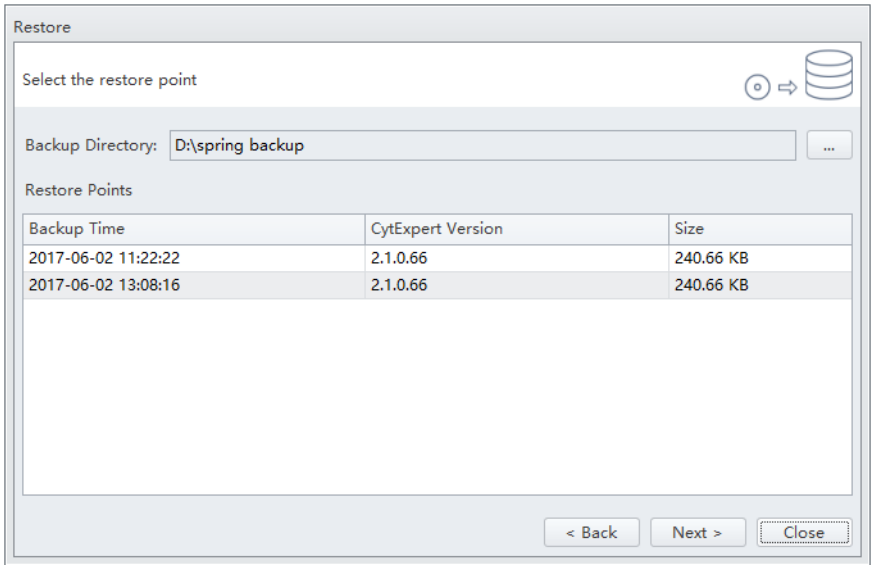
1 Select **Backup/Restore** > **Restore.** The Restore window appears.



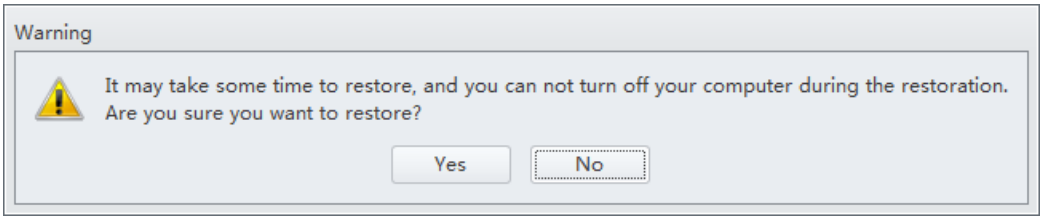
NOTE Ensure the data is backed up before the restoration.

2 Select **Next.**

3 Select ... and browse to the desired directory to restore.

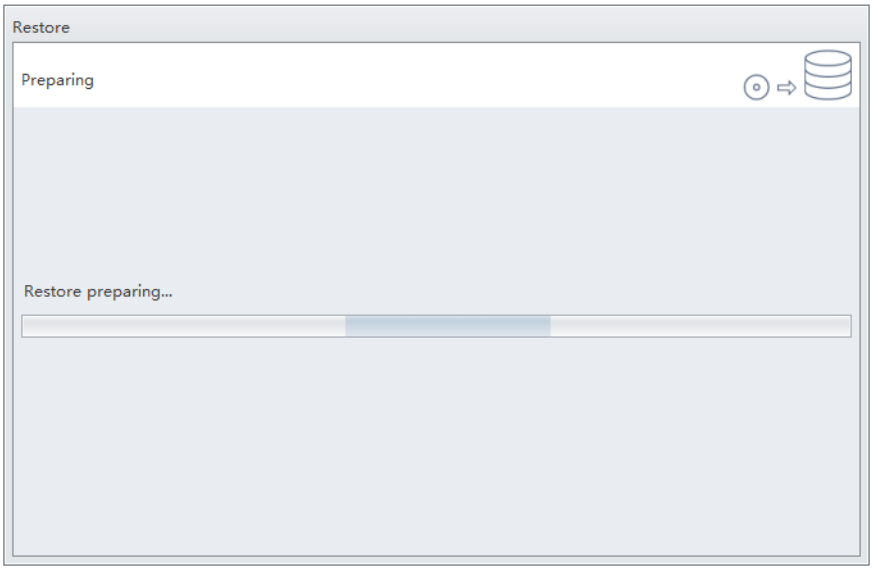


4 Select **Next**. The Warning window appears.

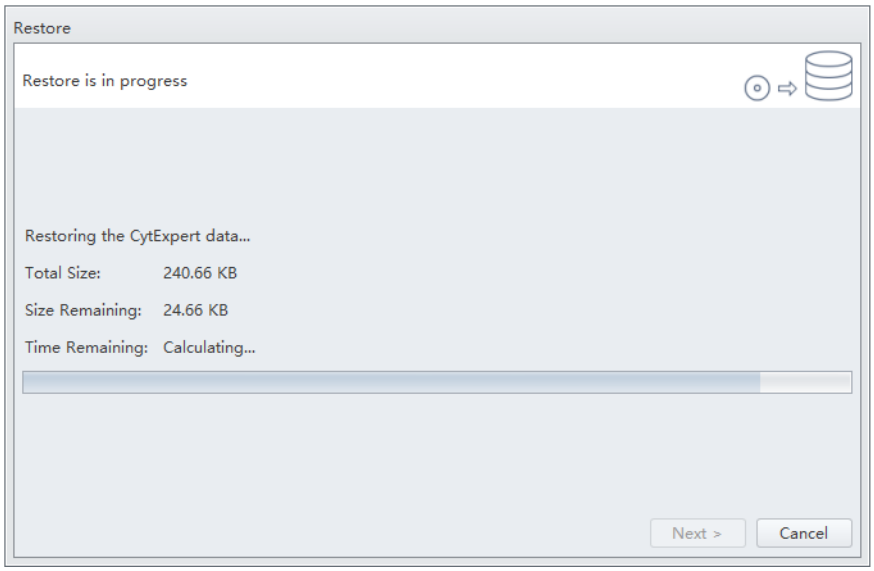


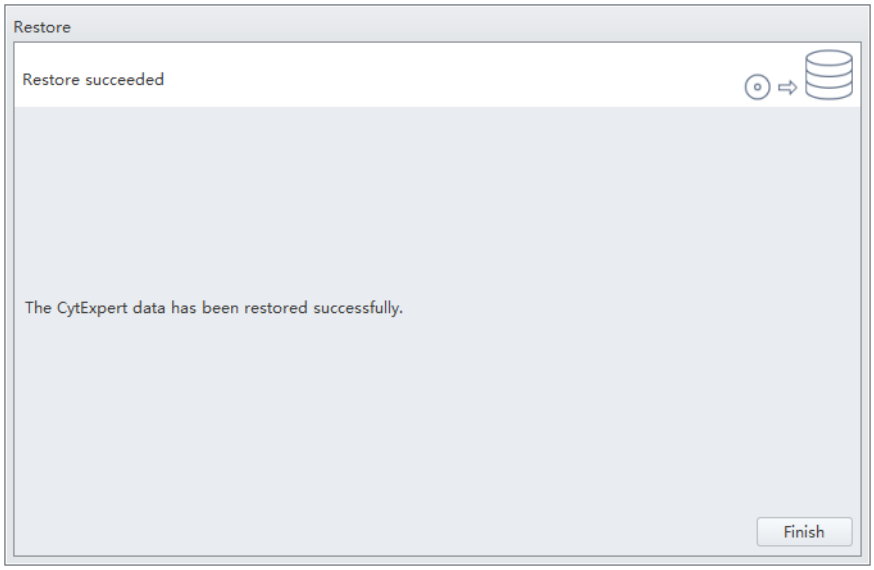
IMPORTANT Do not turn off your computer during the restoration.

5 Select **Yes**. The Preparing window appears.

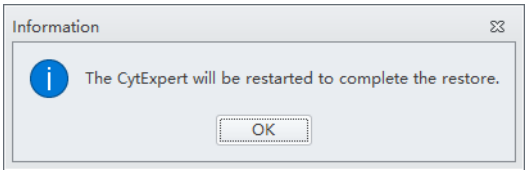


The restoration starts automatically after finishing the preparation.





6 Select **Finish**. The following system prompt appears.



7 Select **OK**.

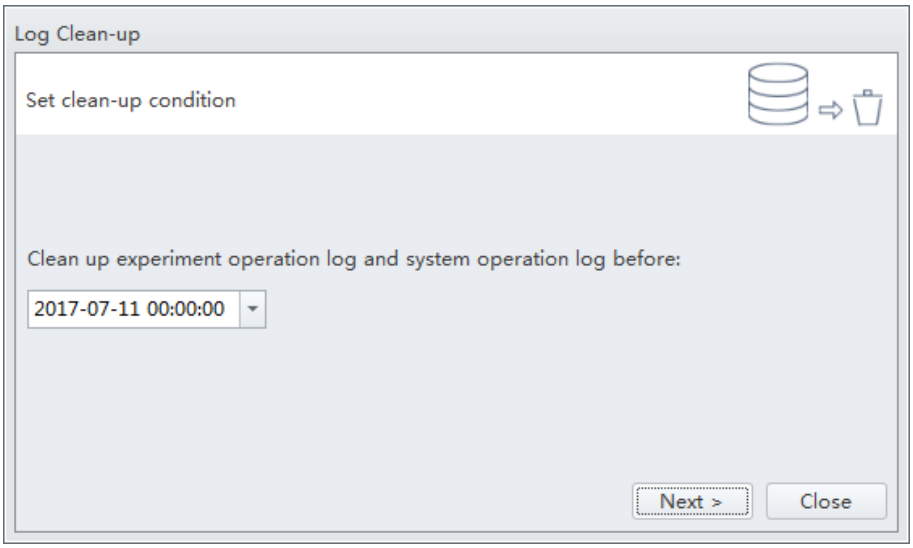
Log Cleanup

Use Log Cleanup to delete Experiment Operation Logs and System Operation Logs before a selected date.

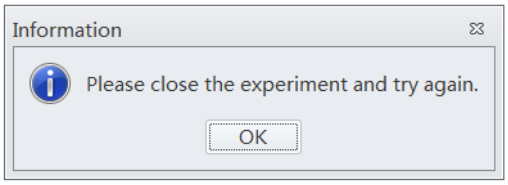
NOTE These procedures are only available if you have either the User Management or Electronic Record Management software option installed.

1 Ensure all experiment files are closed.

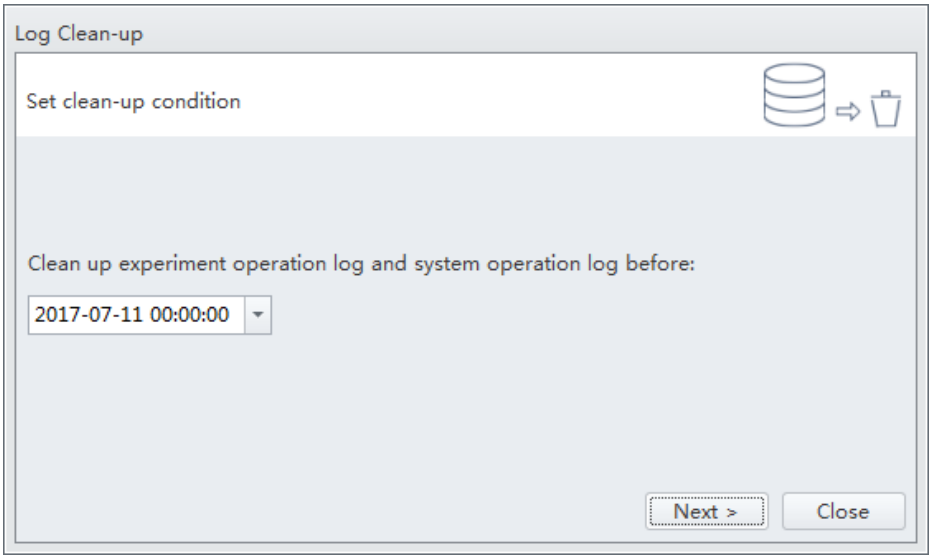
2 Select **Backup/Restore** > **Log Cleanup**. The Log Clean-up window appears.



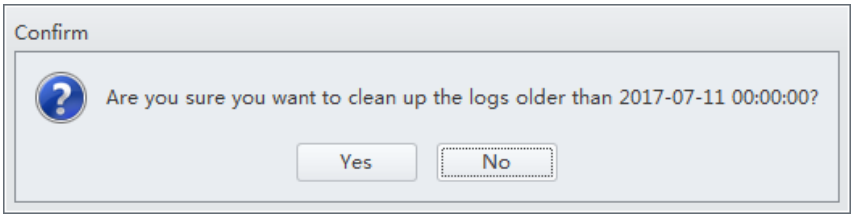
Close the experiment and try again if the operation system prompts the following message:



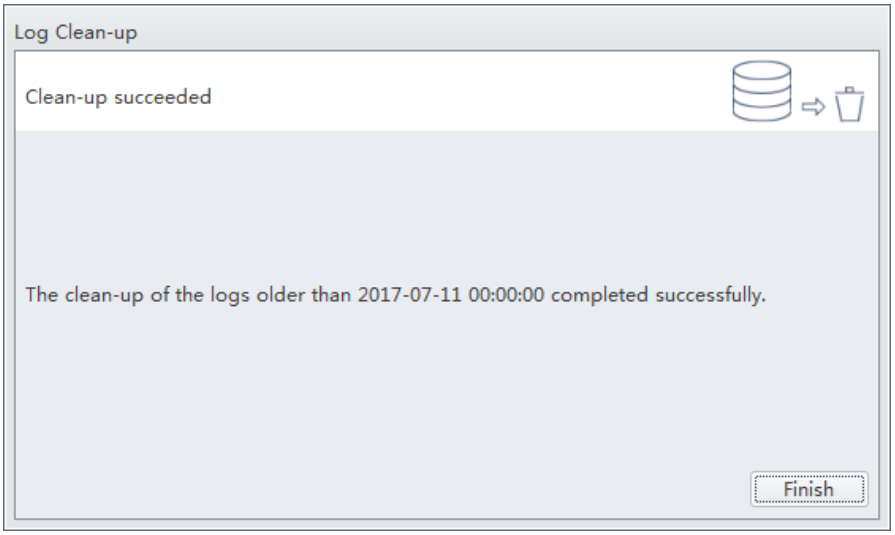
3 Select the desired date and time.



4 Select **Next**. The Confirm window appears.



5 Select **Yes**. The Clean-up succeeded window appears.



6 Select **Finish**.

CHAPTER 10

Cleaning Procedures

Overview

This chapter describes how to carry out certain routine and nonscheduled cleaning procedures. Proper cleaning can help extend the service life of the instrument and ensure experimental accuracy. When conducting any cleaning, take all necessary biosafety precautions and use proper personal protective equipment.

This chapter contains information on:

Routine Cleaning

Daily Clean

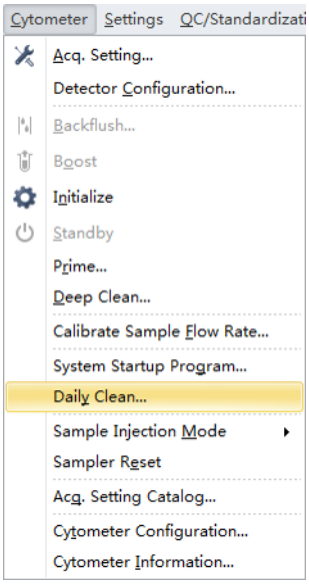
Daily Clean should be performed during instrument startup and instrument shutdown to clean the sample line.

After sampling an excessively large sample or a sample that can easily clog the sample probe, it is recommended to perform the Daily Clean procedure. Daily Clean can also be used to remove residual sample from previous tubes.

---

1 Open the CytExpert software and confirm that the instrument is connected and that it has already been initialized. Refer to Logging Into the Software in CHAPTER 3, Daily Startup.

2 Select **Daily Clean** in the Cytometer menu.

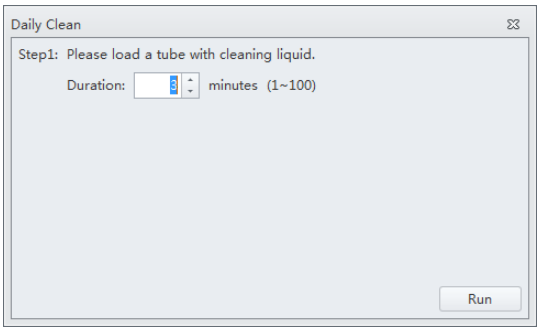


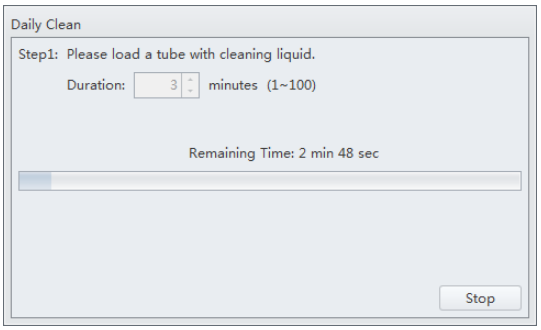
3 Add 2 mL of FlowClean solution to an unused sample tube.

4 Add 3 mL of DI water to an unused sample tube.

5 Insert the sample tube with 2 mL of FlowClean solution into the sample holder and select **Run**.

NOTE The default cleaning time is 3 minutes.

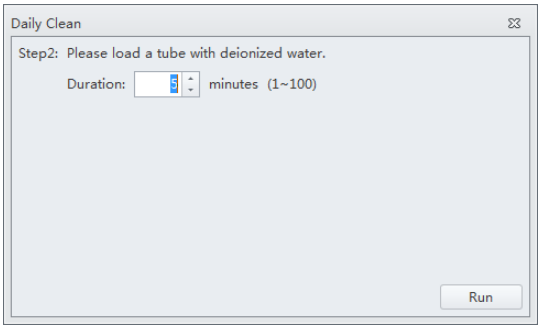


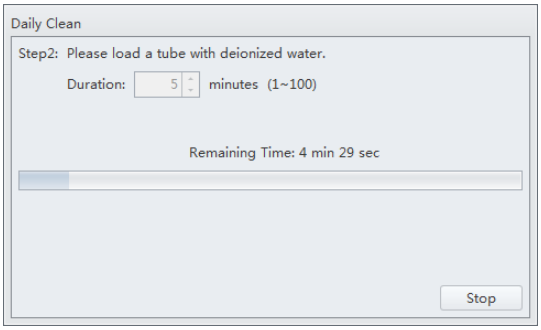


6 Remove the Flow Clean tube.

7 Insert the sample tube with 3 mL of DI water into the sample holder and select **Run** to perform the second step of the cleaning process.

NOTE The default cleaning time is 5 minutes.





8 After the process has been completed, remove the sample tube and close the Daily Clean window.

Daily Clean [With Plate Loader]

Daily Clean should be performed during instrument startup and instrument shutdown to clean the sample line.

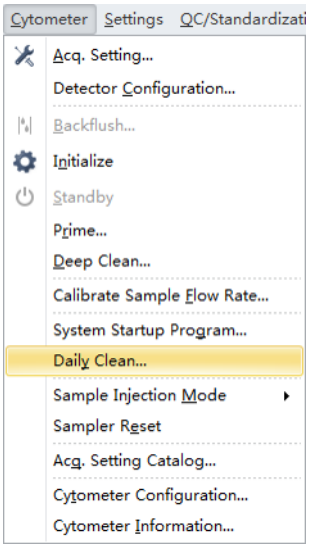
After sampling an excessively large sample or a sample that can easily clog the sample probe, it is recommended to perform the Daily Clean procedure. Daily Clean can also be used to remove residual sample from previous tubes.

---

1 Open the CytExpert software and confirm that the instrument is connected and that it has already been initialized. Refer to Logging Into the Software in CHAPTER 3, Daily Startup.

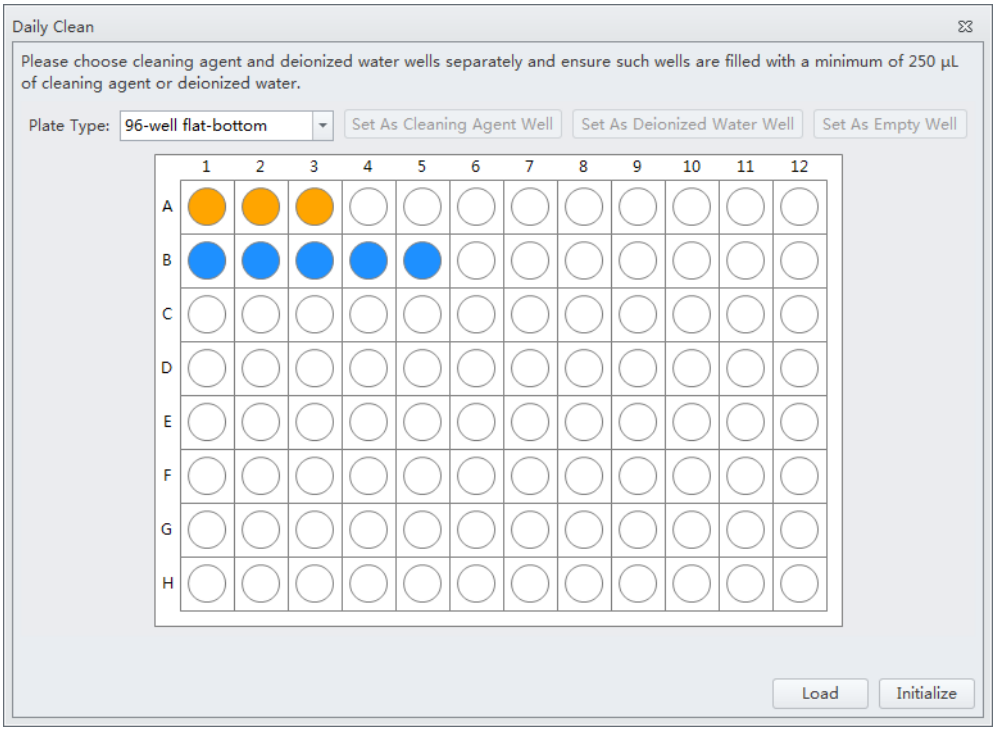
---

2 Select **Daily Clean** in the Cytometer menu. The Daily Clean window appears. The plate loader automatically ejects the plate holder stage.



3 Follow the on screen software prompts and select the desired wells for cleaning agent and deionized water.

[CytoFLEX LX Shown]



IMPORTANT You must select at least one cleaning solution well and one water well.

a. Select the desired wells for the cleaning agent and select **Set As Cleaning Agent Well.**

b. Select the desired wells for the deionized water and select **Set As Deionized Water Well**.

NOTE To deselect water wells, select the desired well and select **Set As Empty Well**.

c. Select the *Turn off cytometer after daily clean* checkbox to automatically shutdown the cytometer after Daily Clean is finished. **[CytoFLEX LX Only]**

4 Select **Start** to start the cleaning procedure. The message *Please confirm that the correct plate is placed properly and press OK* appears. Select **OK**.

5 Select **Close**.

Cleaning the Sample Station

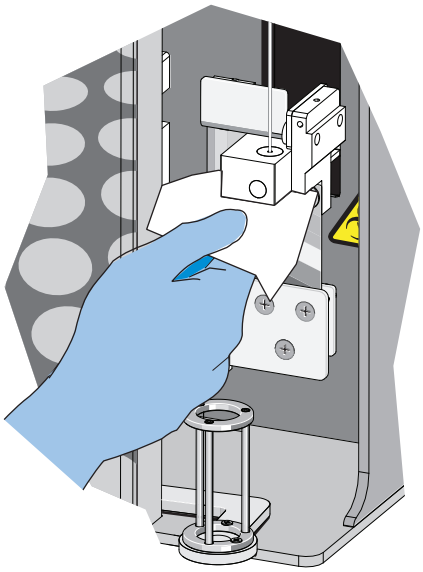
Carry out semi-automatic cleaning for the sample injection device once a week.

1 Ensure that the system has been shut down properly. Refer to Shutting Down the Instrument in CHAPTER 8, Daily Shutdown.

⚠ 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.

2 Use a piece of absorbent material with a 10% bleach solution (1 part bleach [5 to 6% sodium hypochlorite - available chlorine] with 9 parts DI water) to wipe off all surfaces in the sample station, while taking all necessary biological safety precautions.



3 Wipe off the bottom of the semi-automatic sample injection device.

Cleaning the Sample Probe

When problems such as blockage of the sample probe occur, it is required to replace or clean the sample probe.

1 Confirm that the instrument is in the standby state or that the power supply is turned off.

⚠ WARNING

Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample probe and the sample peristaltic pump tubing in accordance with your local regulations and acceptable laboratory procedures.

2 Remove the sample probe. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures.

3 Put the sample probe into a clean container and soak it in clean water. Use an ultrasonic cleaning device to clean for 2 minutes.

4 Reattach the sample probe to the sample peristaltic pump tubing and confirm that the bead on the sample probe touches the sleeve on the end of the sample peristaltic pump tubing.

IMPORTANT To ensure that the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

5 Install the sample pump cover.

6 If ineffective, replace with a new sample probe. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures.

Deep Clean Procedure

Carry out a deep clean once a month to clean the instrument flow cell. If the unit will be shutdown and not used for more than 10 days, it is recommended to complete one deep clean before resuming use.

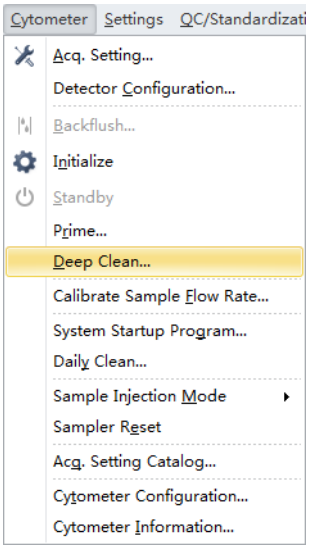
1 Place the instrument in standby state.

2 Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

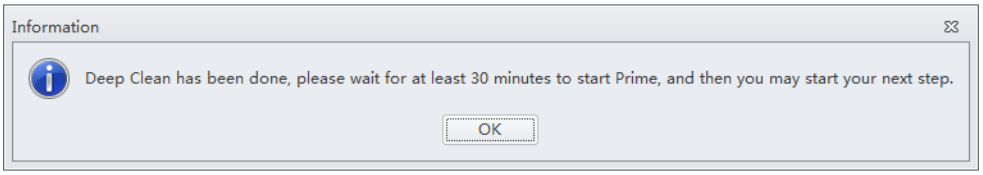
3 Confirm that the Deep Clean solution volume in the bottle located inside the Fluidics module is sufficient.

To prepare and add more Deep Clean solution, refer to Adding the Deep Clean Solution in CHAPTER 11, Replacement/Adjustment Procedures.

4 Select **Deep Clean** in the Cytometer menu. The software message *Are you sure to start deep clean?* appears. Select **Yes** to start the Deep Clean process in the instrument flow cell.



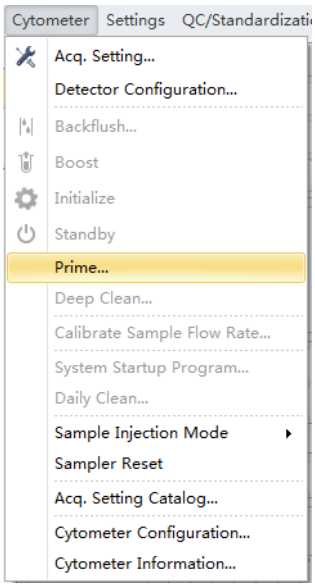
5 The status bar prompts that a deep clean is under way. Wait for the Deep Clean process to finish. The following software message appears:



Select **OK**.

6 Allow the cleaning solution to remain in the flow cell for approximately 30 minutes. If you are required to postpone the cleaning time, do not exceed 24 hours. During the Deep Clean cycle, the power supply of the unit can be turned off, but the instrument cannot be initialized.

7 Select **Prime** in the Cytometer menu. The software message *Are you sure to start Prime?* appears. Select Yes.



8 Run Daily Clean. Refer to Daily Clean.

9 Perform initialization as required (see Initializing the Instrument in CHAPTER 3, Daily Startup) to carry out the next experiment or to turn off the instrument.

10 Reinstall the right-side cover. Refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

Cleaning the 4 L Sheath Fluid Container

Clean the sheath fluid container once a month.

1 Confirm that the instrument is turned off or is in the standby state.

2 Remove the sheath fluid container from the Fluid Container holder.

3 Remove the sheath fluid harness from the sheath fluid container.

4 Empty the residual sheath fluid from the sheath fluid container.

5 Add about 50 to 100 mL of CytoFLEX Sheath Fluid to the sheath fluid container.

6 Insert the sheath fluid harness back into the sheath fluid container and tightly close the sheath fluid container cap.

7 Swirl the sheath fluid in the sheath fluid container, rinsing all surfaces.

8 Empty the sheath fluid container.

9 Refill the sheath fluid container. Refer to Filling the 4 L Sheath Fluid Container [CytoFLEX] in CHAPTER 11, Replacement/Adjustment Procedures.

Cleaning the 4 L Waste Container

Clean the waste container once a month.

1 Confirm that the instrument is turned off or is in the standby state.

2 Remove the waste container from the Fluid Container holder.

⚠ 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.

3 Remove the harness from the waste container.

⚠ 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.

4 Empty the waste container.

⚠ 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.

5 Add one liter of sodium hypochlorite solution with 0.5% active chlorine to the waste container.

6 Insert the waste harness back into the waste container and tightly close the waste container cap.

⚠ CAUTION

Risk of damage to the sheath fluid harness and/or waste harness. Do not leave the sodium hypochlorite solution in the fluid containers longer than 10 minutes.

7 Let stand for 5 to 10 minutes.

⚠ 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.

8 Dispose of the sodium hypochlorite solution in accordance with your local regulations and acceptable laboratory procedures.

9 Use deionized water to rinse the waste container and the waste harness. Ensure that there is no sodium hypochlorite residue.

10 Place the waste container back into the Fluid Container holder.

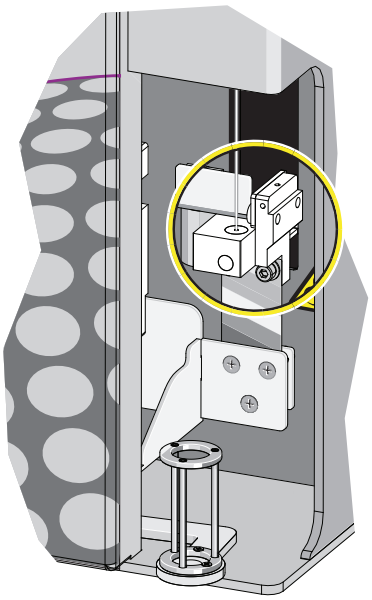
Nonscheduled Cleaning

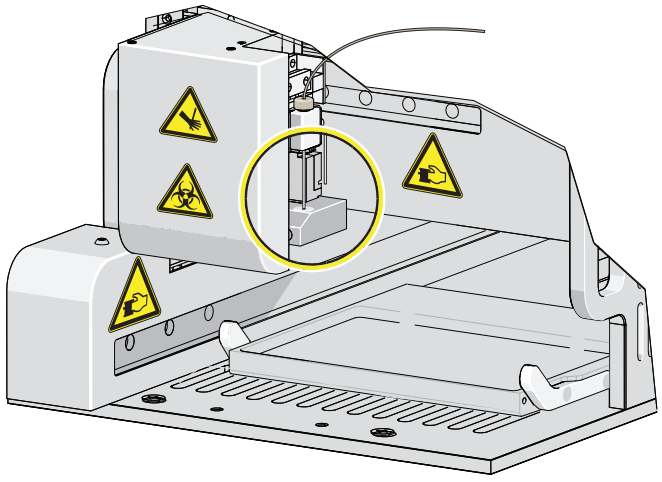
Surface Cleaning and Disinfection

1 Wipe the CytoFLEX Series label of the instrument clean with water and wipe dry immediately.

2 If you have a plate loader installed on your instrument, remove the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

3 Wipe the wash collar modules in the single tube station and the plate loader station with 100% isopropanol and wipe dry immediately.





4 If you have a plate loader installed on your instrument, reinstall the front cover. Refer to Front Cover Removal and Reinstallation 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.

5 Prepare a cleaning solution of 1 part high-quality, fragrance-free bleach (5% or 6% solution of sodium hyphochlorite - available chlorine) and 9 parts distilled water.

⚠ CAUTION

Risk of instrument damage. The instrument label can peel or fade, and the wash collar can become brittle and crack over time if these surfaces are wiped clean using the bleach solution followed by the 70% ethanol. Do not clean the label or the wash collar with the bleach solution followed by the 70% ethanol. Only use the specified cleaning methods in Steps 1 and 3 to clean these surfaces.

⚠ CAUTION

Risk of personal injury if electronic equipment is used near fumes or flammable gases. Ethanol is a flammability hazard. Avoid this risk by never using it in or near operating instruments.

6 Wipe down all exposed surfaces with the bleach solution and then 70% ethanol. Pay special attention to the Sampling area.

Be sure to avoid wiping the instrument label and wash collar module with the bleach solution and 70% ethanol.

Preparing the Instrument for Transport or Storage

When the instrument is to be transported or is not to be used for 30 days or more, complete the emptying processes to prevent instrument damage and to reduce the possibility of biological contamination. Contact us if you have any questions.

1 Run the Deep Clean procedure. Refer to Deep Clean Procedure.

2 Run the Daily Clean procedure. Refer to Daily Clean.

3 Clean the Sample Station. Refer to Cleaning the Sample Station.

4 Empty the sheath fluid container and the waste container (see Emptying the 4 L Waste Container [CytoFLEX] in CHAPTER 11, Replacement/Adjustment Procedures).

5 Clean and disinfect all surfaces. Refer to Surface Cleaning and Disinfection.

⚠ 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.

6 Clean the sheath fluid container and the waste container. Refer to Cleaning the 4 L Sheath Fluid Container.

7 Remove the right-side cover (see Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures).

8 Remove the Deep Clean solution bottle from the bracket, empty the Deep Clean solution bottle, and rinse with DI water. Then, attach the Deep Clean solution bottle to the bracket.

9 Remove the Plate Loader module if applicable (see Plate Loader Module Removal and Reinstallation [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures).

10 Power down and disconnect all the cables and sheath fluid and waste harnesses.

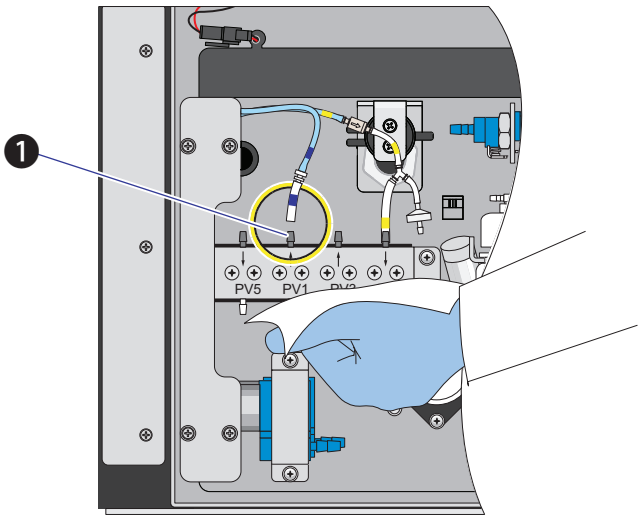
⚠ CAUTION

Risk of instrument damage. The Cytometer can suffer irreparable damage if it is exposed to subfreezing temperatures while it still contains liquid. Always drain the flow cell after cleaning the Cytometer if the Cytometer will be stored or transported in subfreezing temperatures.

⚠ WARNING

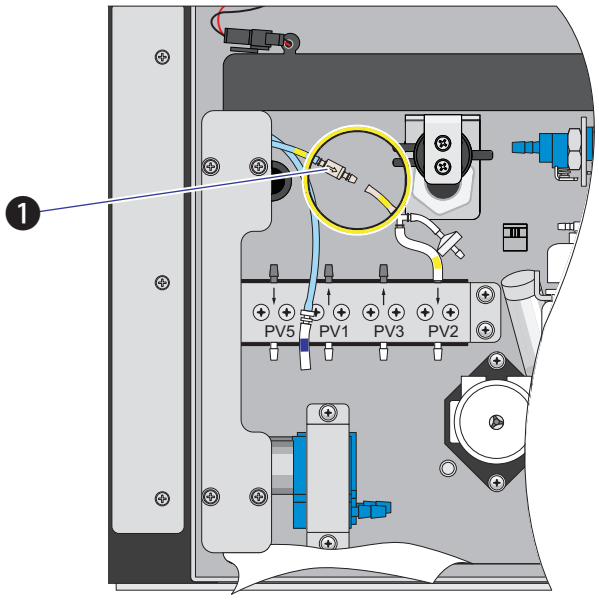
Risk of contamination from biohazardous material. Always wear PPE when performing this procedure as you can contact components with blood residue when handling the Fluidics module. Dispose of any absorbent materials used to collect or clean up leaks in accordance with the local regulations and acceptable laboratory procedures.

11 Disconnect the blue-labeled tubing from the pneumatic valve PV1 and hold the absorbent material under the disconnected tubing to collect any dripping liquid.



1. PV1

12 Disconnect the yellow-labeled tubing connected to the liquid damper to vent the flow cell, allowing it to drain.



1. Liquid damper

13 Verify that liquid has stopped dripping from the blue-labeled tubing.

NOTE The flow cell is empty when liquid stops dripping from the blue-labeled tubing.

14 Dispose of the absorbent material used to collect the liquid in accordance with the local regulations and acceptable laboratory procedures and cleanup any spills.

15 Reconnect the blue-labeled tubing to PV1.

16 Reconnect the yellow-labeled tubing to the liquid damper.

17 Reinstall the right-side cover (see Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures).

18 Ensure that the optical filters are seated properly.

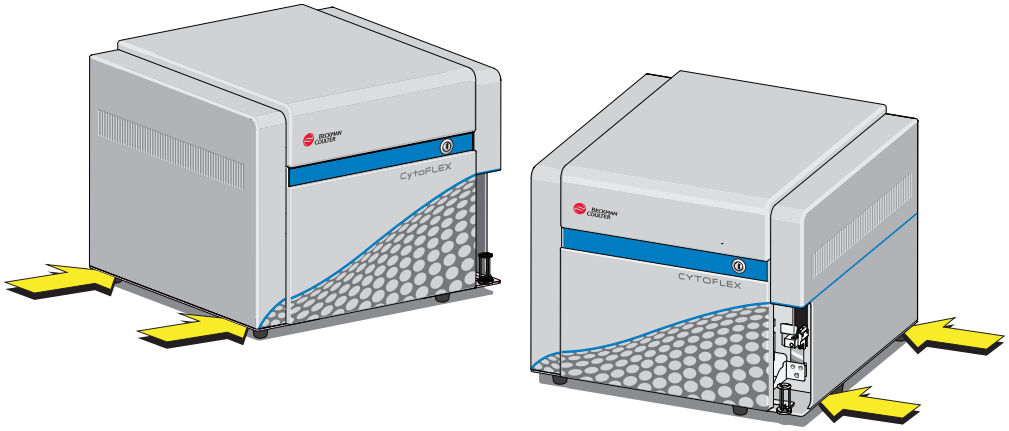
19 Ensure that the top cover is tightly closed.

20 If the instrument is to be transported or stored, put the instrument and the Plate Loader module (if applicable) into the Beckman Coulter supplied packing and comply with the requirements in Instrument Transportation and Storage in APPENDIX A, Instrument Installation, regarding correct placement during transportation and storage.

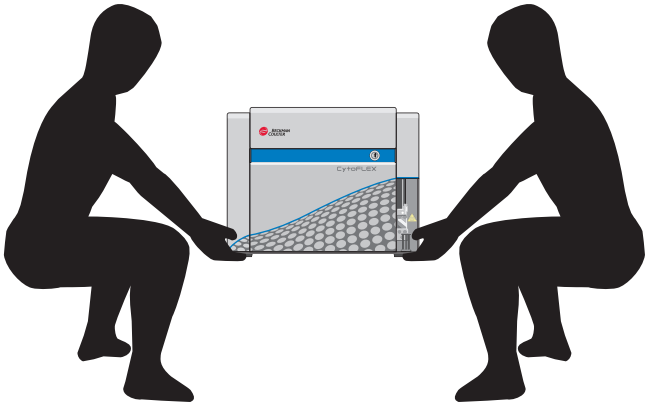
Lifting and Carrying Instructions

1 Position a person on the left and right sides of the Cytometer.

2 Reach under the base of the Cytometer in the areas indicated by the arrows in the figure below.



3 Gently lift the Cytometer as shown in the figure below.



⚠ WARNING

Risk of personal injury. Use caution when lowering the Cytometer to avoid pinching fingers.

4 Lower the Cytometer to its designated location.

CHAPTER 11

Replacement/Adjustment Procedures

Overview

This chapter describes how to carry out certain routine and nonscheduled maintenance procedures. Proper maintenance can help extend the service life of the instrument and ensure experimental accuracy. When conducting any maintenance work, take all necessary biosafety precautions.

IMPORTANT In addition to parts specifically discussed, for all replacement parts, use only parts provided by Beckman Coulter to ensure proper functioning of the instrument. Never disassemble any part of the instrument without prior authorization. Beckman Coulter assumes no responsibility for any instrument problems resulting from the use of any part not authorized by Beckman Coulter for use with the instrument.

This chapter contains information on:

Routine Replacement/Adjustment

Front Cover Removal and Reinstallation

Removal

⚠ WARNING

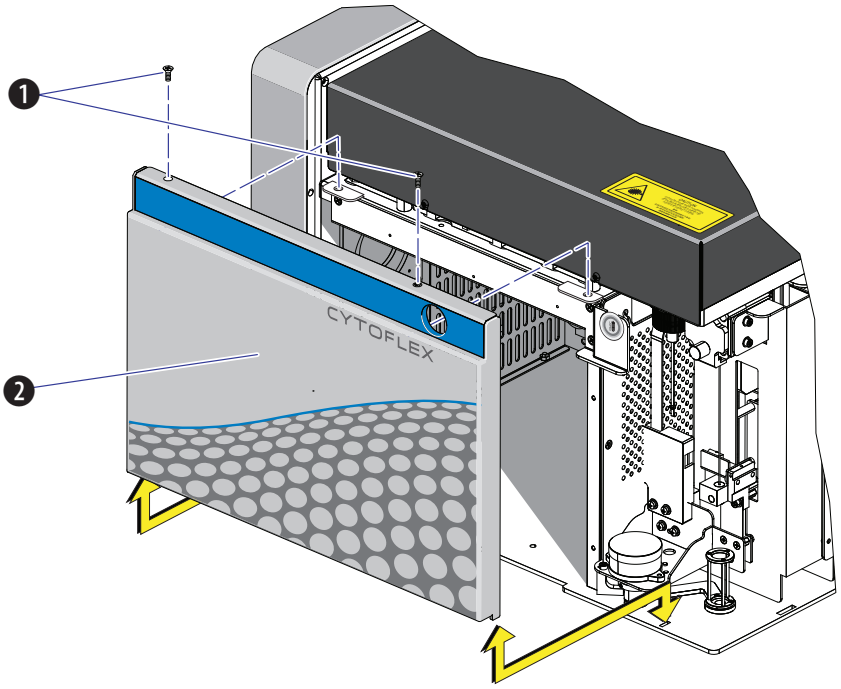
Risk of personal injury from electric shock caused by contacting exposed electronic components. Power down the instrument before removing the front cover of the Cytometer.

1 Exit the system software.

2 Turn off the main power switch on the back of the Cytometer.

3 Open the top cover.

4 Remove the two screws securing the front cover and pull the front cover forward.



1. Securing screws
2. Front cover

5 Lift the front cover up and out of the slots in the frame.

Reinstallation

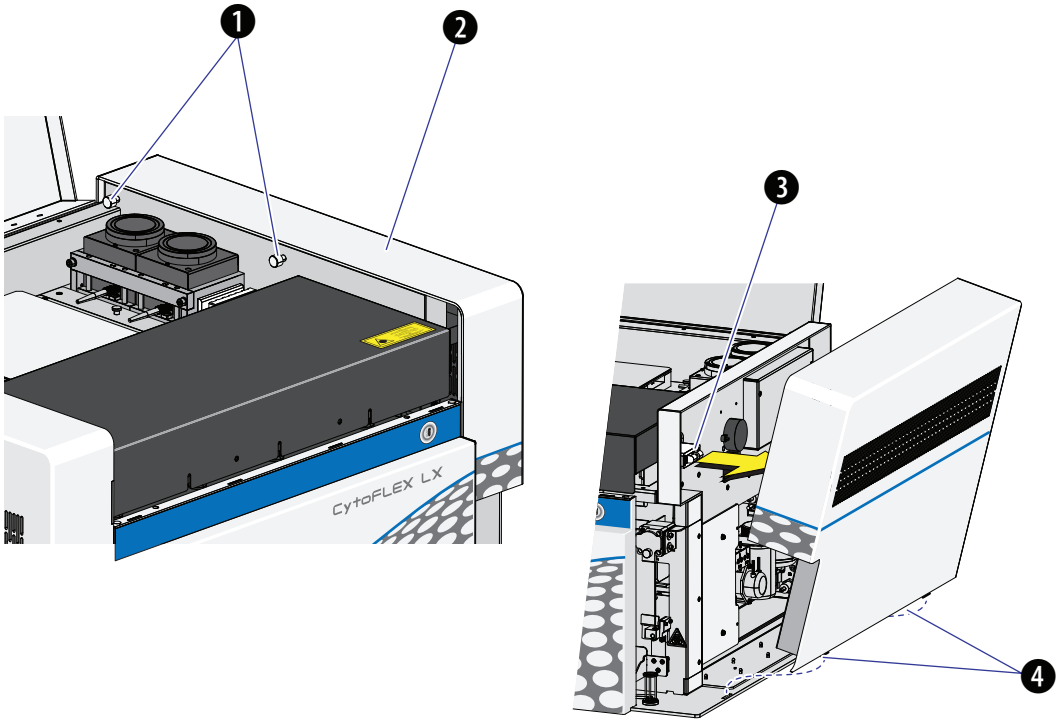
1 Slide the tabs on the bottom of the front cover into the slots in the bottom of the frame.

2 Push in the latches on the front cover to retract the pins, push the front cover into place, and release the latches to secure the cover.

3 Close the top cover.

Right-Side Cover Removal and Reinstallation

IMPORTANT The CytoFLEX LX flow cytometer only requires you to unfasten the thumbscrews the first time. There is a retaining clip that is used to secure the right-side cover without the need for the thumbscrews, if desired. Once the thumbscrews are unfastened, the right-side cover can simply be pulled off of the retaining clip for removal and pushed onto the retaining clip for reinstallation. Ensure the top cover is open before removing the right-side cover from the retaining clip.

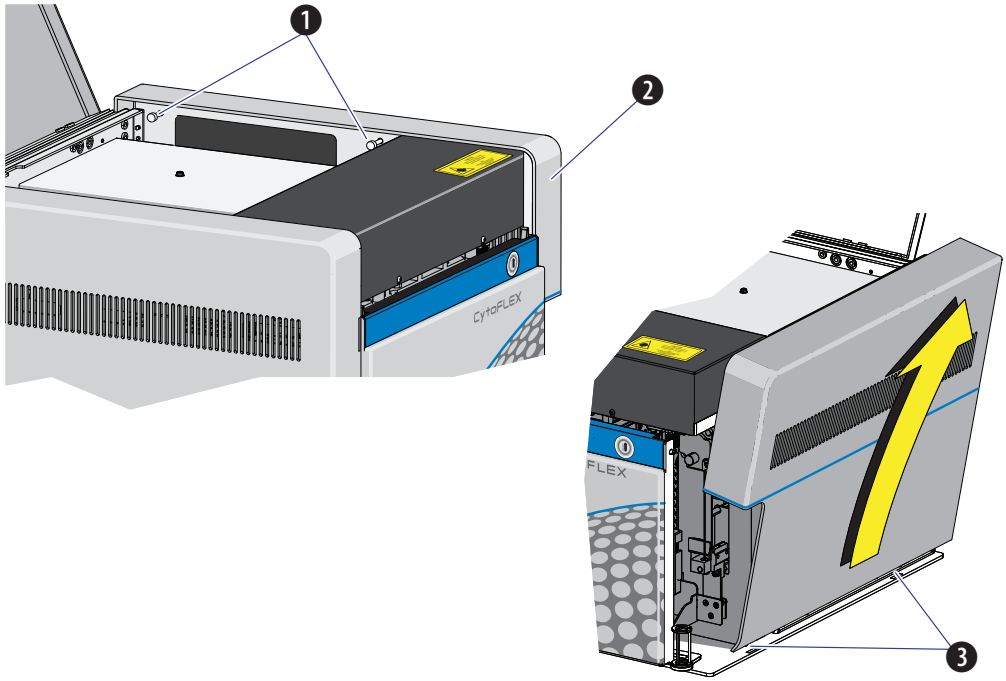


1. Thumbscrews
2. Right-side cover
3. Retaining clip
4. Tabs

Removal

1 Open the top cover.

2 Unfasten the two captive thumbscrews for the right-side cover.



1. Captive thumbscrews
2. Right-side cover
3. Tabs

3 Lift the right-side cover up and out of the slots in the frame.

Reinstallation

1 Slide the tabs on the bottom of the right-side cover into the slots in the bottom of the frame and push the cover into place.

2 Fasten the two captive thumbscrews to secure the right-side cover.

3 Close the top cover.

Filling the 4 L Sheath Fluid Container [CytoFLEX]

1 Confirm that the instrument is turned off or is in the standby state.

2 If necessary, remove any cardboard cutouts from the new CytoFLEX Sheath Fluid cubitainer. If you do not need a new cubitainer, skip to Step 7.

3 Locate the spigot inside the cardboard cutout.

4 Remove the cap and seal from the new sheath cubitainer. Be sure to completely remove the foil seal.

5 Screw on the spigot.

6 Unscrew the sheath fluid harness from the sheath fluid container and place in the fluid sensor holder cutout (refer to Figure 1.7.) to prevent contamination of the sheath fluid harness.

⚠ CAUTION

Risk of instrument damage. Remove the sheath fluid container from the Fluid Container holder and fill away from the instrument to prevent spills that could damage the instrument circuitry.

7 Remove the sheath fluid container from the Fluid Container holder.

8 Hold the Sheath fluid container under the CytoFLEX Sheath Fluid cubitainer ensuring the cubitainer is resting on a stable surface.

9 Fill the sheath fluid container.

10 Reinstall the sheath fluid harness onto the sheath fluid container.

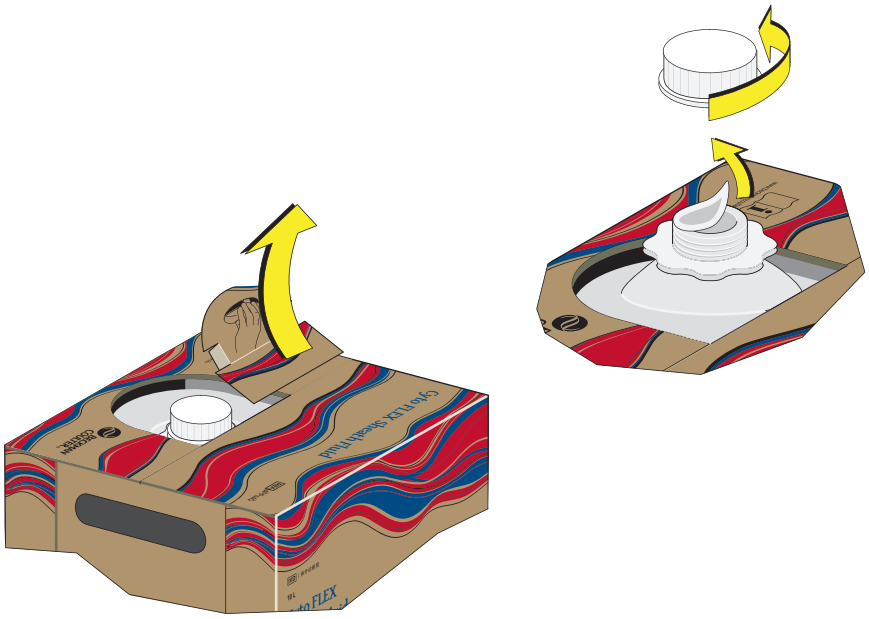
11 Place the sheath fluid container back into the Fluid Container holder.

Replacing the 10 L Sheath Fluid Cubitainer

1 Confirm that the instrument is turned off or is in the standby state.

2 If necessary, remove any cardboard cutouts from the new CytoFLEX Sheath Fluid cubitainer.

Remove the cap and seal from the new sheath fluid cubitainer. Be sure to completely remove the foil seal.

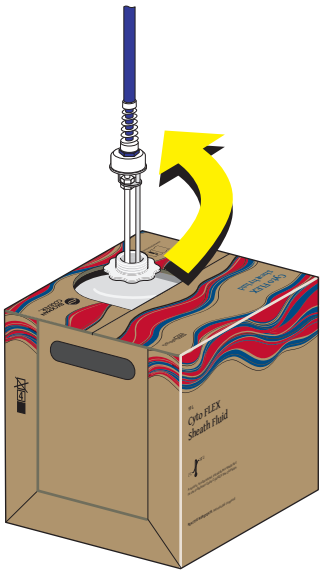


⚠ CAUTION

Misleading results could occur if you contaminate the sheath fluid. Be careful not to contaminate the sheath fluid. Do not let your fingers, paper towels, or other objects touch the pickup tube assembly.

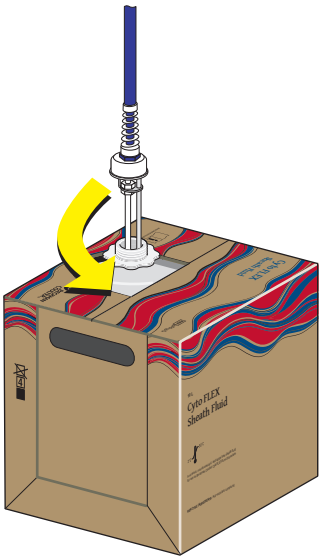
3 Unscrew the plastic cap that secures the pickup tube assembly into the old sheath fluid cubitainer and lay it on a leakproof disposable container, such as a glove or beaker.

Lift the pickup tube assembly straight up and out.



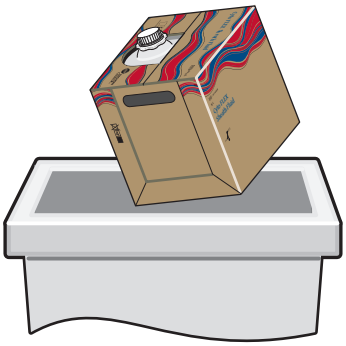
4 Inspect the pickup tube assembly and replace it if necessary.

5 Carefully insert the pickup tube assembly straight into the new sheath fluid cubitainer. Tighten the cap.



6 Place the 10 L sheath fluid cubitainer to the left of the instrument.

7 Put the cap from the new container onto the old container and dispose of the container properly.



Emptying the 4 L Waste Container [CytoFLEX]

1 Confirm that the instrument is turned off or is in the standby state.

2 Remove the waste harness (see Figure 1.7). The waste harness from the Cytometer is connected to a 4-L waste container.

3 Remove the waste container from the Fluid Container holder.

⚠ 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 might 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 proper personal protective equipment.

4 Empty the waste container. Dispose of the waste in accordance with your local regulations and acceptable laboratory 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.

5 Add 400 mL of 5 to 6% bleach to the waste container.

6 Reinstall the waste harness in the waste container.

7 Put the waste container in the Fluid Container holder.

Emptying the 10 L Waste Cubitainer

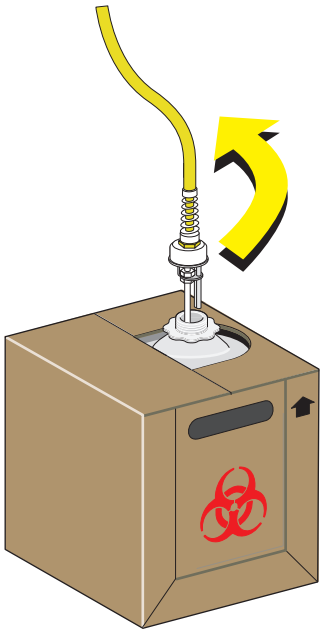
1 Confirm that the instrument is turned off or is in the standby state.

2 Lift the waste cubitainer and swirl it before removing the cap.

⚠ WARNING

Risk of biohazardous contamination if you have skin contact with the waste cubitainer, its contents, and its associated tubing. The waste cubitainer and it associated tubing might container residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of the waste cubitainer in accordance with your local regulations and acceptable laboratory procedures.

3 Unscrew the cap and lay it on a leakproof disposable container, such as a glove or beaker.



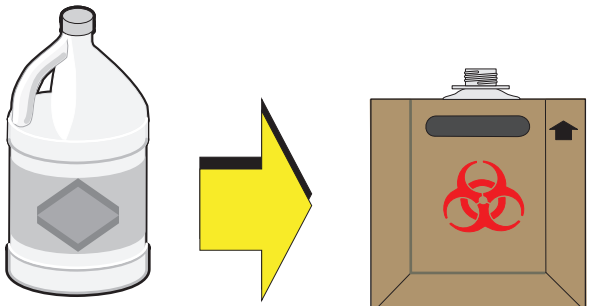
4 Empty the waste cubitainer according to your laboratory's procedures.

NOTE Take proper precautions to avoid spills if you are emptying the waste cubitainer into a sink, drain, or larger container. When moving the waste cubitainer to dispose of its contents, be sure the cap is secure to avoid spills.

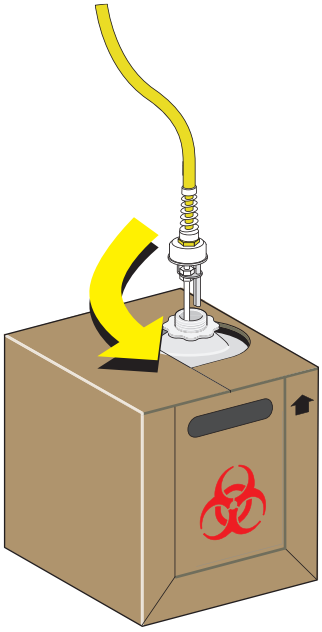
⚠ WARNING

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.

5 Put about 1 L of high-quality, fragrance-free, gel-free bleach (5 to 6% solution of sodium hypochlorite -available chlorine) in the 10-L waste cubitainer to cover the bottom of the cubitainer.



6 Replace the cap on the new waste cubitainer and securely tighten.

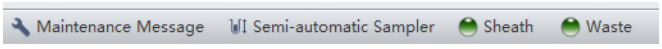


NOTE Properly dispose of the leakproof disposable container used in Step 3 after you screw the cap back on the waste container.

Managing the Maintenance Reminder

The maintenance reminder tracks the last maintenance date and initiates a reminder to complete maintenance for the following three items:

- Refill Deep Clean solution bottle (cleaning solution)
- Replace sheath fluid filter
- Replace sample peristaltic pump tubing

When reagents or parts have reached the designated use time limit in either days or number of uses, the Maintenance Message icon 

appears in the right side of the status bar.

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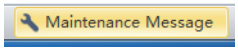
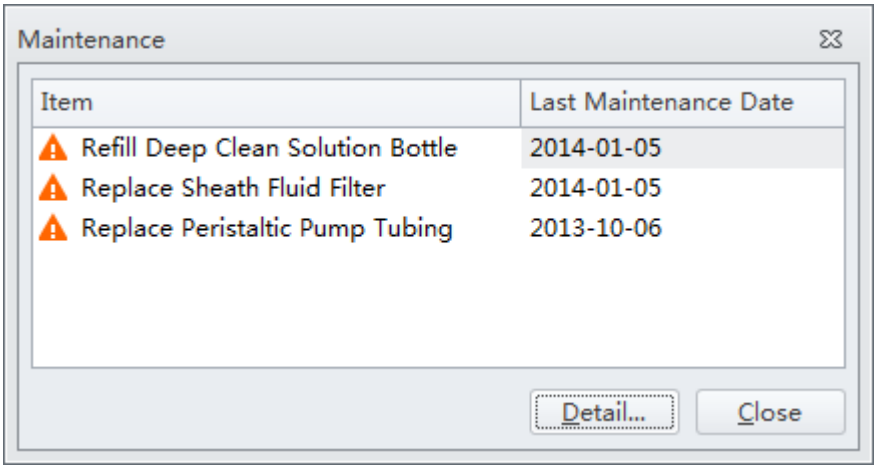
1 Select the Maintenance Message icon 

from the status bar to access the Maintenance window. The expired item appears with a warning triangle  to the left of the item listed.

Or

Select **Maintenance** in the Advanced menu. The Maintenance window appears. The expired item is displayed with a warning triangle  to the left of the item listed.



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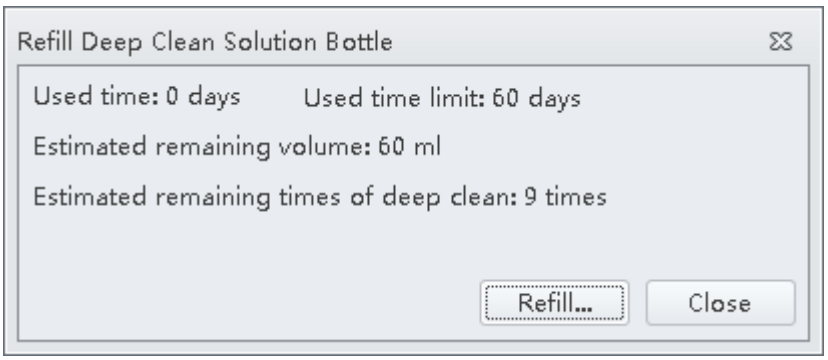
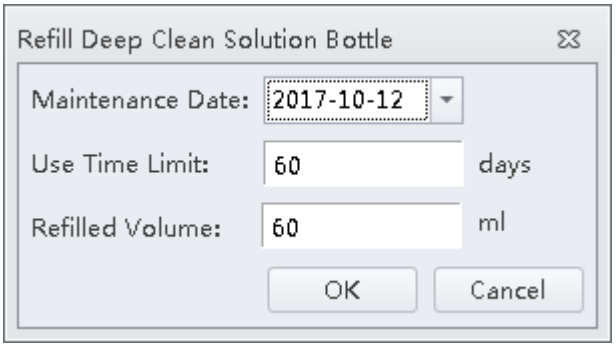
2 Select the desired item to manage, then choose one of the following:

- To manage refilling the Deep Clean solution bottle, go to Step 3.
- To manage replacing the sheath filter, skip to Step 4.
- To manage replacing the sample peristaltic pump tubing, skip to Step 5.

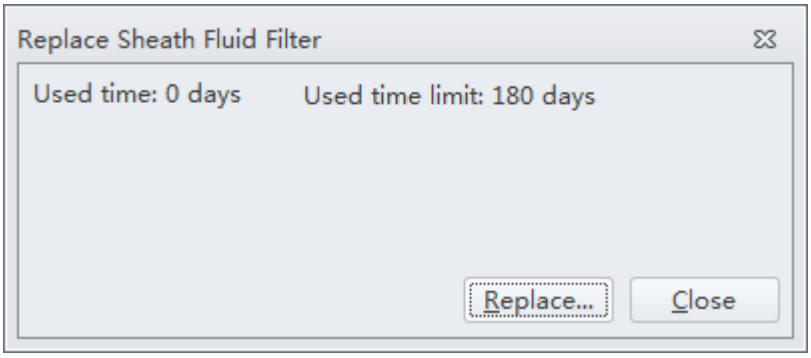
3 Select **Detail**. The Refill Deep Clean Solution Bottle window appears.



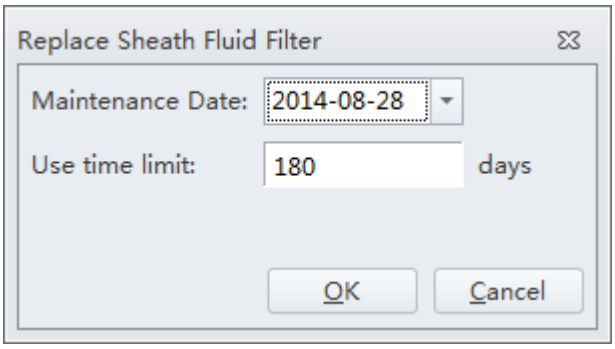
Select **Refill**. A pop-up window appears to reset the maintenance date as the current date that the maintenance was performed.



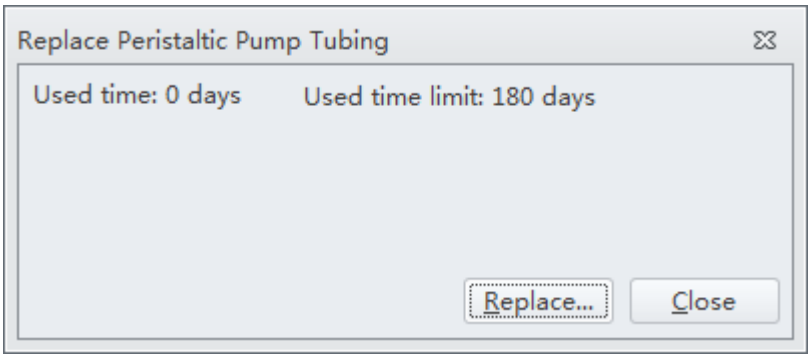
4 Select **Detail**. The Replace Sheath Fluid Filter window appears.



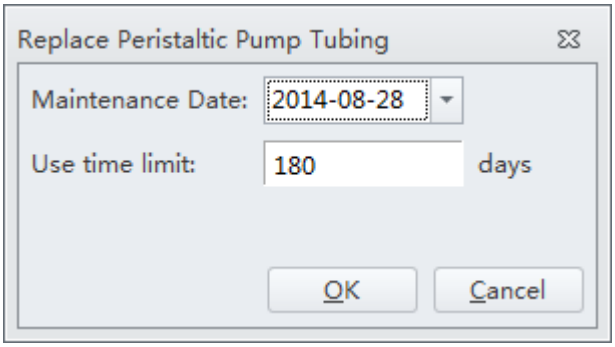
Select **Replace.** A pop-up window appears to reset the maintenance date as the current date that the maintenance was performed.



5 Select **Detail**. The Replace Peristaltic Pump Tubing window appears.



Select **Replace.** A pop-up window appears to reset the maintenance date as the current date that the maintenance was performed.



Adding the Deep Clean Solution

Check occasionally whether the Deep Clean solution in the Deep Clean solution bottle is sufficient. Replace the Deep Clean solution when the maintenance reminder prompts you to do so.

⚠ WARNING

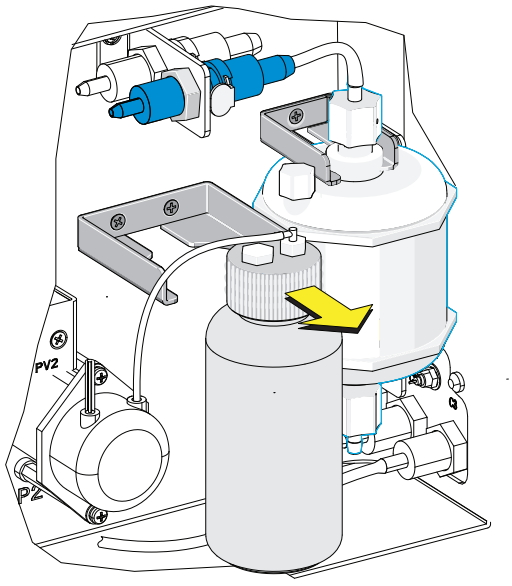
Risk of chemical injury from Contrad® 70 reagent. To avoid contact with the Contrad® 70 reagent, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

1 Make 60 mL of Deep Clean solution by mixing 30 mL of Contrad® 70 and 30 mL DI water in the Deep Clean solution bottle and swirl the solution gently to create the Deep Clean solution.

2 Verify that the Cytometer is in standby state or is turned off.

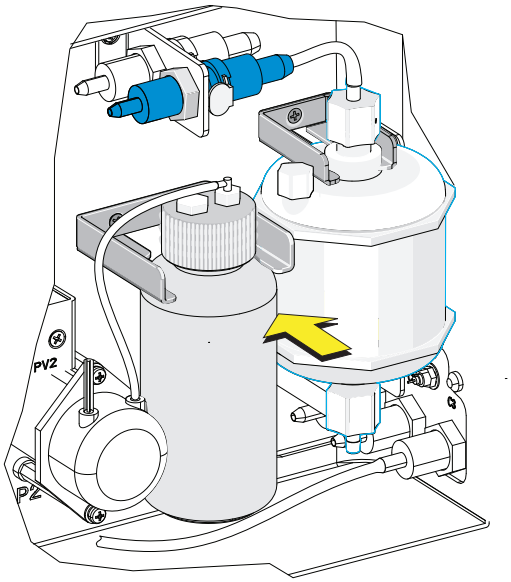
3 Remove the right-side cover of the instrument. Refer to Right-Side Cover Removal and Reinstallation.

4 Remove the Deep Clean solution bottle and open the cap.



5 Add 60 mL Deep Clean solution to the bottle.

6 Tighten the cap, and attach the Deep Clean solution bottle to the bracket.



7 Reinstall the right-side cover (see Right-Side Cover Removal and Reinstallation), and fasten the thumbscrews.

8 Reset the maintenance reminder tracker. Refer to Managing the Maintenance Reminder.

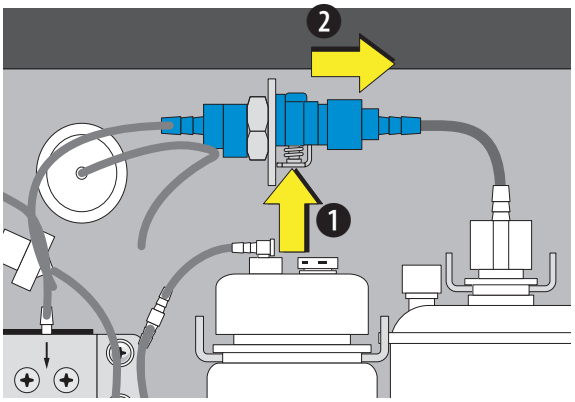
Replacing the Sheath Fluid Filter

It is recommended to replace the sheath fluid filter every six months when the maintenance reminder prompts you to do so. The life of the filter is related to the quality of the sheath fluid used. If it is found that there are impurities in the light scatter pattern, replace the sheath fluid filter.

1 Select **Standby** on the left of the screen to place the instrument in standby state, or shut off the Cytometer's power.

2 Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation.

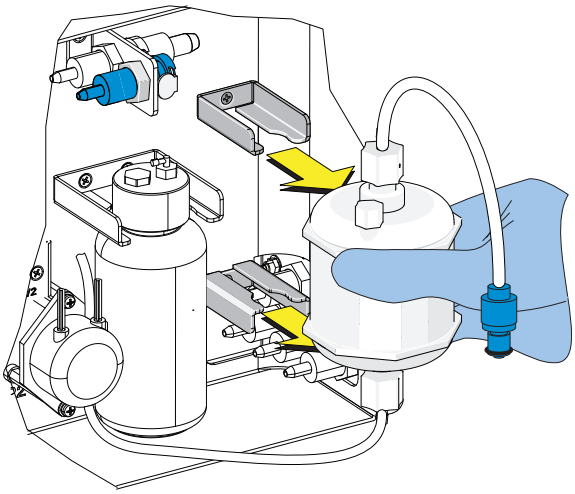
3 Press the spring piece on the quick connector and the Cytometer on the upper side of the filter, and disconnect the quick connector.



4 Repeat Step 3 for the quick connector behind the quick connector removed in the previous step.

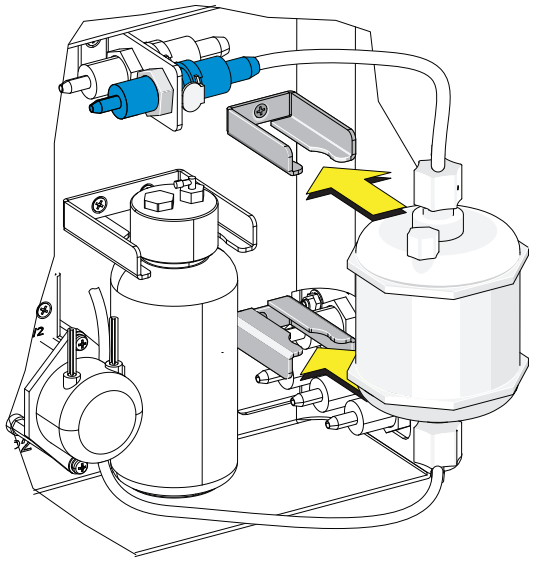
NOTE The spring is on the opposite side.

5 Remove the sheath fluid filter from the bracket.

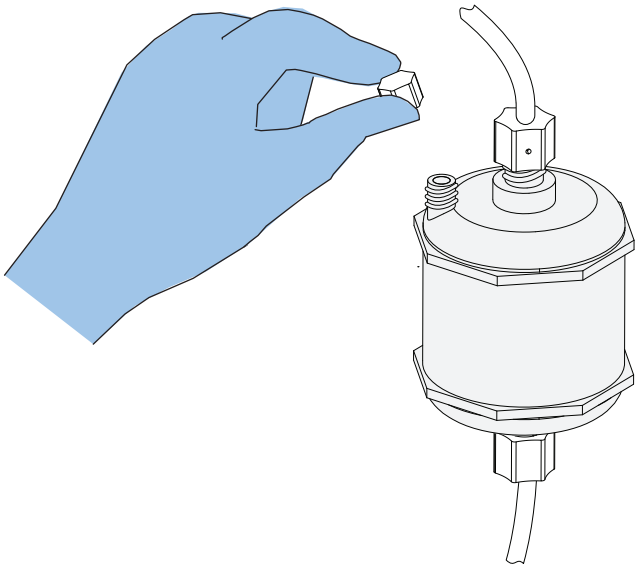


6 Connect the new, unused filter using the quick connector springs.

7 Attach the filter to the filter bracket.



8 Remove the vent cap and set it aside.



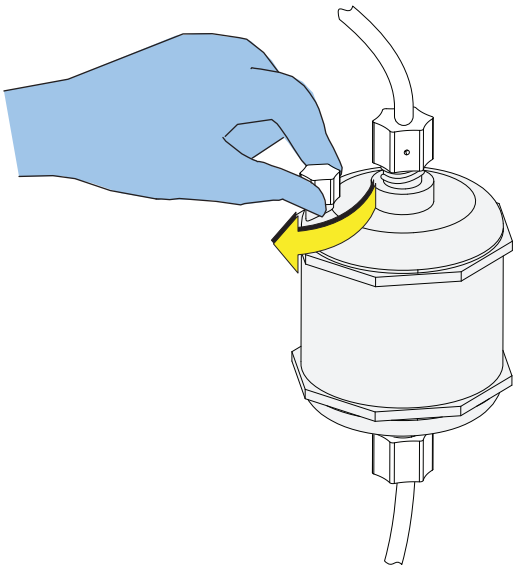
9 Turn on the Cytometer and open the software.

10 Select **Prime** in the Cytometer menu.

⚠ CAUTION

Risk of instrument damage. If the vent cap is not sealed tightly, unstable flow rate and leakage of the sheath fluid can occur.

11 Observe the liquid level in the filter during the prime cycle. When the liquid level reaches the upper section of the filter, reinstall the vent cap to prevent air leakage.



12 Reinstall the right-side cover (see Right-Side Cover Removal and Reinstallation) and lock the screw.

13 If the problem persists, contact us.

14 Run the system startup program. Refer to Running the System Startup Program [with the Single Tube Loader] in CHAPTER 3, Daily Startup.

15 Reset the maintenance reminder tracker. Refer to Managing the Maintenance Reminder.

Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing

⚠ WARNING

Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample probe and the sample peristaltic pump tubing in accordance with your local regulations and acceptable laboratory procedures.

IMPORTANT If you need to replace the single tube sample probe assembly and you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, pull the probe out of the CytoFLEX Sample Injection Mode Control Kit and push the new probe into place.

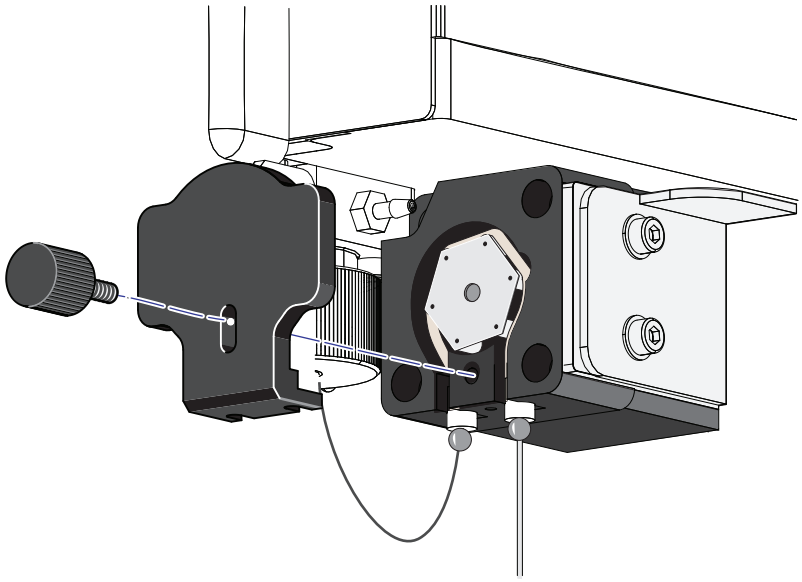
It is recommended to replace the tubing of the sample peristaltic pump every six months, as tubing used for an excessively long time can cause degradation of the stability of the sample flow and increase of the CV detected.

1 Place the instrument in standby state.

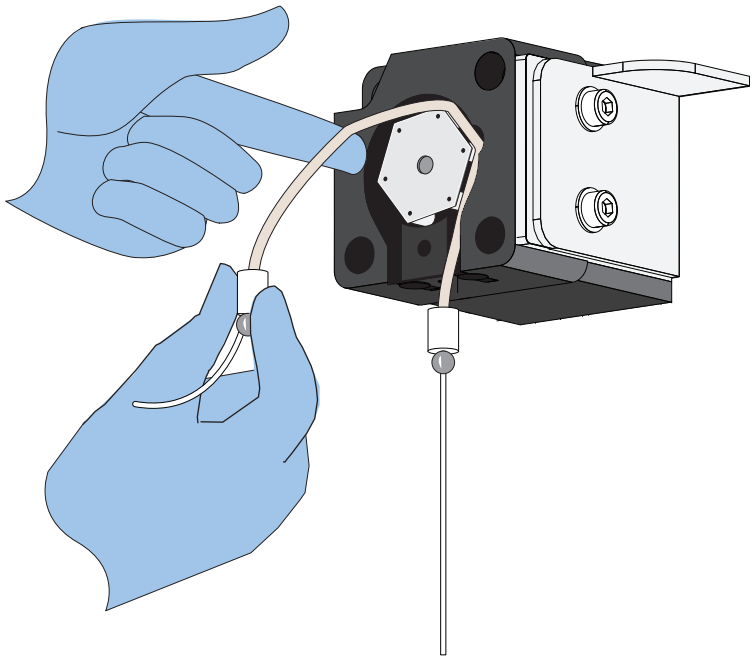
2 Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation.

3 Remove the front cover. Refer to Front Cover Removal and Reinstallation.

4 Remove the sample pump cover thumbscrew and the sample pump cover.



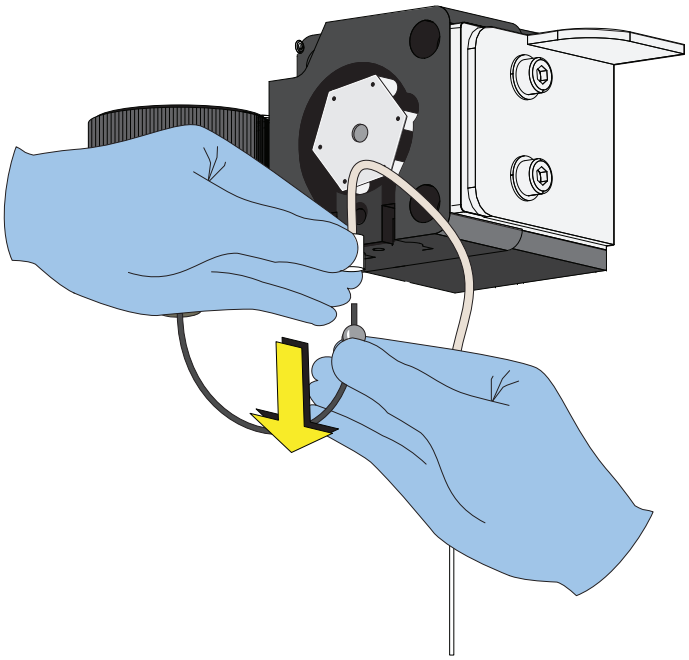
5 Take out the sample peristaltic pump tubing.



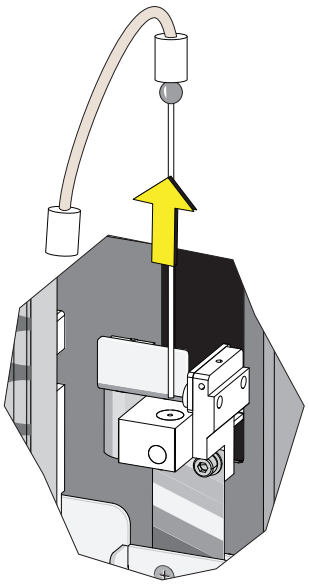
⚠ CAUTION

Risk of optical misalignment. Removing the PEEK tubing from the bottom of the flow cell could cause misalignment of the optical components. Do not remove the PEEK tubing from the bottom of the flow cell.

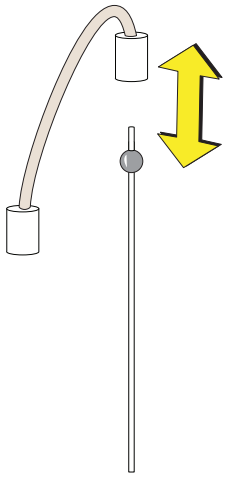
6 Remove the sample PEEK tubing from the sample peristaltic pump tubing.



7 Lift the sample probe out of the wash station.

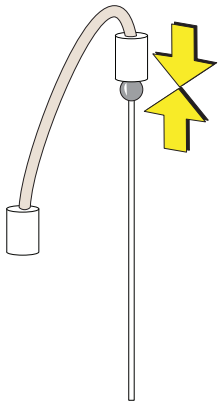


8 Remove the sample peristaltic pump tubing from the sample probe.

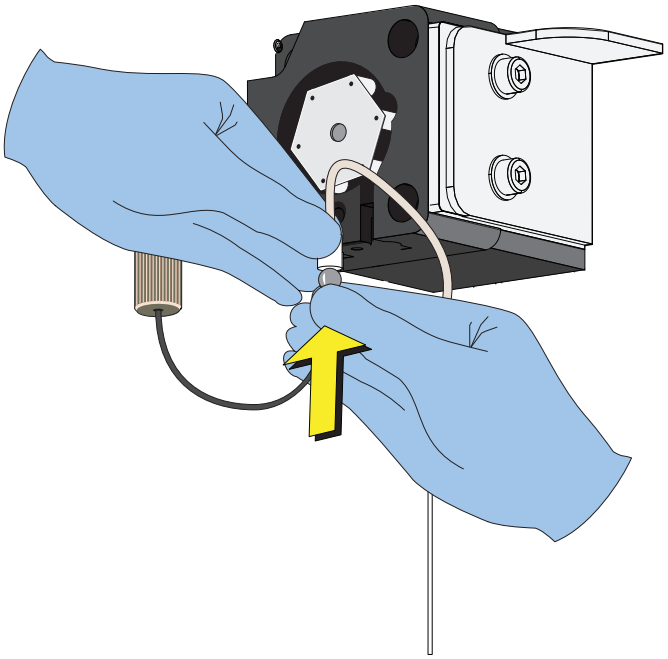


9 Dispose of the old sample probe and/or sample peristaltic pump tubing in accordance with your local regulations and acceptable laboratory procedures.

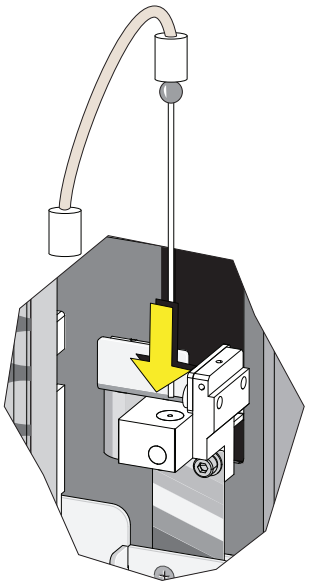
10 Connect the sample peristaltic pump tubing to the sample probe.



11 Connect the sample PEEK tubing to the sample peristaltic pump tubing.

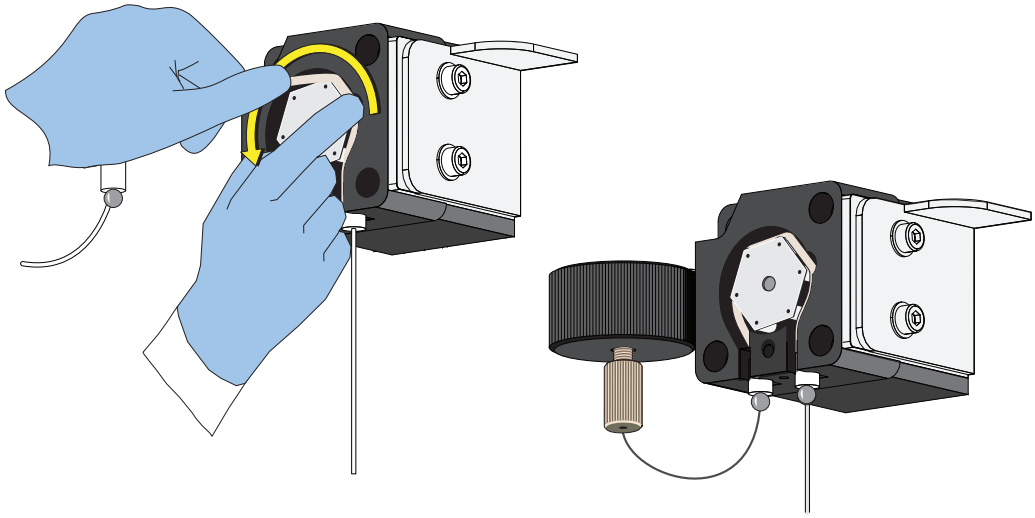


12 Slide the sample probe into the wash station.

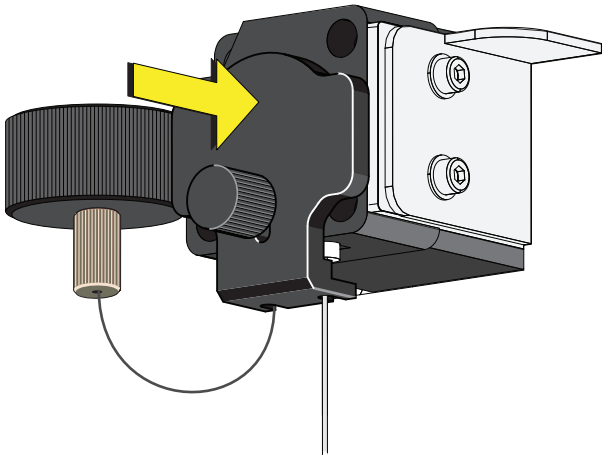


13 Install the sample peristaltic pump tubing, taking care not to use any sharp tools, ensuring that the tube is fully inserted into the groove.

NOTE You can use the sample pump cover thumbscrew to install the sample peristaltic pump tubing.



14 Install the sample pump cover.



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

15 Reinstall the right-side cover (see Right-Side Cover Removal and Reinstallation), and lock with the screw.

Replacing the Sample Probe Assembly [With Plate Loader]

IMPORTANT If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, contact us to replace the sample probe assembly.

⚠ WARNING

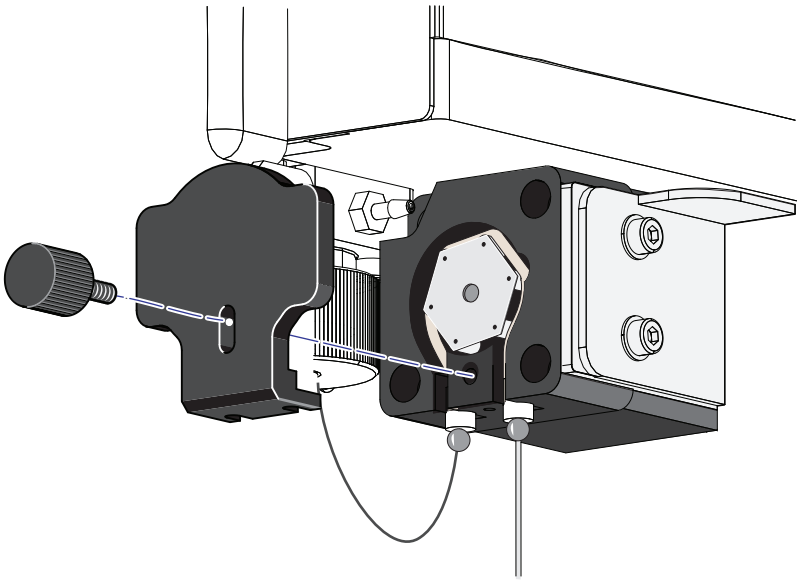
Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample probe and the sample peristaltic pump tubing in accordance with your local regulations and acceptable laboratory procedures.

1 Turn the Cytometer's main power switch off.

2 Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

3 Remove the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

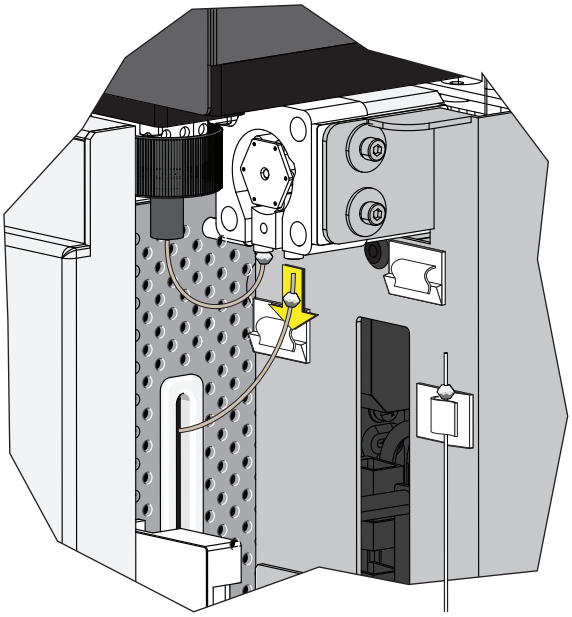
4 Remove the sample pump cover thumbscrew and the sample pump cover.



⚠ CAUTION

Risk of optical misalignment. Removing the PEEK tubing from the bottom of the flow cell could cause misalignment of the optical components. Do not remove the PEEK tubing from the bottom of the flow cell.

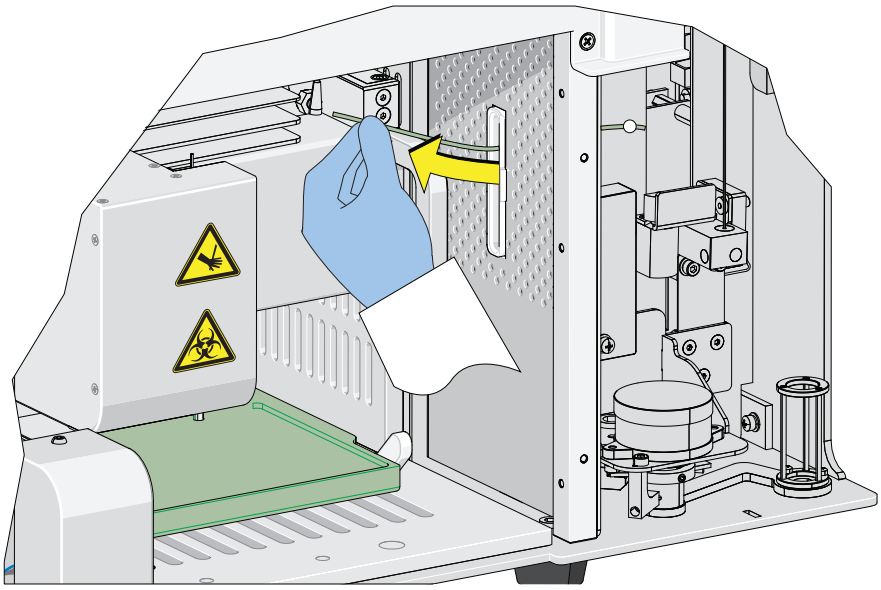
5 Remove the plate loader PEEK tubing from the sample peristaltic pump tubing.



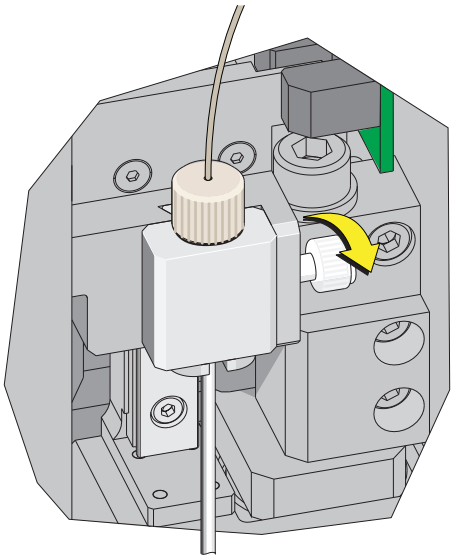
⚠ CAUTION

The plate loader PEEK tubing can be deformed, which could affect sample flow. When routing the plate loader PEEK tubing to and from the Sample Station, be careful not to pinch, crimp, stretch, or break the tubing.

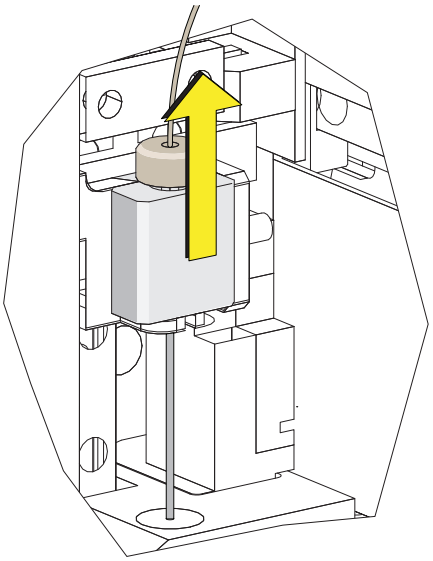
6 Pull the plate loader PEEK tubing through the slot so the tubing sits inside the instrument.



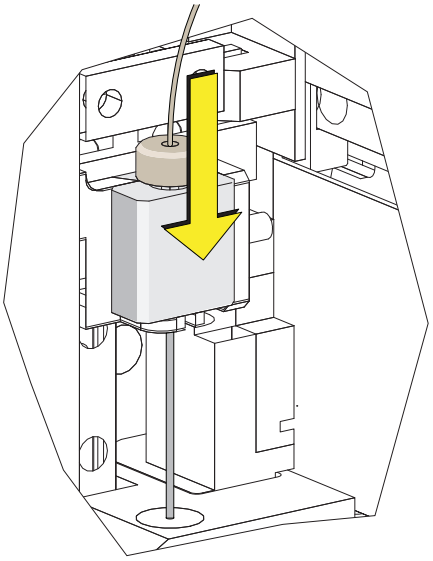
7 Loosen the white plastic thumbscrew behind the plate loader sample probe.



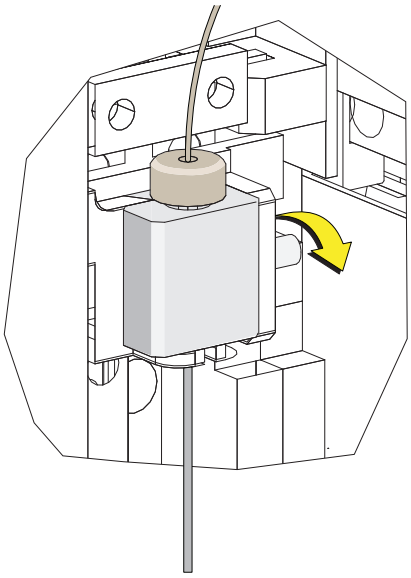
8 Lift the plate loader sample probe assembly straight up to remove it from the probe holder.



9 Align the tongue on the plate loader sample probe assembly with the groove in the probe holder and slide the probe down until it is flush with the probe holder.



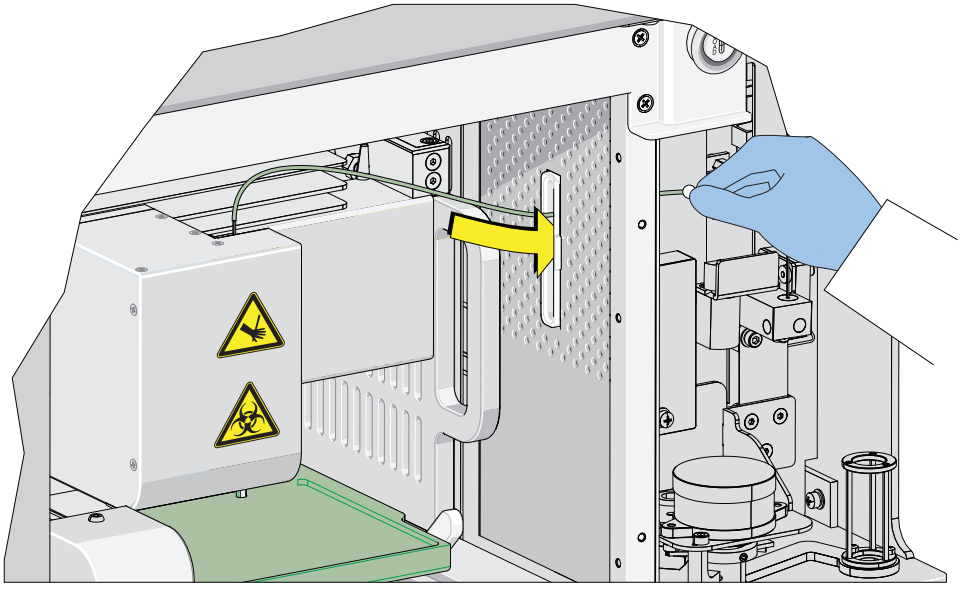
10 Tighten the white plastic thumbscrew.



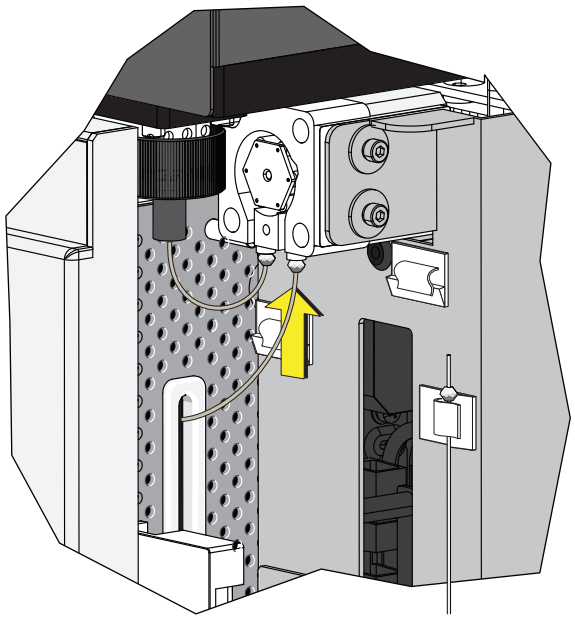
⚠ CAUTION

The plate loader PEEK tubing can be deformed which could affect sample flow. When routing the plate loader PEEK tubing to and from the Sample Station, be careful not to pinch, crimp, stretch, or break the tubing.

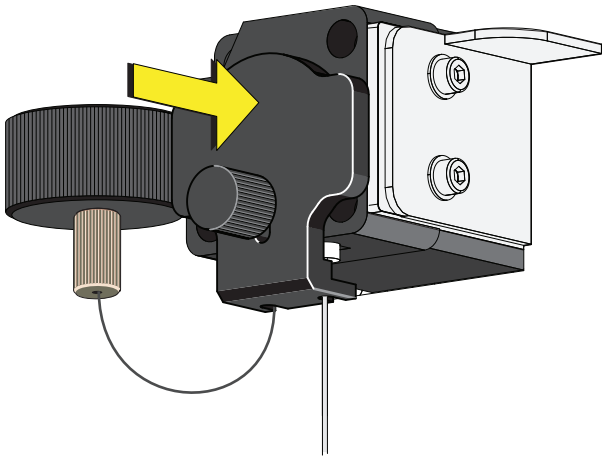
11 Slide the new plate loader PEEK tubing through the slot into the single tube sample station area.



12 Connect the sample peristaltic pump tubing to the plate loader PEEK tubing.



13 Install the sample pump cover.



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

14 Reinstall the right-side cover (refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures), and lock with the screw.

15 Close the top cover.

16 Turn the Cytometer's main power switch on.

Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [CytoFLEX With Plate Loader]

⚠ WARNING

Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately.

NOTE If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, refer to Switching the Sample Probe from the Single Tube Sample Station to the Plate Loader [With Sample Injection Mode Control Knob] in APPENDIX C, Sample Injection Mode Control Kit for instructions on switching between the single tube mode and the plate loader mode.

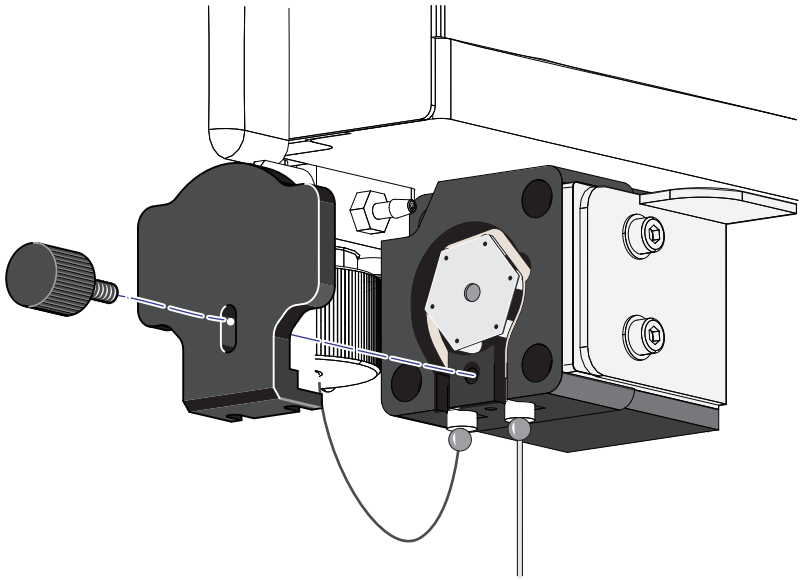
1 Switch to the Plate Loader sample injection mode. Refer to Selecting the Plate Loader Sample Injection Mode [With Plate Loader] in CHAPTER 3, Daily Startup.

2 Lift the top cover.

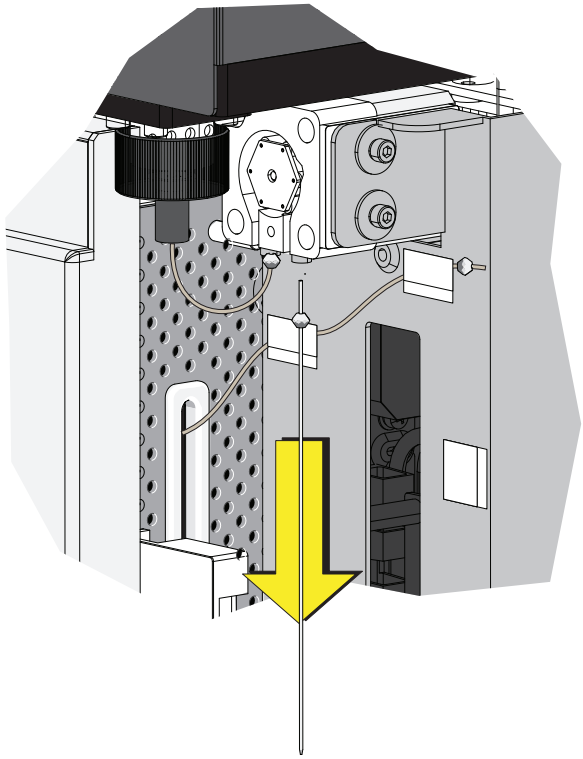
3 Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

4 Remove the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

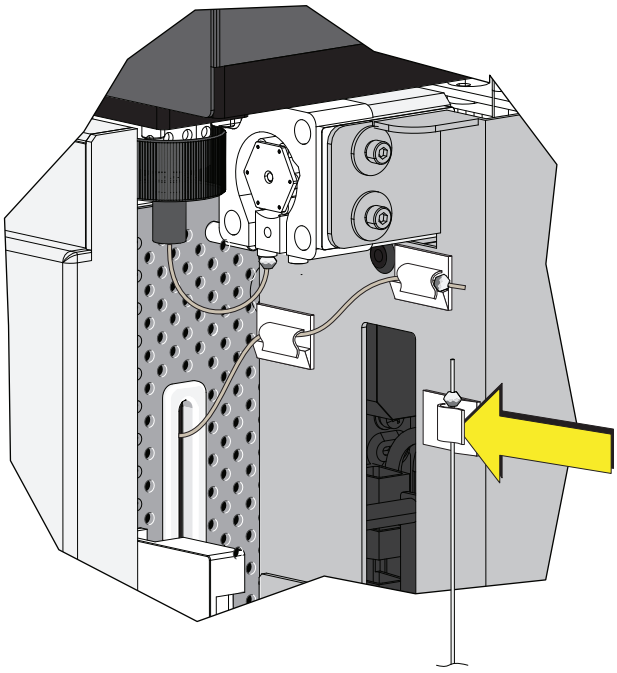
5 Remove the sample pump cover thumbscrew and the sample pump cover.



6 Remove the sample probe from the single tube sample station.



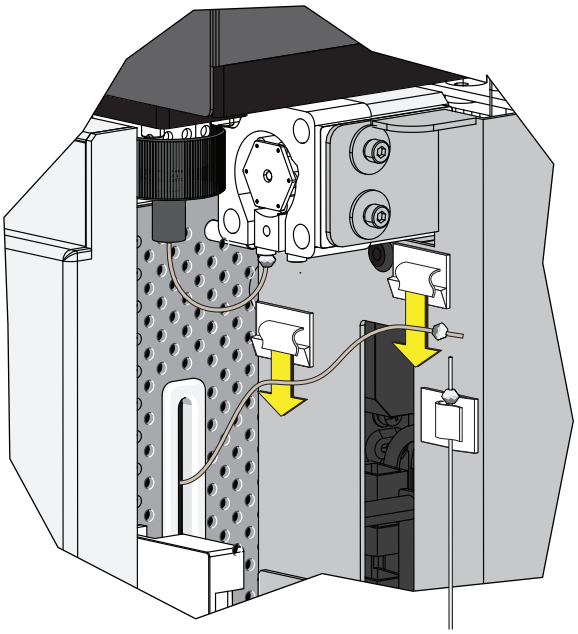
7 Place the sample probe in the white clip located on the right side of the sample station.



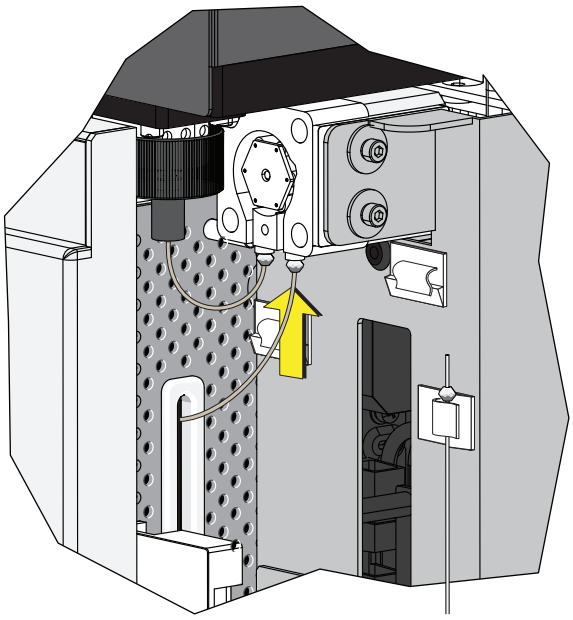
⚠ CAUTION

The plate loader PEEK tubing can be deformed which could affect sample flow. When removing the plate loader PEEK tubing from the white clips, be careful not to pinch, crimp, stretch, or break the tubing.

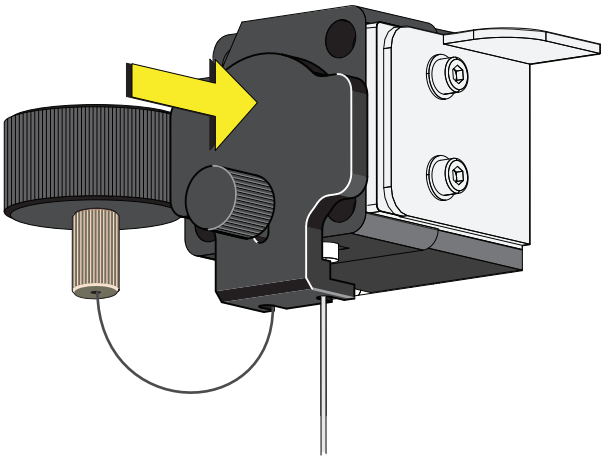
8 Remove the plate loader PEEK tubing from the two white clips located at the top of the sample station.



9 Connect the plate loader PEEK tubing to the sample peristaltic pump tubing.



10 Reinstall the sample pump cover.



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

11 Reinstall the right-side cover (refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures), and lock with the screw.

12 Close the top cover.

13 Turn the Cytometer's main power switch on.

Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [CytoFLEX With Plate Loader]

⚠ WARNING

Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately.

NOTE If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, refer to Switching the Sample Probe from the Plate Loader to the Single Tube Sample Station [With Sample Injection Mode Control Knob] in APPENDIX C, Sample Injection Mode Control Kit for instructions on switching between the single tube mode and the plate loader mode.

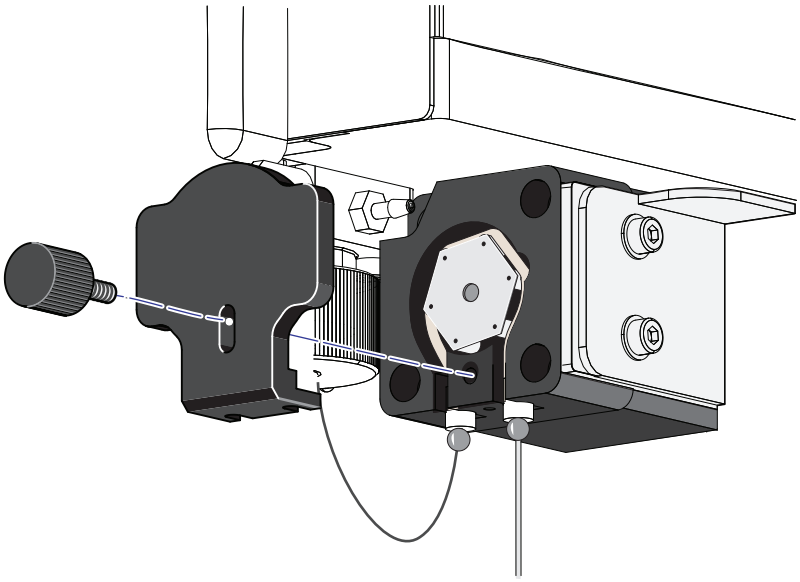
1 Switch to either the Semi-Automatic or manual sample injection mode. Refer to Selecting the Proper Sample Injection Mode in CHAPTER 3, Daily Startup.

2 Lift the top cover.

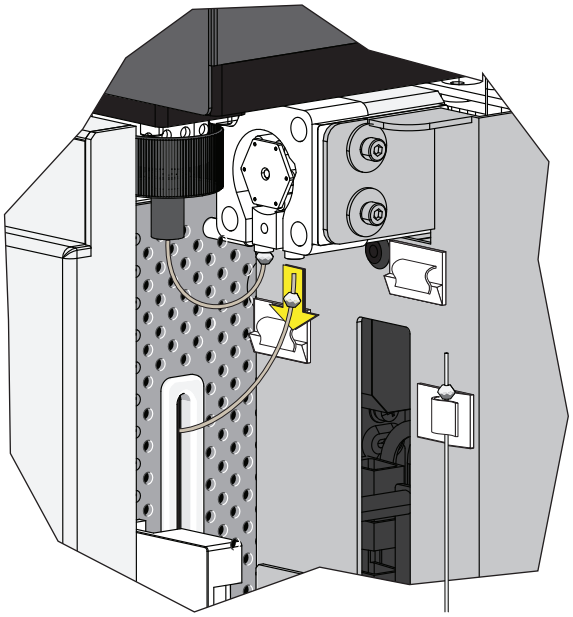
3 Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

4 Remove the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

5 Remove the sample pump cover thumbscrew and the sample pump cover.



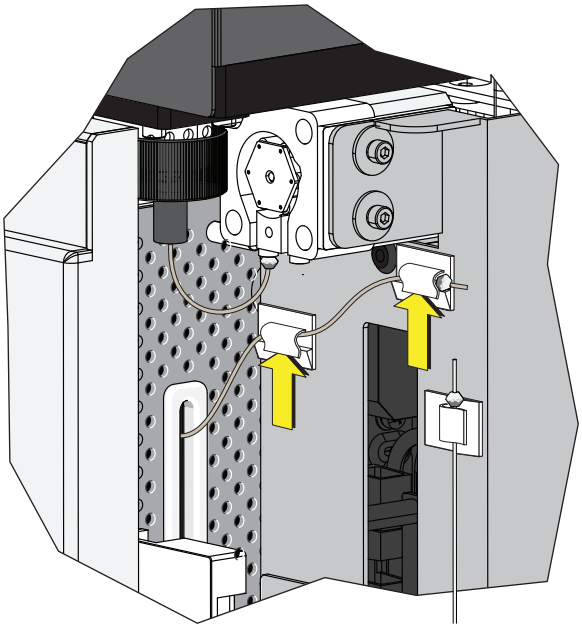
6 Remove the plate loader PEEK tubing from the sample peristaltic pump tubing.



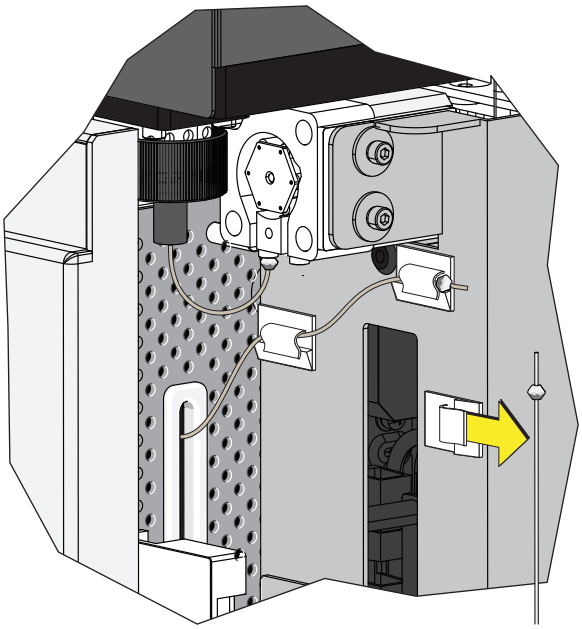
⚠ CAUTION

The plate loader PEEK tubing can be deformed, which could affect sample flow. When placing the plate loader PEEK tubing in the white clips, be careful not to pinch, crimp, stretch, or break the tubing.

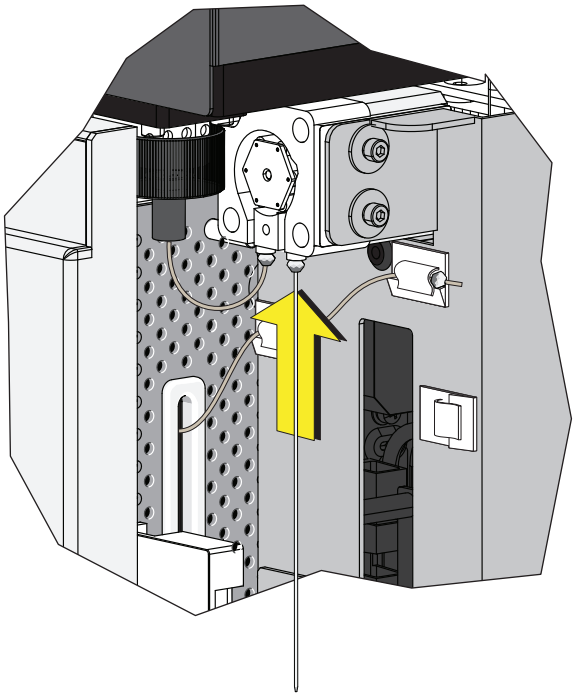
7 Place the plate loader PEEK tubing in the two white clips located at the top of the sample station.



8 Remove the sample probe from the white clip located on the right side of the sample station.



9 Connect the sample probe to the sample peristaltic pump tubing.



10 Run Daily Clean in CHAPTER 10, Cleaning Procedures.

Inspecting the Liquid Flow Path for Leaks

⚠ WARNING

The liquid flow tubing can aged and cracked or the connector can be loosened. Liquid leakage can lead to biological harm. To reduce occurrence of such problems, carry out liquid flow tubing inspection every six months and ensure that the Fluidics module functions without any leaks. If any leaks are found when using the Cytometer, stop the experiment immediately and look for the source of the leak.

1 Remove the right-side cover of the instrument. Refer to Right-Side Cover Removal and Reinstallation.

2 Perform instrument initialization to enable the sheath fluid to flow. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup.

3 Check the connectors and tubes in the Fluidics module, and check whether any liquid leaks out.

4 Check the sheath fluid, backflush, and waste liquid connector on the back of the Cytometer, and check whether any liquid leaks out.

5 Place the instrument in standby state, complete the priming procedure, and check whether the Fluidics module has any liquid leakage.

6 If any liquid leaks out and the point of liquid leakage is from the filter, try to tighten the filter connector and check again. If the problem persists, replace the sheath fluid filter.

7 If any liquid leaks out from any other connector or tube, stop running the instrument and contact us.

Priming the Flow Cell

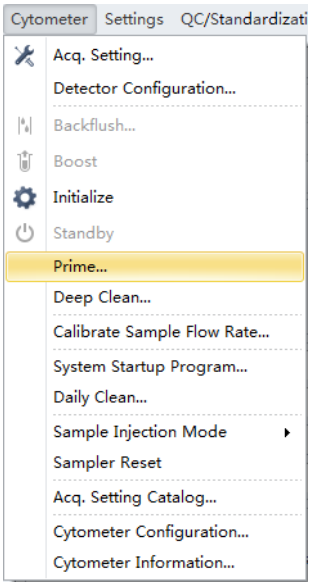
Priming of the flow cell is required when:

- The instrument is not used for a long period of time.
- The sheath fluid is refilled.
- The instrument is being used for the first time.
- The signal of the instrument is poor or the signal drifts.
- The sheath filter is replaced.

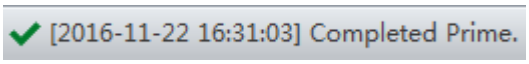
1 Ensure that the instrument is in standby state.

NOTE If the instrument is not already in the standby state, select **Standby** from the Cytometer Menu or select **Standby** in the Data Acquisition Control screen.

2 Select **Prime** from the Cytometer Menu to prime the flow cell. Wait for the beep and for the Instructions window to close.



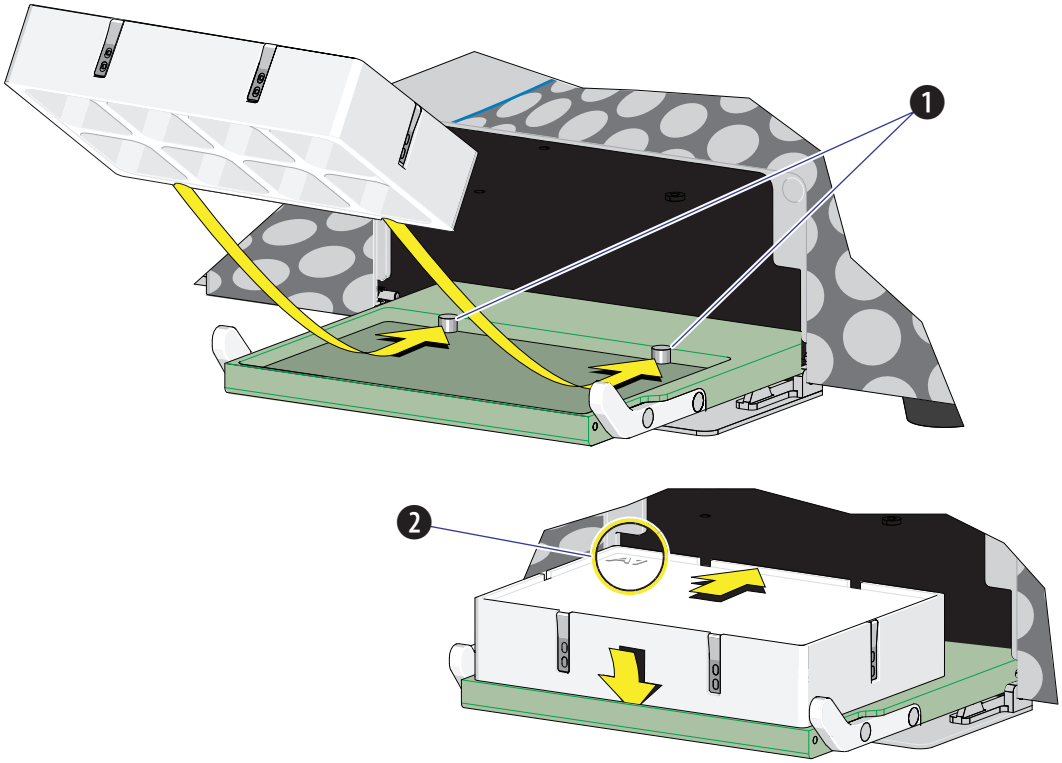
Otherwise, look for the status bar to display that priming has completed.



3 Run Daily Clean to clean the sample line. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures

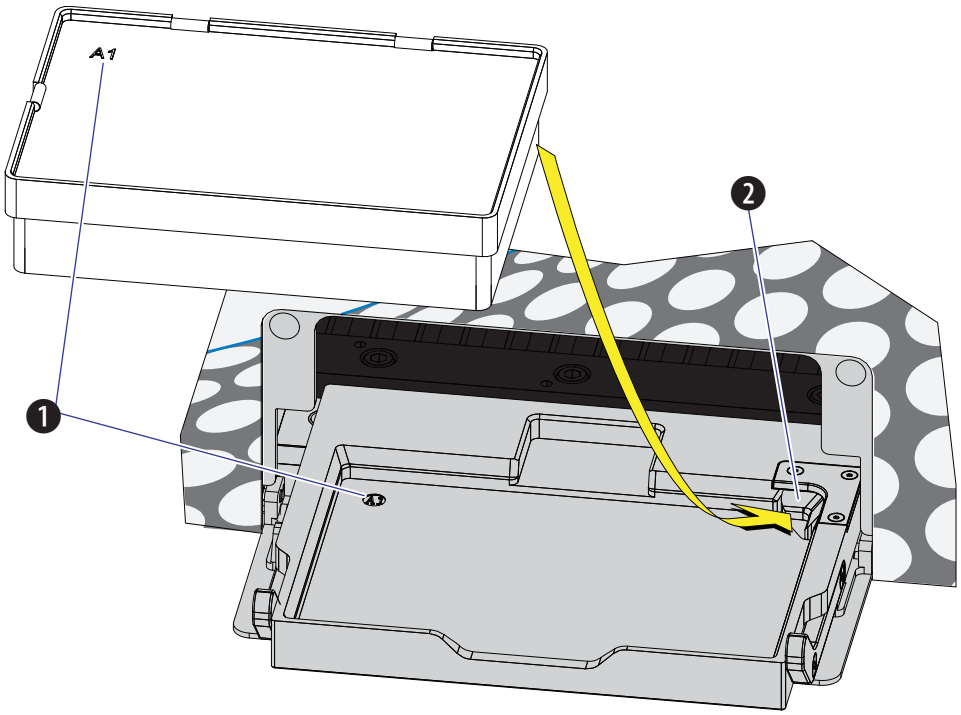
Replacing the Plate Holder [With Plate Loader]

Figure 11.1 Plate Holder Stage (Standard Plate Loader)



1. Pins
2. Position A1

Figure 11.2 Plate Holder Stage (Plate Loader DW)



1. Position A1
2. Spring lock

⚠ CAUTION

The plate holder must be secured tightly to the plate holder stage with position A1 located in the top, left corner of the plate holder stage (refer to Figure 11.1 and Figure 11.2) to prevent instrument damage.

Slide the notches on the bottom of the plate holder (refer to Figure 1.19) over the pins (refer to Figure 11.1, and Figure 11.2).

Plate Loader Module Removal and Reinstallation [With Plate Loader]

⚠ WARNING

Risk of contamination from biohazardous material. Always wear PPE when performing this procedure as you can contact components with blood residue.

Removal

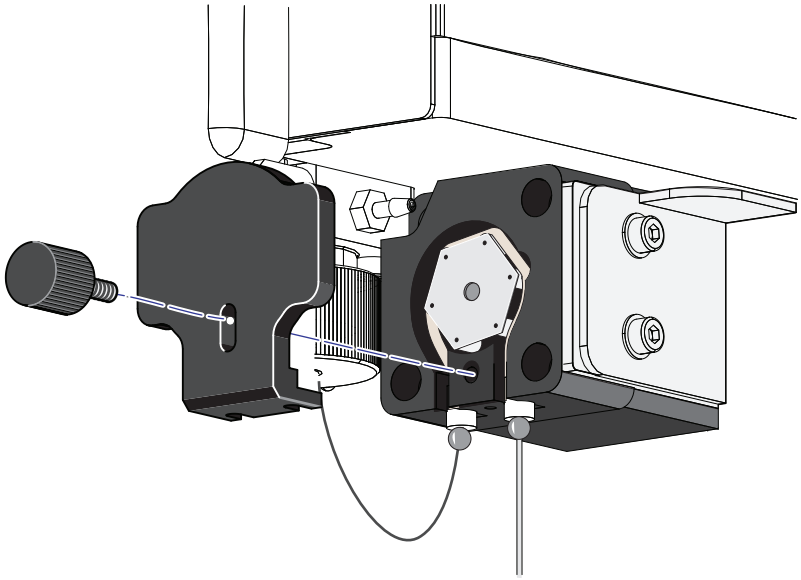
1 Power down the instrument and disconnect the power cable from the wall.

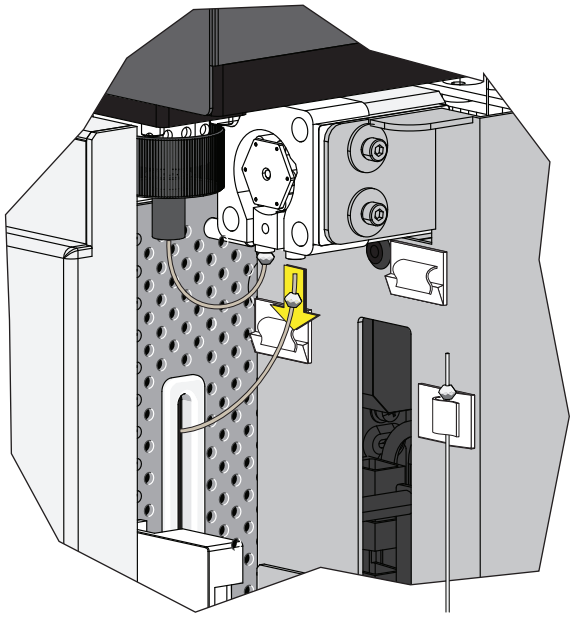
2 Lift the top cover and remove the front cover. Refer to Front Cover Removal and Reinstallation.

3 Place absorbent material on the stage in the Plate Loader module.

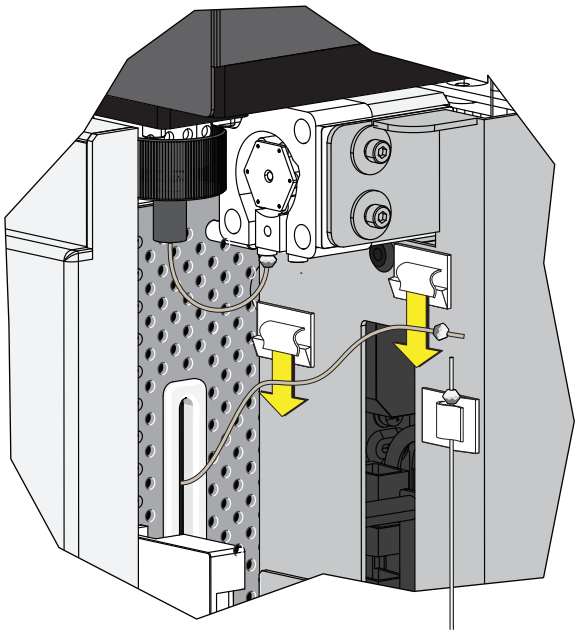
4 In the Sample Station, disconnect that end of the plate loader PEEK tubing.

- If the plate loader PEEK tubing is attached to the sample peristaltic pump tubing, remove the sample pump cover and disconnect the plate loader PEEK tubing.





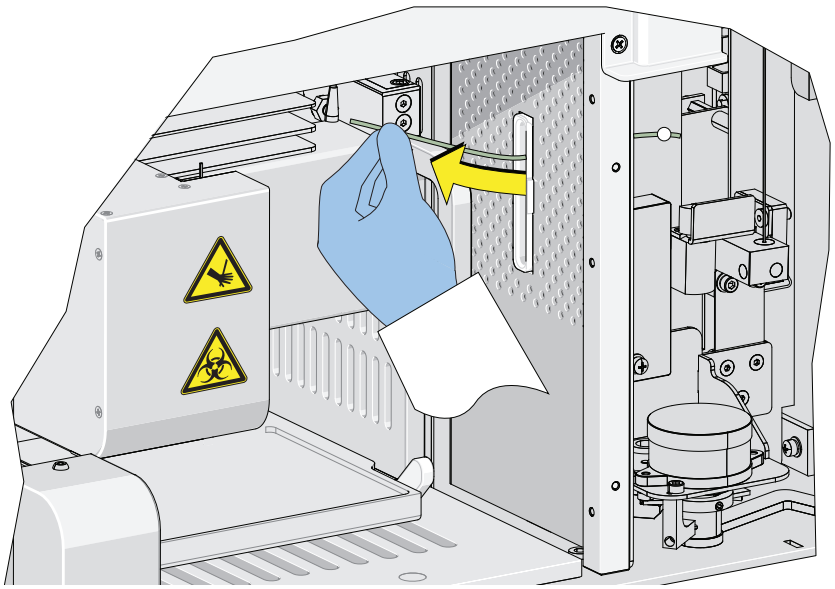
- If the plate loader PEEK tubing is in the plate loader PEEK tubing clips, remove the tubing from the clips.



⚠ CAUTION

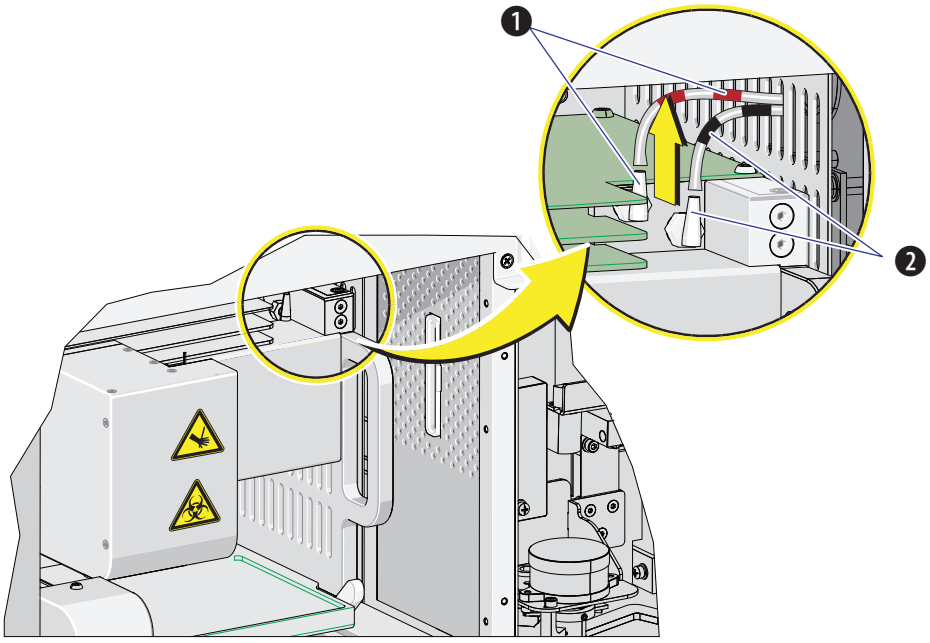
The PEEK tubing can be deformed which could affect sample flow. When routing the Plate Loader PEEK tubing to and from the Sampling Station, be careful not to pinch, crimp, stretch, or break the tubing.

5 Pull the plate loader PEEK tubing through the slot so the tubing sits inside the instrument.



6 Remove the red and black marked tubes that are attached to the left and right connectors in the Plate Loader module, respectively, as shown in Figure 11.3.

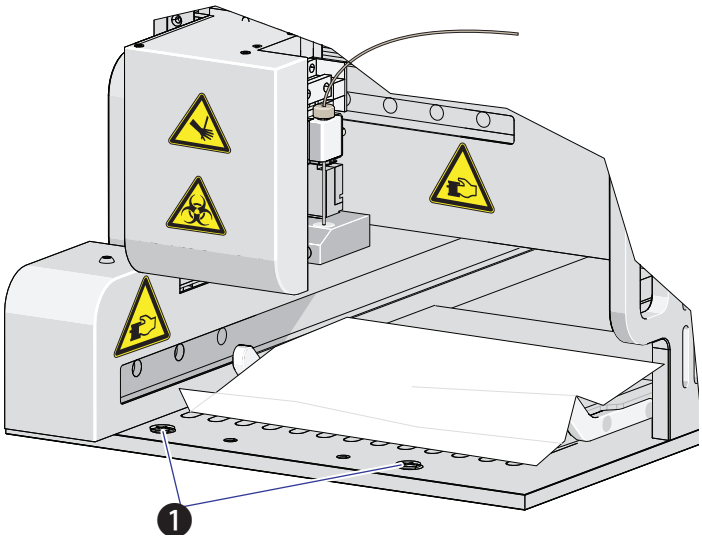
Figure 11.3 Removing the Tubings from the Fluidics Module to the Plate Loader



1. Red
2. Black

7 Remove the two countersunk M4 x 10 Phillips-head screws in the Plate Loader module. Refer to Figure 11.4.

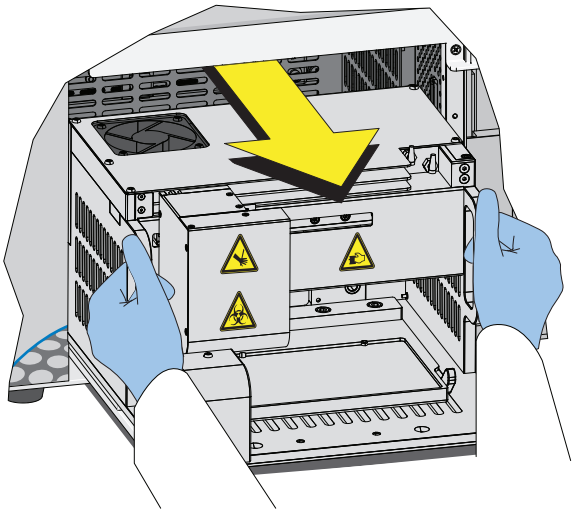
Figure 11.4 Plate Loader Module Securing Screws



1. Securing screws

8 Slide the Plate Loader module out of the Cytometer. Refer to Figure 11.5.

Figure 11.5 Removing the Plate Loader from the Cytometer



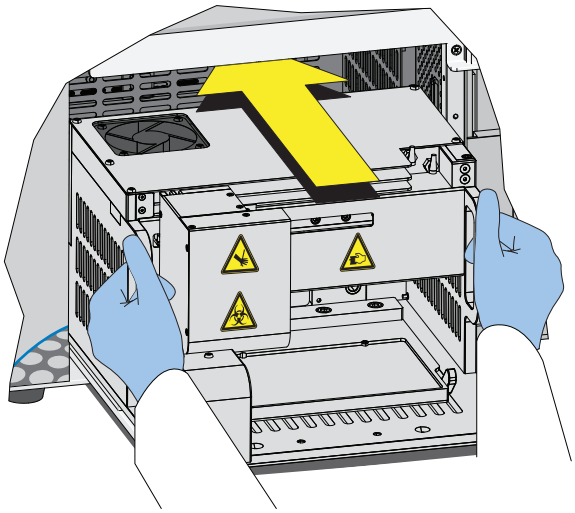
Installation

1 Remove the front cover. Refer to Front Cover Removal and Reinstallation.

2 Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation.

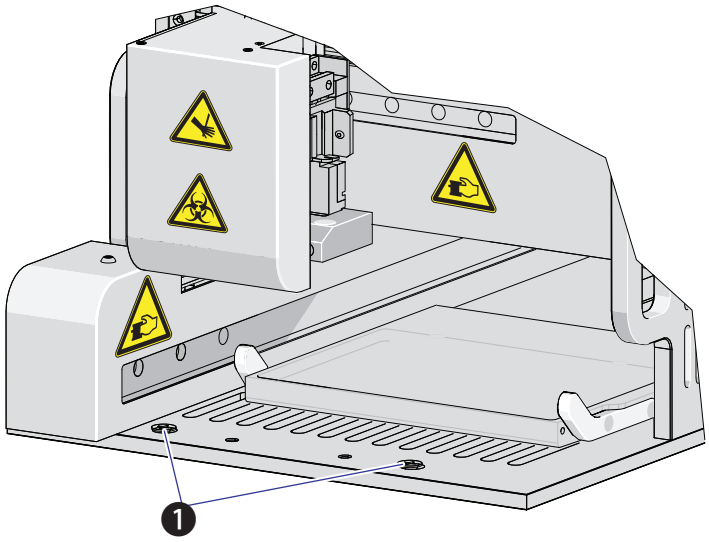
3 Slide the Plate Loader module in to the Cytometer. Refer to Figure 11.6.

Figure 11.6 Installing the Plate Loader in to the Cytometer



4 Install the two countersunk M4 x 10 Phillips-head screws in the Plate Loader module. Refer to Figure 11.7.

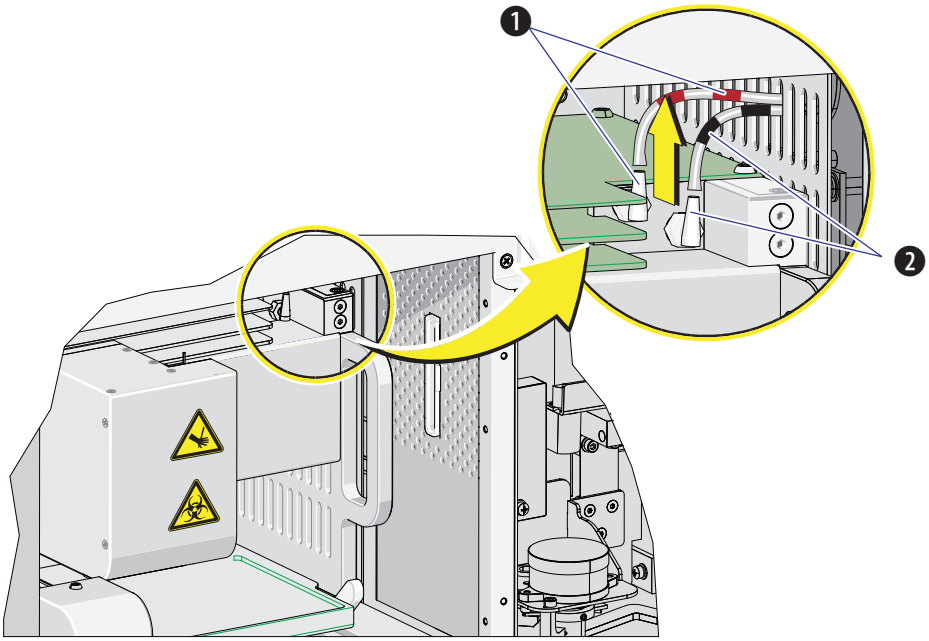
Figure 11.7 Plate Loader Module Securing Screws



1. Securing screws

5 Install the red and black marked tubings that are attached to the left and right connectors in the Plate Loader module, respectively, as shown in Figure 11.8.

Figure 11.8 Connecting the Tubings from the Fluidics Module to the Plate Loader

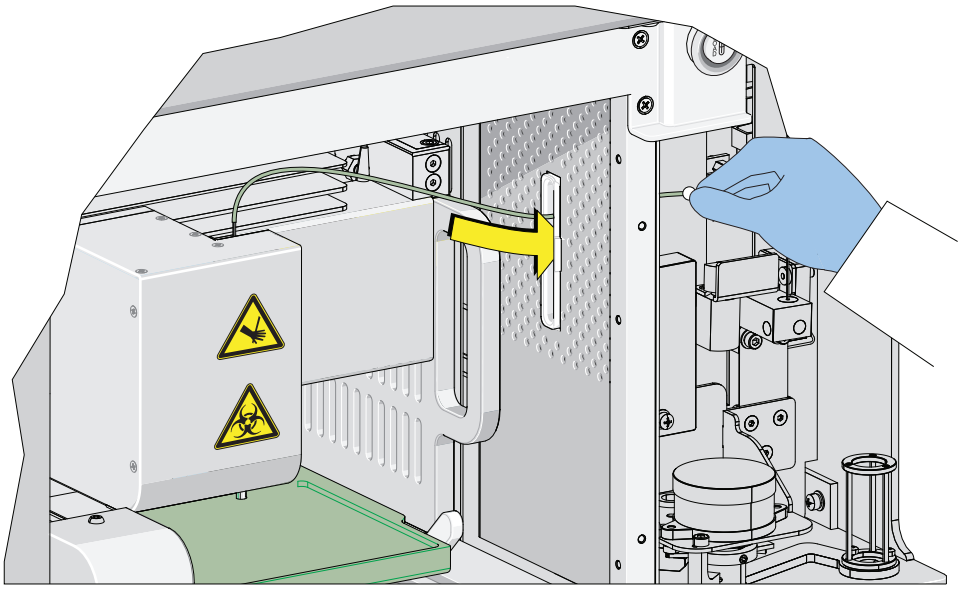


1. Red
2. Black

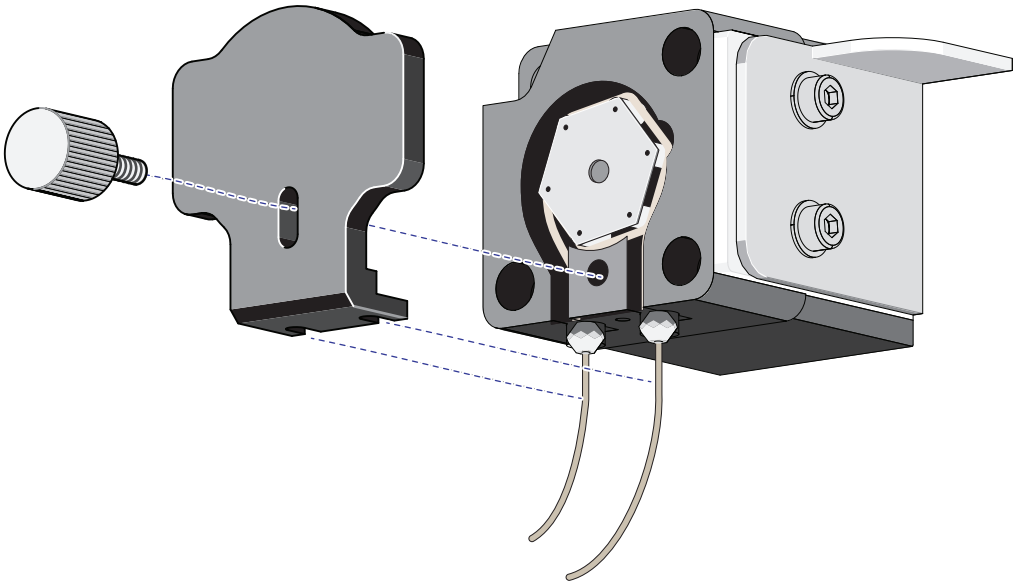
⚠ CAUTION

The PEEK tubing can be deformed, which could affect sample flow. When routing the Plate Loader PEEK tubing to and from the Sampling Station, be careful not to pinch, crimp, stretch, or break the tubing.

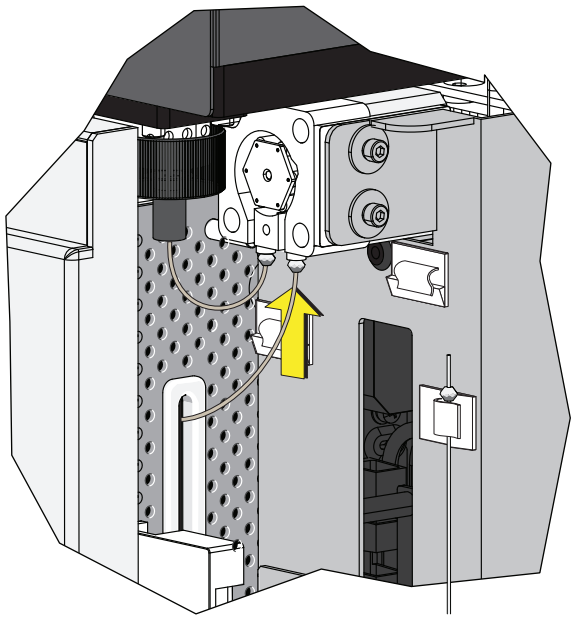
6 Slide the new plate loader PEEK tubing through the slot into the single tube sample station area.



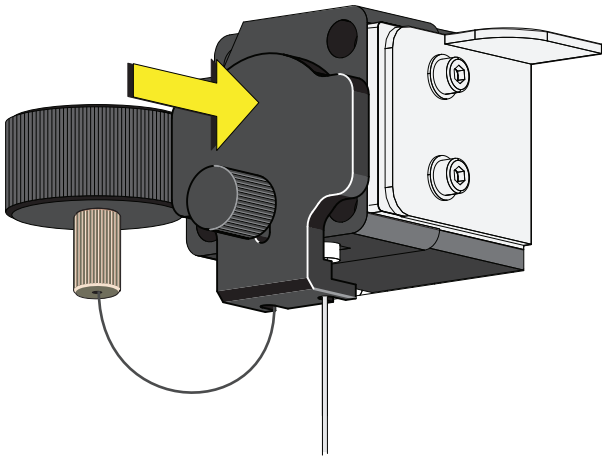
7 Remove the sample pump cover thumbscrew and the sample pump cover.



8 Connect the plate loader PEEK tubing to the sample peristaltic pump tubing.



9 Reinstall the sample pump cover.



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

10 Replace the right-side cover (refer to Right-Side Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures), and lock with the screw.

11 Replace the front cover. Refer to Front Cover Removal and Reinstallation.

12 Close the top cover.

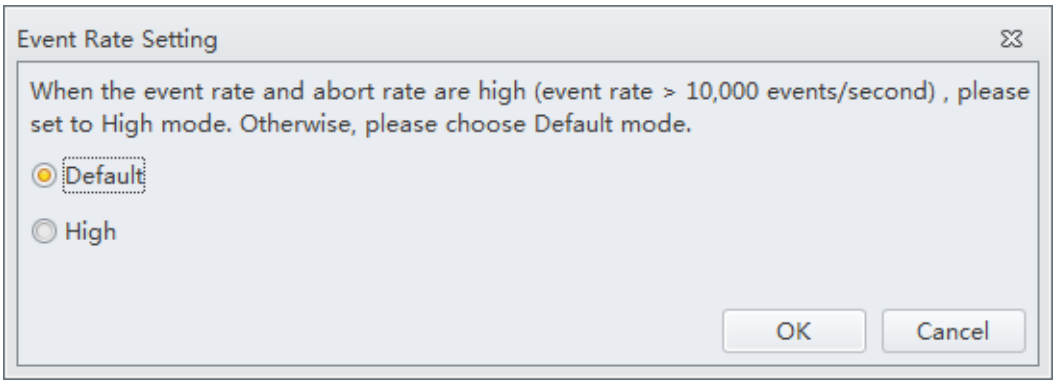
13 Turn the Cytometer's main power switch on.

14 Verify that the system is operating correctly by running QC in the Plate Loader mode. Refer to Preparing the QC Sample [With Plate Loader] and Collecting QC Data [With Plate Loader] in CHAPTER 4, Instrument Quality Control and Standardization.

Changing the Event Rate Setting

The Event Rate Setting adjusts the collection setting around signal measurement so that the system is able to optimize the acquisition of events ensuring optimal sensitivity and abort rates when acquiring at higher event rates.

1 Select **Event Rate Setting** in the Advanced menu. The Event Rate Setting window appears.



2 Select **High** if the event rate is >10,000 events/second.

Or

Select **Default** if the event rate is <10,000 events/second.

3 Select **OK**.

Nonscheduled Replacement/Adjustment

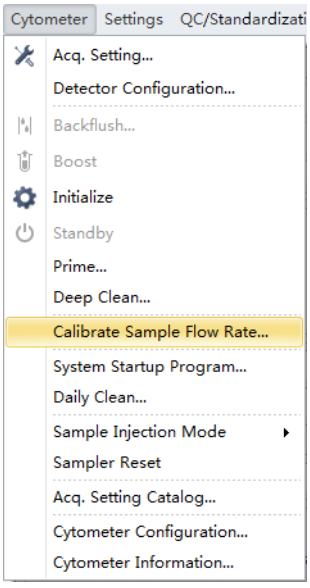
Calibrating the Sample Flow Rate

Calibrate the sample flow rate:

- After replacing the sample peristaltic pump tubing.
- If a precise volumetric measurement is required.
  The accuracy of concentration calculations can be affected by the sample flow rate.

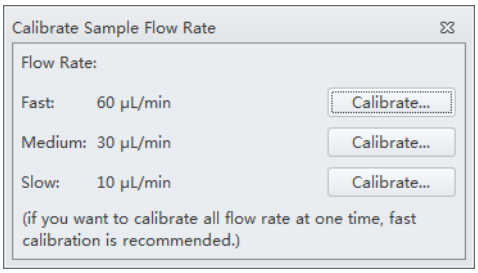
1 Verify that the instrument is in the initialized state.

2 Select **Calibrate Sample Flow Rate** in the Cytometer menu.

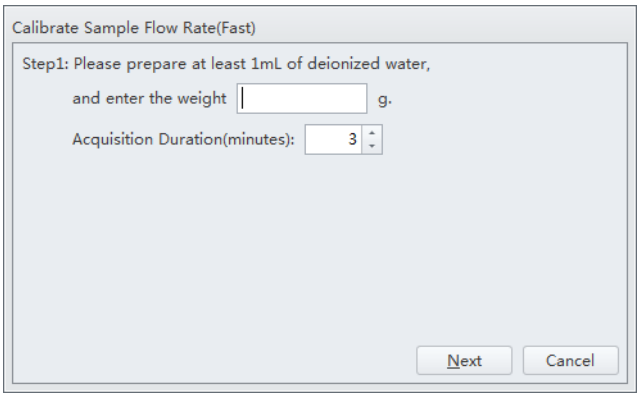


3 Select the flow rate to be calibrated.

If you want to calibrate all flow rates at once, fast calibration is recommended. The rate selected in the Calibrate Sample Flow Rate window overrides the rate selected in the Acquisition window.



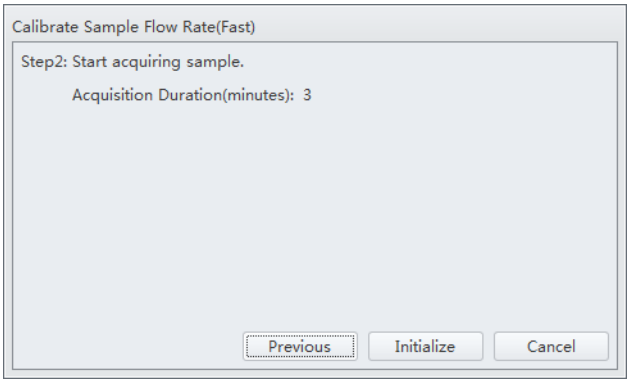
4 Prepare one sample tube with 1 mL of clean deionized water then use a calibrated analytical balance to measure the weight of the prepared sample tube. Record the weight and enter it into the software.



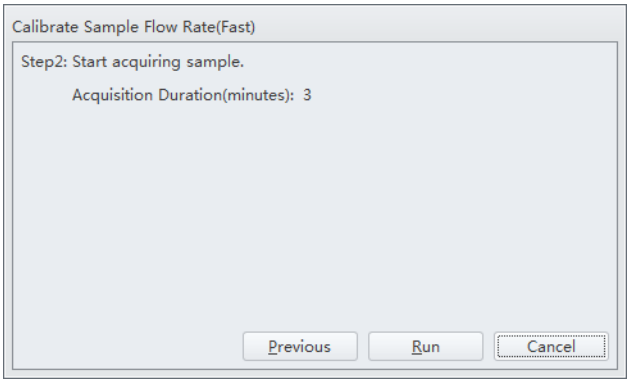
NOTE The weight section accepts up to four decimal places.

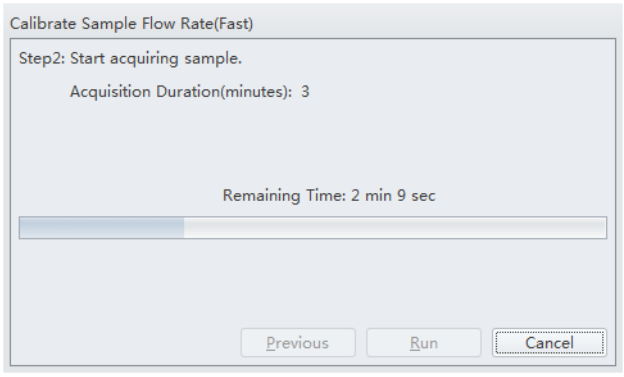
5 Select **Next** and put the sample tube in the sample loading position (see Figure 1.13).

6 Select **Initialize** to start the sample run.

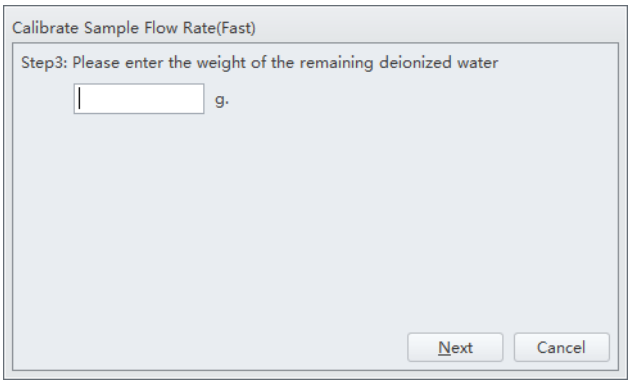


7 Select **Run** to begin acquiring the sample.



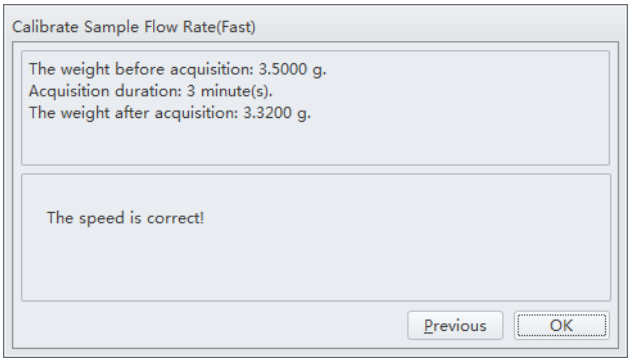


8 Wait for the sample run to finish, remove the sample tube, and use the analytical balance to measure the weight and record the value.

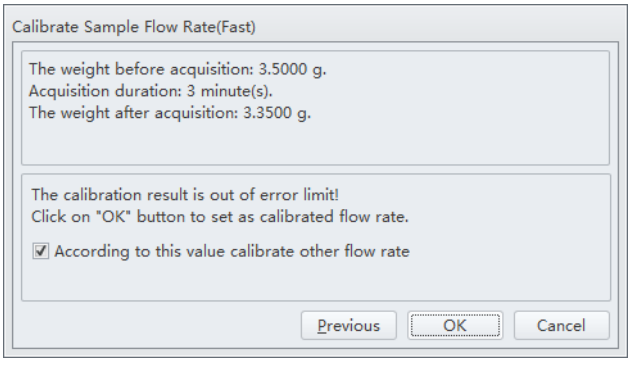


9 Select **Next** to determine if the results fall within the acceptable range.

If the results fall within the acceptable range, the current setting is kept.



If deviation occurs, the setting is automatically corrected.



Calibrating the Sample Flow Rate [With Plate Loader]

Calibrate the sample flow rate:

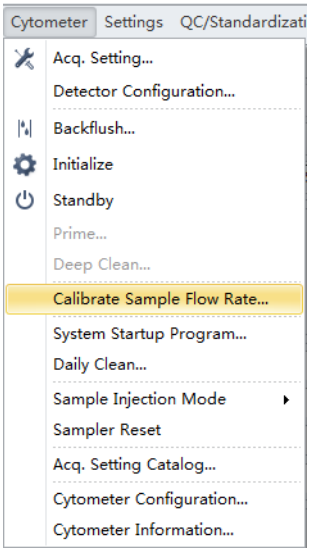
- After replacing the sample peristaltic pump tubing.
- If a precise volumetric measurement is required.
  The accuracy of concentration calculations can be affected by the sample flow rate.

---

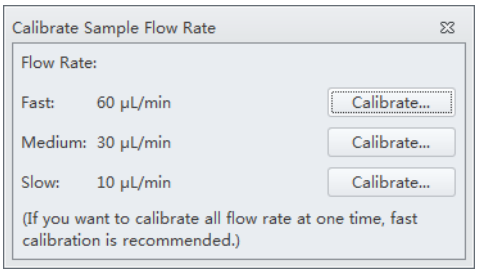
1 Verify that the instrument is in the initialized state.

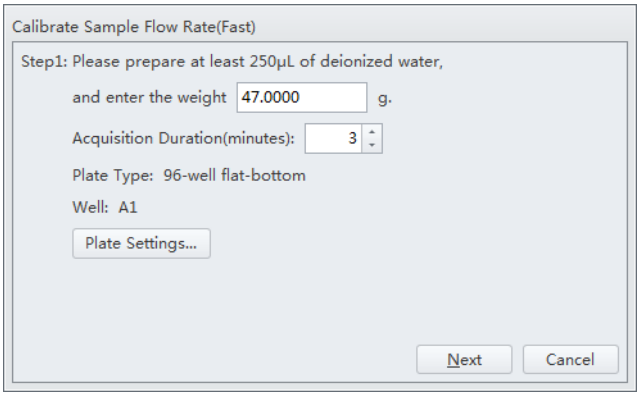
---

2 Select **Calibrate Sample Flow Rate** in the Cytometer menu. The plate loader automatically ejects the plate holder stage.



3 Select the sampling speed that needs to be calibrated and select **Calibrate**. The Calibrate Sample Flow Rate window appears.

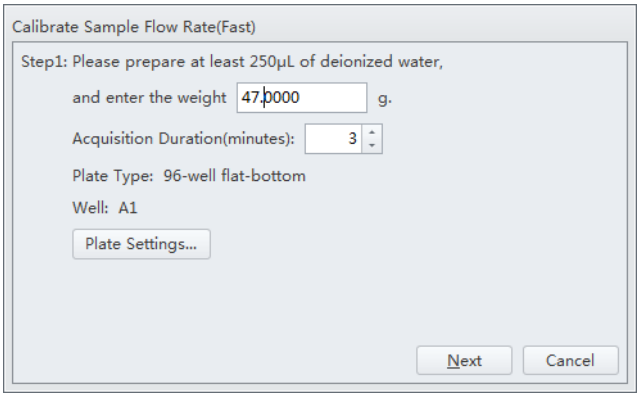




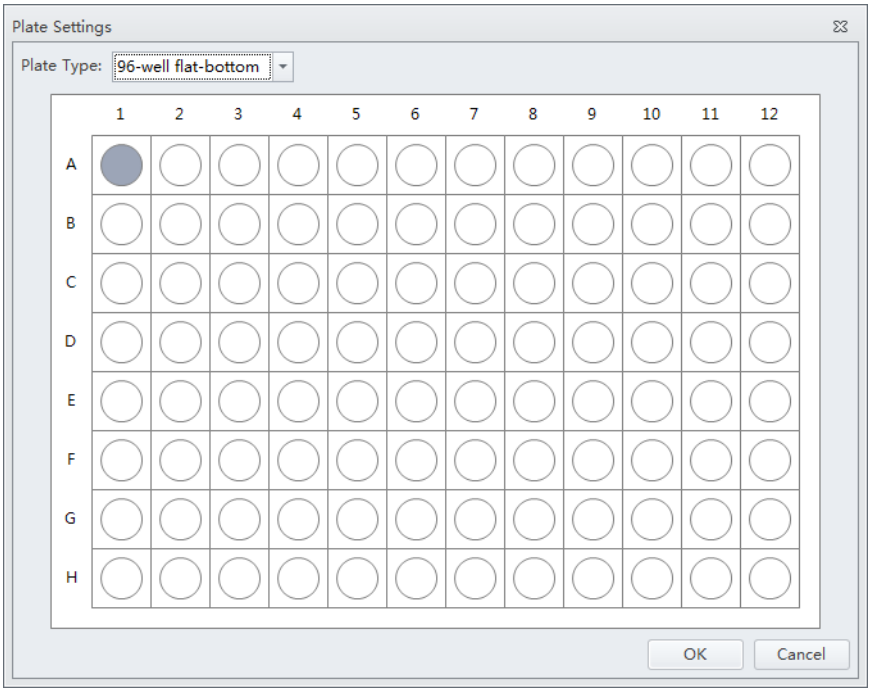
4 Follow the on-screen software prompts, then weigh the sample plate.

5 Enter the weight of the sample plate and set the acquisition time.

NOTE Do not exceed three minutes when using the fast sampling rate.

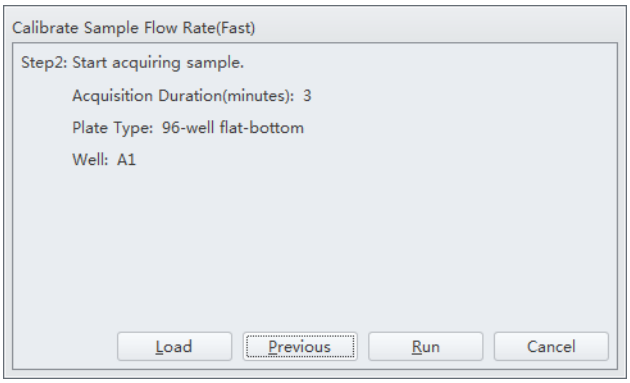


6 Select **Plate Settings** to set the sample well and plate type.



7 Select **OK** to save the settings.

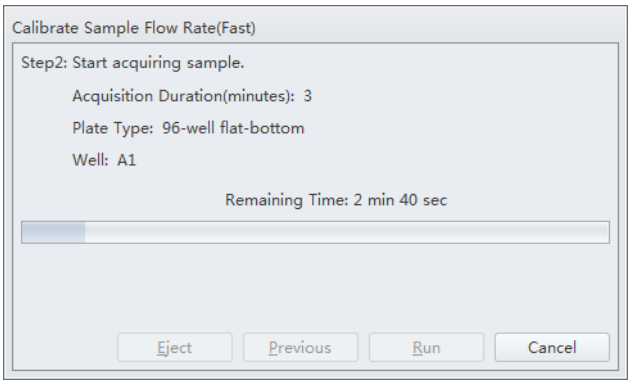
8 Select **Next** to proceed to the next step. The plate loader automatically ejects the plate holder stage.



9 Place the sample plate onto the plate loader.

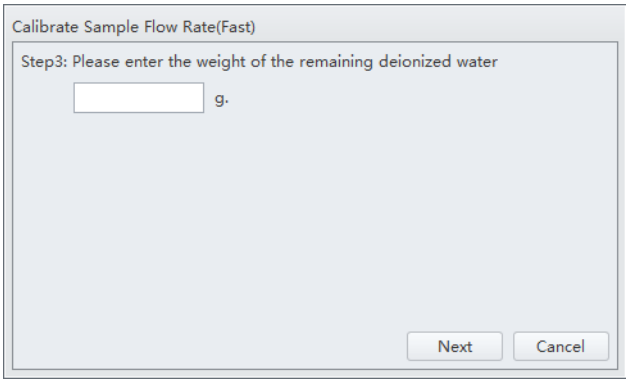
10 Verify the settings and select **Load** to load the plate.

11 Select **Run**. The message *Please confirm that the correct plate is placed properly and press OK* appears. Select **OK**. The system begins acquiring the sample.

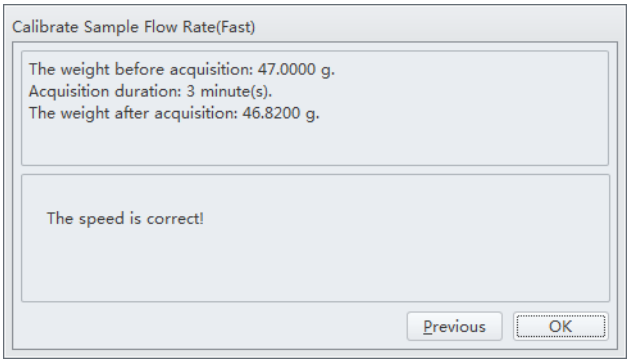


12 The plate loader automatically ejects the plate holder stage after the sample is acquired. Weigh the plate.

13 Enter the remaining weight value and select **Next** to confirm the calibration.

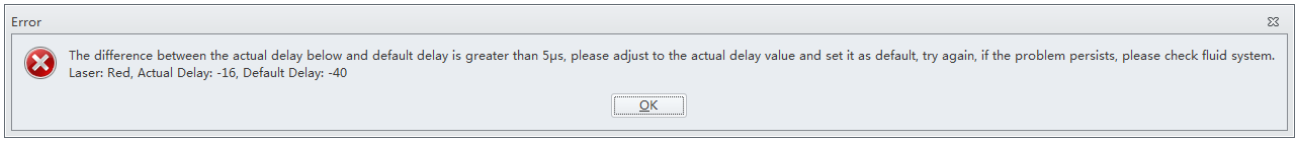


14 Select **OK**.

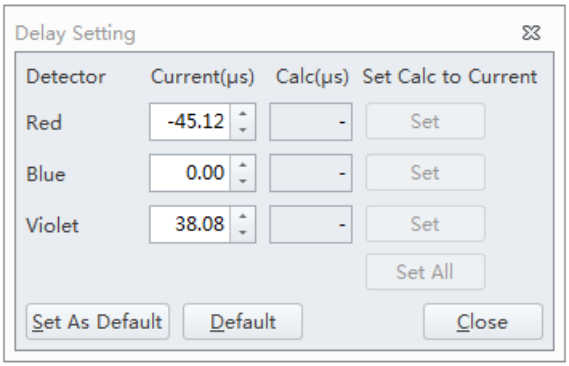


Setting Laser Delay

Laser delay is preset for QC. Only change the laser delay if the software prompts you that there is a difference in the actual delay and the default delay.



1 Select **Delay Setting** from the Advanced menu. The Delay Setting window appears.



2 Set the current to the actual delay for the specified detector stated in the error message received.

3 Select **Set as Default**.

4 Select **Close**.

Replacing the Optical Filter

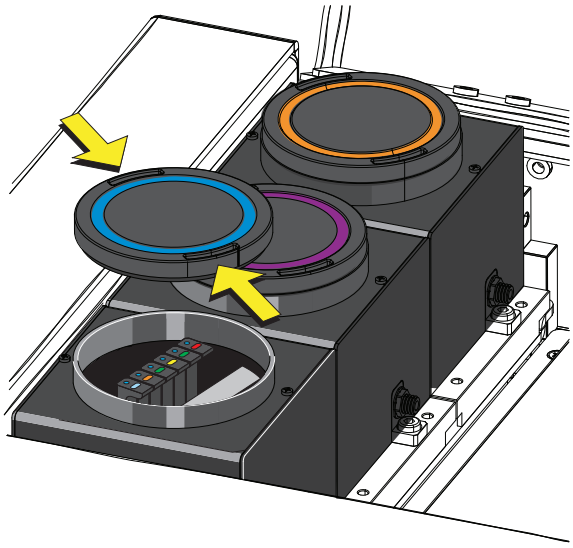
When the optical filter is damaged or it is required to use an optical filter with a non-standard wavelength, you must replace the optical filter yourself. For the specific part number of the optical filter, consult your Beckman Coulter Representative or your local dealer.

1 Confirm that the instrument is in the standby state or that the instrument is turned off.

2 Confirm the laser corresponds to the channel in which the optical filter is to be replaced.

3 Open the top cover of the instrument.

4 Press the spring piece of the WDM cap corresponding to the laser, and open the WDM cap.



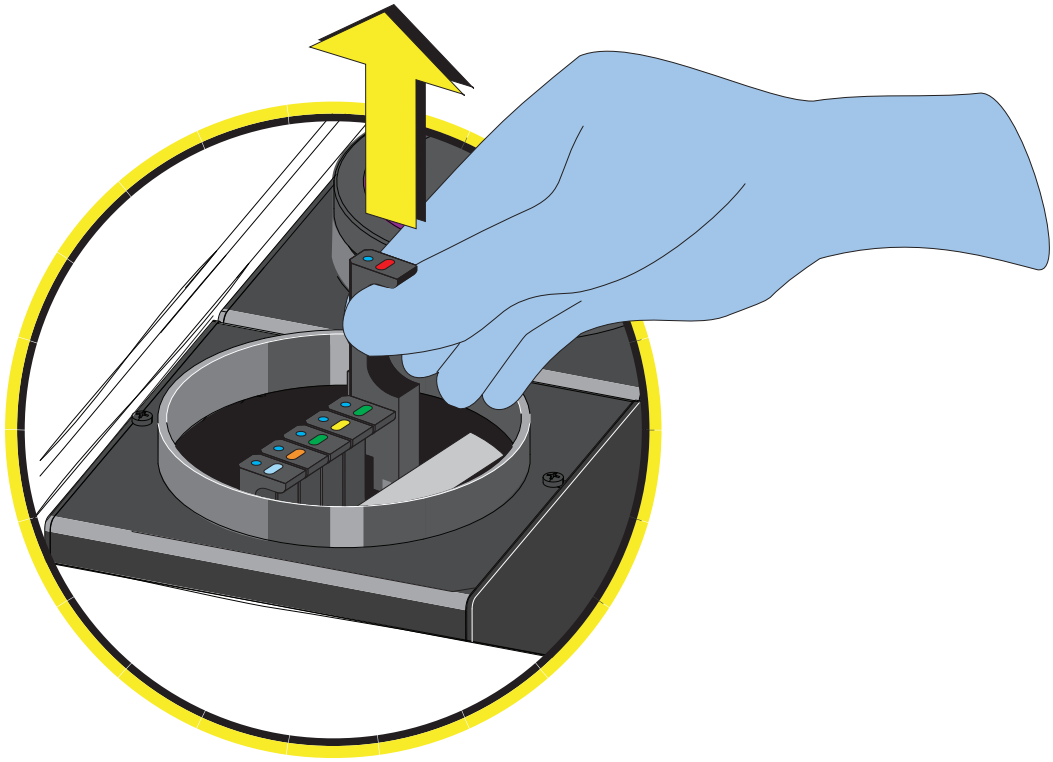
⚠ CAUTION

Risk of damage to the optical filter. Do not touch the optical filter glass piece. Touching the optical filter glass piece can obscure and/or scratch the optical filter glass piece.

⚠ CAUTION

Risk of damage to the optical filter. Pull the filter straight up when removing it from the WDM. Removing the filter at an angle could chip the edge of the filter glass.

5 Use vertical force to remove the optical filter to be replaced, and note the color identification and wavelength identification on the optical filter bracket.



⚠ CAUTION

Risk of damage to the optical filter. Push the filter straight down when inserting it into the WDM. Inserting the filter at an angle could chip the edge of the filter glass.

6 Insert the optical filter to be installed vertically into the corresponding position, taking care to align the wavelength identification with the left, and that the bracket is inserted into the bottom.

7 Close the WDM cap and the instrument top cover.

8 Turn on the Cytometer and open the software.

9 Select **Detector Configuration** in the Cytometer menu and create a new instrument configuration based on the settings of the new optical filter. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Data Acquisition and Sample Analysis.

Set this new configuration as the current configuration.

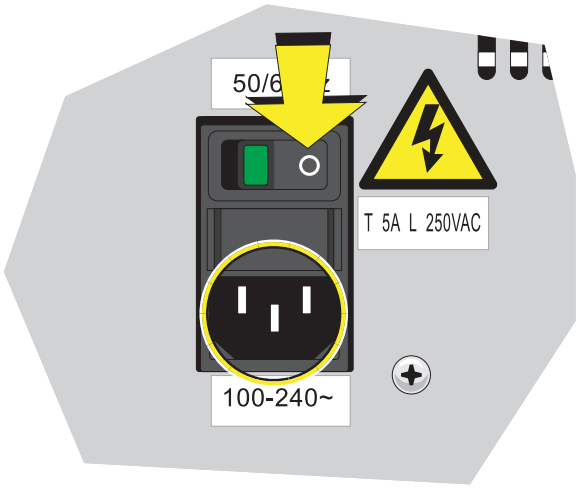
Replacing the Fuse

Use a 5 A, time delay, T 5 AL, 250 VAC fuse.

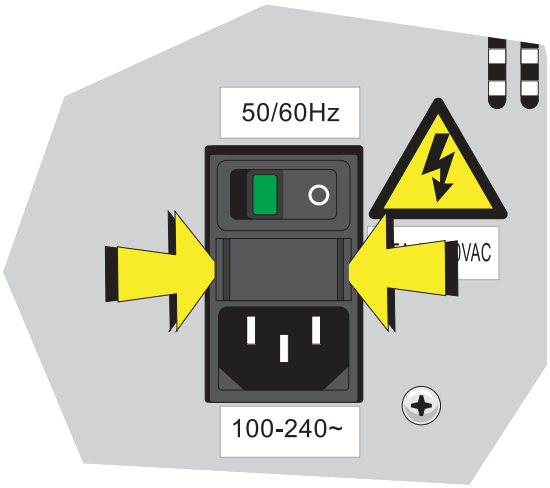
⚠ WARNING

Risk of personal injury. A shock hazard exists if the power cable is connected. Turn off the Cytometer and disconnect the primary power cable before performing these procedures.

1 Turn off the Cytometer, and disconnect the power cable.



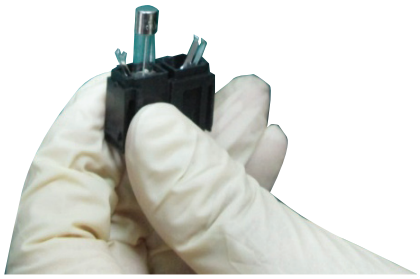
2 Press both sides of the fuse holder of the instrument inwards using a flat head screwdriver, and pull out the fuse holder.



IMPORTANT Select well-performing products that comply with the specifications required, to ensure that the instrument can function normally and safely.

3 Check whether the fuse installed is blown, and replace the blown fuse with a new one.

The specifications of the fuse required are: T 5 AL 250 VAC, delay blow fuse, 5A, 250 VAC, 5 x 20 mm. Beckman Coulter recommends using SCHURTER 0034.3124.



4 Insert the fuse holder.

5 Reconnect the power cable.

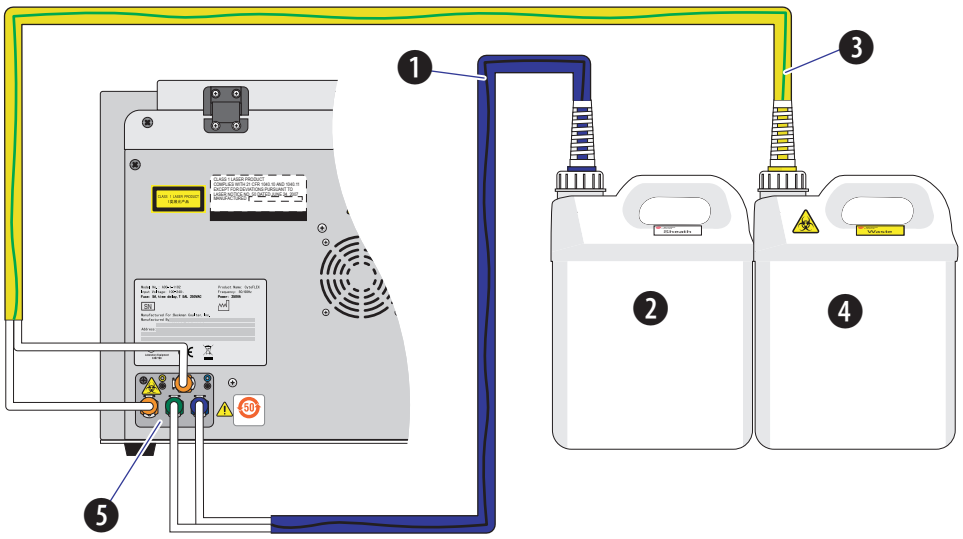
Replacing the Sheath Fluid Harness and/or Waste Harness

Replace the sheath fluid harness and/or the waste harness if you have a faulty sheath fluid sensor and/or waste sensor.

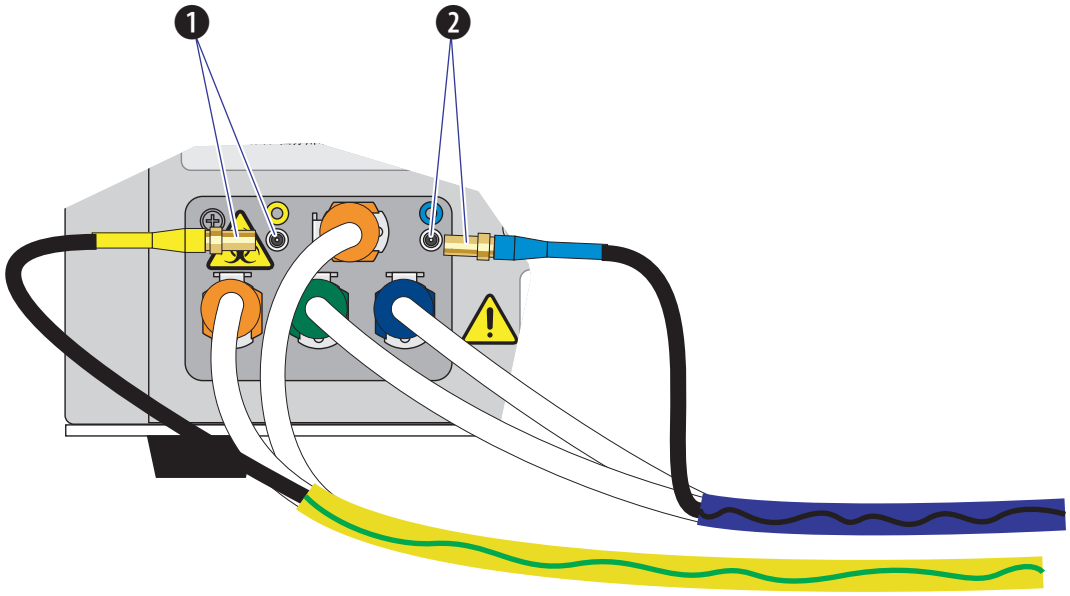
1 Confirm that the instrument is turned off or is in the standby state.

2 Remove sheath and/or waste pickup tubing from the appropriate container.

3 Disconnect the blue harness **(1)** from the sheath fluid container **(2)** and/or the yellow harness **(3)** from the waste container **(4)** from the fluid connector panel **(5)** on the back right corner of the instrument according to the color code.



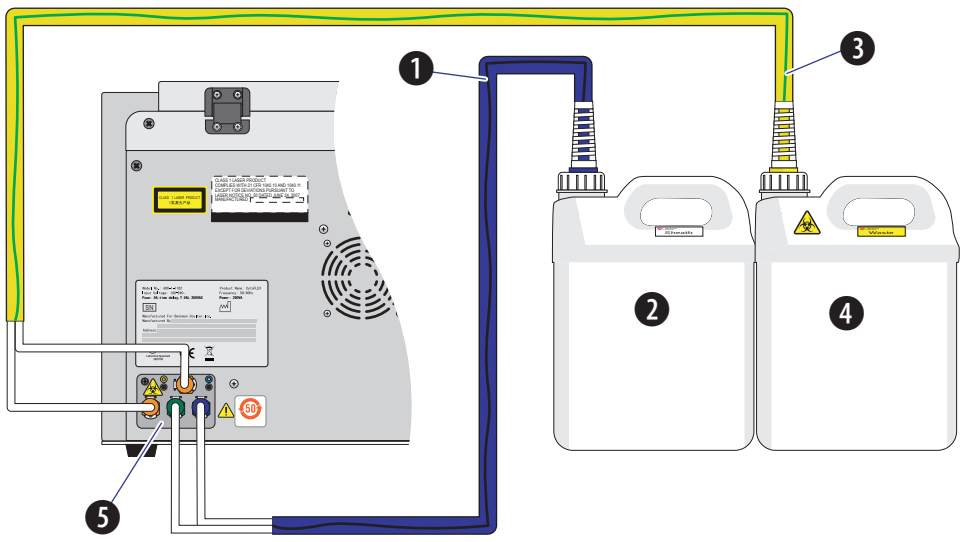
Disconnect the waste **(1)** and/or sheath **(2)** level sensors.



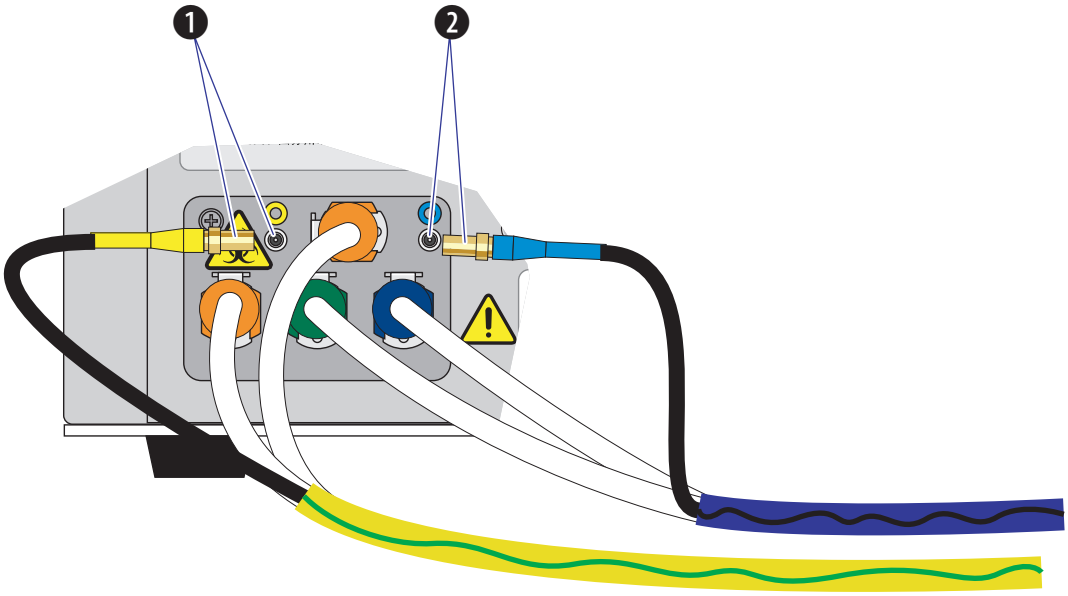
4 Dispose of the sheath fluid harness and/or the waste harness according to your laboratory procedures.

5 Insert the new sheath and/or waste pickup tubing into the appropriate container.

6 Connect the blue harness **(1)** from the sheath fluid container **(2)** and/or the yellow harness **(3)** from the waste container **(4)** to the fluid connector panel **(5)** on the back right corner of the instrument according to the color code.



Connect the waste **(1)** and/or sheath **(2)** level sensors.



Changing Sample Mixing and Backflush Settings

⚠ WARNING

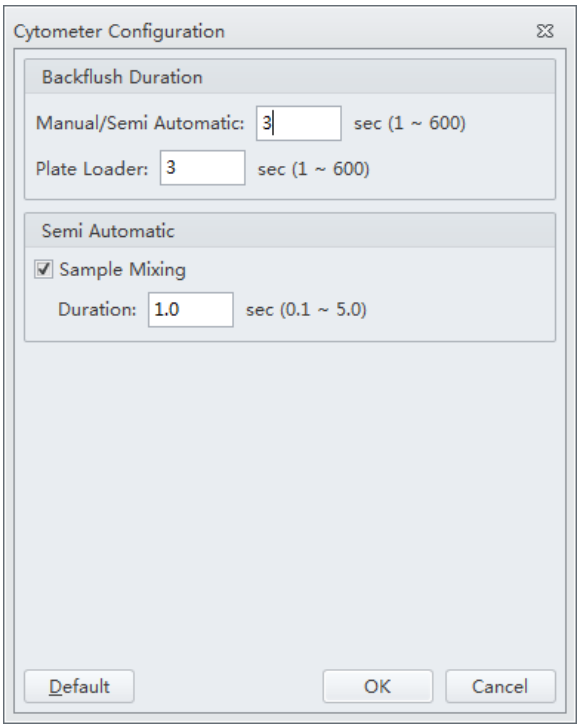
Risk of biohazardous contamination. Enabling sample mixing for 1.5-mL and 2-mL sample tubes in the semi-automatic sample injection mode can result in sample splashing. Exceeding 300-µL sample volume when using 1.5-mL and/or 2-mL sample tubes can also result in sample splashing. Disable sample mixing in the semi-automatic sample injection mode when using 1.5-mL and 2-mL sample tubes and do not exceed 300-µL sample volume.

The sample mixer can be enabled or disabled if necessary. The sample mixing duration can also be increased or decreased if necessary.

Whenever a sample is likely to leave residue or cause contamination, the backflush time can be increased to reduce cross contamination.

1 Open the CytExpert software and confirm that the instrument is connected. Refer to Logging Into the Software in CHAPTER 3, Daily Startup.

2 Select **Cytometer Configuration** in the Cytometer menu. The Cytometer Configuration window appears.



3 Select the Sample Mixing checkbox to enable sample mixing.

Or

Deselect the Sample Mixing checkbox to disable sample mixing.

4 Change the sample mixing duration to the desired time.

NOTE The default setting is 1 second. Select **Default** to set the Cytometer configuration settings back to the factory default settings.

5 Change the backflush duration to the desired time for either the Manual/Semi-Automatic sample injection mode or the Plate Loader sample injection mode depending on the current sample injection mode selected.

NOTE The default setting is 3 seconds without the Sample Injection Mode Control Kit installed or 4 seconds **[CytoFLEX and CytoFLEX S]** or 6 seconds **[CytoFLEX LX]** with the Sample Injection Mode Control Kit installed. Select **Default** to set the Cytometer configuration settings back to the factory default settings.

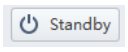
6 Select **OK**.

Calibrating the Plate Position [With Plate Loader]

Use the following procedure to calibrate the plate position and the sample probe position:

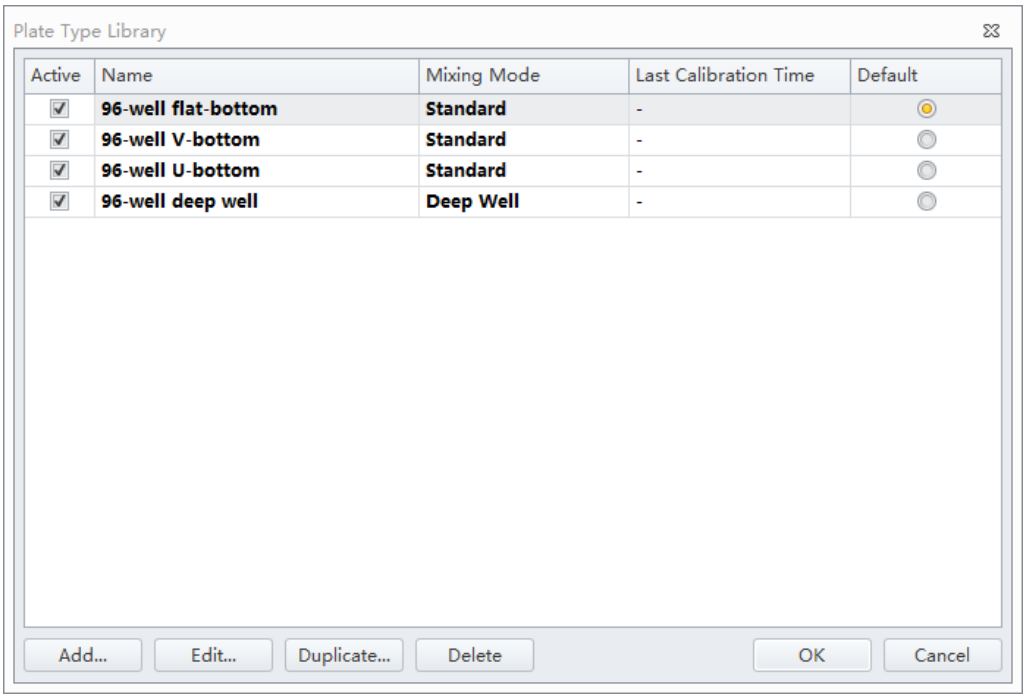
- Upon installation
- After a new plate type is defined for first use
- After changing plate manufacturers of the same previously calibrated plate type
- When optimizing plate performance

1 Remove the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

2 Select 

.

3 Select **Plate Type Library** in the Advanced menu. The plate loader automatically ejects the plate holder stage and the Plate Type Library window appears.



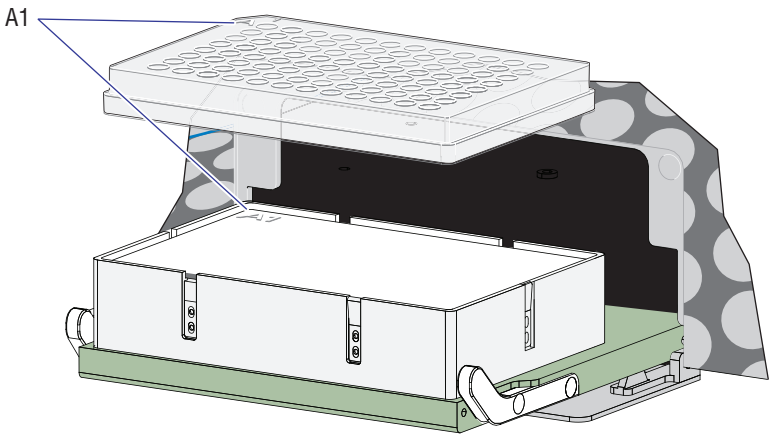
NOTE The 96-well deep well plate is only available for use if the Plate Loader DW is installed.

[Standard 96-Well Plate]: Proceed to Step 4.

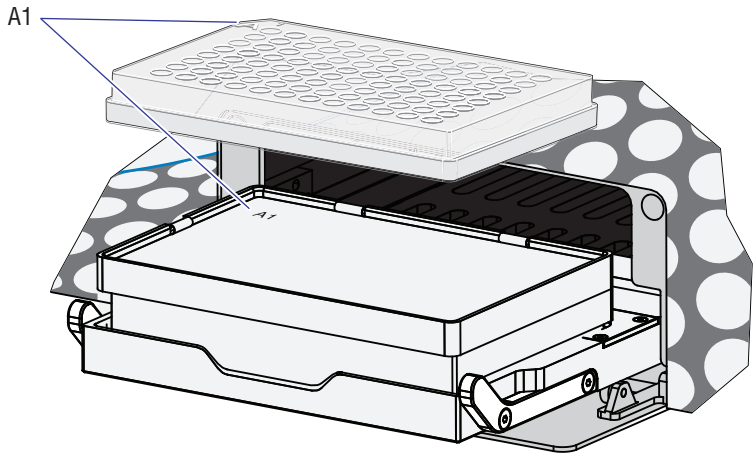
[96-Well Deep Well Plate]: Skip to Step 5.

4 **[Standard 96-Well Plate]:** Select the plate type and place the plate on the plate holder. Skip to Step 11.

[Standard 96-Well Plate in the Plate Holder (Standard Plate Loader -Without groove)]



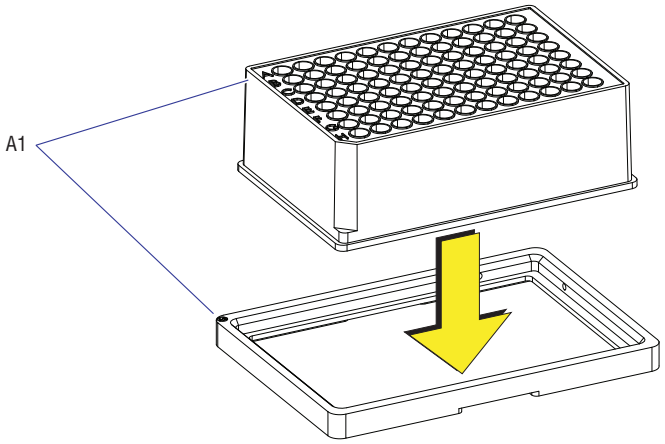
[Standard 96-Well Plate in the Plate Holder (Plate Loader DW -With groove)]



NOTE Ensure plate well A1 aligns with position A1.

5 **[96-Well Deep Well Plate]:** Select the 96-Well Deep Well plate type.

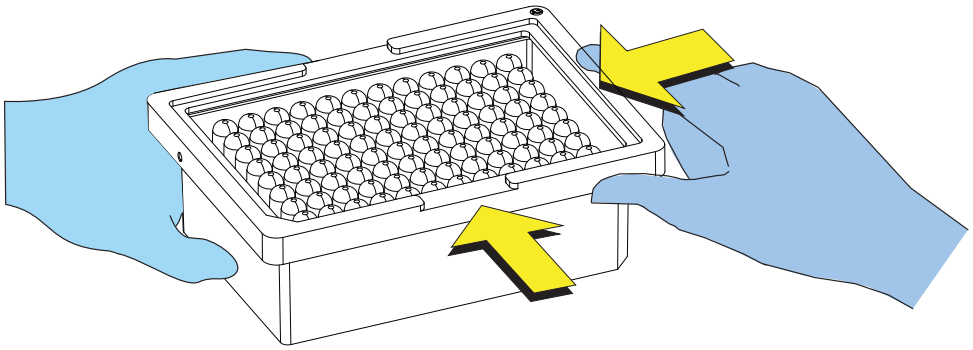
6 **[96-Well Deep Well Plate]:** Place a 96-well deep well plate into the calibration frame.



NOTE Ensure plate well A1 aligns with position A1.

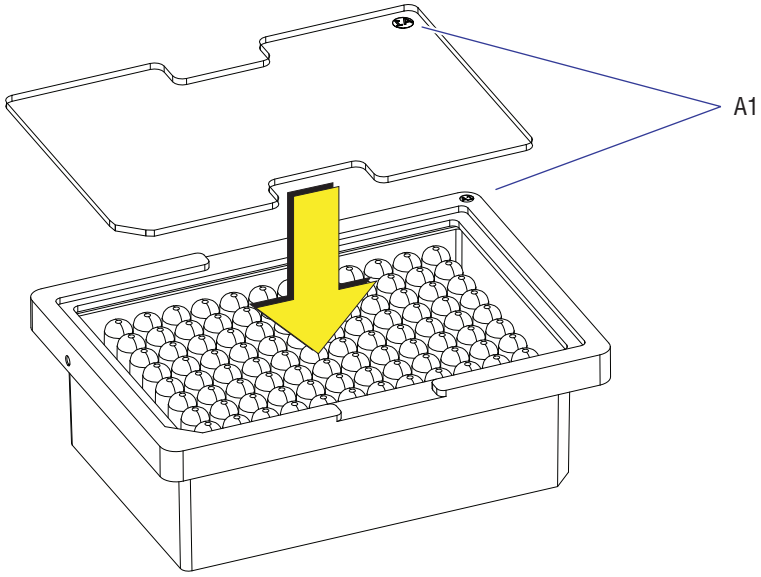
NOTE The calibration frame and the transparent plate are used to assist the calibration in X-axis and Y-axis. They are delivered together with the Plate Loader DW.

7 **[96-Well Deep Well Plate]:** Turn the calibration frame together with the deep well plate upside down. Push the right corner of the frame into place against the lower right corner of the plate.



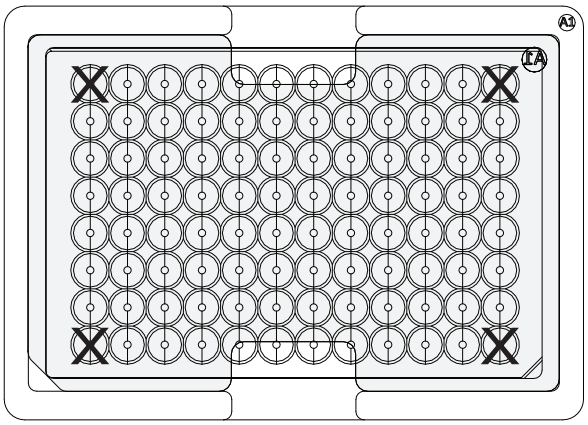
NOTE Ensure the deep well plate is correctly placed into the calibration frame.

8 **[96-Well Deep Well Plate]:** Place the transparent plate on the frame.

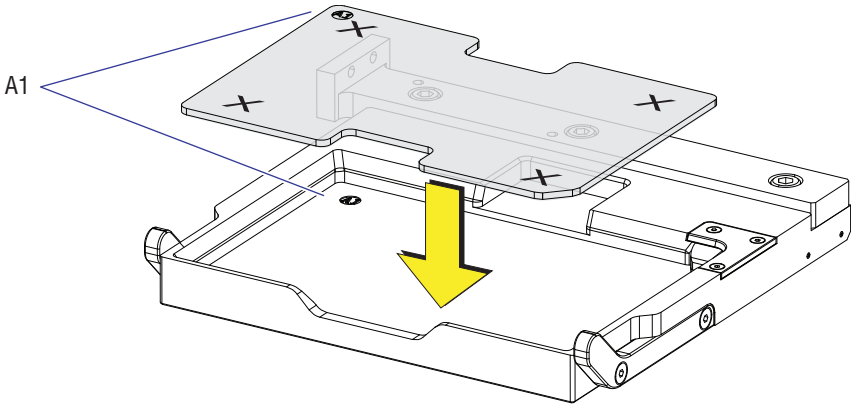


NOTE Ensure the A1 of the transparent plate aligns with position well A1.

9 **[96-Well Deep Well Plate Shown]:** Mark the center position of well A1, A12, H1, H12 on the transparent plate.



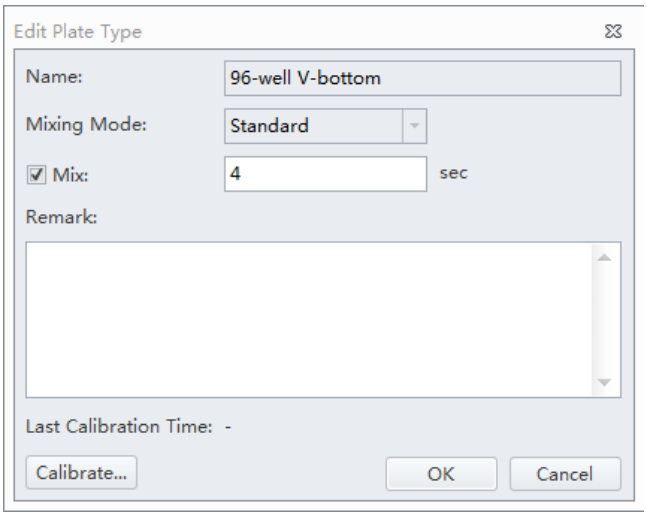
10 **[96-Well Deep Well Plate]:** Place the transparent plate on the plate holder stage.



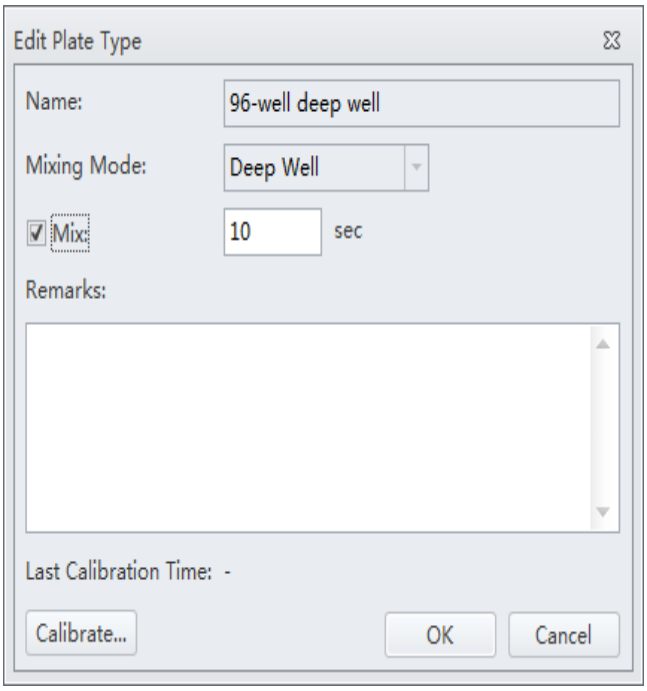
NOTE Ensure plate well A1 aligns with position A1.

11 Select **Edit** to access the calibrate icon. The Edit Plate Type window appears.

[Standard 96-Well Plate]



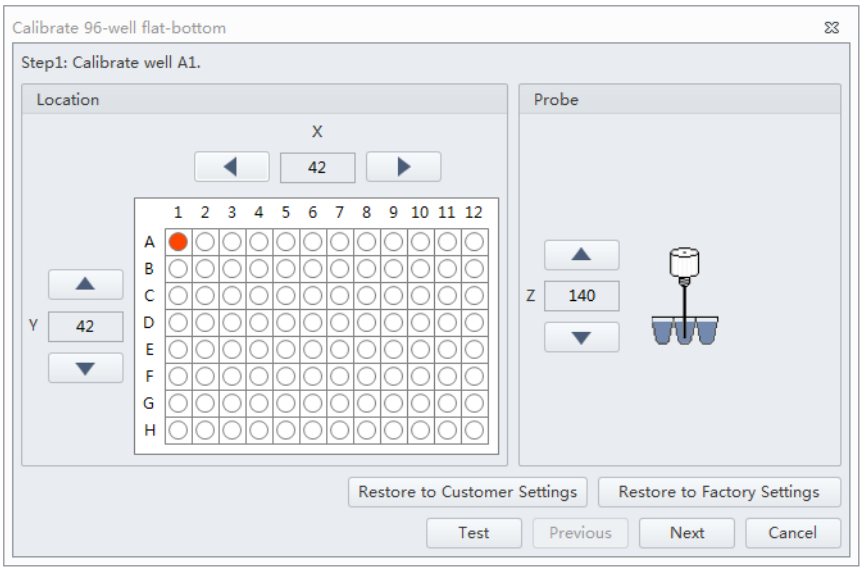
[96-Well Deep Well Plate]



NOTE You can access the calibrate icon by either adding a plate or duplicating a plate. Refer to Plate Type Library in CHAPTER 2, Using the CytExpert Software.

12 Set the Mix settings.

13 Select **Calibrate.** The message *Please confirm that the plate is placed properly and Press Ok.* appears. Select **OK.** The plate loader loads the plate holder stage and the sample probe moves to the sample aspiration position of well A1.



[Standard 96-Well Plate]: Proceed to Step 14.

[96-Well Deep Well Plate]: Skip to Step 15.

⚠ CAUTION

Risk of instrument damage. Do not crash the probe into the bottom of the plate as this will cause irreparable damage to the probe. Move the probe one step at a time when lowering the probe on the Z-axis toward the bottom of the plate. You will hear a click when the probe makes contact with the bottom of the well, this is the extreme position for the Z-axis of the probe. Do not move the probe any lower after you hear the click.

14 **[Standard 96-Well Plate]:** Select ▲ and ▼ or ◀ and ▶ to adjust the sample probe positions in the X-, Y-, and Z-axes.

Ensure the sample probe is centered and touches the bottom of the well.

NOTE The sample probe should just make contact with the bottom of the well.

NOTE The X-axis arrows moves the sample probe well position left and right. The Y-axis arrows moves the sample probe well position forward and back. The Z-axis arrows moves the sample probe up and down.

Skip to Step 16.

15 **[96-Well Deep Well Plate]:** Select ▲ and ▼ or ◀ and ▶ to adjust the sample probe positions in the X-, and Y-axes.

Ensure the sample probe is centered on the mark of transparent plate.

NOTE The X-axis arrows moves the sample probe well position left and right. The Y-axis arrows moves the sample probe well position forward and back. The Z-axis arrows moves the sample probe up and down.

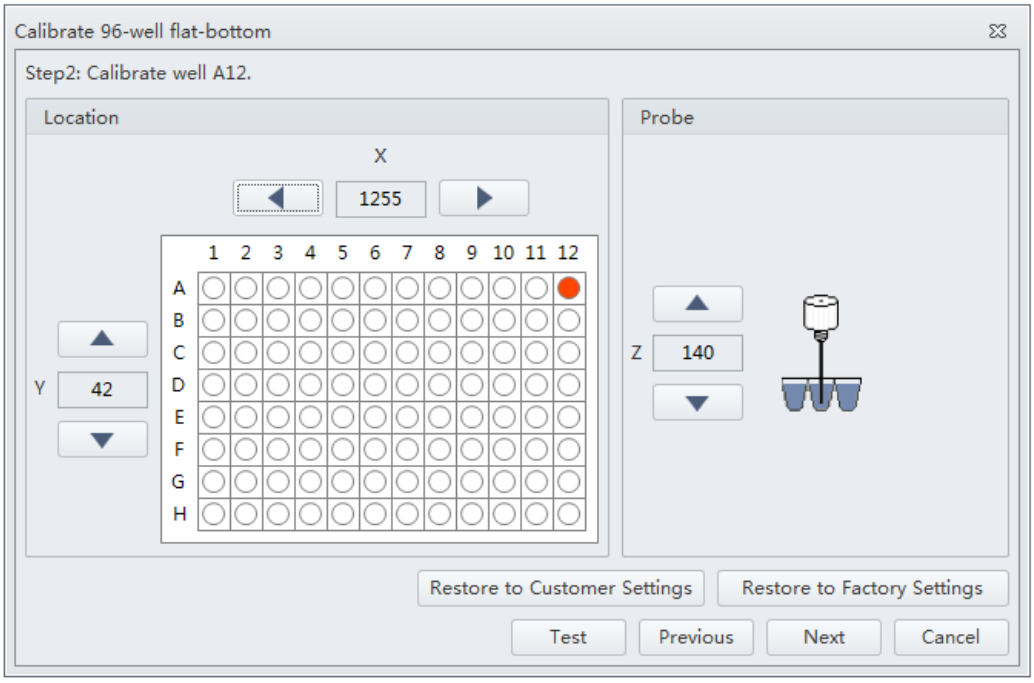
16 Select **Test** to verify the sample probe position.

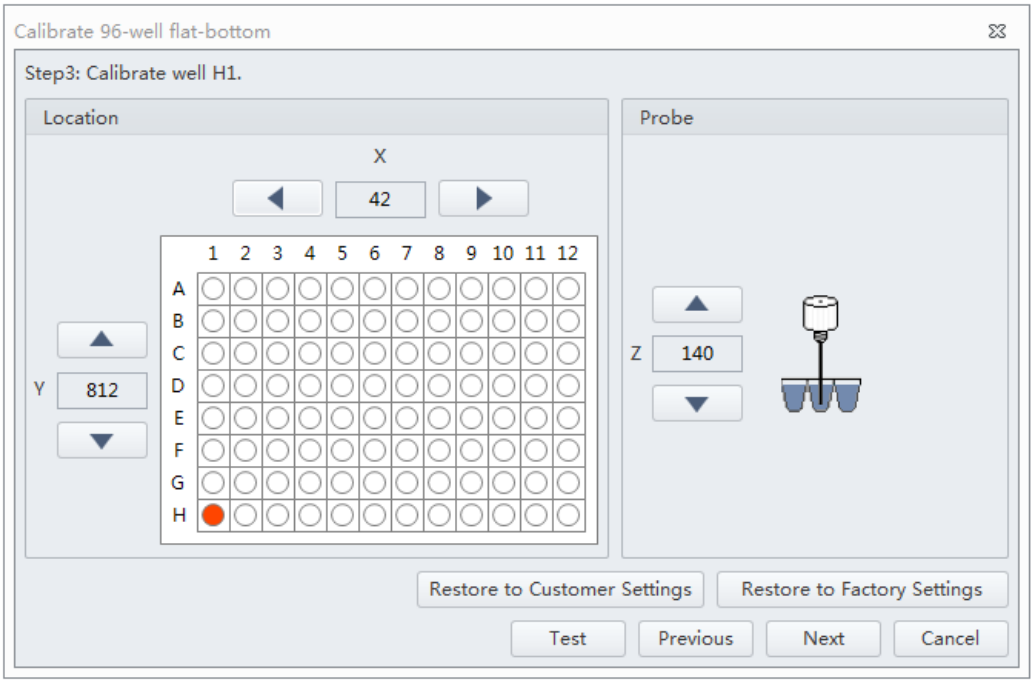
Listen for the click to ensure the probe has made contact with the bottom of the well. Readjust the sample probe position by moving the probe 3 steps up. This is the correct position for the Z-axis of the probe.

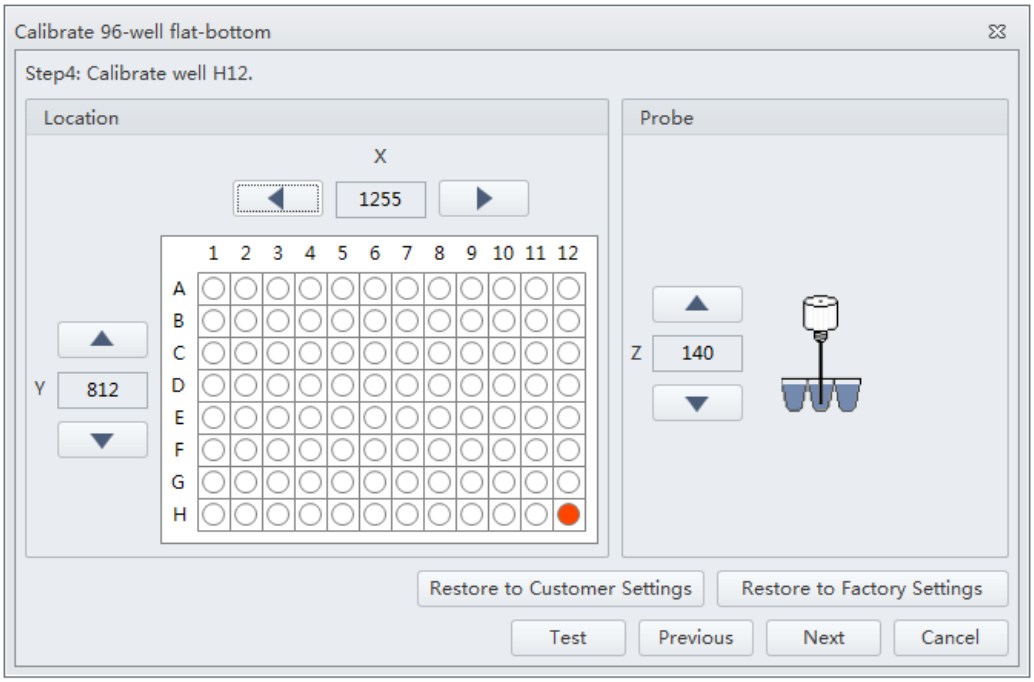
NOTE **[Transparent Plate]** The sample probe cannot come in contact with the surface of the transparent plate in the Z-axis. This is normal. Do not adjust the sample probe positions in the X-axis. The probe position in the Z-axis will be calibrated using a deep well plate.

17 Select **Next** to move to the next well.

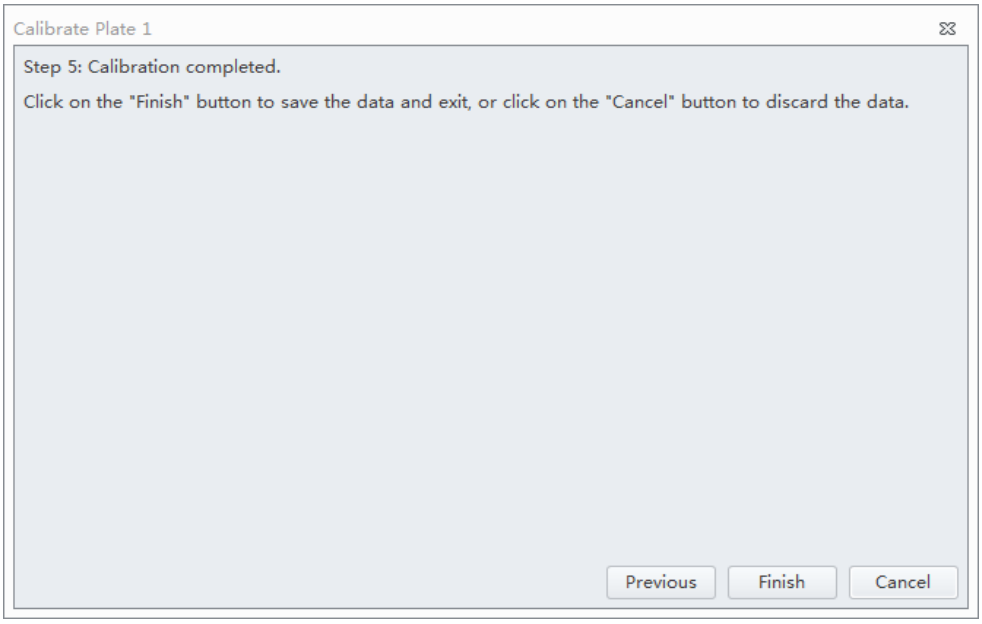
18 Repeat Steps 14-17 for wells A12, H1, and H12.







19 Select **Next**. The calibration completion window appears.



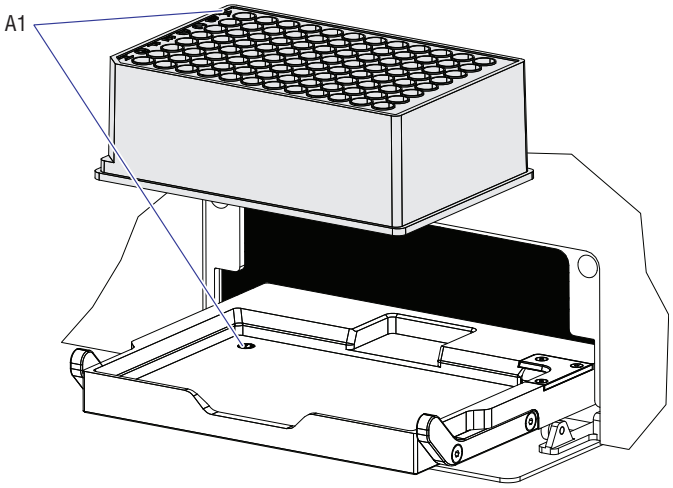
20 **[Standard 96-Well Plate]:** Select **Finish** to save the settings and exit. Skip to Step 30.

[96-Well Deep Well Plate]: Select **Finish** to save the settings and exit. Continue to calibrate the probe position of Z-axis for the deep well plate. Proceed to Step 21.

21 **[96-Well Deep Well Plate]:** Remove the transparent plate from the plate holder stage.

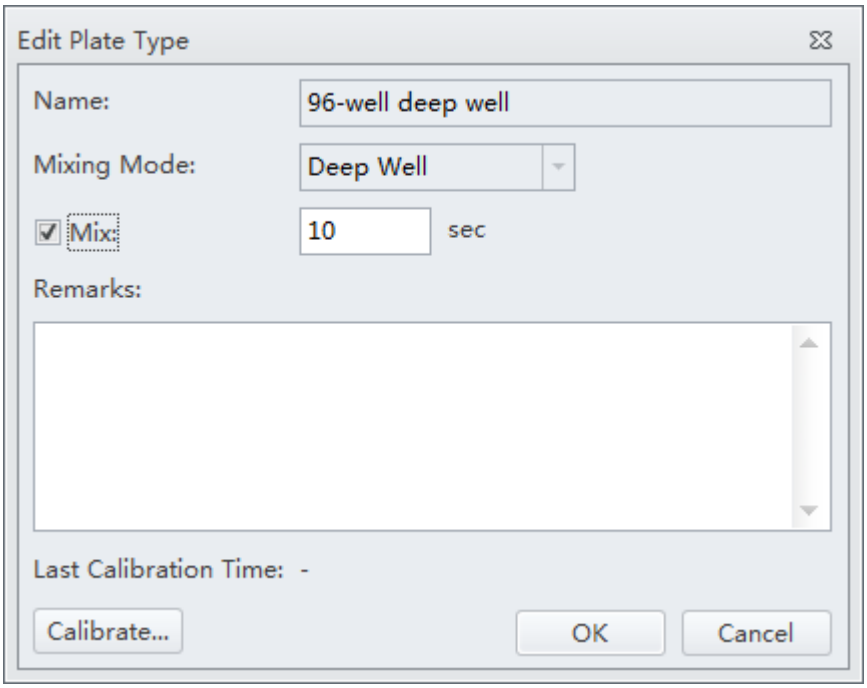
22 **[96-Well Deep Well Plate]:** Select the plate type which has already been calibrated on the X-axis and Y-axis above on the Plate Type Library window, and place the deep well plate on the plate holder stage.

[96-Well Deep Well Plate]

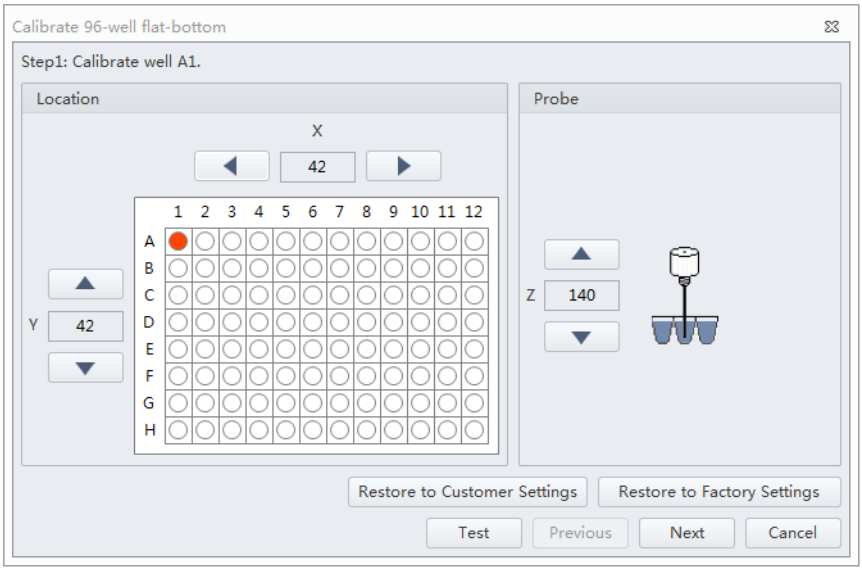


NOTE Ensure plate well A1 aligns with position A1.

23 **[96-Well Deep Well Plate]:** Select **Edit** to access the calibrate icon again. The Edit plate window appears.



24 Select **Calibrate.** The message *Please confirm that the plate is placed properly and Press Ok.* appears. Select **OK.** The plate loader loads the plate holder stage and the sample probe moves to the sample aspiration position of well A1.



⚠ CAUTION

Risk of instrument damage. Do not crash the probe into the bottom of the plate as this will cause irreparable damage to the probe. Move the probe one step at a time when lowering the probe on the Z-axis toward the bottom of the plate. You will hear a click when the probe makes contact with the bottom of the well, this is the extreme position for the Z-axis of the probe. Do not move the probe any lower after you hear the click.

25 **[96-well Deep Well Plate]:** Select ▲ and ▼ to adjust the sample probe positions in the Z-axis.

Ensure the sample probe is centered and touches the bottom of the well.

NOTE The sample probe should just make contact with the bottom of the well.

NOTE The probe positions of X-axis and Y-axis have already been calibrated on the transparent plate. Do not continue to adjust the sample probe positions in X-axis and Y-axis.

26 **[Deep-96 Well Plate]:** Select **Test** to verify the sample probe position.

Listen for the click to ensure the probe has made contact with the bottom of the well. Readjust the sample probe position by moving the probe 3 steps upper. This is the correct position for the Z-axis of the probe.

27 **[96-Well Deep Well Plate]:** Select **Next** to move to the next well.

28 **[96-Well Deep Well Plate]:** Repeat Steps 25-27 for wells A12, H1, and H12.

29 **[96-Well Deep Well Plate]:** Select **Finish** to save the settings and exit.

30 Reinstall the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 11, Replacement/Adjustment Procedures.

APPENDIX A

Instrument Installation

Overview

[CytoFLEX]: Your instrument may have been shipped directly to your laboratory, in which case you will need to set up and connect the Cytometer and the Workstation. Refer to this chapter for the instrument installation procedure.

Validate the environment	→	Open the package	→	Install the instrument	→	Prepare to boot

[CytoFLEX LX]: The CytoFLEX LX is installed by your Beckman Coulter Service Representative. Do not open the box or crate. Wait for a qualified Beckman Coulter Service Representative.

This chapter contains information on:

Instrument Transportation and Storage

Refer to Preparing the Instrument for Transport or Storage in CHAPTER 10, Cleaning Procedures, prior to transportation or storage.

Attention to the following items is required when transporting or storing the instrument:

- Take caution to protect the instrument from exposure to rain or sunlight.
- Always place the instrument on a flat, stable surface, and take note of the symbol for *this side up*.
- Temperature range: see Temperature and Humidity.
- To prevent extrusion, the load on top cannot exceed 100 kg.
- CytoFLEX Cytometer net weight 23 kg, gross weight 27 kg; transport the instrument using only appropriate equipment so as to guard against personal injury.
- CytoFLEX LX Cytometer net weight 23 kg, gross weight 27 kg; transport the instrument using only appropriate equipment so as to guard against personal injury.

Installation Environment Validation

IMPORTANT This instrument is intended for indoor use only.

Verify whether the installation environment satisfies the following requirements:

Worktable

⚠ CAUTION

Risk of instrument damage. Place the instrument on a level surface. Failing to do so places the system is in danger of toppling and can result in damage. Take all necessary precautions throughout the process of storing or transporting the instrument.

- The tabletop must be smooth and level.
- Minimum tabletop load bearing capacity [CytoFLEX]: 50 kg
- Minimum tabletop load bearing capacity [CytoFLEX LX]: 100 kg.
- The tabletop must not vibrate or shake.
- Minimum tabletop dimensions [CytoFLEX]: 120 cm x 80 cm; minimum vertical space above tabletop: 80 cm
- Minimum tabletop dimensions [CytoFLEX LX]: 200 cm x 80 cm; minimum vertical space above tabletop: 100 cm
- Position the instrument in such a manner that it will facilitate disconnection of the power cable at the instrument end.

Ventilation and Cleaning

IMPORTANT If necessary, use ventilation equipment, but airflow must not be allowed to blow directly on the system, as it can affect the reliability of the data.

- Ensure that the working environment is well ventilated for proper heat dissipation.
- Maintain a clearance of at least 20 cm from the back of the instrument for heat dissipation.
- Keep the environment as dust free as possible.
- Avoid direct exposure to sunlight.
- Avoid placing near heat sources or exposing to drafts.
- Avoid corrosives or flammable gases.

Power Source

DANGER

Risk of electric shock and/or instrument damage. Ensure that the power source is properly grounded. Improper grounding can cause electric shock and damage the system. Verify that the output voltage of the power outlet conforms to the system requirements and that a 5 A, time delay, T 5 AL 250 VAC fuse is installed. To prevent personal injury, Beckman Coulter recommends using a power source designed to protect against electrical shock.

CAUTION

Possible instrument damage could occur if you use an extension cord or a power strip to connect the Cytometer. Always plug the Cytometer into a dedicated outlet with an isolated ground.

The power source requirements are as follows:

- This instrument has been tested to and meets all applicable requirements for CE Marking.
- This instrument complies with the emission and immunity requirements described in IEC 61326-1.
- This equipment has been designated and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case, you may need to take measures to mitigate the interference.
- It is advised that the electromagnetic environment should be evaluated prior to operation of the device.
- Do not use this device in close proximity to sources of strong electromagnetic radiation (unshielded intentional RF sources), as these may interfere with the proper operation.
- 100-240 volts, 50/60 Hz, 3-wire power cable, well grounded.
- Amperage not less than 10 A.
- The system requires a well-grounded power outlet (150 VA normal, 250 VA max) to provide the necessary power.
- Distance from system to socket less than 1.5 m.

Power consumption of the Plate Loader is <30 W.

Temperature and Humidity

⚠ CAUTION

Risk of instrument damage and/or erroneous results. To ensure reliability, the system must be operated in the specified environment, within the required temperature and humidity ranges. If the ambient temperature or humidity level falls outside the ranges mentioned above, use appropriate air conditioning.

- **CytoFLEX:** Ambient temperature: 15-27°C with fluctuations of no more than <±2°C per hour.
- **CytoFLEX LX:** Ambient temperature: 15-30°C with fluctuations of no more than <±2°C per hour.
- Relative humidity: 15% RH-80% RH, non-condensing.

Waste Disposal

⚠ 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 might 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.

The waste line from the Cytometer is connected to a waste container/cubitainer. Dispose of the system's waste in accordance with your local regulations and acceptable laboratory procedures.

The waste line supplied with the instrument can be connected to an open drain. If you use an open drain, mechanically secure the waste tube into the drain so the tube cannot accidentally come out of the drain. This prevents spillage.

Unpacking the Instrument and Inspecting the Materials for Defects or Omissions [CytoFLEX]

Take care to store the instrument in a suitable environment where it can remain in the proper position.

Check that the following components on the packing list are present:

- Cytometer
- Cables
- Computer

- Mouse
- Keyboard
- Monitor
- Fluid Container holder [CytoFLEX]
- Sheath fluid container
- Waste container
- Sheath fluid tubing
- Waste tubing
- USB configuration key
- Software USB

Installing the Instrument and Connecting the Equipment [CytoFLEX]

IMPORTANT Use the appropriate power cable plug for your geographic region.

⚠ CAUTION

Risk of erroneous results. Place the Fluid Containers and the instrument on the same, level surface. An excessive difference in height can alter the flow velocity.

⚠ CAUTION

Possible instrument damage could occur if you use an extension cord or a power strip to connect the Cytometer. Always plug the Cytometer into a dedicated outlet with an isolated ground.

---

1 Remove the Cytometer, the Fluid Containers, the accompanying holder, the computer, the monitor, and the keyboard and mouse from the each respective box placing them flat on the instrument worktable.

NOTE The Fluid Container holder must be on the same plane as the Cytometer.

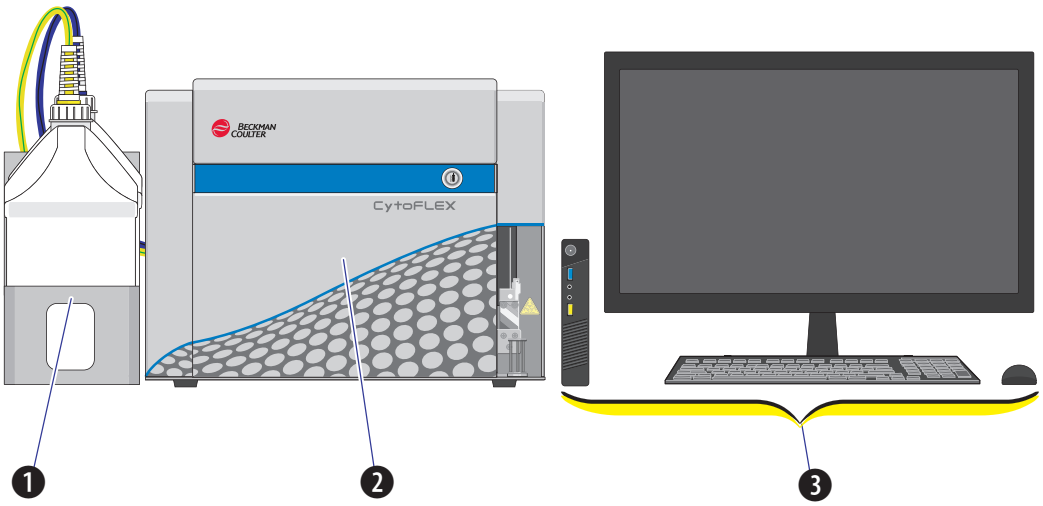
Reach under the base of the instrument to lift the Cytometer out of the package. Beckman Coulter recommends that two people lift the Cytometer out together.

---

2 Ensure a minimum clearance of 20 cm on both sides and to the back of the Cytometer to maintain enough room to access the on/off controls for the Cytometer devices.

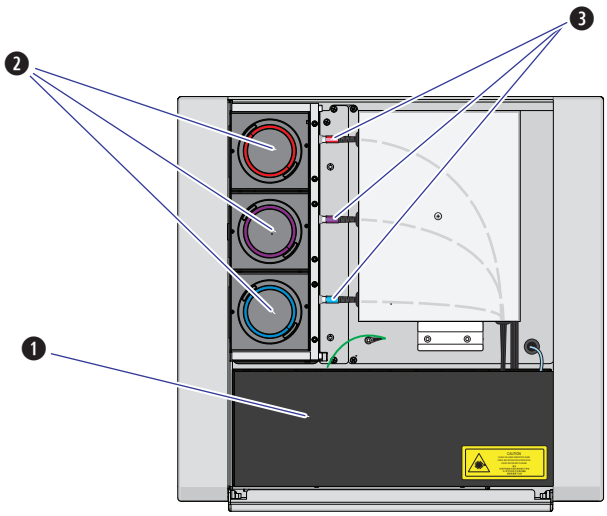
3 After removing the monitor and attaching the base, place them on the worktable with the computer.

[CytoFLEX Without Plate Loader Shown]

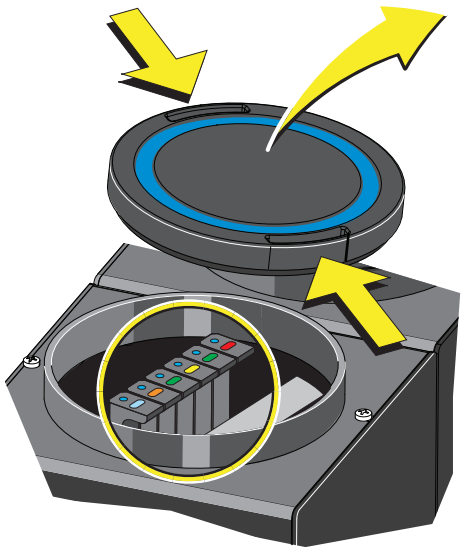


1. **Fluid Containers.** Place on the left side of the Cytometer.
2. **Cytometer.** Place between the Fluid Containers and the Workstation.
3. **Workstation.** Place on the right side of the Cytometer.

4 Open the top cover of the Cytometer. Check inside to verify that the optical bench cover **(1)** is tightly closed and that the optical fibers **(3)** and WDMs **(2)** are securely connected.

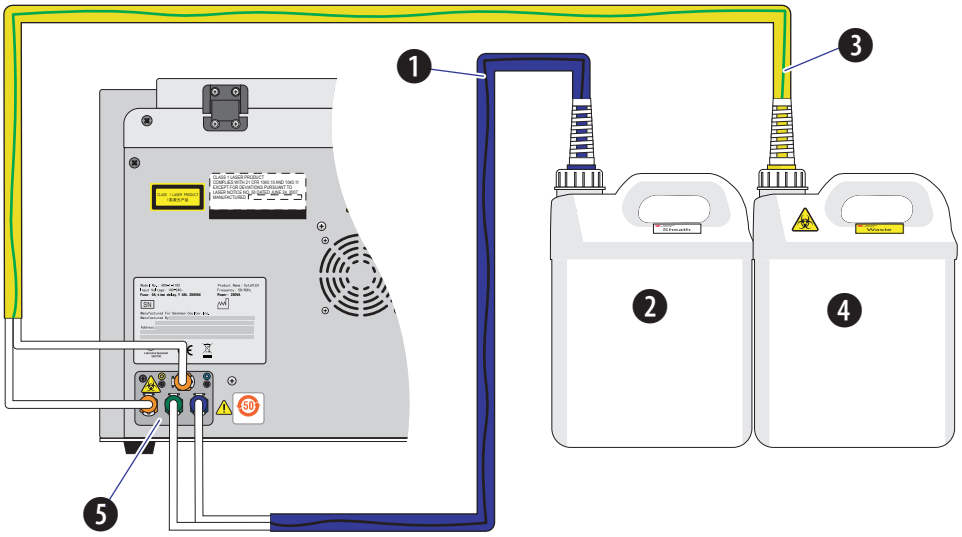


5 Remove any shipping tape and open the cap of each WDM. Verify that the light filters inside are in place.

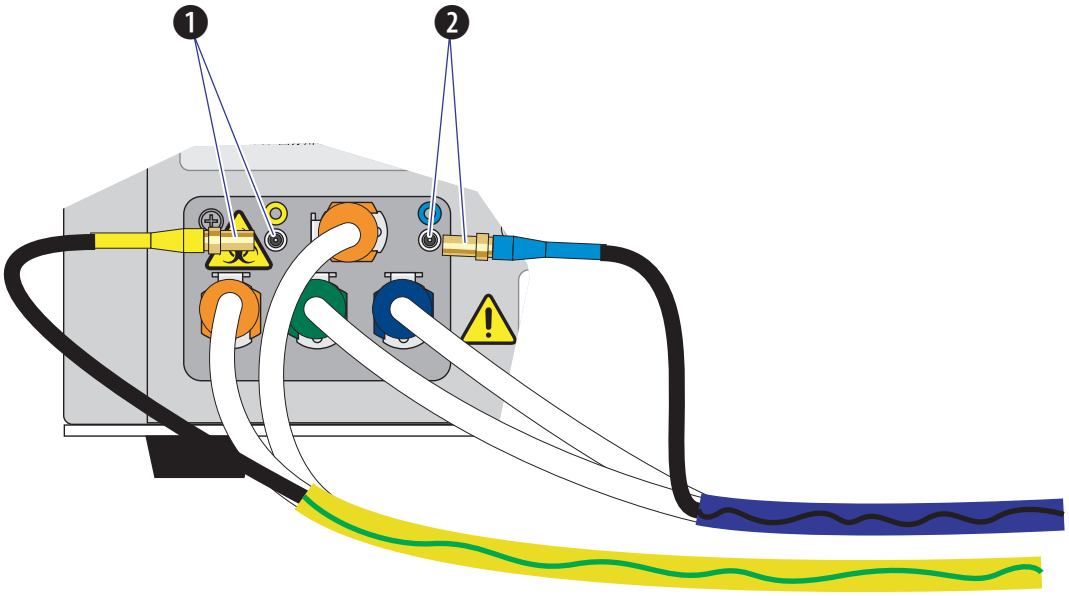


6 Insert sheath and waste pickup tubing into the appropriate container.

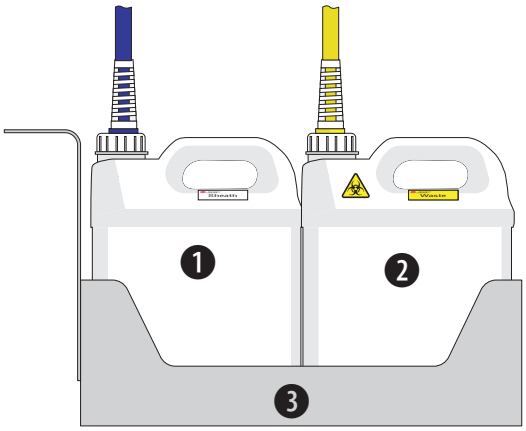
7 Connect the blue harness **(1)** from the sheath fluid container **(2)** and the yellow harness **(3)** from the waste container **(4)** to the fluid connector panel **(5)** on the back right corner of the Cytometer according to the color code.



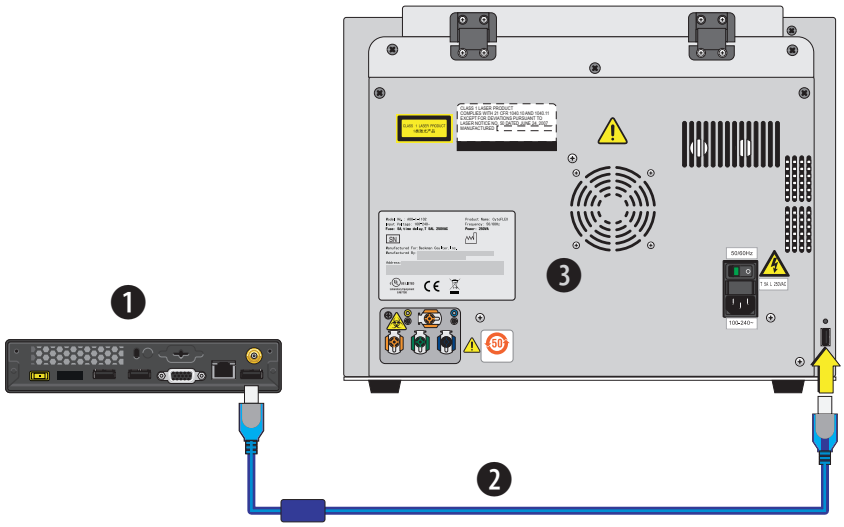
8 Connect the waste **(1)** and sheath **(2)** level sensors.



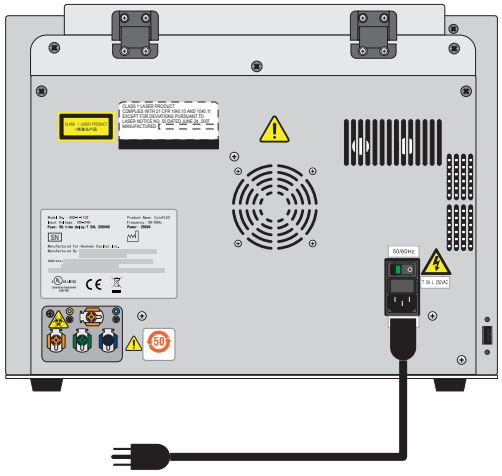
9 Place the sheath fluid container **(1)** and the waste container **(2)** in the Fluid Container holder **(3)**.



10 Set up the supplied computer **(1)** and connect the USB cable **(2)** from the back of the Cytometer **(3)** to a USB port on the back of the computer.



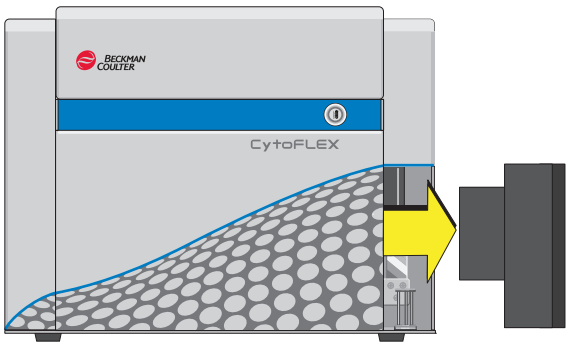
11 Plug the Cytometer power cable in to the back of the Cytometer.



12 Connect the computer keyboard, mouse, and monitor.

13 Remove the foam support holding the sample loader in the front-right corner of the instrument.

[CytoFLEX Without Plate Loader Shown]



14 Add Deep Clean solution to the Deep Clean solution bottle. Refer to Adding the Deep Clean Solution in CHAPTER 11, Replacement/Adjustment Procedures.

15 Clean the sheath fluid container. Refer to Cleaning the 4 L Sheath Fluid Container in CHAPTER 10, Cleaning Procedures.

16 Fill the Sheath fluid container. Refer to Filling the 4 L Sheath Fluid Container [CytoFLEX] 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.

17 Add 400 mL of 5 to 6% bleach to the waste container.

18 Install the CytExpert software. Refer to Installing the Software [CytoFLEX].

19 Turn on the instrument. Refer to Turning On the Instrument in CHAPTER 3, Daily Startup.

20 Open the CytExpert software. Refer to Logging Into the Software in CHAPTER 3, Daily Startup.

21 Run the System Startup Program. Refer to Running the System Startup Program [with the Single Tube Loader] in CHAPTER 3, Daily Startup.

22 Prime the instrument three times.

23 Prepare a QC sample. Refer to Preparing the QC Sample in CHAPTER 4, Instrument Quality Control and Standardization.

24 Import the lot-specific target values files. Refer to Importing Lot-Specific Target Values in CHAPTER 4, Instrument Quality Control and Standardization.

25 Perform a QC to establish the target gain values for your instrument. Refer to Collecting QC Data in CHAPTER 4, Instrument Quality Control and Standardization.

26 If QC fails, the following software message appears. Select **Yes**.

NOTE Target gain values must be established upon installation. QC could fail up to three times upon installation until target gain values are established.

27 Repeat Steps 23-26 until the target gain values are established and QC has passed.

NOTE If QC fails more than three times, contact us.

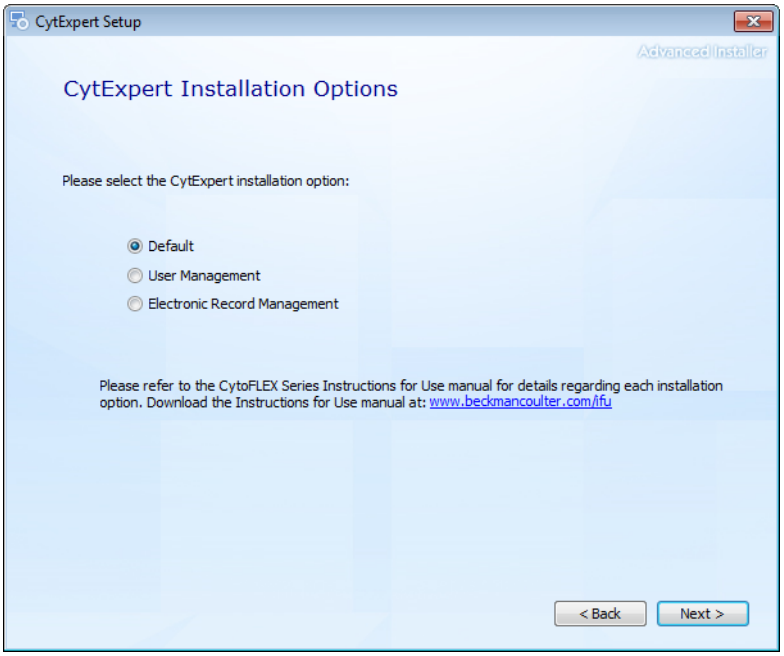
28 Within five business days, activate your Warranty by contacting us and providing them with your latest QC run results.

Installing the Instrument and Connecting the Equipment [CytoFLEX LX]

The CytoFLEX LX is installed by Beckman Coulter service.

CytExpert Software Installation Options

CytExpert software version 2.0 and higher has three installation options upon install.



- **CytExpert Default software option.** User Login is not required to run the system.
- **CytExpert User Management software option.** User Login is required to run the system. Contains features and functionality that facilitates user and role management.
- **CytExpert Electronic Record Management software option.** User Login is required to run the system. Contains features and functionality that facilitates compliance with 21 CFR Part 11 guidelines for Electronic Records and Signatures.

Installing the Software [CytoFLEX]

The installation process workflow is as follows:

Install the CytExpert software	→	Install instrument configuration files	→	Start the software

The CytExpert software can be installed on any computer that meets the minimum specifications (see Instrument Specifications in CHAPTER 1, System Overview) for analysis-only use.

Required Materials

The following materials are required to install the CytExpert software:

- CytoFLEX Series flow cytometer.
- CytoFLEX Series Workstation.
- CytExpert software installation USB.
- Authorized Beckman Coulter USB configuration key.

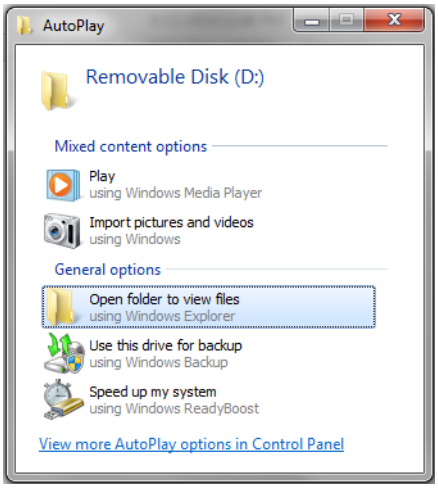
Installing the CytExpert Software

IMPORTANT Follow this procedure when installing the CytExpert software for the first time.

---

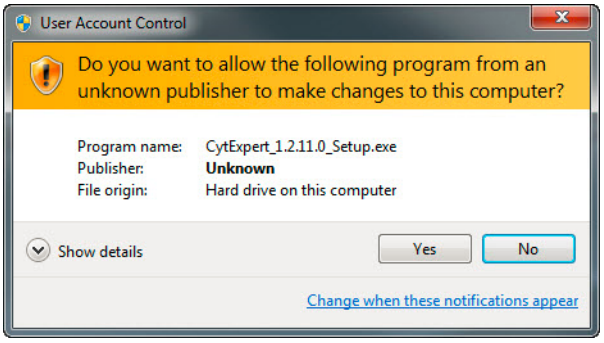
1 Insert the software USB into the computer.

NOTE If the Autoplay window appears, select Open folder to view files.

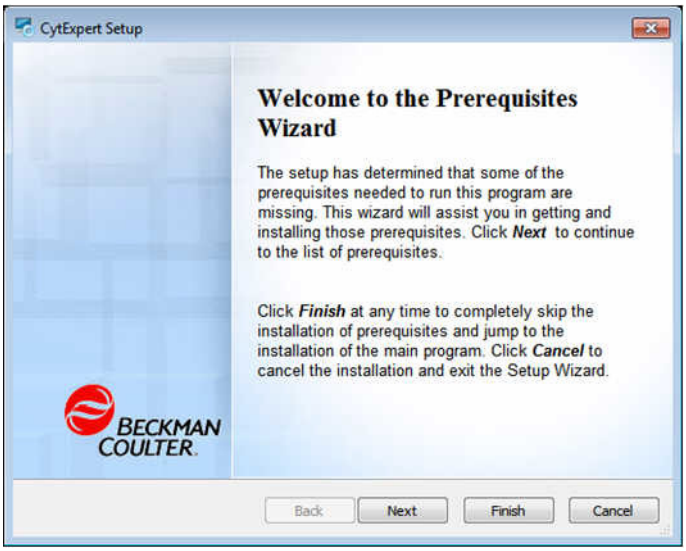


---

2 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.

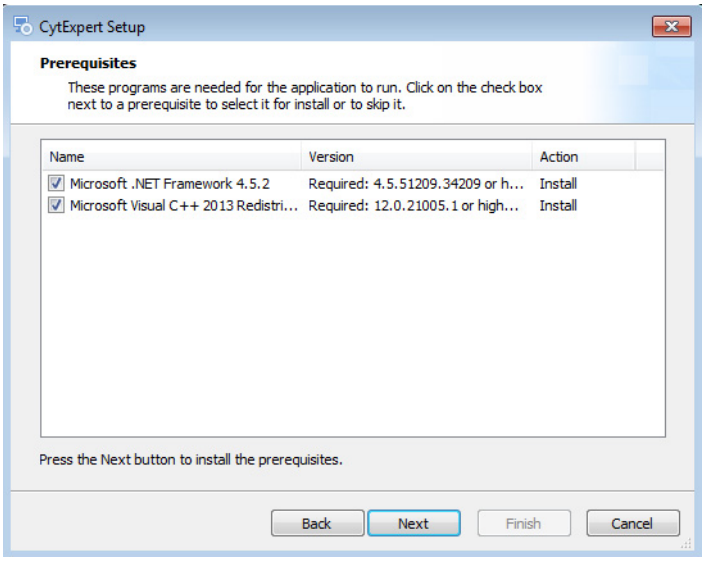


3 Select **Yes**. The CytExpert Setup Welcome window appears.

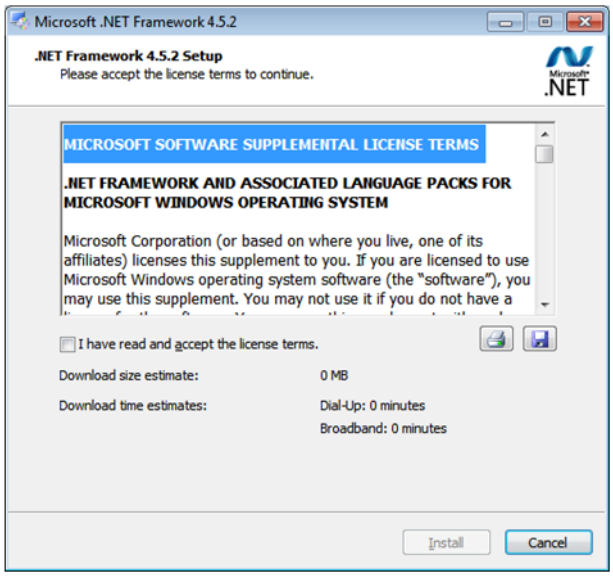


4 Select **Next**.

5 Select both support program checkboxes in the CytExpert Setup Prerequisites window.

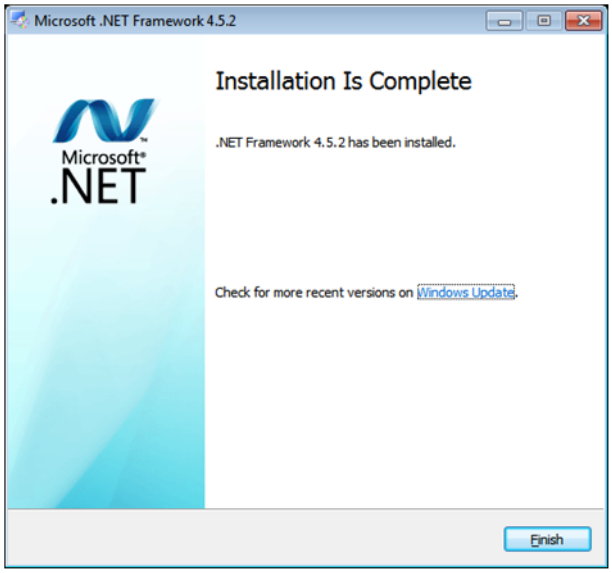


6 Select **Next**. The Microsoft .NET Framework window appears.



7 Select the *I have read and accept the license terms* checkbox.

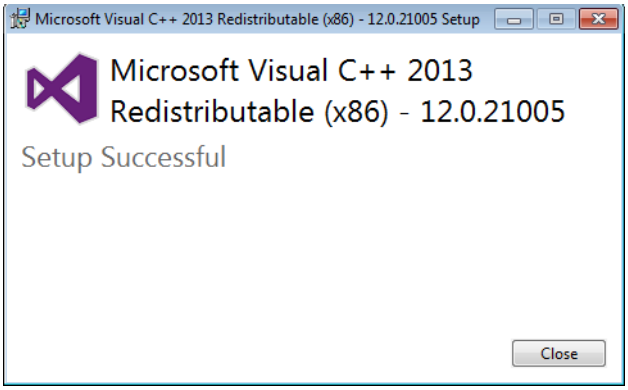
8 Select **Install**. The Installation is Complete window appears when installation has finished.



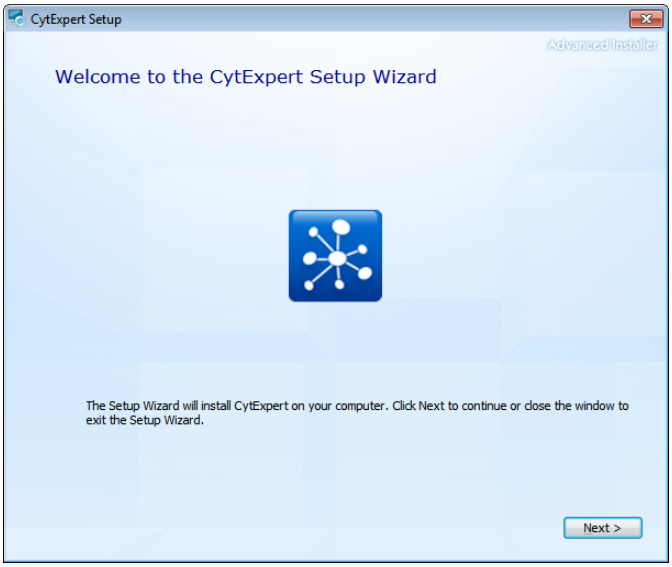
9 Select **Finish**. The Microsoft Visual C++ 2010 Redistributable Setup window appears.

10 Select the *I have read and accept the license terms* checkbox.

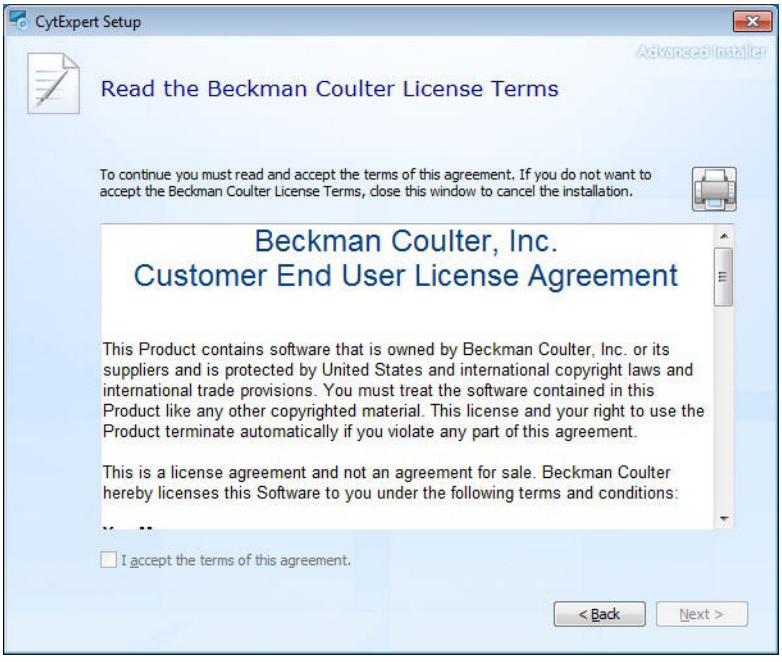
11 Select **Install**. The Setup Successful window appears when installation has finished.



12 Select **Close**. The Welcome to the CytExpert Setup Wizard window appears.



13 Select **Next**. The Beckman Coulter License Terms window appears.

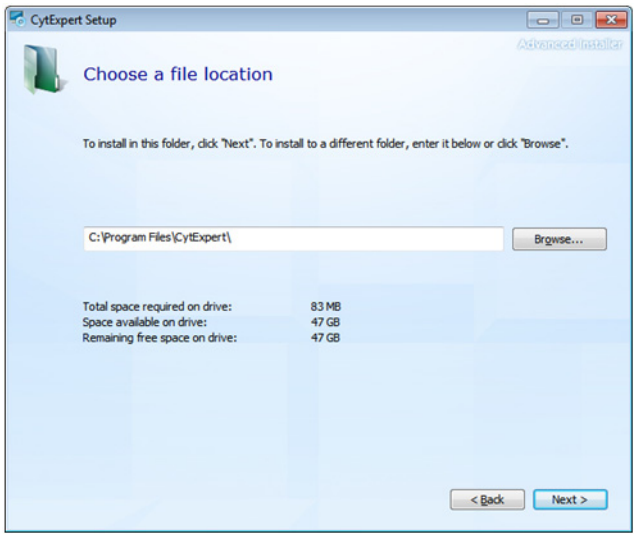


14 Read the Beckman Coulter Customer End User License Agreement.

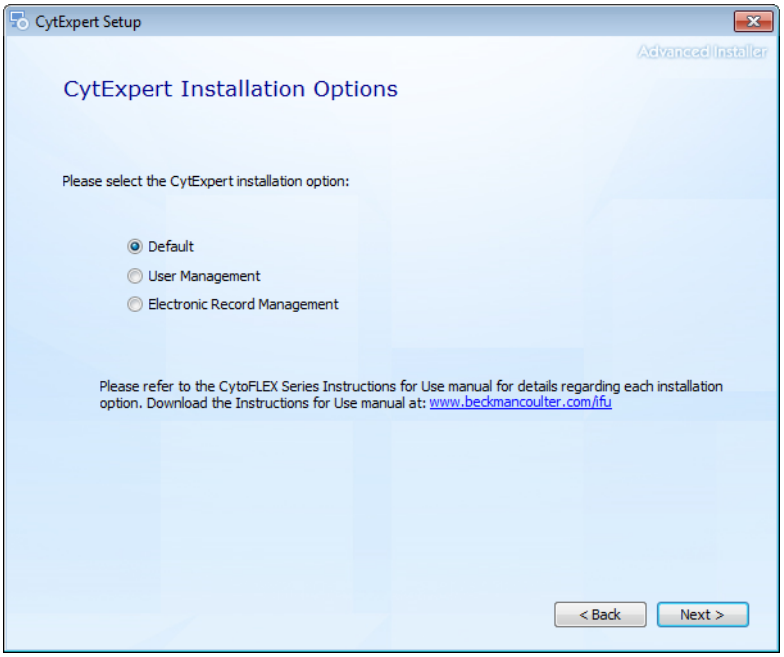
15 Select the *I accept the terms of this agreement* checkbox.

NOTE The checkbox is not selectable until you scroll all the way to the end of the agreement.

16 Select **Next**. The Choose a file location window appears.

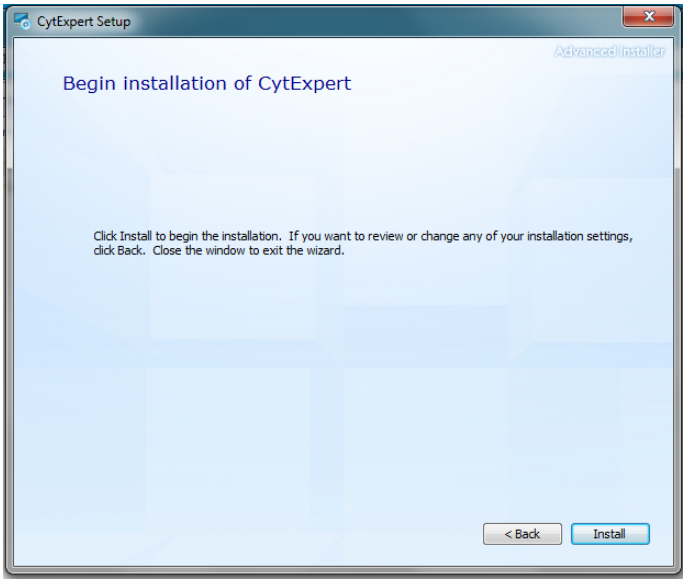


17 Select **Next**. The Installation Options window appears.

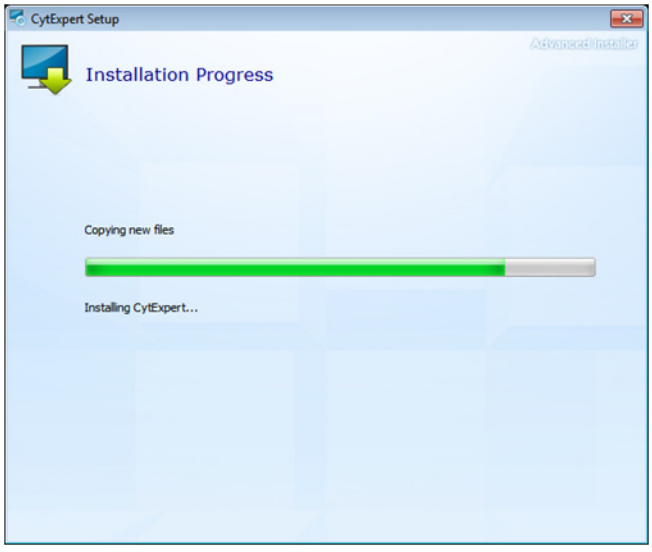


18 Select the desired installation option. Refer to CytExpert Software Installation Options.

19 Select **Next**. The Begin installation of CytExpert window appears.

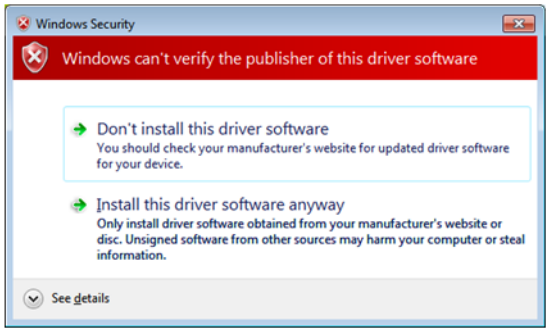


20 Select **Install** to begin installing the software. The Installation Progress window appears.

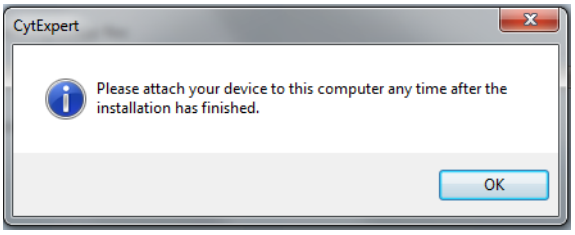


NOTE The software will install into the default file path provided unless otherwise specified.

21 If a Windows Security window appears. Select *Install this driver software anyway* to install the USB drive.

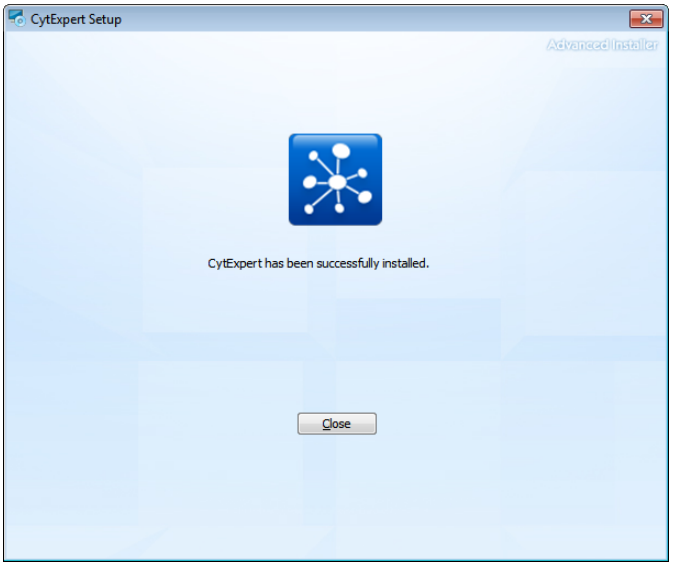


22 The following software prompt appears. Select **OK**.



NOTE The term “device” in this message refers to the cytometer.

23 Wait for the software to finish installing. The install complete window appears.



24 Select **Close** to finish the CytExpert software installation.

25 Install the instrument configuration file. Refer to Installing the Instrument Configuration File.

Installing the Instrument Configuration File

⚠ CAUTION

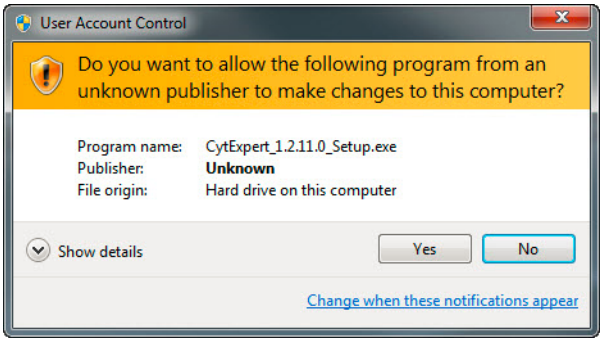
Risk of erroneous results or instrument damage. Only install the configuration file that matches your instrument. Installing an incorrect configuration file could cause erroneous results or instrument damage.

Use this procedure to install the configuration settings for the instrument. If the CytExpert software will not be connected to a Cytometer, this step can be skipped.

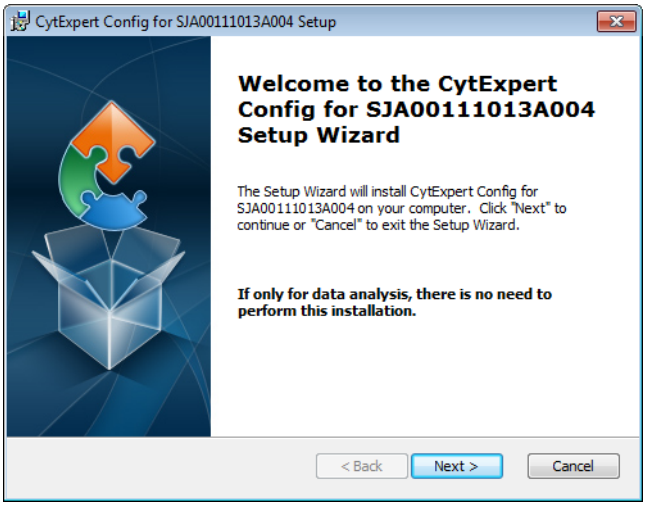
IMPORTANT You must install the CytExpert software before installing the instrument configuration file. Refer to Installing the Software [CytoFLEX].

1 Select and run **CytExpert_X.X_Config_Setup_XXXX.exe**. The User Account Control window appears.

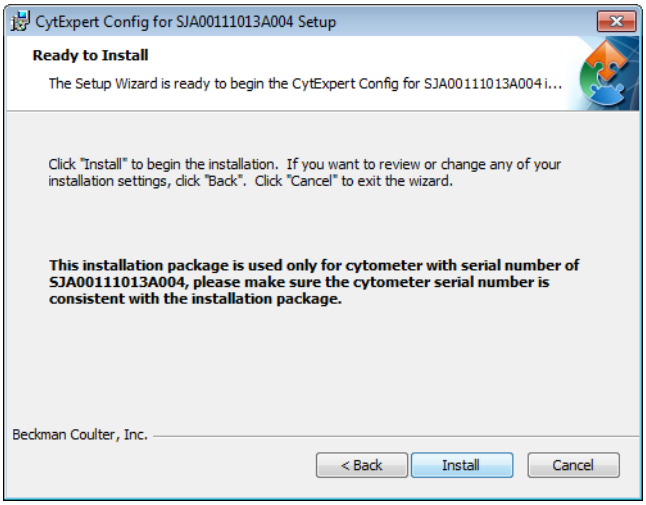
NOTE XXXX refers to the serial number of the instrument.



2 Select **Yes**. The CytExpert Config Welcome window appears.

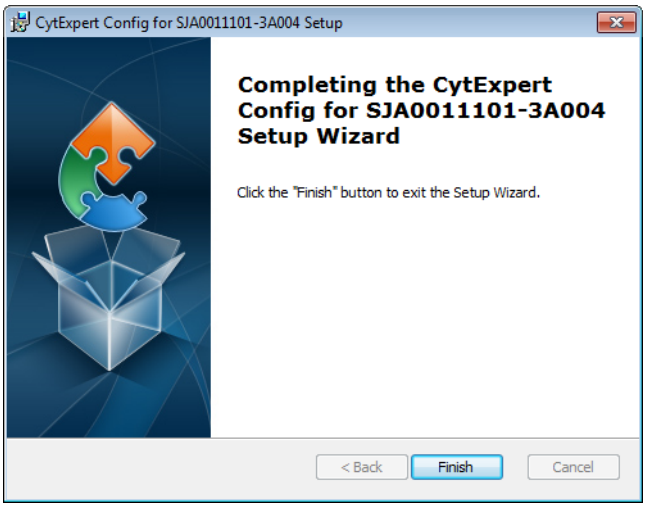


3 Select **Next**. The CytExpert Config Ready to Install window appears.



4 Verify that the serial name displayed at the top of the window is correct.

5 Select **Install**. When the installation has finished, the Completing the CytExpert Config Setup Wizard window appears.



6 Select **Finish**.

7 If you get the following message, select **OK**. When you launch CytExpert, the system will automatically upgrade your configuration file. Proceed to Step 8.

Starting the Software

IMPORTANT The default username is *admin*. The default password is *password*.

1 Insert the USB configuration key into the USB port of the computer.

2 Start the software. Refer to Logging Into the Software in CHAPTER 3, Daily Startup, for detailed instructions on opening the software and confirming the connection status.

NOTE If the software shows *Connected*, data collection and analysis can be completed.

Upgrading the CytExpert Software

Use this procedure to upgrade to software version 2.0 or higher from any software version prior to software version 2.0.

If you only need to upgrade to the CytExpert Default software option, you should follow the procedure below.

If you need to install either the CytExpert User Management software option or the CytExpert Electronic Record Management software option, you should first follow the procedure below then follow the reinstallation procedure. Refer to Reinstalling the CytExpert Software.

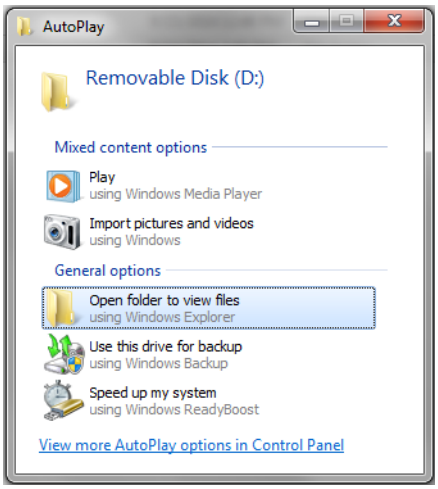
Refer to CytExpert Software Installation Options for the differences between each software option available.

⚠ CAUTION

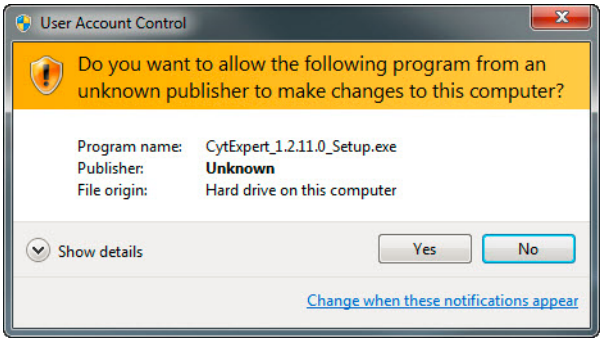
Risk of data loss. Reinstalling the CytExpert Software could overwrite your database. Ensure you backup your database prior to software reinstallation.

1 Insert the software USB into the computer.

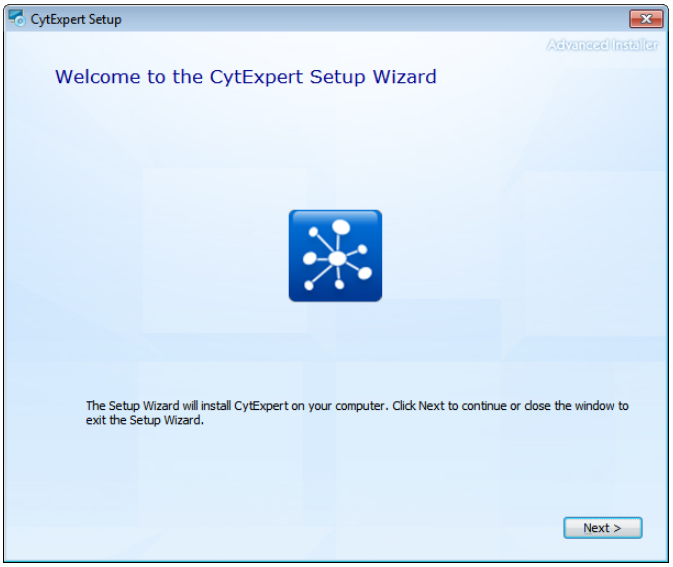
NOTE If the Autoplay window appears, select Open folder to view files.



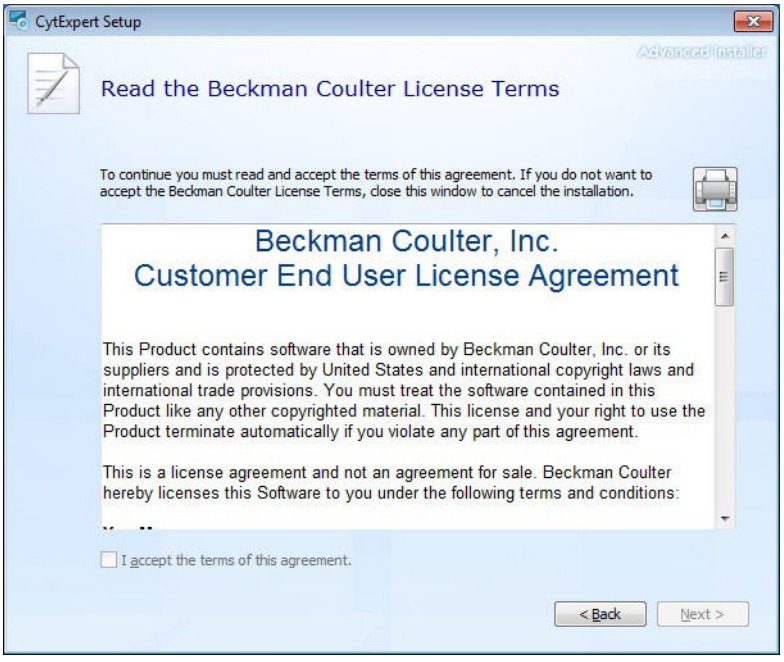
2 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.



3 Select **Yes**. The Welcome to CytExpert Setup Wizard window appears.



4 Select **Next**. The Beckman Coulter License Terms window appears.

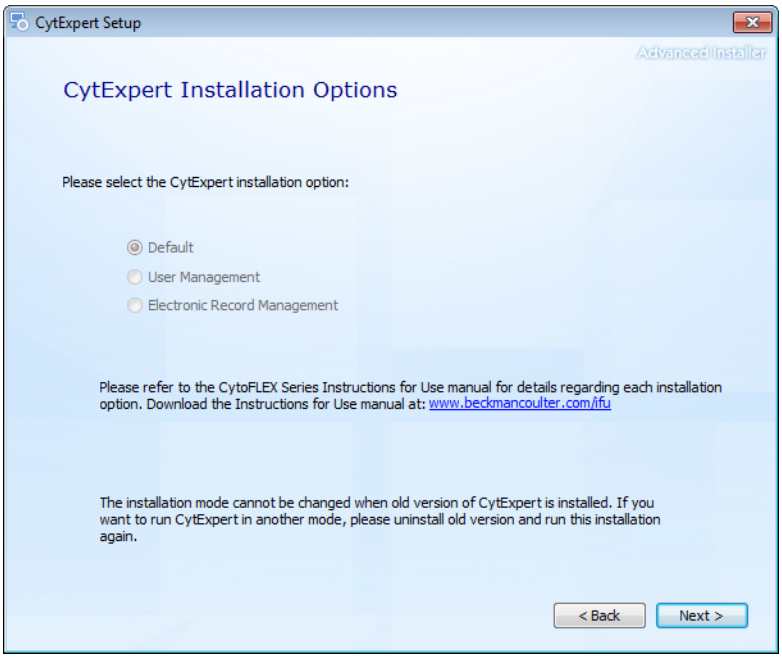


5 Read the Beckman Coulter Customer End User License Agreement.

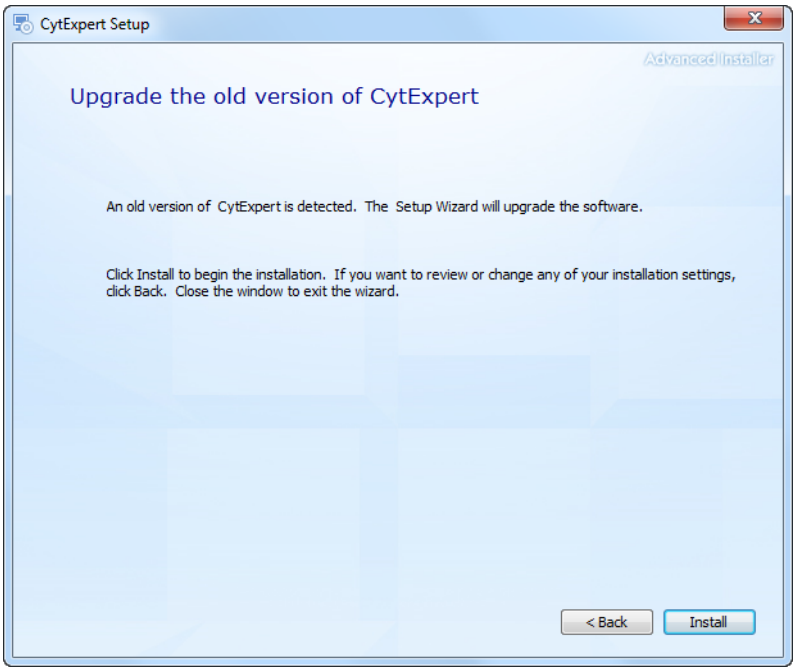
6 Select the *I accept the terms of this agreement* checkbox.

NOTE The checkbox is not selectable until you scroll all the way to the end of the agreement.

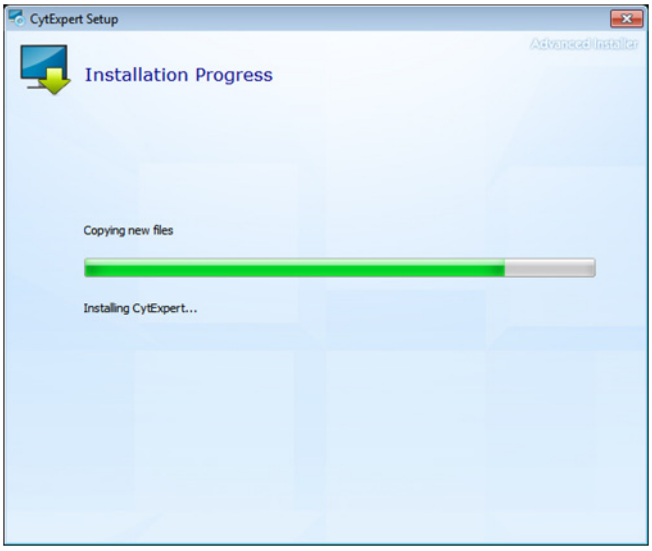
7 Select **Next**. The Installation Options window appears.



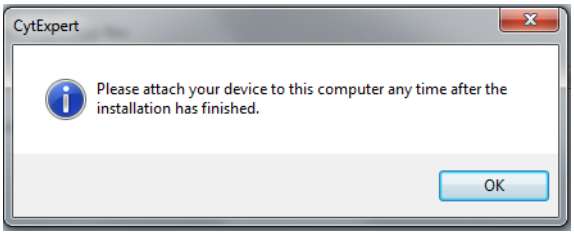
8 Select **Next**. If you are upgrading your software from a previous version and the previous version is not uninstalled first, the following window appears.



9 Select **Install** to begin installing the software. The Installation Progress window appears.

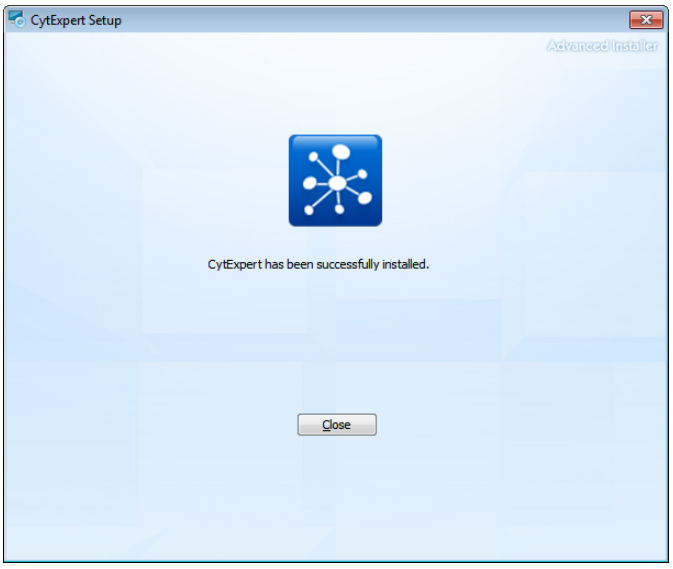


10 If the following software prompt appears, select **OK**.



NOTE The term “device” in this message refers to the cytometer.

11 Wait for the software to finish installing. The install complete window appears.



12 Select **Close** to finish the CytExpert software installation.

Reinstalling the CytExpert Software

Use this procedure to:

- Change the software option installed in all software versions 2.0 and higher. Refer to CytExpert Software Installation Options for the differences between each software option available.

  NOTE If you are upgrading to software version 2.0 or higher from any software version prior to software version 2.0, refer to Upgrading the CytExpert Software.

- Reinstall the same version of software.
- Upgrade your software to a version newer than software version 2.0.

⚠ CAUTION

Risk of data loss. Reinstalling the CytExpert Software could overwrite your database. Ensure you backup your database prior to software reinstallation.

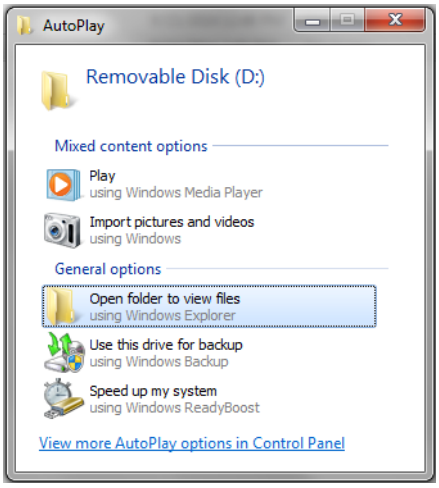
---

1 Backup your CytExpert data if you previously had a software option other than the Default software option installed. Refer to Backup and Restore in CHAPTER 9, Troubleshooting.

---

2 Insert the software USB into the computer.

NOTE If the Autoplay window appears, select Open folder to view files.



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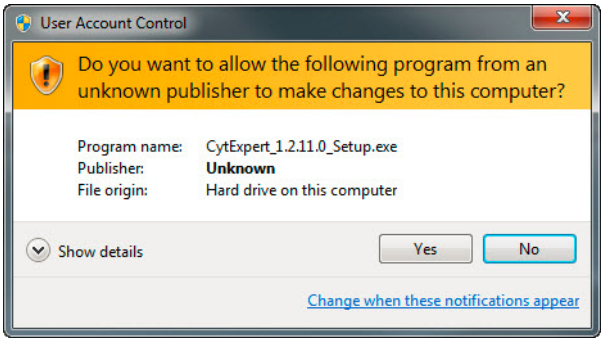
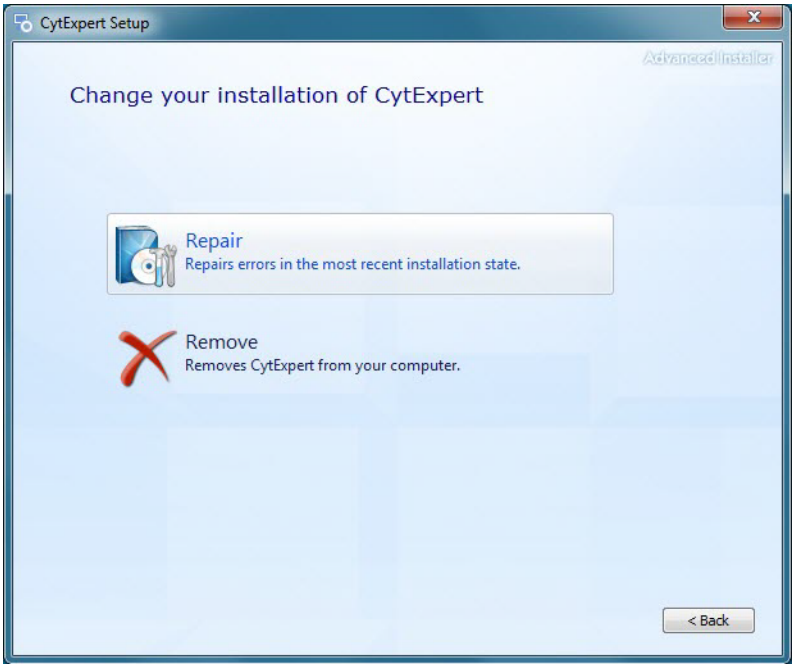
3 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.

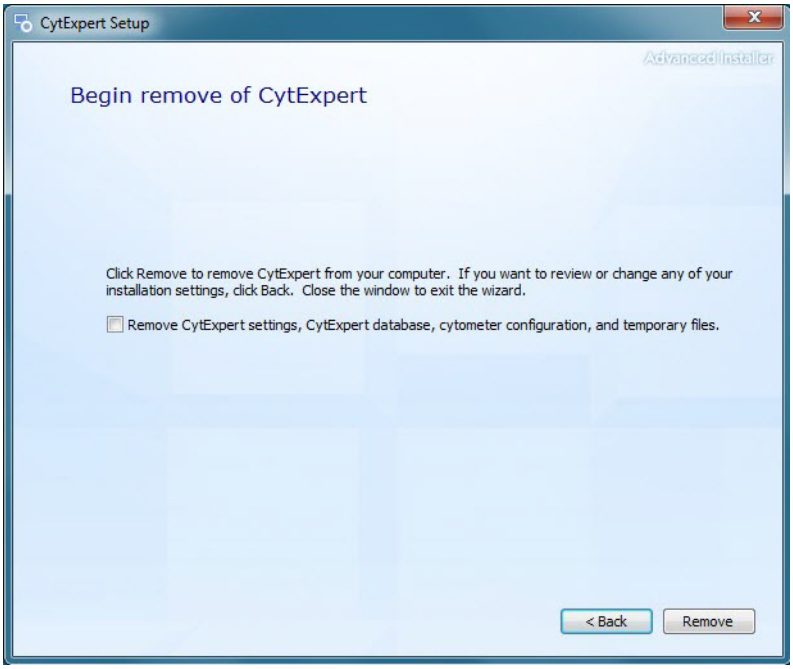


4 Select **Yes**. The Welcome to CytExpert Setup Wizard window appears.

5 Select **Next**. The Change your installation of CytExpert screen appears.



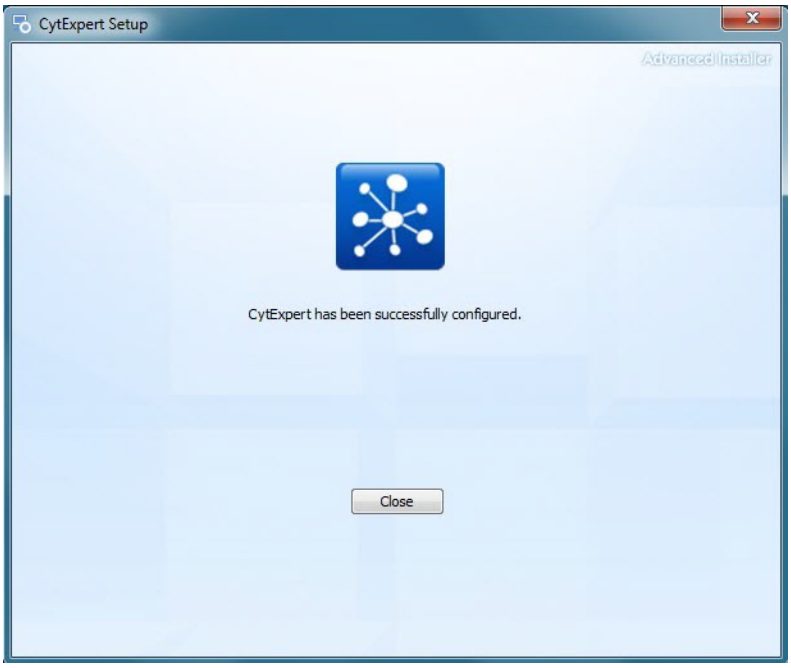
6 Select **Remove**. The Begin Remove of CytExpert screen appears.



IMPORTANT If the *Remove CytExpert settings, CytExpert database, cytometer configuration, and temporary files* checkbox is checked, your settings, database, configuration files, and temporary files will be overwritten and you will need to reinstall your cytometer configuration and restore any databases you might have.

7 Ensure the *Remove CytExpert settings, CytExpert database, cytometer configuration, and temporary files* checkbox is unchecked.

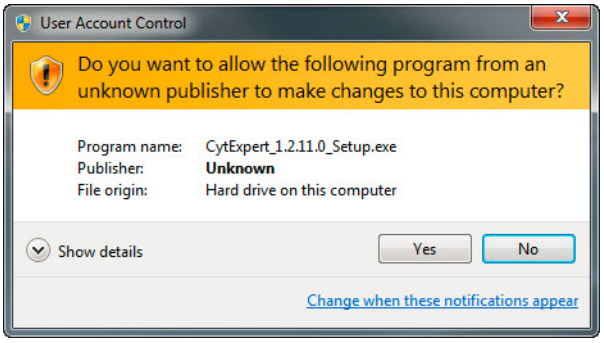
8 Select **Remove**. When software removal is complete, the software displays the message *CytExpert has been successfully configured.*



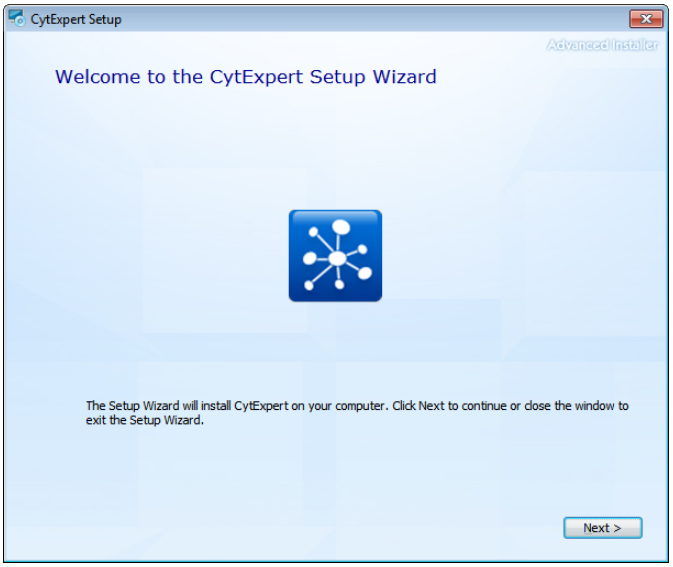
9 Select **Close**.

10 Navigate back to the software USB folder.

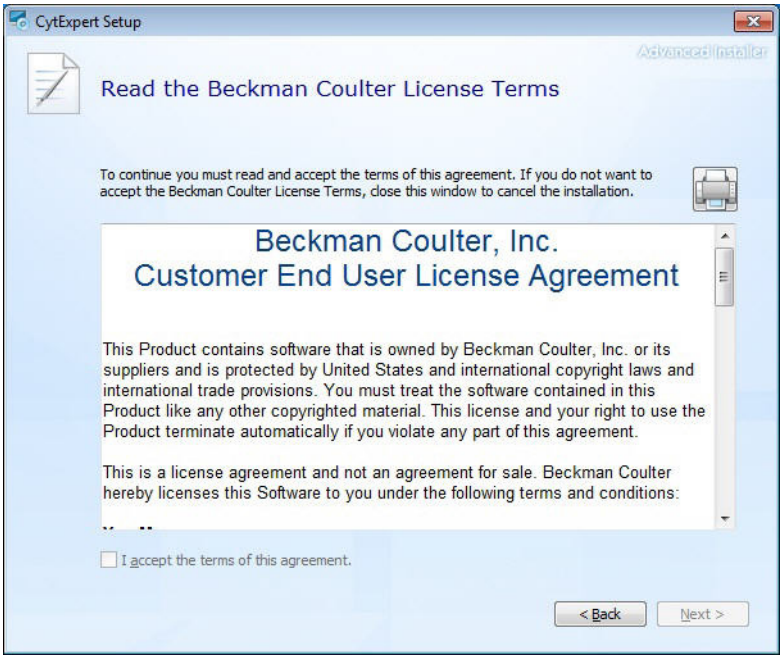
11 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.



12 Select **Yes**. The Welcome to CytExpert Setup Wizard window appears.



13 Select **Next**. The Beckman Coulter License Terms window appears.

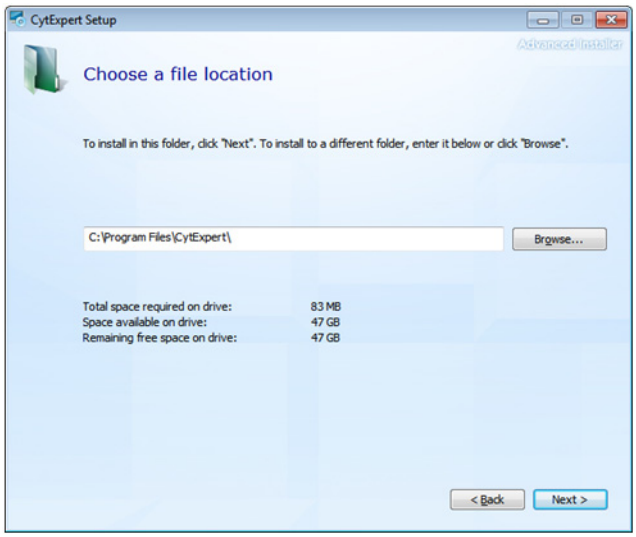


14 Read the Beckman Coulter Customer End User License Agreement.

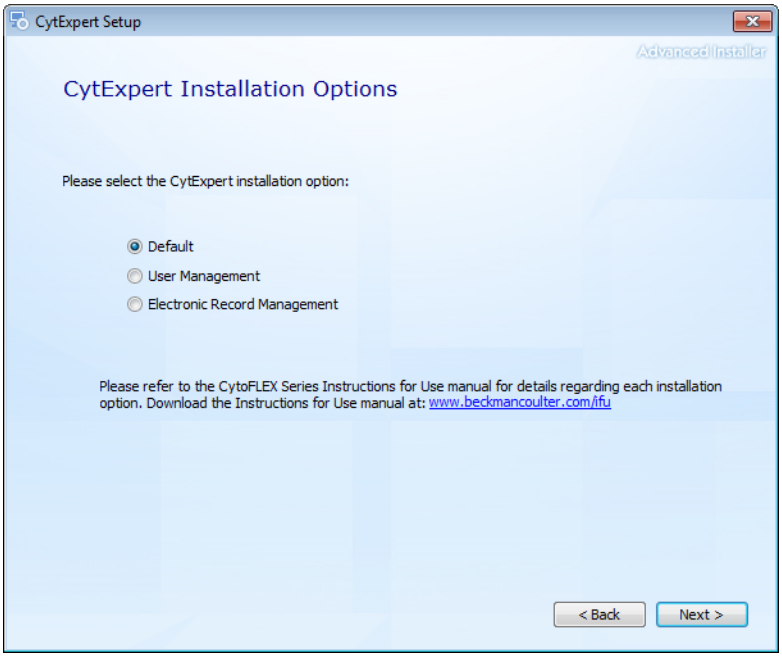
15 Select the *I accept the terms of this agreement* checkbox.

NOTE The checkbox is not selectable until you scroll all the way to the end of the agreement.

16 Select **Next**. The Choose a file location window appears.

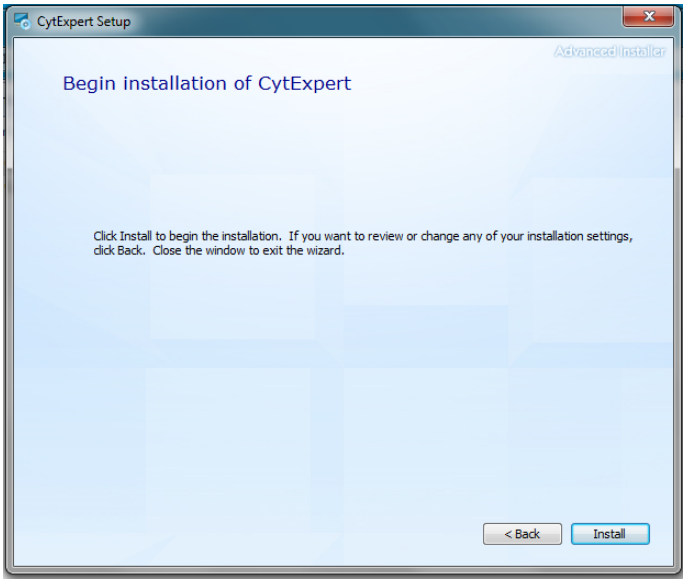


17 Select **Next**. The Installation Options window appears.

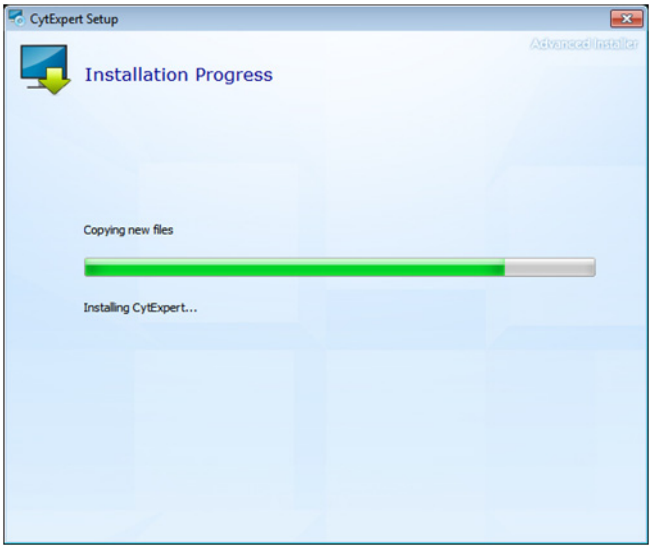


18 Select the desired installation option. Refer to CytExpert Software Installation Options.

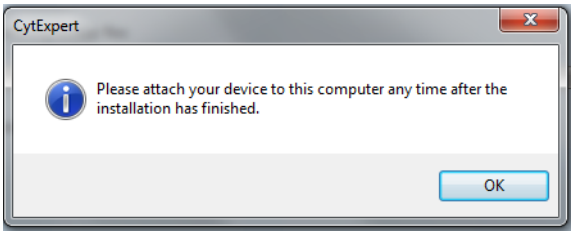
19 Select **Next**. The Begin installation of CytExpert window appears.



20 Select **Install** to begin installing the software. The Installation Progress window appears.

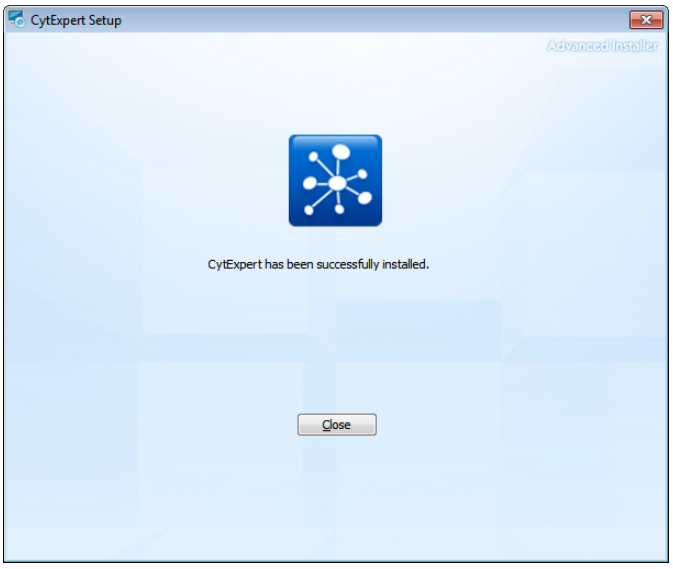


21 If the following software prompt appears, select **OK**.



NOTE The term “device” in this message refers to the cytometer.

22 Wait for the software to finish installing. The install complete window appears.



23 Select **Close** to finish the CytExpert software installation.

APPENDIX B

CytExpert Electronic Record Management

Overview

IMPORTANT You must have the CytExpert Electronic Record Management software option installed to use the features listed below. Refer to CytExpert Software Installation Options in APPENDIX A, Instrument Installation.

Beckman Coulter's CytoFLEX with CytExpert software version 2.0 and higher contains features and functionality that facilitates compliance with 21 CFR Part 11 guidelines for Electronic Records and Signatures. This electronic record management includes controls for user identification, permissions, electronic signatures, data integrity, operation and experiment logs and audit trails. CytExpert software version 2.0 and higher contains a database that uses checksum matching to prevent tampering of the records and files that are indexed in the Closed File System.

This chapter contains information on:

- Software Menu
- Experiment Management
- Log
- Electronic Signature
- User Management

Software Menu

The CytExpert Electronic Record Management software option includes additional software menu items that are not necessarily available in the CytExpert Default software option or the CytExpert User Management software option. Refer to Figure 2.3 and Figure 2.4 for comprehensive software menu trees and details on which menu item applies to each software option.

Experiment Management

⚠ CAUTION

Risk of file corruption. Do not add, delete, or modify data from the Windows Explorer directory. Manage all data changes using Experiment Explorer to ensure file indexing remains intact.

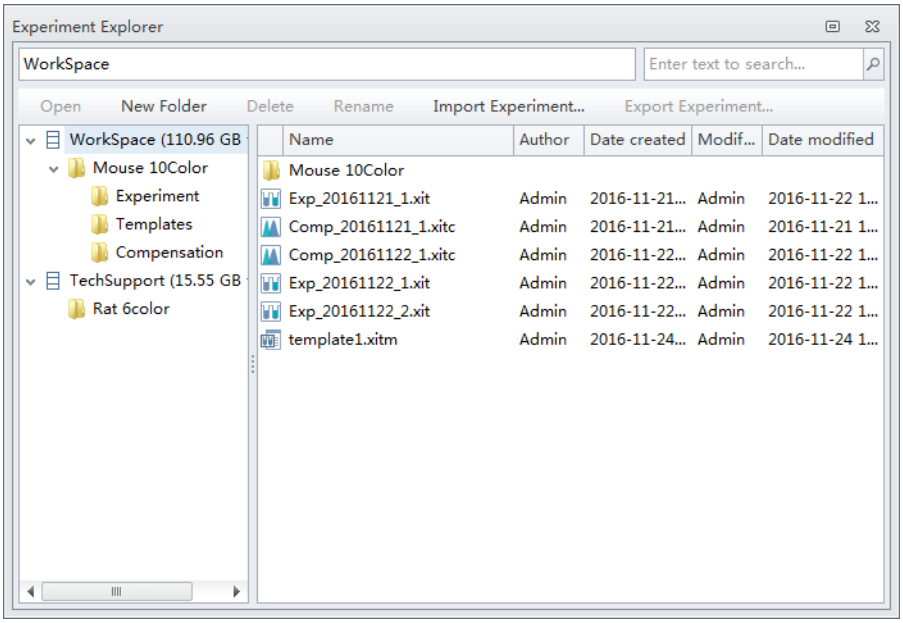
Closed File System

The closed file system provides audit trail capability for CytExpert experiment files. The closed file system provides a secure layer between the actual Windows Explorer files and the CytExpert users to retain file integrity.

Three file types are managed by the closed file system:

- Experiment files
- Compensation experiment files
- Experiment template files

The Experiment Explorer dialog can be accessed by selecting **File > Experiment Explorer**. The Experiment Explorer dialog functions the same as Windows file explorer.



Other mode experiment, compensation experiment, and experiment template files can be imported into the closed file system. These closed file system experiments can also be exported. Refer to Importing an Experiment/Template and Exporting an Experiment/Template.

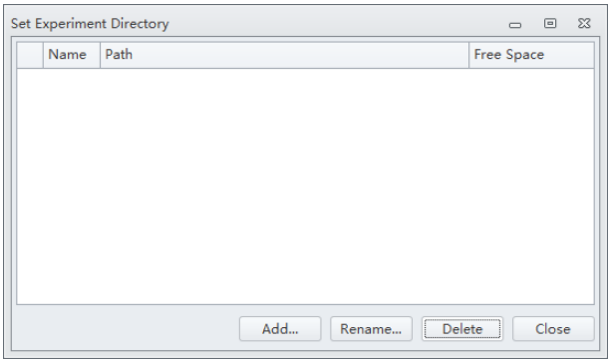
Experiment Directory Management

When launching CytExpert for the first time after installing the software, the Administrator must setup at least one experiment directory for experiment files.

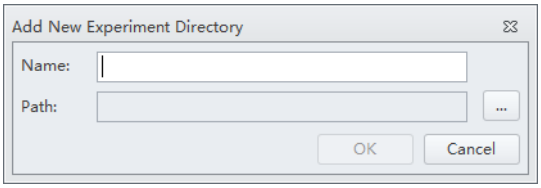
NOTE Only one experiment directory can be created per disk.

Setting Up the Experiment Directory

1 Select **Settings** > **Set Experiment Directory**. The Set Experiment Directory window appears.



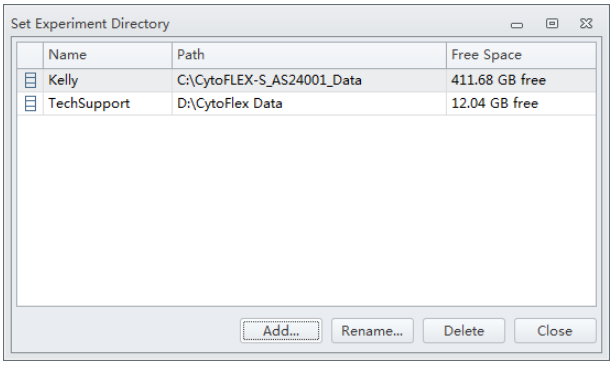
2 Select **Add**. The Add New Experiment Directory window appears.



3 Enter a name for the experiment directory.

4 Select ... and browse to the desired Windows file system folder.

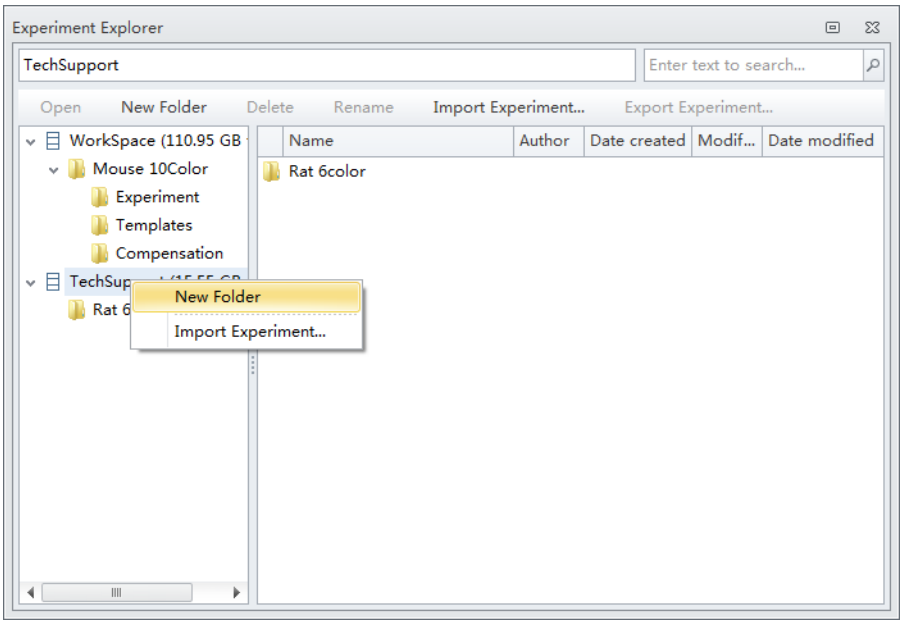
5 Select **OK**. The specified folder appears in the Set Experiment Directory window.



NOTE Select **Rename** to rename the experiment directory. Select **Delete** to delete an experiment directory.

Folder Hierarchy Management

Select **File > Experiment Explorer** to view Experiment Explorer. Select **New Folder** from the right-click drop down menu or from the Experiment Explorer toolbar to create a new subfolder.

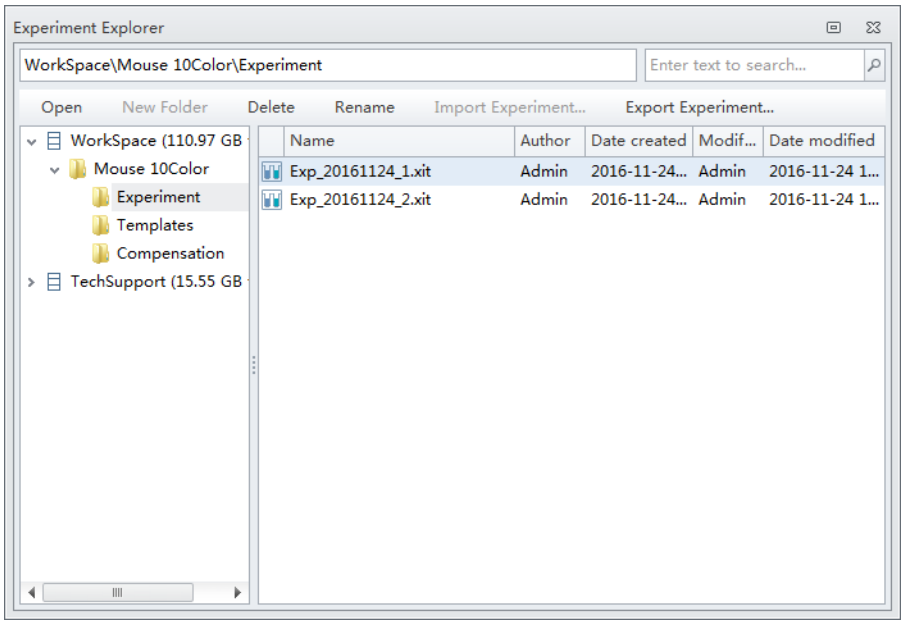


Select **Rename** from the right-click drop down menu or from the Experiment Explorer toolbar to rename a subfolder.

Select **Delete** from the right-click drop down menu or from the Experiment Explorer toolbar to delete a subfolder.

Experiment Related Operations

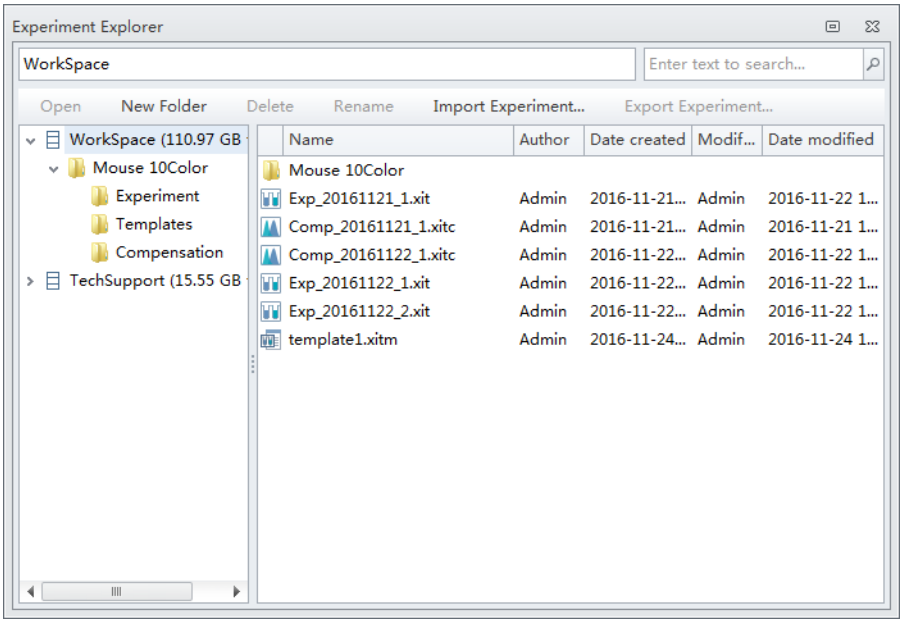
The Experiment Explorer dialog appears in place of the Windows File Explorer in the following operations: New/Open Experiment, New/Open Compensation Experiment, Save As, Save Experiment As Template, Recent Template, and New Experiment from Template.



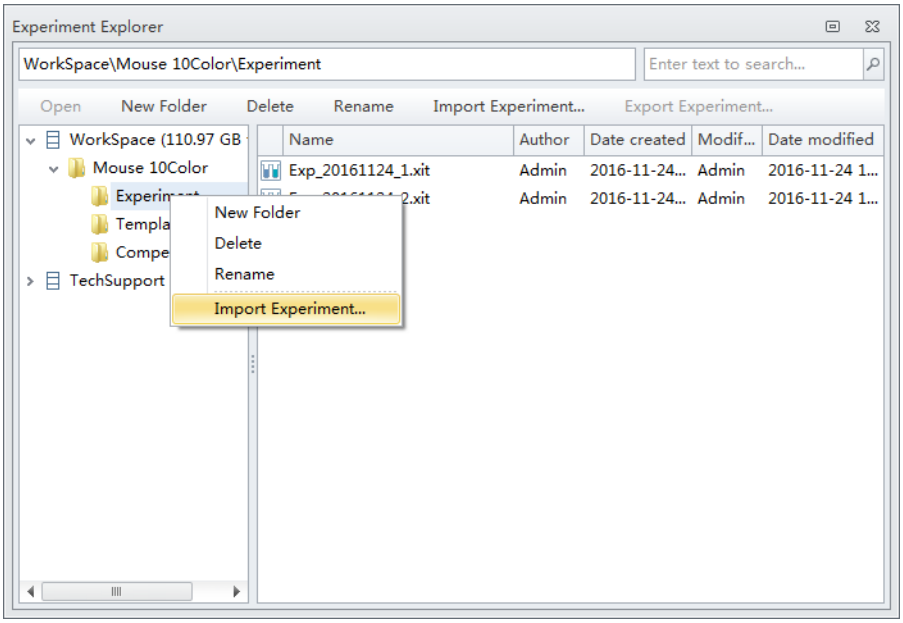
Importing an Experiment/Template

Use the following procedure to import experiment (.xit), compensation (.xitc), or experiment from template (.xitm) files into the system.

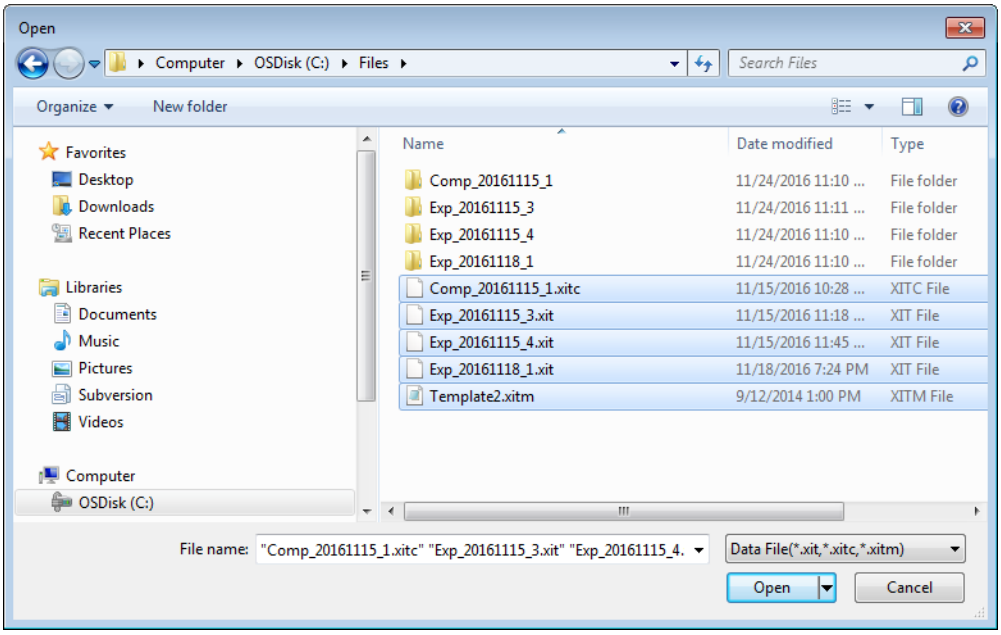
1 Select **File > Experiment Explorer**. The Experiment Explorer window appears.



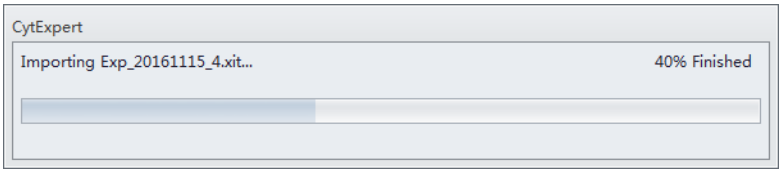
2 Select I**mport Experiment** from the right-click drop down menu or from the Experiment Explorer toolbar.



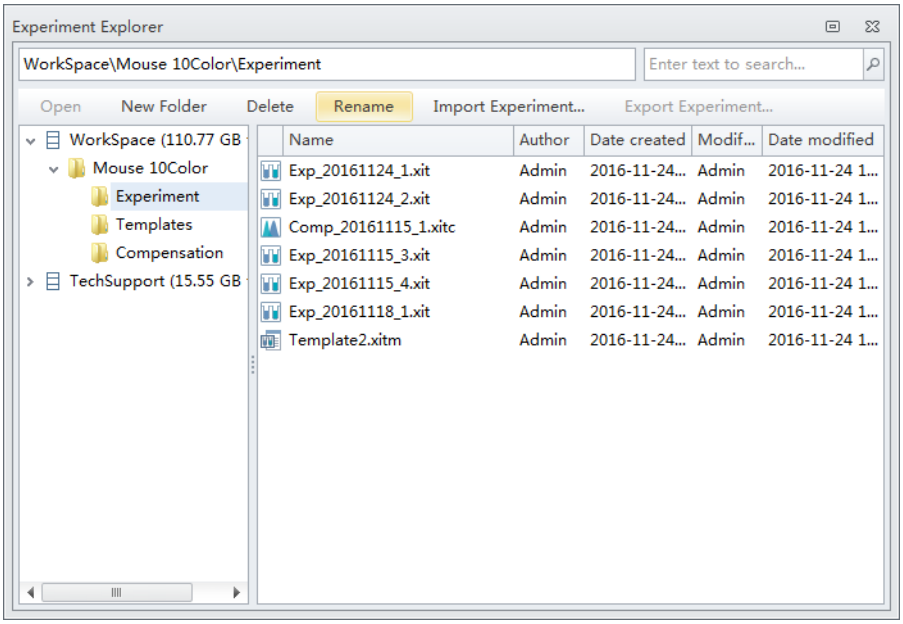
3 Browse to the desired file path to import and select **Open**.



A progress bar appears when importing files.



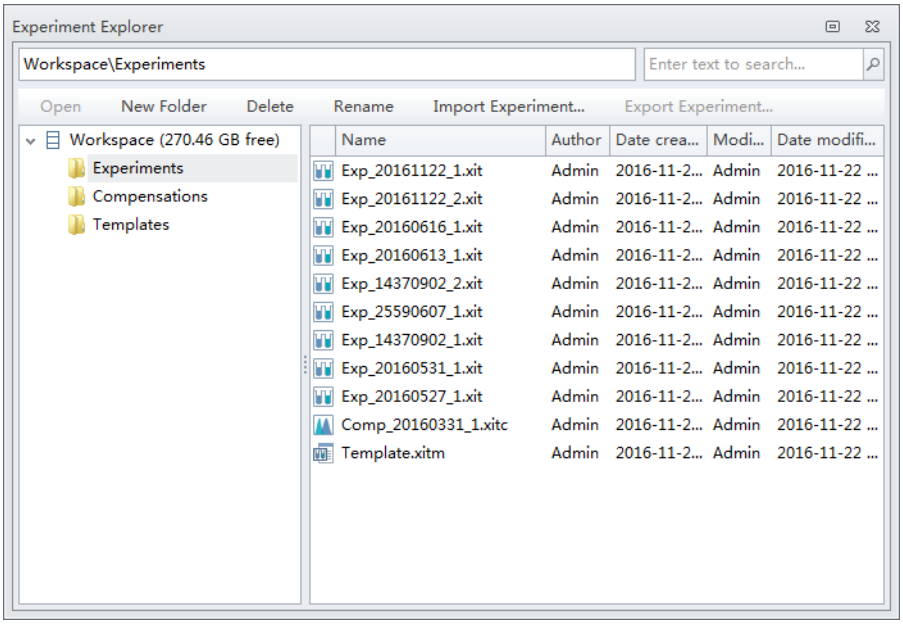
Once the import is complete, the imported files display in the Experiment Explorer.



NOTE Users can import .xit, .xitc, and .xitm files individually or multiple files at a time.

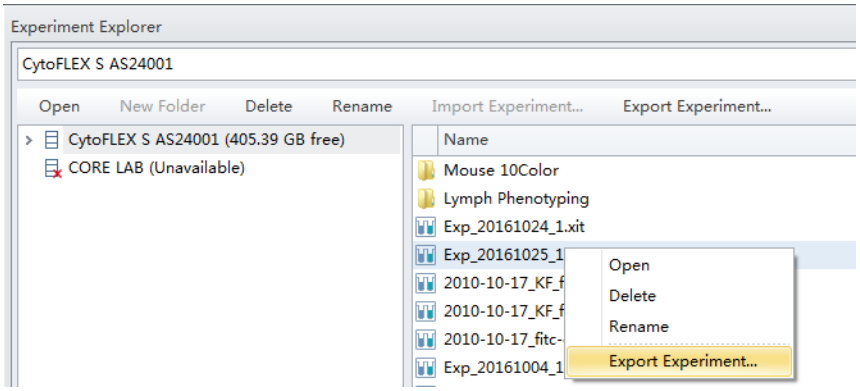
Exporting an Experiment/Template

1 Select **File > Experiment Explorer.** The Experiment Explorer window appears.

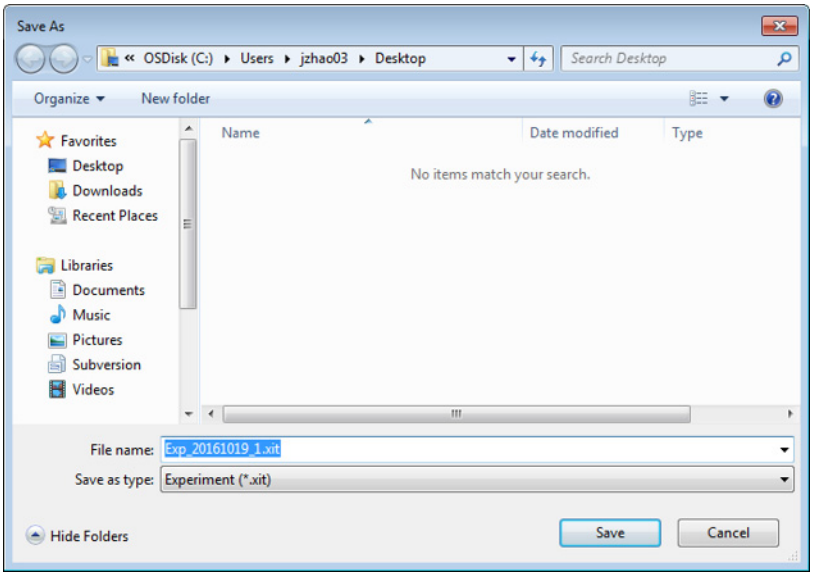


2 To export a single experiment:

a. Select **Export Experiment** from the right-click drop down menu or from the Experiment Explorer toolbar.



b. Browse to the desired file path for export and select **Save**.



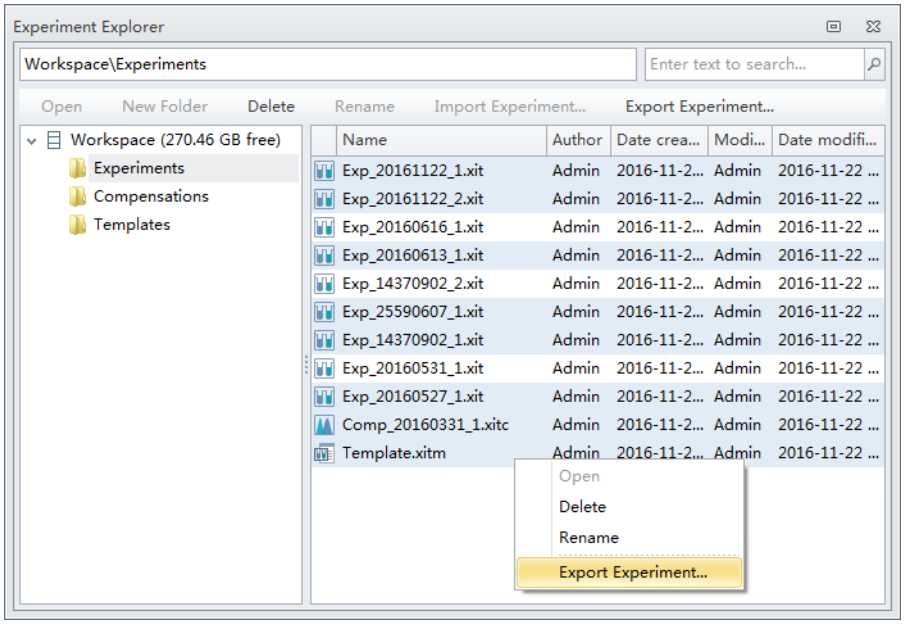
A progress bar appears when exporting files.

Once the export is complete, the exported file displays in the target folder.

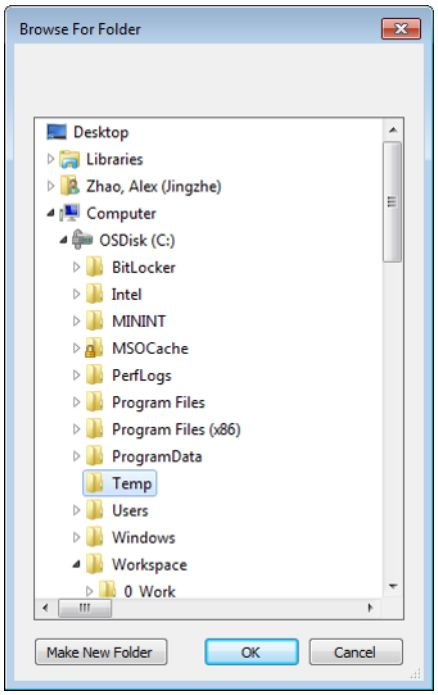
OR

To export multiple experiments:

a. Select all of the experiments to be exported then select **Export Experiment** from the right-click drop down menu or from the Experiment Explorer toolbar.



b. Browse to the desired file path for export and select **OK**.



A progress bar appears when exporting files.

Once the export is complete, the exported file displays in the target folder.

Log

The CytExpert Electronic Record Management software option includes three logs:

Experiment Operation Log — The experiment operation log lists the audit trail records related to the experiment operations based on query criteria.

System Operation Log — The system operation log lists the log records associated with the system configuration changes based on query criteria.

User Management Operation Log — The user management operation log lists all of the log records related to the user management operations based on query criteria.

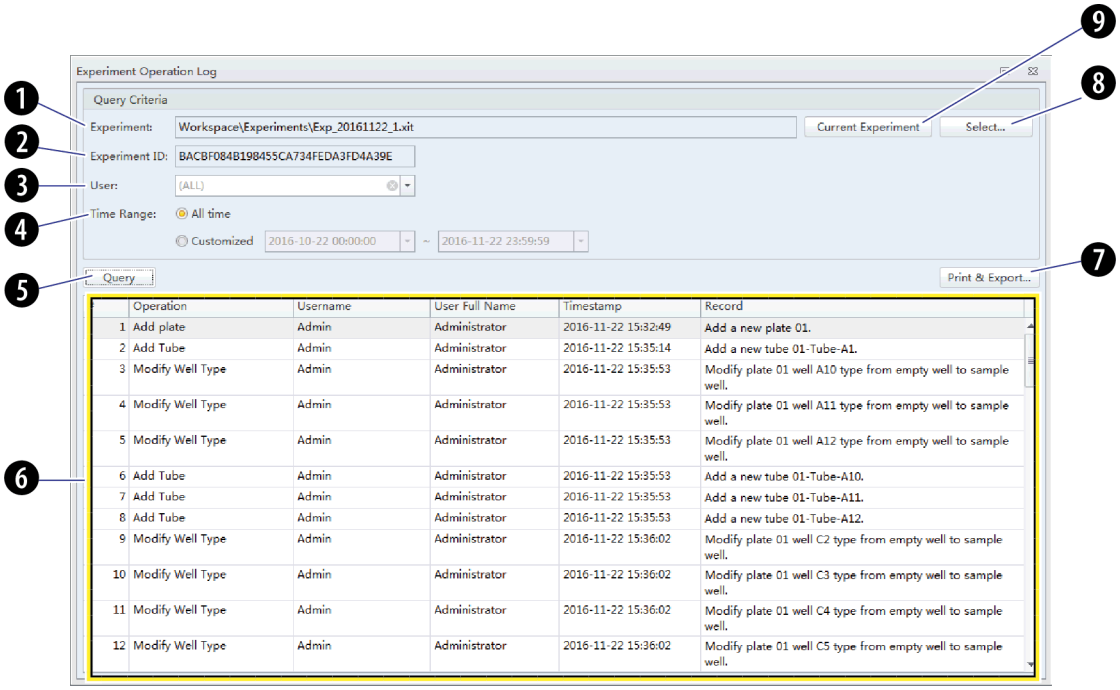
NOTE All three logs support print and export to PDF or CSV file functions.

Experiment Operation Log

The Experiment Operation Log generates an experiment audit trail record when performing experiment operations.

Select **Log > Experiment Operation Log** to open the Experiment Operation Log window. Refer to Figure B.1.

Figure B.1 Experiment Operation Log Window



1. **Experiment:** Used to specify the experiment file criteria.

   NOTE If an experiment is open, the current experiment displays in the Experiment section.

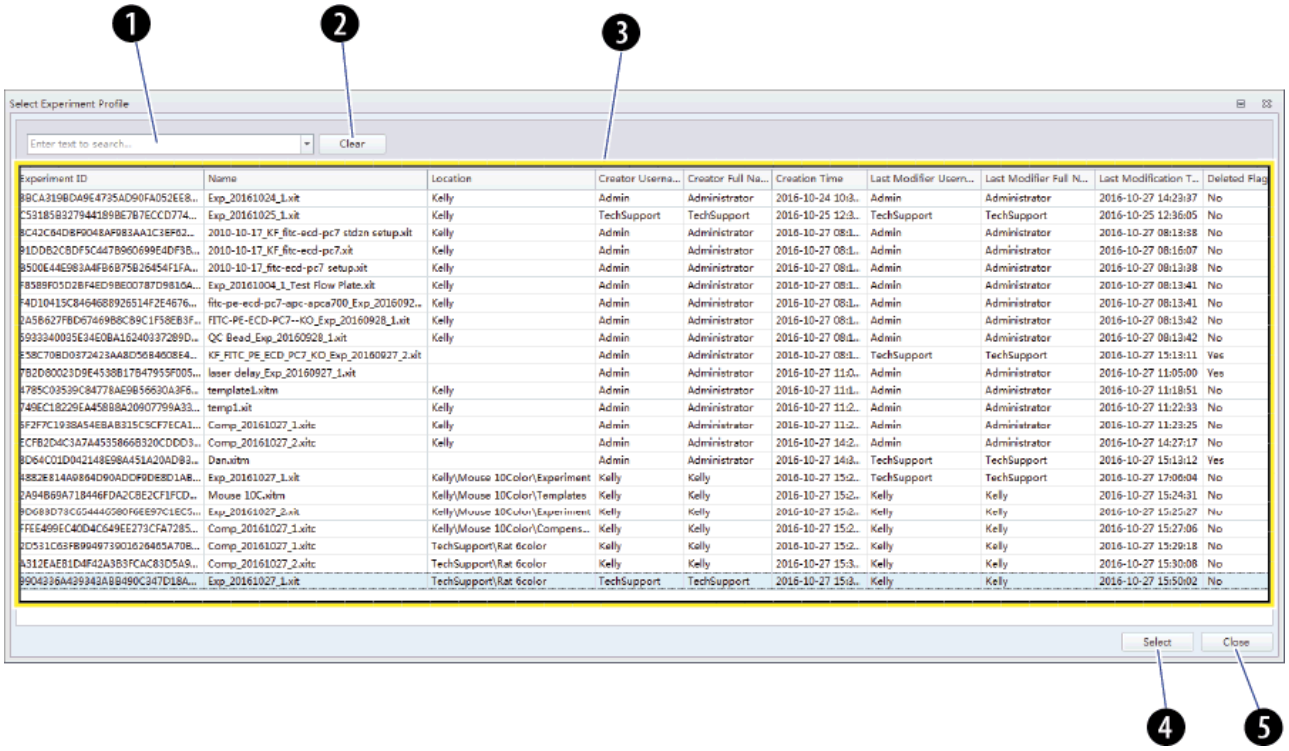
2. **Experiment ID:** Used to specify the experiment ID query criteria.
3. **User:** Used to specify the user query criteria.

   NOTE The default selection all users.

4. **Time Range:** Used to specify the operation time range query criteria.
5. Query : Runs the query on the specified query criteria.
6. **Query display:** Displays the queried results.
7. Print & Export... : Displays the print and export preview dialog. Refer to Figure B.3.
8. Select... : Selects an experiment from the Select Experiment Profile window. Refer to Figure B.2.
9. Current Experiment : Selects the experiment that is currently open.

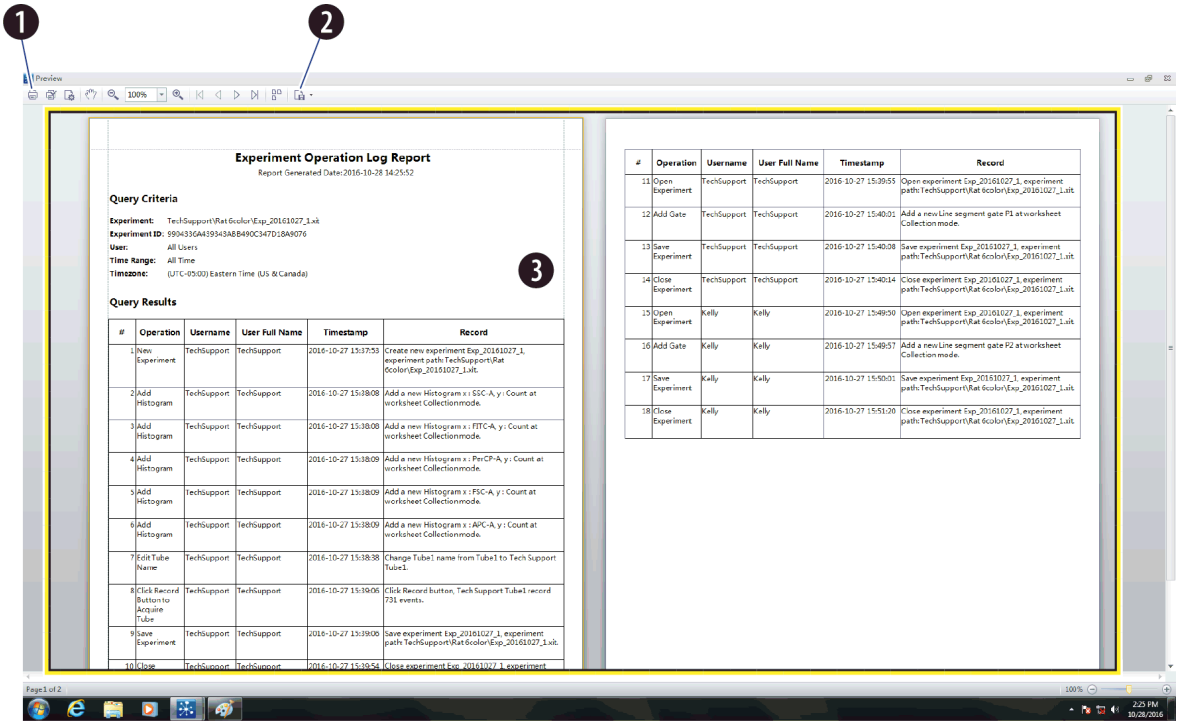
   NOTE An experiment must be open for the button to be selectable.

Figure B.2 Select Experiment Profile Window



1. **Keyword search:** Used to search for keywords in the experiment display list.
2. **Clear:** Clears the keyword search.
3. **Experiment display list:** Displays the experiments in a list.
4. **Select:** Selects an experiment.
5. **Close:** Closes the Select Experiment Profile window.

Figure B.3 Print and Export Preview Window



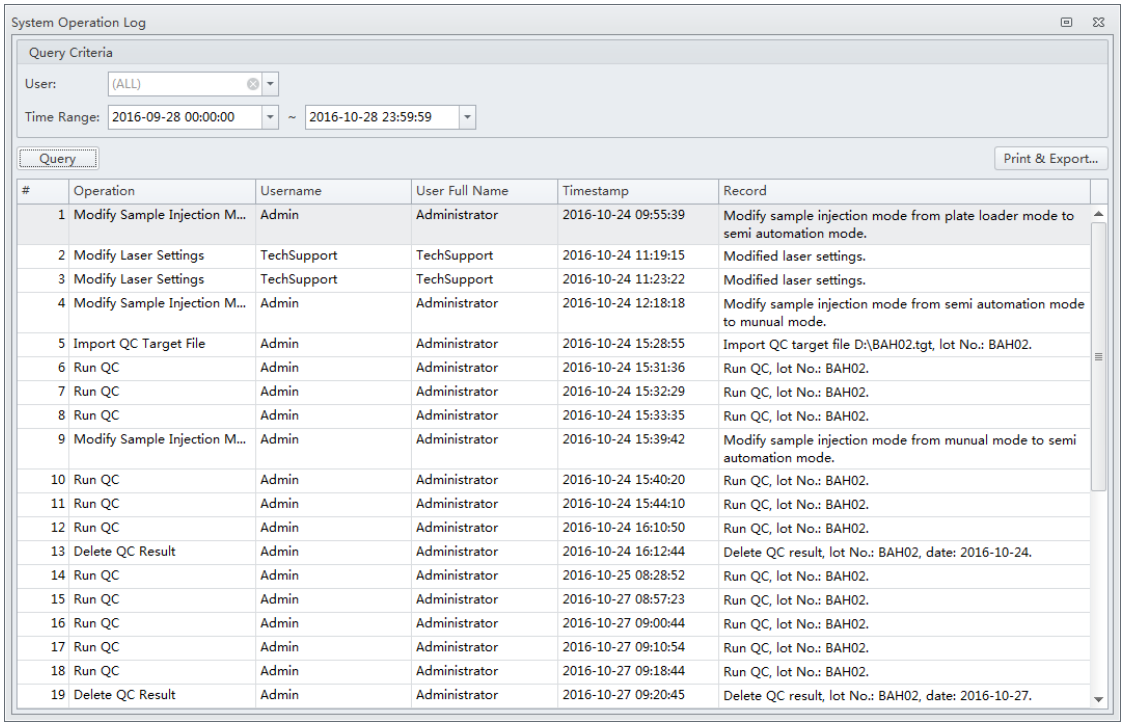
1. **Print:** Prints the report.
2. **Export to PDF or CSV:** Exports the report as a PDF or CSV file.
3. **Report View:** Displays the detailed report view.

System Operation Log

The System Operation Log generates system log records for settings, configuration, maintenance, and QC.

Select **Log > System Operation Log** to open the System Operation Log window. Refer to Figure B.4.

Figure B.4 System Operation Log Window



#	Operation	Username	User Full Name	Timestamp	Record
1	Modify Sample Injection M...	Admin	Administrator	2016-10-24 09:55:39	Modify sample injection mode from plate loader mode to semi automation mode.
2	Modify Laser Settings	TechSupport	TechSupport	2016-10-24 11:19:15	Modified laser settings.
3	Modify Laser Settings	TechSupport	TechSupport	2016-10-24 11:23:22	Modified laser settings.
4	Modify Sample Injection M...	Admin	Administrator	2016-10-24 12:18:18	Modify sample injection mode from semi automation mode to munual mode.
5	Import QC Target File	Admin	Administrator	2016-10-24 15:28:55	Import QC target file D:\BAH02.tgt, lot No.: BAH02.
6	Run QC	Admin	Administrator	2016-10-24 15:31:36	Run QC, lot No.: BAH02.
7	Run QC	Admin	Administrator	2016-10-24 15:32:29	Run QC, lot No.: BAH02.
8	Run QC	Admin	Administrator	2016-10-24 15:33:35	Run QC, lot No.: BAH02.
9	Modify Sample Injection M...	Admin	Administrator	2016-10-24 15:39:42	Modify sample injection mode from munual mode to semi automation mode.
10	Run QC	Admin	Administrator	2016-10-24 15:40:20	Run QC, lot No.: BAH02.
11	Run QC	Admin	Administrator	2016-10-24 15:44:10	Run QC, lot No.: BAH02.
12	Run QC	Admin	Administrator	2016-10-24 16:10:50	Run QC, lot No.: BAH02.
13	Delete QC Result	Admin	Administrator	2016-10-24 16:12:44	Delete QC result, lot No.: BAH02, date: 2016-10-24.
14	Run QC	Admin	Administrator	2016-10-25 08:28:52	Run QC, lot No.: BAH02.
15	Run QC	Admin	Administrator	2016-10-27 08:57:23	Run QC, lot No.: BAH02.
16	Run QC	Admin	Administrator	2016-10-27 09:00:44	Run QC, lot No.: BAH02.
17	Run QC	Admin	Administrator	2016-10-27 09:10:54	Run QC, lot No.: BAH02.
18	Run QC	Admin	Administrator	2016-10-27 09:18:44	Run QC, lot No.: BAH02.
19	Delete QC Result	Admin	Administrator	2016-10-27 09:20:45	Delete QC result, lot No.: BAH02, date: 2016-10-27.

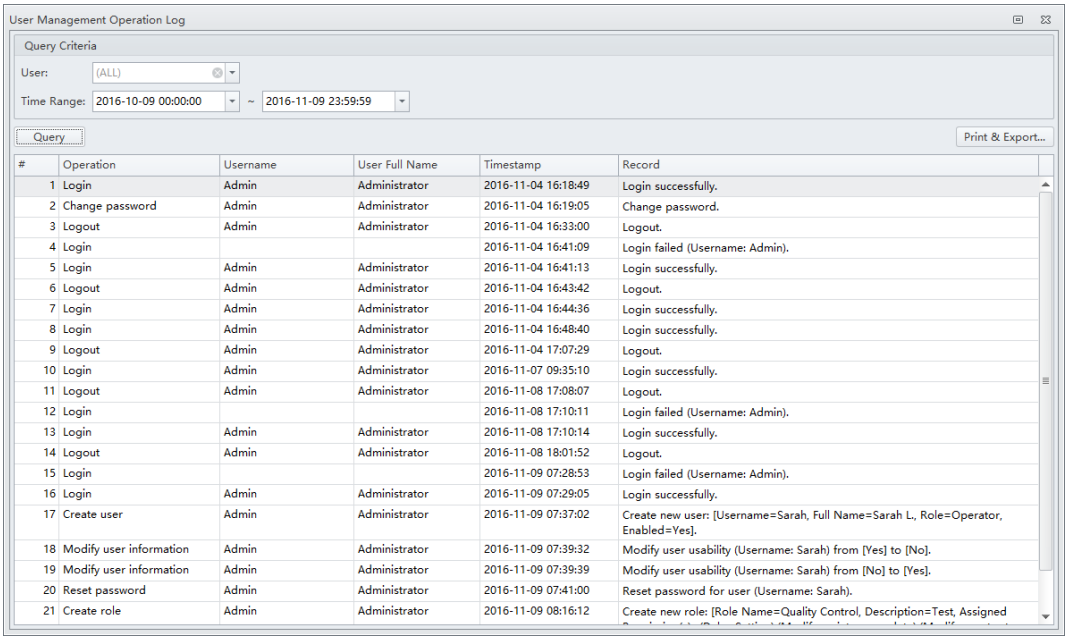
NOTE The System Operation Log uses the same query criteria and functions as the Experiment Operation Log. Refer to Figure B.1.

User Management Operation Log

The User Management Operation Log generates audit log records for user management, role management, account policies, password changes, and login/logout records.

Select **Log > User Management Operation Log** to open the User Management Operation Log window. Refer to Figure B.5.

Figure B.5 User Management Operation Log Window



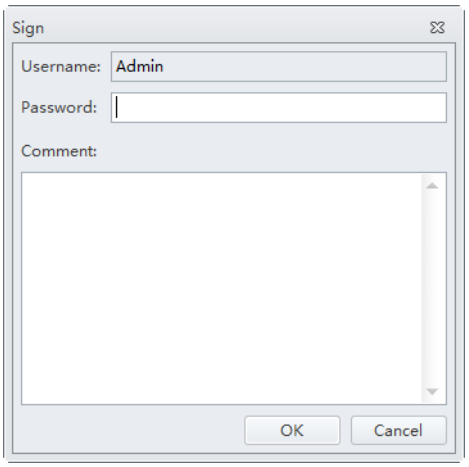
#	Operation	Username	User Full Name	Timestamp	Record
1	Login	Admin	Administrator	2016-11-04 16:18:49	Login successfully.
2	Change password	Admin	Administrator	2016-11-04 16:19:05	Change password.
3	Logout	Admin	Administrator	2016-11-04 16:33:00	Logout.
4	Login			2016-11-04 16:41:09	Login failed (Username: Admin).
5	Login	Admin	Administrator	2016-11-04 16:41:13	Login successfully.
6	Logout	Admin	Administrator	2016-11-04 16:43:42	Logout.
7	Login	Admin	Administrator	2016-11-04 16:44:36	Login successfully.
8	Login	Admin	Administrator	2016-11-04 16:48:40	Login successfully.
9	Logout	Admin	Administrator	2016-11-04 17:07:29	Logout.
10	Login	Admin	Administrator	2016-11-07 09:35:10	Login successfully.
11	Logout	Admin	Administrator	2016-11-08 17:08:07	Logout.
12	Login			2016-11-08 17:10:11	Login failed (Username: Admin).
13	Login	Admin	Administrator	2016-11-08 17:10:14	Login successfully.
14	Logout	Admin	Administrator	2016-11-08 18:01:52	Logout.
15	Login			2016-11-09 07:28:53	Login failed (Username: Admin).
16	Login	Admin	Administrator	2016-11-09 07:29:05	Login successfully.
17	Create user	Admin	Administrator	2016-11-09 07:37:02	Create new user: [Username=Sarah, Full Name=Sarah L, Role=Operator, Enabled=Yes].
18	Modify user information	Admin	Administrator	2016-11-09 07:39:32	Modify user usability (Username: Sarah) from [Yes] to [No].
19	Modify user information	Admin	Administrator	2016-11-09 07:39:39	Modify user usability (Username: Sarah) from [No] to [Yes].
20	Reset password	Admin	Administrator	2016-11-09 07:41:00	Reset password for user (Username: Sarah).
21	Create role	Admin	Administrator	2016-11-09 08:16:12	Create new role: [Role Name=Quality Control, Description=Test, Assigned

NOTE The User Management Operation Log uses the same query criteria and functions as the Experiment Operation Log. Refer to Figure B.1.

Electronic Signature

Signing an Experiment

1 Select **Signature > Sign** to sign an experiment. The Sign window appears.

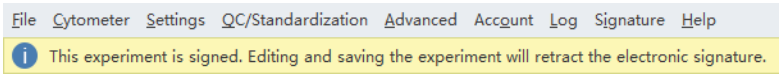


2 If necessary, enter comments related to the experiment.

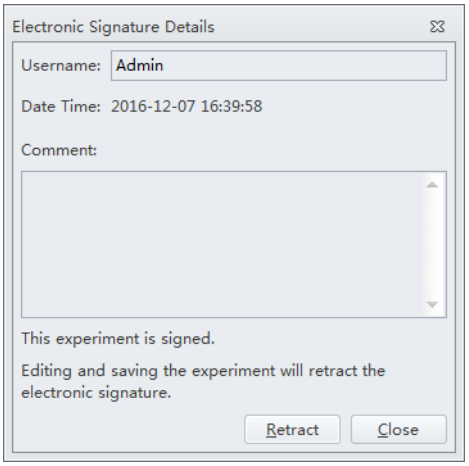
3 Enter your password.

4 Select **OK**.

The signature status displays in the top left corner of the main UI.

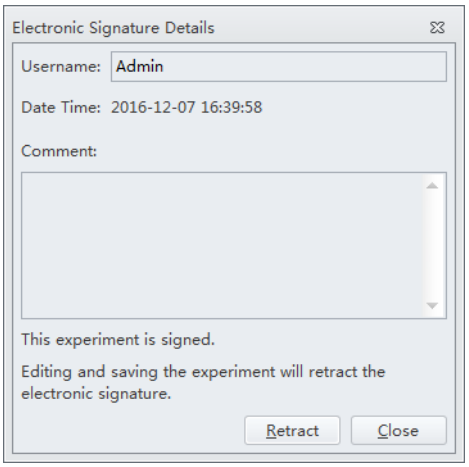


5 Select **Signature > Electronic Signature Details** to view the electronic signature details.



Retracting an Experiment Signature

1 Select **Signature > Electronic Signature Details** to view the electronic signature details.



2 Select Retract. The following message appears: *Are you sure you want to remove the electronic signature?*

3 Select **Yes** to confirm the retraction of your signature or **No** to cancel.

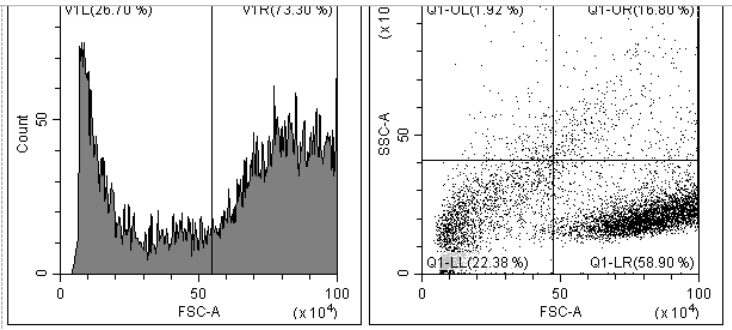
The following message appears after editing and saving a signed experiment: *Saving the experiment will retract the electronic signature of this experiment, continue?* Select **Yes** to remove the signature or **No** to cancel.

The following message appears before starting data acquisition: *Acquiring data will retract the electronic signature of this experiment, continue?* Select **Yes** to remove the signature or **No** to cancel.

Printing an Experiment Signature

IMPORTANT Ensure an experiment is signed prior to attempting to print the experiment signature.

To print an experiment with a signature, refer to CHAPTER 5, Printing Graphics in CHAPTER 5, Data Acquisition and Sample Analysis.



Tube Name: 01-N1G-A3
Sample ID:

Population	Events	% Total	% Parent
All Events	10064	100.00 %	100.00 %
Q1-UR	1691	16.80 %	16.80 %
Q1-UL	193	1.92 %	1.92 %
Q1-LL	2252	22.38 %	22.38 %
Q1-LR	5928	58.90 %	58.90 %
V1L	2687	26.70 %	26.70 %
V1R	7377	73.30 %	73.30 %

Tube Name: 01-N1G-A3
Sample ID:

Population	Events	% Total	% Parent
All Events	10064	100.00 %	100.00 %
Q1-UR	1691	16.80 %	16.80 %
Q1-UL	193	1.92 %	1.92 %
Q1-LL	2252	22.38 %	22.38 %
Q1-LR	5928	58.90 %	58.90 %
V1L	2687	26.70 %	26.70 %
V1R	7377	73.30 %	73.30 %

Signed by Admin at 2017-06-22 10:49:46. Comment: Approved.

User Management

User Administration

Logging In and Out of the Software

Refer to Logging Into the Software and Logging Out of the Software in CHAPTER 3, Daily Startup.

Role Management

Refer to Role Management in CHAPTER 2, Using the CytExpert Software.

User Management

Refer to User Management in CHAPTER 2, Using the CytExpert Software.

Account Policies

Refer to Account Policies in CHAPTER 2, Using the CytExpert Software.

APPENDIX C

Sample Injection Mode Control Kit

Overview

The Sample Injection Mode Control Kit is a mechanical knob installed by your service engineer that enables users to switch between the Plate Loader sample injection mode and the Semi-Automatic or manual sample injection mode. The switch eliminates the need to manually re-route the tubing.

This chapter contains information on:

Performance Characteristics [With the Sample Injection Mode Control Knob]

Performance [CytoFLEX With Standard Plate Loader]	
Throughput [With Standard Plate Loader][a]	10 second acquisition without mixing or backflush: <35 min.
	10 second acquisition with 3 second mixing and 4 second backflush: < 50 min.

a. This performance characteristic is different if you do not have the Sample Injection Mode Control Kit installed on your CytoFLEX flow cytometer. Refer to Performance Characteristics in CHAPTER 1, System Overview.

Performance [CytoFLEX With Plate Loader DW]		
Carryover	**Plate Loader format**	<0.5%
Throughput [With Plate Loader DW][a]	Standard 96-well plate, 10 second acquisition without mixing or backflush: <36 min. 96-well deep well plate, 10 second acquisition without mixing or backflush: <37 min	
	Standard 96-well plate, 10 second acquisition with 5 second mixing and 4 second backflush: <54 min. 96-well deep well plate, 10 second acquisition with 10 second mixing and 4 second backflush: <64 min.	

a. The Plate Loader DW is equipped with the Sample Injection Mode Control Kit. Refer to APPENDIX C, Sample Injection Mode Control Kit.

Performance [CytoFLEX LX With Standard Plate Loader]	
Throughput [With Standard Plate Loader][a]	10 second acquisition without mixing or backflush: <38 min.
	10 second acquisition with 3 second mixing and 6 second backflush: < 56 min.

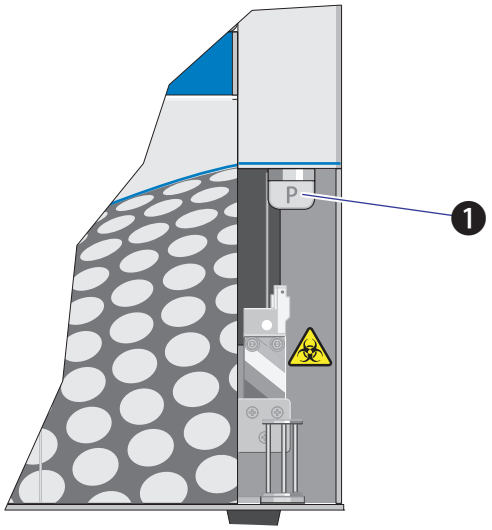
a. This performance characteristic is different if you do not have the Sample Injection Mode Control Kit installed on your CytoFLEX LX flow cytometer. Refer to Performance Characteristics in CHAPTER 1, System Overview.

Performance [CytoFLEX LX With Plate Loader DW]		
Carryover	**Plate Loader format**	<0.5%
Throughput [With Plate Loader DW][a]	Standard 96-well plate, 10 second acquisition without mixing or backflush: <39 min. 96-well deep-well plate, 10 second acquisition without mixing or backflush: <40 min	
	Standard 96-well plate, 5 second acquisition with 6 second mixing and 4 second backflush: <60 min. 96-well deep well plate, 10 second acquisition with 6 second mixing and 4 second backflush: <69 min.	

a. The Plate Loader DW is equipped with the Sample Injection Mode Control Kit. Refer to APPENDIX C, Sample Injection Mode Control Kit.

Sample Injection Mode Control Kit Components

Figure C.1 Sample Injection Mode Control Knob



1. Switch module knob

NOTE The **P** indicates the instrument is set to Plate Loader sample injection mode. The **T** indicates the instrument is set to the Semi-Automatic or manual sample injection mode.

Switching the Sample Probe from the Single Tube Sample Station to the Plate Loader [With Sample Injection Mode Control Knob]

⚠ WARNING

Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. 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 biohazardous contamination and/or sample dilution when performing a manual backflush. If the CytExpert Sample Injection Mode does not match the Sample Injection Control Knob position, a manual backflush can cause backflush fluid to flow through the path set by the Sample Injection Mode Control knob potentially contaminating the sample tube/plate and/or the sample station. Ensure the Sample Injection Mode Control Knob is positioned to match the correct CytExpert Sample Injection Mode. 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.

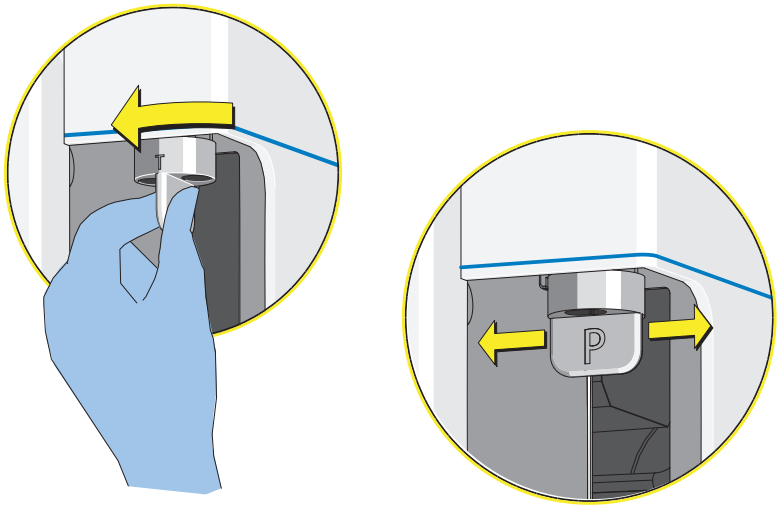
⚠ CAUTION

Risk of instrument damage. The sample probe can easily become damaged or deformed. To avoid damage to the sample probe, turn the switch module knob carefully and avoid contacting the sample probe.

NOTE If you do not have the Sample Injection Mode Control Kit installed, refer to Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [CytoFLEX With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.

1 Switch to the Plate Loader sample injection mode. Refer to Selecting the Plate Loader Sample Injection Mode [With Plate Loader] in CHAPTER 3, Daily Startup.

2 Turn the switch module knob to the right (clockwise) until the flat side of the knob is parallel to the front panel and the **P** is facing you. Ensure that the knob is turned as far as it will go.



The system is now ready for use in the Plate Loader sample injection mode.

NOTE If you do not see any events upon running an acquisition:

1. Select Stop to stop the acquisition.
2. Ensure that the switch module knob is turned as far as it will go.
3. Rerun the acquisition.
4. If the problem persists, contact us.

Switching the Sample Probe from the Plate Loader to the Single Tube Sample Station [With Sample Injection Mode Control Knob]

⚠ WARNING

Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. 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 biohazardous contamination and/or sample dilution when performing a manual backflush. If the CytExpert Sample Injection Mode does not match the Sample Injection Control Knob position, a manual backflush can cause backflush fluid to flow through the path set by the Sample Injection Mode Control knob potentially contaminating the sample tube/plate and/or the sample station. Ensure the Sample Injection Mode Control Knob is positioned to match the correct CytExpert Sample Injection Mode. 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.

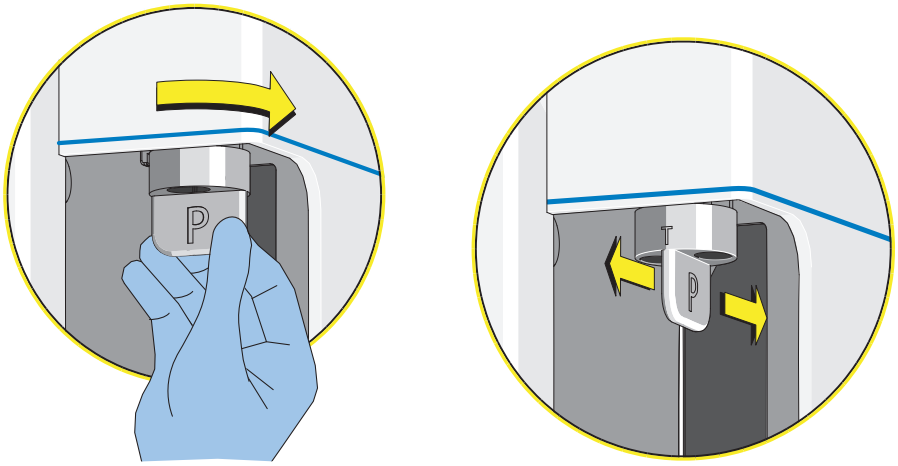
⚠ CAUTION

Risk of instrument damage. The sample probe can easily become damage or deformed. To avoid damage to the sample probe, turn the switch module knob carefully and avoid contacting the sample probe.

NOTE If you do not have the Sample Injection Mode Control Kit installed, refer to Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [CytoFLEX With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.

1 Switch to either the Semi-Automatic or manual sample injection mode. Refer to Selecting the Proper Sample Injection Mode in CHAPTER 3, Daily Startup.

2 Turn the switch module knob to the left (counterclockwise) until the flat side of knob is perpendicular to the front panel and the **T** is facing you. Ensure that the knob is turned as far as it will go.



The system is now ready for use in either the Semi-Automatic or manual sample injection mode.

NOTE If you do not see any events upon running an acquisition:

1. Select Stop to stop the acquisition.
2. Ensure that the switch module knob is turned as far as it will go.
3. Rerun the acquisition.
4. If the problem persists, contact us.

APPENDIX D

Deep Well Plate

Specimen Collection Plate Specifications

Beckman Coulter does not recommend the use of one plate in preference to another nor guarantee the acceptability of the plates to produce quality results. If you need information on a plate not listed in Table D.1, ensure that the plate size and characteristics conform to the specifications listed below. Calibrate the plate position for any new plate types prior to acquisition. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.

Table D.1 Deep Well Plate **[with Plate Loader DW]**

Name	Volume	Material	Manufacturer	PN
96-well deep well plate	1 mL	Polystyrene	Beckman Coulter	267001
96-well deep well plate	1 mL	Polypropylene	Beckman Coulter	267006
96-well deep well plate	2 mL	Polypropylene	Beckman Coulter	140504

NOTE Ensure that the following specifications are met when you select the 96-well deep well plates:

- The inner well diameter is ≥ 5.8 mm.
- The plate height is ≤ 45.5 mm.

APPENDIX E

Table of Hazardous Substances

Table of Hazardous Substances

The Hazardous Substances Names and Concentration are shown in Table E.1, Table E.2, and Table E.3

Table E.1 有毒有害物质名称及含量的标识格式 Table of Hazardous Substances Name and Concentration [CytoFLEX]

电子电气产品号码 **EEP Part Number: A00-1-1102**	**产品名称 Product Name: CytoFLEX** **产品型号 Product Model Number: A00-1-1102**					
部件名称 Component Name	有毒有害物质或元素 Hazardous Substances Name					
	铅 (Pb)	汞 (Hg)	镉 (Cd)	六价铬 (Cr^{6+})	多溴联苯 (PBB)	多溴二苯醚 (PBDE)
印刷电路板组件 Circuit Boards	X	O	O	O	O	O
电源组件 Power Supplies	O	O	O	O	O	O
计算机 Computer	O	O	O	O	O	O
功率调节器 Power Conditioner	O	O	O	O	O	O
光量传感器 Optical Sensors	O	O	O	O	O	O
激光 Laser	O	O	O	O	O	O
发动机/泵/阀门/ Motors/Pumps/Valves	O	O	O	O	O	O
电线 Cables	X	O	O	O	O	O
管路及橡胶 Tubing & Rubber	O	O	O	O	O	O
塑料部件 Plastic	O	O	O	O	O	O
连接部件 Hardware	O	O	O	O	O	O
包装材料 Packing Material	O	O	O	O	O	O

This table is prepared in accordance with the provisions of SJ/T 11364

O：表示该有毒有害物质在该部件所有均质材料中的含量均在GB/T 26572标准规定的限量要求以下

x: 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出GB/T 26572标准规定的限量要求

（企业可在此处，根据实际情况对上表中打“x”的技术原因进行进一步说明）

O: Indicates that the toxic or hazardous substances contained in all of the homogenous materials for this part is below the limit requirements in GB/T 26572.

X: Indicates that the toxic or hazardous substance contained in at least one of the homogenous materials used for this part in above the limit requirement in GB/T 26572.

Table E.2 有毒有害物质名称及含量的标识格式 Table of Hazardous Substances Name and Concentration [CytoFLEX LX]

电子电气产品号码 **EEP Part Number: B90883**	**产品名称 Product Name: CytoFLEX LX** **产品型号 Product Model Number: B90883**					
部件名称 Component Name	有毒有害物质或元素 Hazardous Substances Name					
	铅 (Pb)	汞 (Hg)	镉 (Cd)	六价铬 (Cr^{6+})	多溴联苯 (PBB)	多溴二苯醚 (PBDE)
印刷电路板组件 Circuit Boards	X	O	O	O	O	O
电源组件 Power Supplies	O	O	O	O	O	O
计算机 Computer	O	O	O	O	O	O
功率调节器 Power Conditioner	O	O	O	O	O	O
光量传感器 Optical Sensors	O	O	O	O	O	O
激光 Laser	X	O	O	O	O	O
发动机/泵/阀门/ Motors/Pumps/Valves	O	O	O	O	O	O
电线 Cables	X	O	O	O	O	O
管路及橡胶 Tubing & Rubber	O	O	O	O	O	O
塑料部件 Plastic	O	O	O	O	O	O
连接部件 Hardware	X	O	O	O	O	O
包装材料 Packing Material	O	O	O	O	O	O

This table is prepared in accordance with the provisions of SJ/T 11364

O：表示该有毒有害物质在该部件所有均质材料中的含量均在GB/T 26572标准规定的限量要求以下

x: 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出GB/T 26572标准规定的限量要求

（企业可在此处，根据实际情况对上表中打“×”的技术原因进行进一步说明）

O: Indicates that the toxic or hazardous substances contained in all of the homogenous materials for this part is below the limit requirements in GB/T 26572.

X: Indicates that the toxic or hazardous substance contained in at least one of the homogenous materials used for this part in above the limit requirement in GB/T 26572.

Table E.3 有毒有害物质名称及含量的标识格式 Table of Hazardous Substances Name and Concentration [CytoFLEX LX 355]

电子电气产品号码 **EEP Part Number: C06779**	**产品名称 Product Name: CytoFLEX LX 355** **产品型号 Product Model Number: C06779**					
部件名称 Component Name	有毒有害物质或元素 Hazardous Substances Name					
	铅 (Pb)	汞 (Hg)	镉 (Cd)	六价铬 (Cr^{6+})	多溴联苯 (PBB)	多溴二苯醚 (PBDE)
印刷电路板组件 Circuit Boards	X	O	O	O	O	O
电源组件 Power Supplies	O	O	O	O	O	O
计算机 Computer	O	O	O	O	O	O
功率调节器 Power Conditioner	O	O	O	O	O	O
光量传感器 Optical Sensors	O	O	O	O	O	O
激光 Laser	X	O	O	O	O	O
发动机/泵/阀门/ Motors/Pumps/Valves	O	O	O	O	O	O
电线 Cables	X	O	O	O	O	O
管路及橡胶 Tubing & Rubber	O	O	O	O	O	O
塑料部件 Plastic	O	O	O	O	O	O
连接部件 Hardware	X	O	O	O	O	O
包装材料 Packing Material	O	O	O	O	O	O

This table is prepared in accordance with the provisions of SJ/T 11364

○：表示该有毒有害物质在该部件所有均质材料中的含量均在GB/T 26572标准规定的限量要求以下

x: 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出GB/T 26572标准规定的限量要求

（企业可在此处，根据实际情况对上表中打“×”的技术原因进行进一步说明）

O: Indicates that the toxic or hazardous substances contained in all of the homogenous materials for this part is below the limit requirements in GB/T 26572.

X: Indicates that the toxic or hazardous substance contained in at least one of the homogenous materials used for this part in above the limit requirement in GB/T 26572.

Abbreviations

The following list is a composite of the symbols, abbreviations, acronyms, and reference designators either used in this manual or related to the information in it. When the same abbreviation (or reference designator) is used for more than one word (or type of component), all meanings relevant to this manual are included, separated by semicolons.

' — foot

“ — inch

% — percent

°C — degrees Celsius

°F — degrees Fahrenheit

± — plus or minus

< — less than

> — greater than

≤ — less than or equal to

µ — micron

µL — microliters

µm — micrometer

A — ampere

AC — alternating current

APC — Allophycocyanin

APC-A700 — Allophycocyanin-Alexa Fluor™ 700 tandem dye

APC-A7500 — Allophycocyanin-Alexa Fluor™ 750

APC-Cy7 — Allophycocyanin-Cyanin 7

API — Application Programming Interface

Acq. — Acquisition

BCI — Beckman Coulter Incorporated

BMP — bitmap

BP — band-pass filter

CDRH — Center for Devices and Radiological Health

CFSE — carboxyfluorescein succinmidyl ester

cm — centimeters

CSV — comma separated value

CV — coefficient of variation

DNA — deoxyribonucleic acid

DW — deep well

ECD — Energy Coupled Dye

EFUP — Environmentally friendly Use Period

EMF — enhanced metafile format

EMR — electromagnetic radiation

FAPD — Fiber Array Photo Detector

FCS — flow cytometry standard

FITC — Fluorescein isothiocyanate

FSC — forward scatter

GB — gigabyte

GHz — gigahertz

Gr Wt — gross weight

H — humidity

Hz — hertz

IEC — International Electrotechnical Commission

IR — infrared

kg — kilograms

KO — Krome Orange

LED — light emitting diode

L — liter

LJ — Levey-Jennings

LWH — length, width, height

m — meter

MB — megabyte

MFI — median fluorescence intensity

MHz — megahertz

min — minute

mL — milliliter

mm — millimeter

mW — milliwatt

NA — numerical aperture

NaClO — sodium hypochlorite solution

NaN_3 — sodium azide

nm — nanometer

Nt Wt — net weight

PB — Pacific Blue™ dye

PC5 — Phycoerythrin-Cy™5 tandem dye

PC5.5 — Phycoerythrin-Cy™5.5 tandem dye

PC7 — Phycoerythrin-Cy™7 tandem dye

PE — Phycoerythrin

PEEK — polyether ether ketone

PerCP — Peridinin-Chlorophyll

PI — Propidium Iodide

PN — part number

QC — quality control

RAM — random access memory

rCV — robust coefficient of variation

RH — relative humidity

RoHS — Restriction of Hazardous Substances Directive

RPTM — real-time messaging protocol

S/N — serial number

SNR — signal to noise ratio

SSC — side scatter

USB — universal serial bus

UV — ultraviolet

V — volts

VA — volt-ampere

VAC — voltage alternating current

VSSC — violet side scatter

WDM — wavelength division multiplexer

W — watts

Index

Symbols

Numerics

A

B

C

D

E

F

M

N

O

P

Q

R

S

T

U

V

W

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Related Documents

Operating Instructions
PN B49006

- Introduction
- System Overview
- Using the CytExpert Software
- Daily Startup
- Instrument Quality Control and Standardization
- Data Acquisition and Sample Analysis
- Compensation
- Data Review
- Daily Shutdown
- Troubleshooting
- Cleaning Procedures
- Replacement/Adjustment Procedures
- Instrument Installation
- CytExpert Electronic Record Management
- Sample Injection Mode Control Kit
- Table of Hazardous Substances
- Abbreviations

CytoFLEX Setup Guide
PN B53767

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