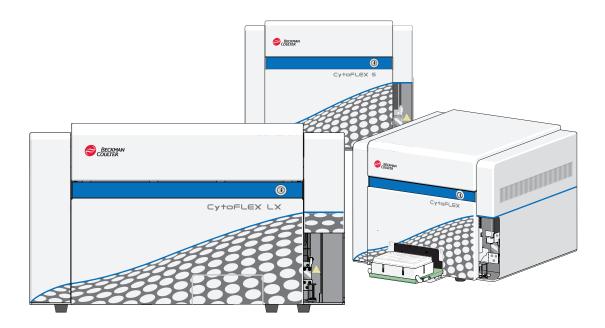
Instructions for Use

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)

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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

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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

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

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

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

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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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]

Issue AL, 11/2017 Software Version 2.2 Safety Notices Symbol Explanations Introduction **Conventions Used** CHAPTER 1, System Overview **Product Description** Sample Station Plate Loader Components **Instrument Specifications** Performance Characteristics CHAPTER 2, Using the CytExpert Software Start Page Software Menu Advanced Menu Plate Type Library CHAPTER 3, Daily Startup Selecting the Plate Loader Sample Injection Mode [With Plate Loader] Running the System Startup Program [with the Single Tube Loader] Running the System Startup Program [With Plate Loader] CHAPTER 4, Instrument Quality Control and Standardization Preparing the QC Sample [With Plate Loader] Collecting QC Data [With Plate Loader] **Confirming Results** CHAPTER 9, Troubleshooting Plate Loader Hazard Labels and Location **Troubleshooting Table CHAPTER 10, Cleaning Procedures** Surface Cleaning and Disinfection CHAPTER 11, Replacement/Adjustment Procedures Managing the Maintenance Reminder **Replacing the Sheath Fluid Filter** Replacing the Plate Holder [With Plate Loader] Calibrating the Plate Position [With Plate Loader] **APPENDIX A, Instrument Installation Power Source**

APPENDIX C, Sample Injection Mode Control Kit

Performance Characteristics [With the Sample Injection Mode Control Knob]

APPENDIX D, Deep Well Plate

Specimen Collection Plate Specifications

Revision History

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

A 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 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.

<u>/</u> 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.

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.

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
4	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
(6	CE = Conformité Européenne
CE	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
50	hazardous substances or elements, and can be used safely during its environmental
制造。日期 / M/g. Date	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.
\sim	Date of Manufacture indicates the date when the medical device was manufactured.
GLASS I LASER PRODUCT COMPLES WITH 21 CPT 100 0.NID 100.11 EXCEPT FOR EVANIONS PURSUANT TO LASER NOTICE NO. 50 04TED JUNE 24, 2007 MANUFACTURED [] LASEL PRI D02520AC BROKING GOBIER, INC. MARE RULS A MARCANES	Laser Compliance
LISE ROUTION CARE PROJECT OF COME CARE BY CARE BE A BY CARE IN REACH AND A CARE AND A CA	Class 3B Laser Caution, do not stare into the beam.
CLASS 1 LASER PRODUCT PRODUIT LASER CLASE 1 1类激光产品	Class 1 Laser Caution
BAR STREAM ST	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.

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Introduction

Overview

This introduction contains the following information:

- How to Use Your Manual
- About this Manual
- Conventions Used
- Graphics

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.

Introduction Graphics

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
- Main Components
- Optical Components
- Fluidics System
- Sample Station
- Plate Loader Components
- System Configuration
- Consumables and Supplies
- Instrument Specifications
- Performance Characteristics
- Reagent Limitations

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

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.

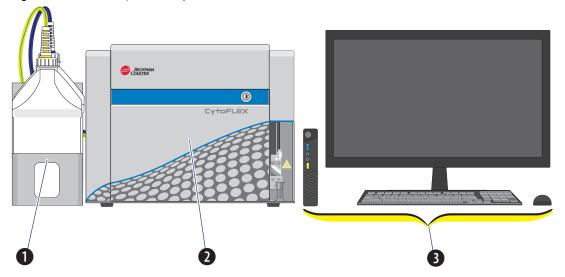
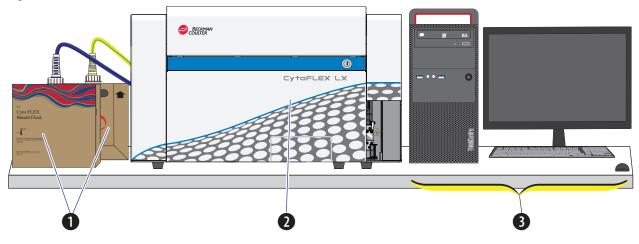


Figure 1.1 Main Components [CytoFLEX Without Plate Loader]

- 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.

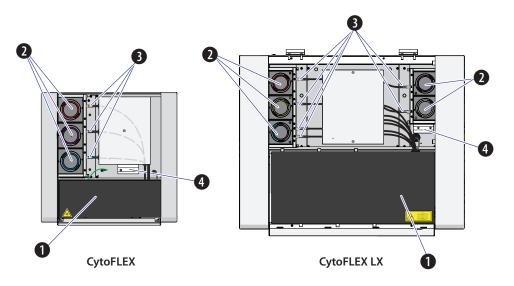
- 2. Cytometer. Provides signal generation and collection.
- **3.** Workstation. Displays and manipulates the contents of the Workstation and displays data generated by the Cytometer.

Optical Components

A 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.

<u>A</u> CAUTION

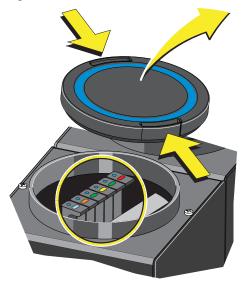
Risk of instrument damage. Do not place sample tubes in the optical filter holder. Liquid spills can damage instrument components. Use a tube rack to hold any sample tubes.

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

Wavelength Division Multiplexer (WDM)

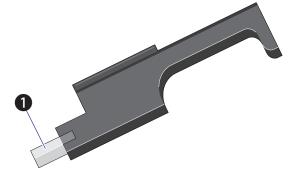
Each WDM corresponds to a different laser, or in some cases two lasers. The color of the ring on each cap corresponds to the color of the respective laser. Pressing the two release buttons on opposite edges of the cap allows you to open the WDM and replace the filters inside. See Figure 1.3. All optical 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



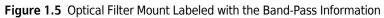
Each optical filter mount has an optical filter glass piece. See Figure 1.4.

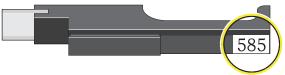
Figure 1.4 Optical Filter Mount with Optical Filter



1. Optical filter glass piece

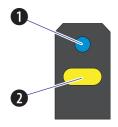
Each optical filter mount is labeled with the corresponding laser and band-pass information. See Figure 1.5.





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.





- 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.

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

 Table 1.1
 WDM Optical Filter Mount Color Codes [CytoFLEX]

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

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, Pl
	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

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

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

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

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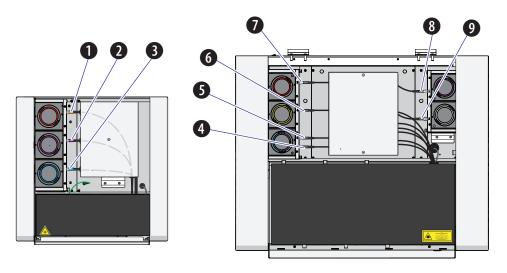
Optical Fiber

Risk of data integrity damage.

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

Fluorescence emitted by laser-excited fluorochromes is picked up and delivered by each optical fiber to the corresponding detector module. Each optical fiber has 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 LX



CytoFLEX

- 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]

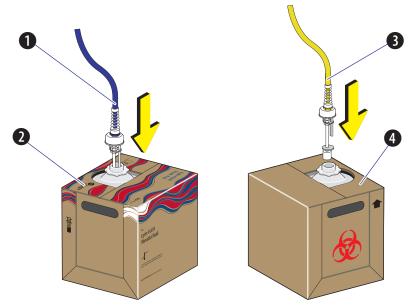
- 1. Fluid Container holder. Holds the Fluid Containers.
- 2. Fluid sensor holder cutout. Holds the sheath fluid harness and the waste harness when removed from their respective containers to protect the sensors from damage and/or contamination.
- **3. Sheath fluid harness.** Connects to the sheath fluid 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.
- 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.





- 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.

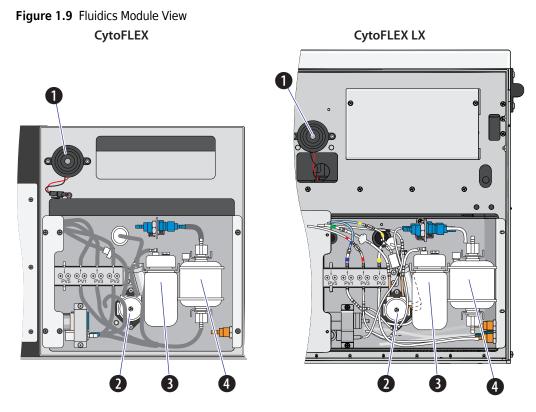
NOTE The waste cubitainer must be on the same level as the Cytometer.

Fluid Containers/Cubitainers

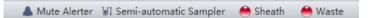
On the CytoFLEX, two Fluid Containers are placed in the Fluid Container holder: a sheath fluid container and a waste container. See Figure 1.7. On the CytoFLEX LX, two 10-L Fluid Cubitainers are placed to the left side of the instrument: a sheath fluid cubitainer and a waste cubitainer. Each container/cubitainer 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).

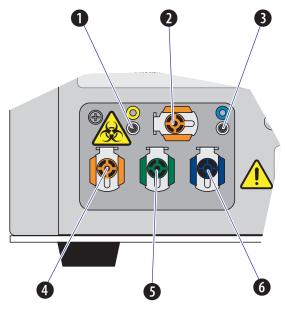


- 1. Alarm. Emits a warning sound when there is a problem with the Fluid Container/Cubitainer capacity or with the performance of certain operations.
 - **NOTE** When the alarm sounds, the Mute Alerter icon appears in the status bar. The alarm continues for about 30 seconds. To mute the alarm temporarily 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.

1

Sample Station

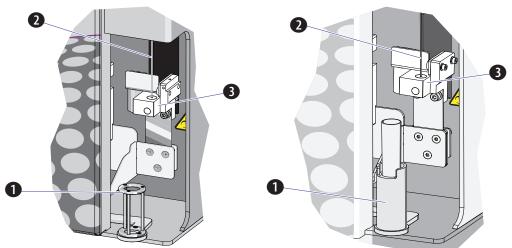




Risk of biohazardous contamination and/or instrument damage. When running samples, it is important to insert the sample tube all the way down into the sample tube holder, until the bottom of the sample tube touches the base of the holder. Failing to do this could cause the sample probe to bend or break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

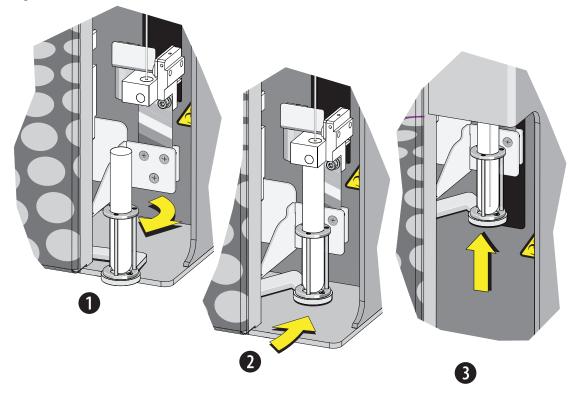


- **1. Sample tube holder**. Supports sample tubes for testing, such 12 x 75 mm, 1.5-mL, and 2-mL microtubes.
- 2. Sample probe. Draws and transfers samples into the flow cell.
- **3.** Wash station and mixer. During the sampling process, samples are automatically shaken and mixed for a default time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



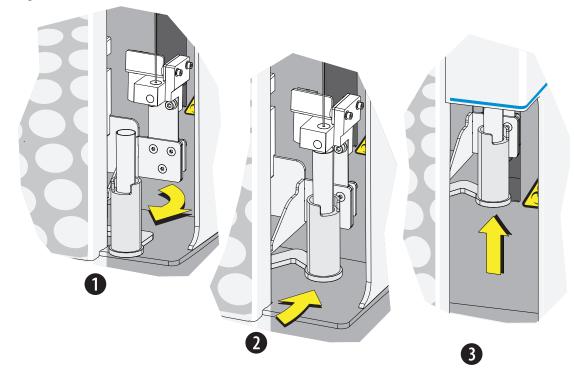
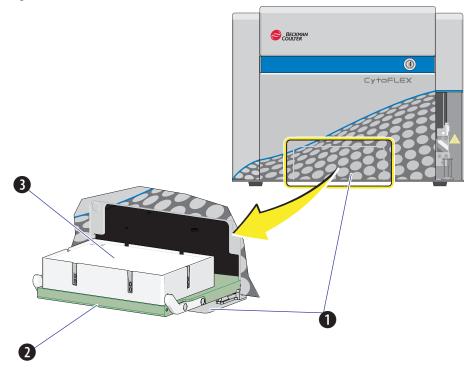


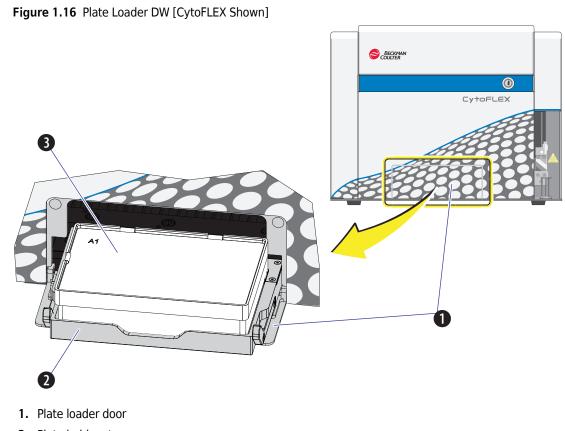
Figure 1.14 Sample Tube Holder Positions

Plate Loader Components





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



- 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.

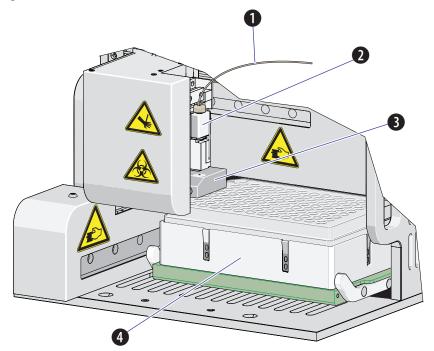


Figure 1.17 Standard Plate Loader (Front Cover Removed)

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

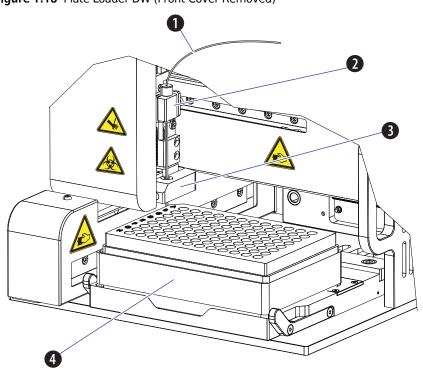


Figure 1.18 Plate Loader DW (Front Cover Removed)

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

Plate Holder Components

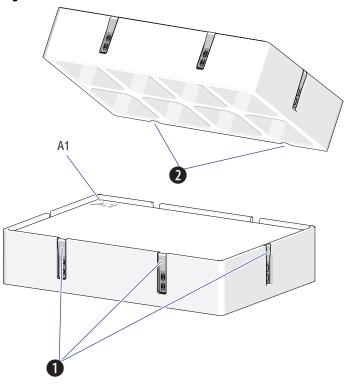


Figure 1.19 Standard Plate Holder without Groove (Standard Plate Loader)

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

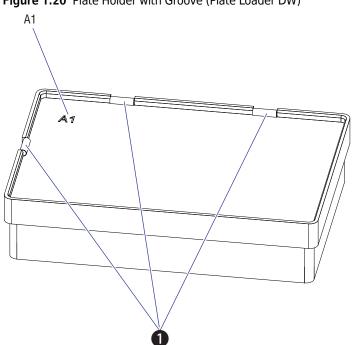
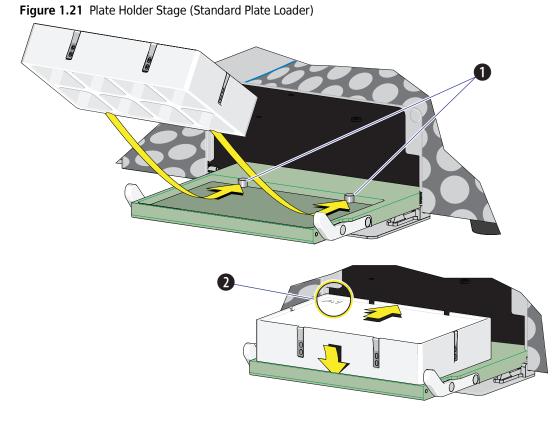


Figure 1.20 Plate Holder with Groove (Plate Loader DW)

- 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.

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- 1. Pins
- 2. Position A1

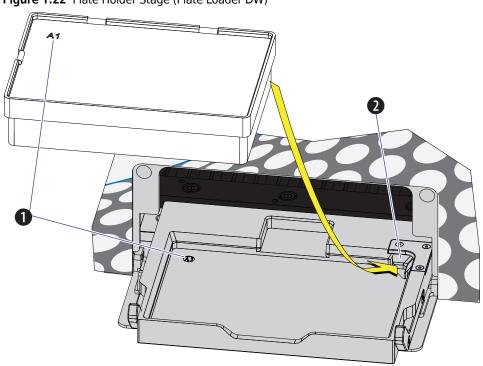


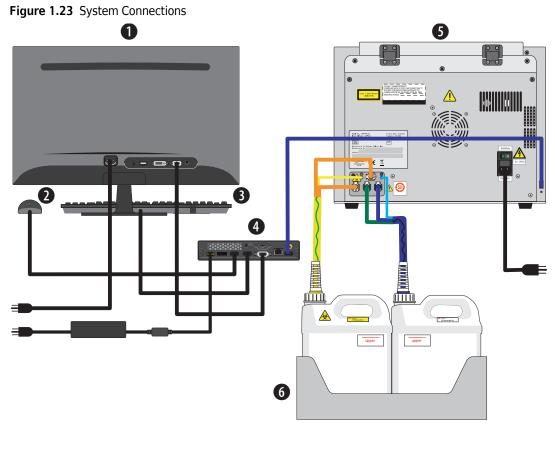
Figure 1.22 Plate Holder Stage (Plate Loader DW)

- 1. Position A1
- 2. Spring Lock

System Configuration

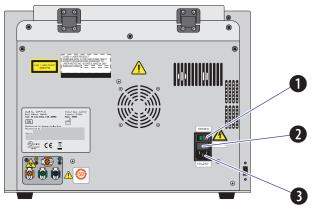
Risk of data loss and/or instrument damage. Never shut off the power or disconnect a data cable while the Cytometer is in the process of performing a task. This could cause data loss or damage to the system.

System Configuration [CytoFLEX and CytoFLEX S]



- 1. Monitor
- Computer
 Cytometer
- 2. Mouse
- 3. Keyboard
- 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]

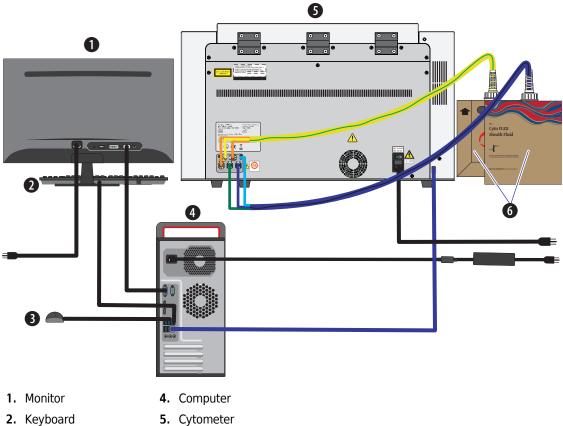
1. Load button. In addition to the software controls, this button can be used for automatic sample loading and data recording.

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

System Configuration [CytoFLEX LX]

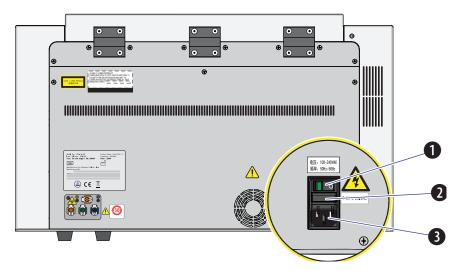
IMPORTANT The fluid cubitainers must be on the same level as the Cytometer. Do not place the fluid cubitainers on the floor.

Figure 1.26 System Connections



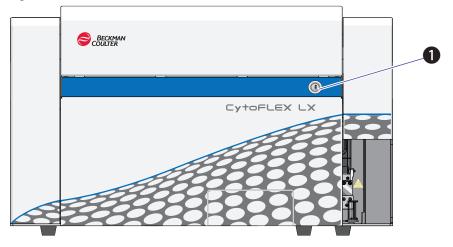
- 3. Mouse
- Cytometer
 Fluid Cubitainers
- **6.** Fluid

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 alignmen >1.3 NA.	t-free integrated optics quartz flow cell design with	
	Flow Cell dimensions: 430	0-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	Yellow laser	
	wavelengths [CytoFLEX S or	• Wavelength: 561 nm, 30 mW	
	CytoFLEX LX]	• 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 	
	Cilicon photodiada with h		
Forward scatter detection	Silicon photoaloae with b	uilt-in 488/8 band-pass filter.	

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	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.
Violet side scatter configuration (VSSC)	Reflective optics with a single transmission band-pass filter in front of each detector. 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	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.

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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	Operating system	Windows [®] 7, 8, 10 Professional 64-bit
Workstation/ computer	Processor	4th Gen Intel [®] Core™ i3 (3MB Cache, 2.90 GHz)
requirements	Memory	4 GB RAM
[CytoFLEX]	Storage	256 GB
	Port	1 GB Ethernet port
	USB	5 USB 2.0 and above ports
Minimum	Operating system	Windows®7, 8, 10 Professional 64-bit
Workstation/ computer requirements [CytoFLEX LX]	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 easildetermine the correct compensation matrix with new gain settings.	

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	rCV <3%	
resolution	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 \leq 5% for the Violet, Blue, Yellow, and Red lasers while the pass criteria is \leq 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	10 second acquisition without mixing or backflush: <32 min.		
Plate Loader] ^a	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 sec second backflush: <64 min.	cond acquisition with 10 second mixing and 4	

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

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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 \leq 5% for the Violet, Blue, Yellow, and Red lasers while the pass criteria is \leq 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	10 second acquisition without mixing or backflush: <34 min.10 second acquisition with 3 second mixing and 3 second backflush: < 47 min.		
Standard Plate Loader] ^a			

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			

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.

System Overview Reagent Limitations

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 of 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.

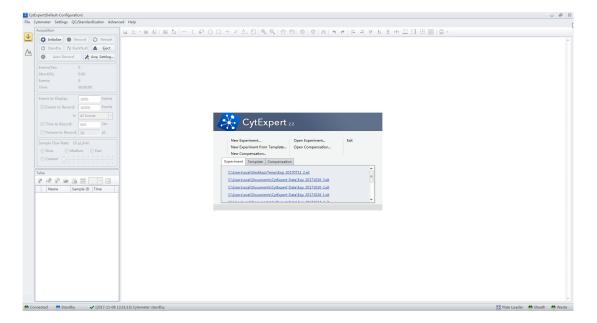
Refer to Logging Into the Software in CHAPTER 3, Daily Startup, for detailed instructions on opening the software and confirming the connection status.

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 Exp	eriment eriment fron mpensation	and a set a set of the set of the set	Open Experiment Open Compensation	Exit
xperiment	Template	Compensation		
Root\Exp Root\Exp	20171102 4.: 20171102 3.: 20171102 2.: 20171102 1.:	<u>kit</u>		

- **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

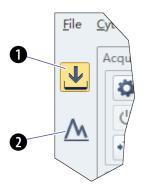
the Acquisition screen. The Acquisition screen can be accessed by selecting 🖄 on the left side of the page.

Cytometer Settings OC/Dandardication Advers	nd no	17 ¹ /16,14 1a − 1 P O D +	* 4 5 4 4 6 6	0 0 0 A •	- 12 - 2 - P	ь ÷ н Ш	11 H m 0	÷	0.8
Control of the second of	2 0 100 A10 1 0 1		50 (1 FBCA (115	a	form:	0 Tetal 0 0	10 Shared	0	

- 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

Star	ndby state	9	Initialized stat	е
cquisition			Acquisition	
🔯 Initialize 🔵 R	ecord O	Restart	Run Record	O Resta
🖒 Standby 🕅 Ba	ckflush 👔	Boost	U Standby 🕅 Backflush	🖞 Boos
+ Next Tube	Acq. Setting		🕕 Next Tube 🔀 Acq. Se	tting
Events/Sec; Abort(%):	0		Events/Sec: 0.0 Abort(%): 0.00	
Events:	0		Events: 0	
Time:	00:00:00		Time: 00:00:	00
Events to Display:	1000	Events	Events to Display: 1000	Even
Events to Record:	10000	Events	Events to Record: 1000	0 Even
in	All Events	-	in All Ev	ents
📝 Time to Record:	600	Sec	☑ Time to Record: 600	Sec
Volume to Record:	10	μί	Volume to Record: 10	μL
Custom	µL/min lium © Fas	4	4 Sample Flow Rate: 10 μL/mir	O Fast

- 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]

Stand	by state	initialized state
Acquisition		Acquisition
🔹 Initialize 🔵 Re	cord 🛛 🕄 Restart	t Run 🗨 Record 💭 Rest
🖒 Standby 🕅 Bad	kflush 🔺 Eject	🕐 Standby 👫 Backflush 🔺 Eje
Auto Record	🔀 Acq. Setting.	. Auto Record 🗶 Acq. Settin
Events/Sec:	0.0	Events/Sec: 0.0
Abort(%):	0.00	Abort(%): 0.00
Events:	0	Events: 0
Time:	00:00:00	Time: 00:00:00
Events to Display:	1000 Events	Events to Display: 1000 Eve
Events to Record:	10000 Events	Events to Record: 10000 Events
in	All Events -	in All Events
☑ Time to Record:	600 Sec	✓ Time to Record: 600 Sec
Volume to Record:	10 µL	□ Volume to Record: 10 µL
Sample Flow Rate: 10	µL/min	Sample Flow Rate: 10 µL/min
O Slow ○ Media	um 🔘 Fast	● Slow ○ Medium ○ Fast
Custom	1 1 1 1 1 1 1 1 1 1 1 1 1 1	4 © Custom

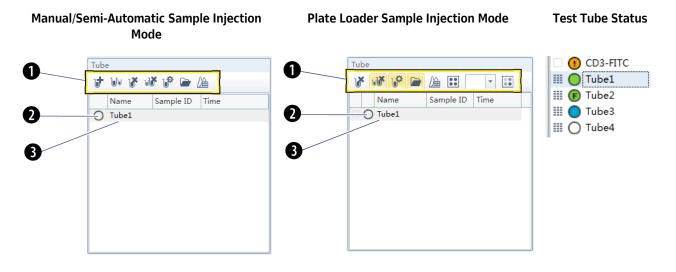
Standby state

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



- 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.
 - O indicates that the tube data was not collected.
 - Indicates that the tube data was acquired by selecting Run but can be overwritten.
 - O indicates that the tube data was saved by selecting **Record** or **Auto Record** and that this data cannot be overwritten.
 - (F) indicates imported FCS data.
 - **NOTE** iii to the left of the test tube status indication symbol indicates that the sample has been compensated.
 - . U 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

2 D 8 መወ -1 6 8 82 0 HH. Θ, (Th) 4 *** + 0 (401×) M • 41 N-OR Ð : ***** O Tube!

- 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.



- 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.

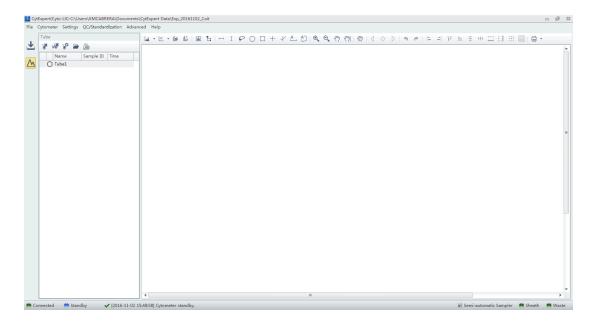
4. Sampler status. Displays the sample injection mode state. There are two sample injection modes: Semi-automatic sample injection mode and manual sample injection mode.

NOTE CytoFLEX Cytometers equipped with a plate loader have three sample injection modes: Semiautomatic 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.



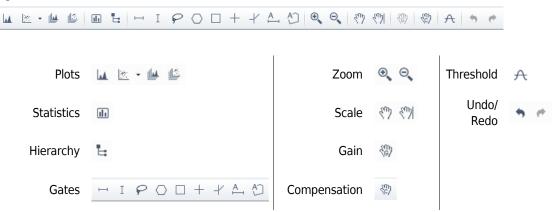
The Tube management module cannot add new sample tubes. Return to the Acquisition screen to add new sample tubes.

Semi-Automatic Sample Injection Mode Shown

Tube								
U†	Մա	ř	u č	ľ		Ď	Þ	
	Nan	ne		Sam	ple II)	Time	
C) Tub	e1						

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

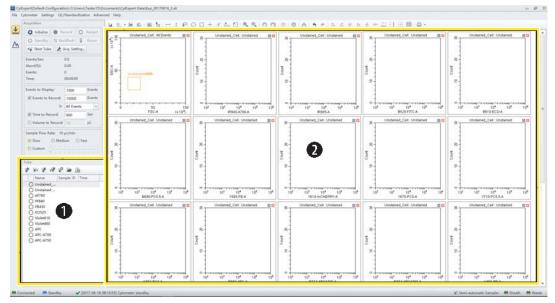
Figure 2.1 Drawing Controls Toolbar (Top of Screen)



Compensation Experiment Screen

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

Semi-Automatic Sample Injection Mode Shown

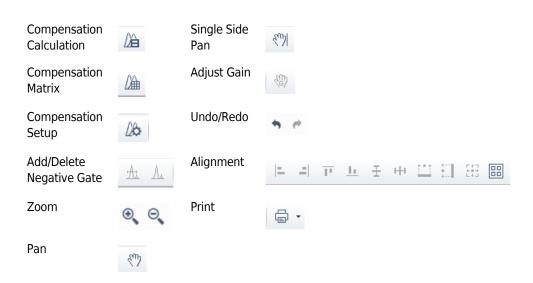


- 1. Tube management. Displays sample tubes required for the compensation experiment.
- 2. Plot area. Displays compensation plots and gating.

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.



QC Experiment Screen

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

QC Report Screen

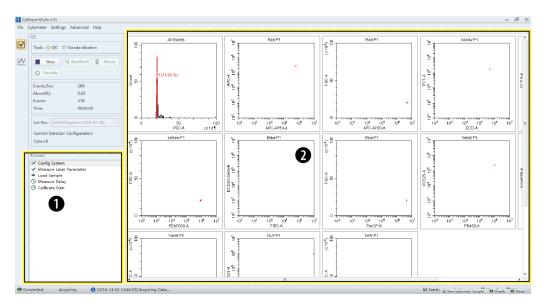
Before starting the QC routine, a Settings screen appears.

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

00	ß										
xper (Cyto-LX)											0
tometer Settings Advance, Help											
QC	PARG	ə •									-
	F eed										
Task: QC Stande dization	1			~							
🔯 Initialize				Qu	Report						
	Bead Lot N		AH02_SE								
🖒 Standby	Bead Lot N Bead Expir		AHU2_SE 019-07-20				DC Date:	2	016-11-0;	2 15:07	
	Cytometer		013-07-20				lytomete		010-11-0	2 13/07	
Events/Sec: 0.0		onfiguration: C	vto-LX			_	,				
Abort(%): 0.00	Loader Typ		emi Automatic		(R					
Events: 0	Threshold										
Time: 00:00:00											
		(Height) Mod	e: Manual Valu	e: 50000							
Lot No.:	Laser										
Current Detector Configuration:	Laser	Delay(µs)	Default Del		rence Delay(µs)			get Power		Result	
Cyto+LX	NUV		2.80	72.96	-0.1		60		50-70	0	
	Blue		0.00	0.00	0.0		49		40-60	0	
Report	Red Yellow		6.16 5.68	36.32	-0.1		49 30		40-60	0	
Detector Configuration:	Violet		0.24	-35.84	0.1		88		70-120	0	
			0.24	-70,40	0.1	·	00		70-120		
Cyto-LX -	Signal Value										
Date: 2016-10-03 × ~ 2016-11-02 ×	Parameter	Gain Targ Gain	et %Difference Target Gain	Median	Target Median	%Difference Target Median		Target rCV(%)	Width	Result	
Process Date Lot No. Result	FSC	252 25	2 0.00	206408.8	209181.4	-1.33			802.0		
2016-11-02 15:02 BAH02 🚫 ~	SSC		1 0.00	604528.9		3.17			883.2		
2016-11-02 15:05 BAH02_SE	PB450		6 0.00	910944.4		-1.05	2.13	5.00	1316.2		
2016-11-02 15:07 BAH02_SE	KO525		4 0.00	234576.1		0.43	2.03	5.00	1315.0		
2016-11-02 15:17 BAH02 Ø	Violet610	202 20		413936.1		-0.55	2.23	5.00	1318.8		
	Violet660	185 18		108776.3		-0.15	2.32	5.00	1312.5		
U	Violet780		2 0.00	63120.5		0.43	2.03	5.00	1294.6		
-	DAPI NUV525		9 0.00	8750280.0		-0.61	0.80	7.00	1604.2		
			1 0.00 0 0.00	951872.7 1364065.0		1.53	0.78	7.00	802.9 820.7		
				1304005.0	1001/15.0						
	HoechstRed	160 16		226824.1	2281425						
		341 34 329 32	1 0.00	326824.1 5459777.0		-0.40	3.55	5.00	1063.2		

- 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

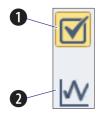


When acquiring QC samples, the software opens the QC screen.

- 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*

File	Cytometer		Settings		QC/Standardization	Advanced	Account ⁺⁺	Logtt	Signature***	Backup/Restore††	Help
					Start			Experiment	Ĭ		
New Experiment	Acq.Setting		Set Channel		QC/Standardization	Delay Setting	User Manager††	Operation Log***	Sign***	Backup††	View Help Fi
New Experiment									Electronic		
From Template								System	Signature		
	Detector Configu	ration	Set Label			Laser Setting	Role Manager††	Operation Log***	Details***	Restore††	About
New Compensation								User Management			
	Backflush		Set Customi	zed Parameter		Maintenance	Account Policies**	Operation Log††		Log Cleanup††	
Open Experiment	Boost† Compensation Matrix				Event Rate Setting	Change Password††					
Open Compensation	Initialize		Compensation Library			Plate Type Library	ŧ				
Save	Standby		Events Disp	lay Setting							
Save As	Prime		Set Experim	ent Directory***							
Save As Template	Deep Clean		Language Setting								
Import FCS File	Calibrate Sample	Flow Rate									
Export FCS File	System Startup P	rogram	Options	Experiment##							
Recent	Daily Clean			Tube							
Recent Template	Sample Injection	Manual		Plot							
Recent Compensation	Mode	Semi Automatic		Gate							
Close Experiment	Wode	Plate Loader**		Page Setup							
Experiment Explorer**	Sampler Reset			Plate Loader							
Exit	Turn On†††										
	Turn Off†††	Turn Off†††									
	Acq.Setting Catal	og									
	Cytometer Confi	guration									
	Cytometer Inform	nation									

* 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

File	Cytometer		Settings		Advanced	Account‡‡	Log#	Signature**	Backup/Restore‡‡	Help
							Experiment			
New Experiment			QC/Standar	dization Setting	Delay Setting	User Manager‡‡	Operation Log**	Sign**	Backup‡‡	View Help Fil
New Experiment								Electronic		
From Template							System	Signature		
	Detector Configu	ration	Target Libra	ry	Laser Setting	Role Manager‡‡	Operation Log**	Details**	Restore##	About
New Compensation							User			
							Management			
	Backflush		Standardiza	tion Target Libra	Maintenance	Account Policies##	Operation Log‡‡		Log Cleanup‡‡	
Open Experiment	Boost*					Change Password	ŧ			
Open Compensation			Language Se	etting	Plate Type Librar	y†				
Recent	Standby		Options	Experiment††						
Recent Template	Prime			Tube						
Recent Compensatio	Deep Clean			Plot						
Close										
QC/Standardization										
	Calibrate Sample	Flow Rate		Gate						
Experiment										
Explorer**	System Startup P	rogram		Page Setup						
Exit	Daily Clean			Plate Loader‡						
	Sample	Manual								
	Injection Mode	Semi Automat	ic							
	F	Plate Loader								
	Sampler Reset									
	Turn On***									
	Turn Off***									
	Acq.Setting Catal	log								
	Cytometer Config	guration								
	Cytometer Inforn	nation								

* **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

File Cytometer Settings QC/Standardization Advanced Help

CytExpert User Management Software Option

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

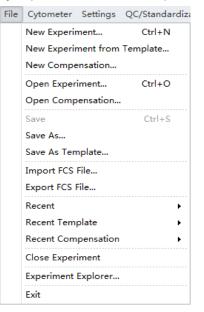
CytExpert Electronic Record Management Software Option

File Cytometer Settings QC/Standardization Advanced Account Log Signature Backup/Restore Help

File Menu

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

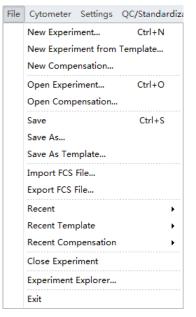
CytExpert Default Software Option



CytExpert User Management Software Option

File Cytometer Settings QC/Standardiza Ctrl+N New Experiment... New Experiment from Template... New Compensation... Open Experiment... Ctrl+O Open Compensation... Ctrl+S Save Save As... Save As Template... Import FCS File... Export FCS File... Recent • Recent Template ۲ Recent Compensation ۲ Close Experiment Exit

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 -

Initialized state

CytExpert Default Software Option -Standby state

Cytor	neter	Settings	QC/Standardizati
×	Acq. 9	Setting	
	Detec	tor Config	guration
(*_b)	Backf	lush	
Û	Boost	t	
Ø.	Initiali	ize	
Ċ	Stand	by	
	Prime		
	Deep	Clean	
	Calibr	rate Samp	le Flow Rate
	Syster	m Startup	Program
	Daily	Clean	
	Samp	le Injectio	n Mode 🛛 🕨
	Samp	ler Reset	
	Turn (On	
	Turn (Off	
	Acq. 9	Setting Ca	talog
	Cyton	neter Conf	figuration
	Cyton	neter Info	rmation

CytExpert User Management Software Option -Standby state

CytExpert User Management Software Option -Initialized state

	Acq. Setting	🔀 Acq. Setting	Acq. Setting		
	Detector Configuration	Detector Configuration			
	Backflush	🕯 Backflush			
Γ	Boost	🔯 Initialize			
Ł	Initialize	也 Standby			
)	Standby	Prime			
	Prime	Deep Clean	Deep Clean		
	Deep Clean	Calibrate Sample Flow Rate			
	Calibrate Sample Flow Rate	System Startup Program	System Startup Program Daily Clean Sample Injection Mode Sampler Reset Turn On		
	System Startup Program	Daily Clean			
	Daily Clean	Sample Injection Mode			
	Sample Injection Mode	Sampler Reset			
	Sampler Reset	Turn On			
	Turn On	Turn Off			
	Turn Off	Acq. Setting Catalog			
	Acq. Setting Catalog	Cytometer Configuration	Cytometer Configuration Cytometer Information		
	Cytometer Configuration	Cytometer Information			

CytExpert Electronic Record Management Software Option - Standby state

CytExpert Electronic Record Management Software Option - Initialized state

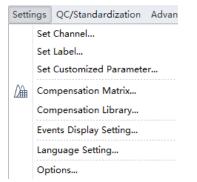
Cyto	meter Settings QC/Standardizat	Cyte	ometer Settings QC/Standardizat
×	Acq. Setting	×	Acq. Setting
	Detector Configuration		Detector Configuration
⁰	Backflush	**)	Backflush
Û	Boost	Û.	Boost
•	Initialize	φ.	Initialize
Ċ	Standby		Standby
	Prime		Prime
	Deep Clean		Deep Clean
	Calibrate Sample Flow Rate		Calibrate Sample Flow Rate
	System Startup Program		System Startup Program
	Daily Clean		Daily Clean
	Sample Injection Mode		Sample Injection Mode
	Sampler Reset		Sampler Reset
	Turn On		Turn On
	Turn Off		Turn Off
	Acq. Setting Catalog		Acq. Setting Catalog
	Cytometer Configuration		Cytometer Configuration
	Cytometer Information		Cytometer Information

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

Setti	ngs	QC/Standardization	Advan						
	Set	Channel							
	Set Label								
	Set Customized Parameter								
<i>D</i> `≙	Compensation Matrix								
	Compensation Library								
	Events Display Setting								
	Language Setting								
	Ор	Options							

CytExpert Electronic Record Management Software Option

Setti	ngs	QC/Standardization	Advan	
	Set	Set Channel		
	Set Label			
	Set Customized Paramet		r	
<i>[</i>]	Cor	mpensation Matrix		
	Cor	mpensation Library		
	Eve	nts Display Setting		
	Lan	guage Setting		
	Set	Experiment Directory.		
	Op	tions		

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

Advanced	Help
Delay	Setting
Laser	Setting
Maint	enance
Event	Rate Setting

٩dv	anced Help
	Delay Setting
	Laser Setting
	Maintenance
	Event Rate Setting
	Plate Type Library

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

Advan	ced	Account	Log
C	Delay	Setting	
L	aser	Setting	
N	Maint	enance	
E	vent	Rate Settir	ng

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

Adv	anced	Account	Log	S
	Delay	Setting		
	Laser	Setting		
	Maint	enance		
	Event Rate Setting		ıg	

CytExpert User Management Software Option -Plate Loader Sample Injection Mode

Advanced		Account	Log
	Delay	Setting	
	Laser	Setting	
	Maint	enance	
	Event	Rate Settir	ıg
	Plate	Type Libra	ry

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

Advanced	Account Log
Delay	Setting
Laser	Setting
Maint	enance
Event	Rate Setting
Plate	Type Library

Account Menu

Used to for user account management settings.

CytExpert User Management Software Option

Account Log Backup/Rest User Manager... Role Manager... Account Policies... Change Password...

CytExpert Electronic Record Management Software Option

ount	Log	Signature	B
User	Man	ager	
Role	Mana	ager	
Acco	ount P	olicies	
Char	nge Pa	assword	
	User Role Acco	User Man Role Man Account P	Log Signature User Manager Role Manager Account Policies Change Password

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

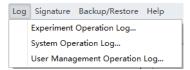
Log Menu

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

CytExpert User Management Software Option

```
Log Backup/Restore Help
User Management Operation Log...
```

CytExpert Electronic Record Management Software Option

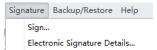


NOTE The Log menu is not available in the CytExpert Default software option.

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

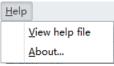
Used to backup/restore databases. Refer to Backup and Restore in CHAPTER 9, Troubleshooting.

B	ackup/Restore	lelp
	Backup	
	Restore	
1	Log Cleanup	

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.



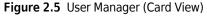
NOTE The Help menu is the same for the CytExpert Default, CytExpert User Management, and CytExpert Electronic Record Management software options.

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.



User [1 of 2]	User [2 of 2]	
Username: Admin	Username: Sarah	
Full Name: Administrator Enabled: Yes	Full Name: Sarah L. Enabled: Ves	
Unlocked: Yes	Unlocked: Ves	
Role: Administrator	Role: Operator	
Creation Time:	Creation Time: 2016-11-09 07:37:01	
Last Login Time: 2016-11-09 07:29:05	Last Login Time:	
	New Modify Delete Unlock Reset Pass	word Close

Figure 2.6 User Manager (Grid View)

Administrator Administrator Constraint C		Photo	Username	Full Name	Role	Creation Time	Last Login Time	Enabled	Unlocked
Ω Sarah Sarah L Operator 2016-11-09 07:37:01 Ves Ves	A Sarah Sarah L Operator 2016-11-09 07:37:01 Ves Ves	<u>L</u> .	Admin	Administrator	Administrator		2016-11-09 07:29:05	Yes	Yes
		L	Sarah	Sarah L.	Operator	2016-11-09 07:37:01		Yes	Yes

- 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.

		23
Username:		
Full Name:		
Password:	password	
Role:	Operator	-
Enabled:		
	OK Cance	
	Full Name: Password: Role:	Full Name: Password: Role: Dperator Enabled: Image: Image: Description: Descri

- **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.

Modify			23
	Username:	Sarah	
	Full Name:	Sarah L.	
25	Role:	Operator	-
	Enabled:		
		OK Cancel	

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.

Change Password	23
Username:	Admin
Old Password:	
New Password:	
Confirm New Password:	
	OK Cancel

- **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

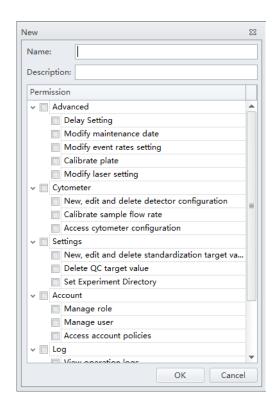
Administrator Operator	Administrator Operator	v √ Advanced	
Operator	Operator		
		✓ Delay Setting	
		✓ Modify maintenance date	
		✓ Modify event rates setting	
		✓ Calibrate plate	
		✓ Modify laser setting	
		✓ ✓ Cytometer	
		✓ New, edit and delete detector configuration	
		✓ Calibrate sample flow rate	=
		✓ Access cytometer configuration	
		✓ ✓ Settings	
		 New, edit and delete standardization target value 	
		✓ Delete QC target value	
		Set Experiment Directory	
		V V Account	
		✓ Manage role	
		✓ Manage user	
		✓ Access account policies	
		v √ Log	
		✓ View operation logs	-
		New Modify Delete Close	\langle

- 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 $\bigcirc \mathsf{K}$. 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 <a>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.

odify			2
Name		Quality Control	
Descr	iption:	Quality Control	
Perm	ission		
~ 🗖	Advan	ced	-
	V De	lay Setting	
	V Mo	odify maintenance date	
	✓ Mo	odify event rates setting	
	🗸 Ca	ibrate plate	
	🔲 Mo	odify laser setting	
~ V	Cytom	neter	
	✓ Ne	w, edit and delete detector configuration	
	✓ Ca	ibrate sample flow rate	=
	Acc	cess cytometer configuration	
¥ 📃	Setting	gs	
	✓ Ne	w, edit and delete standardization target va	
	De	lete QC target value	
	Set	Experiment Directory	
× 目	Accou	nt	
	Ma	inage role	
	_	inage user	
	_	cess account policies	
v 🗸			
	Vie Vie	w operation logs	Ŧ
		OK Cancel	

2 Modify the role information as necessary.

З Select ок

|--|

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

Account Policies	23
Password Expiration Policy	
✓ Enforce password expiration	
Password expires in 90 days	
Reminder for expiration before 15 days	
Account Lockout Policy	
☑ Enforce account lockout	
Invalid login attempts: 3 times	
Lockout time: 30 minutes	
OK Cance	

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.

-						
Query	Criteria					
Jser:	(ALL)					
lime B	ange: 2016-10-09 00:00:00	· ~ 2016-11-0	9 23:59:59 -			
Que	ry				Print & Exp	00
¢	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.	
	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...** .

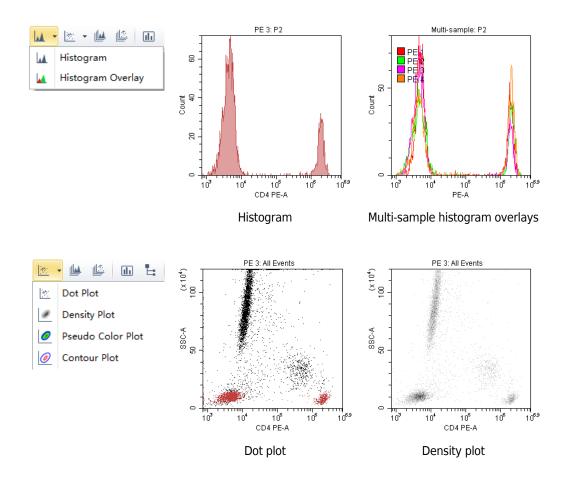
NOTE User logs are exported as a .pdf or .csv file.

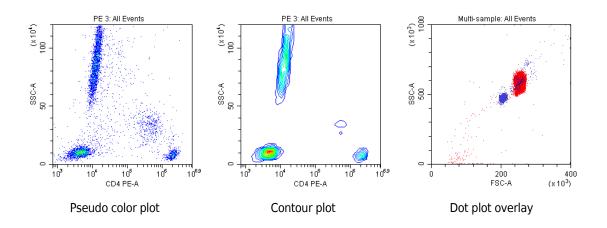
Graphic and Gating Styles

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.



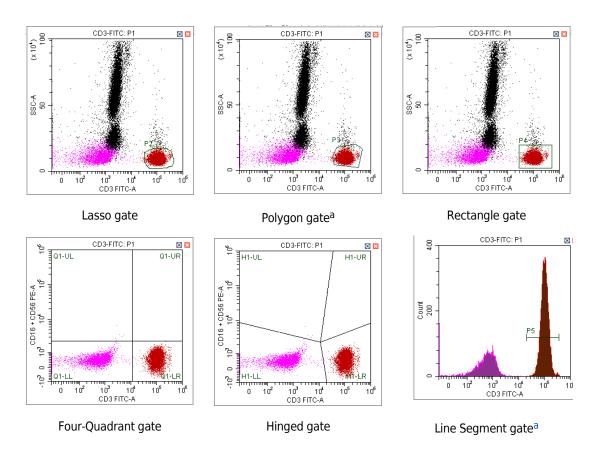


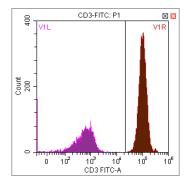
Gates

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





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.

ctive	Name	Mixing Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard		0
1	96-well V-bottom	Standard		0
1	96-well U-bottom	Standard		0
1	96-well deep well	Deep Well	-	0

Figure 2.9 Plate Type Library

- 1. Active. Indicates that the plate type is available 2. Default. Applies the default settings. for use.
- 3. Add. Creates a new plate.
- 5. Duplicate. Duplicates a plate.
- 7. OK. Saves the plate type.

- 4. Edit. Edits a plate.
- 6. Delete. Deletes an existing plate.
- 8. Cancel. Cancels the settings.

Adding a Plate Type

- Select U Standby
- 2 Select Advanced > Plate Type Library. The Plate Type Library window appears.

Active	Name	Mixing Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard	-	0
1	96-well V-bottom	Standard	-	0
1	96-well U-bottom	Standard	-	0
1	96-well deep well	Deep Well	-	0

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

Add Plate Type				Σ	3
Name:]
Mixing Mode:	Standard		Ŧ		
V Mix:	5	sec			
Remarks:					
]
Last Calibration Time:	-				
Calibrate			ОК	Cancel]

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

Add Plate Type		23
Name:	Plate type 1	
Mixing Mode:	Standard	-
I Mix:	5 sec	
Remarks:		
Last Calibration Time:		
Last Calibration Time:	-	
Calibrate		OK Cancel

5 Select the mixing mode.

Add Plate Type		Σ	3
Name:	Plate type 1		
Mixing Mode:	Standard	*	
Mix:	Standard Deep Well		
Remarks:			
		*	
Last Calibration Tim	e: -		
Calibrate		OK Cancel	

6 Enter the Mix time.

Add Plate Type		23
Name:	Plate type 1	
Mixing Mode:	Standard	Ŧ
Mix:	5 sec	
Remarks:		
		A
		Y
Last Calibration Time:	-	
Calibrate		OK Cancel

- **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.

Active	Name	Mixing Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard		0
\checkmark	96-well V-bottom	Standard		0
\checkmark	96-well U-bottom	Standard	-	0
1	96-well deep well	Deep Well	-	0
1	Plate type 1	Standard	2017-10-26 10:41:52	0

10 Select **ок**.

Editing a Plate Type

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

Active	Name	Mixing Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard	-	0
1	96-well V-bottom	Standard	-	0
1	96-well U-bottom	Standard	-	0
1	96-well deep well	Deep Well	-	0

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

Edit Plate Type			23
Name:	96-well V-bot	ttom	
Mixing Mode:	Standard	~	
Mix:	4	sec	
Remark:			
Last Calibration Time:			Y
Last Calibration Time:	-		
Calibrate		ОК	Cancel

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

ctive	Name	Mixing Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard	-	0
1	96-well V-bottom	Standard	-	0
1	96-well U-bottom	Standard	-	0
1	96-well deep well	Deep Well	-	0

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.

Duplicate Plate Type fro	m 96-well V-bottom		23
Name:	96-well V-bottom-Cop	у	
Mixing Mode:	Standard -		
V Mix:	4	sec	
Remark:			
			-
Last Calibration Time:	2017-09-07 13:21:21		
Calibrate		OK Cancel	

- **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.

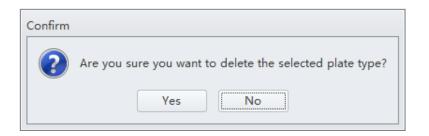
6 Select OK.

Deleting a Plate Type

Active	Name	Mixing Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard	-	0
1	96-well V-bottom	Standard	-	0
1	96-well U-bottom	Standard	-	0
1	96 deep well plate	Deep Well	-	0
1	Plate type 1	Deep Well	2017-10-11 15:28:15	0

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.

Options	5	23
Experiment	Default Path	
Tube	C:\Users\KMCABRERA\Documents\CytExpert Data	
Plot	Default	
Gate		1
Page Setup		
Plate Loader		
	OK Cancel	

)ptions		23
Experiment Tube Plot Gate	Select Columns Select the columns that will appear on the tube list. Sample ID Time Events	
Page Setup Plate Loader	Sample ID Time	
	ОК	Cancel

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.

Options		23
Experiment	Background	
Tube	Plot Background Show Grid ·	
Plot	Figure Background	
Gate	Histogram	
Page Setup	Plot Mode: O Border O Fill O Border and Fill	
Plate Loader	Opacity: 50 %	_
	Signal	
	Main Channel: 🔘 Height 🛛 💿 Area	
	Axis Default Scale	
	FSC/SSC: Min 100 Max 1000000	
	Fluorescence: Min 100 Max 1000000	
	Time: Min 0 Max 120000	
	Width: Min 0 Max 4096	
	OK Car	icel

Options		23
Experiment	Display Options	
Tube	☑ Display population percentage on all plots except overlay.	
Plot		
Gate		
Page Setup		
Plate Loader		
		-
	OK Canc	el

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.

Options	X
Experiment	Paper
Tube	Size: A4: 210mm X 297mm -
Plot	Orientation Margins(millimeters)
Gate	Portrait Left: 5.08 Right: 5.08
Page Setup	C Landscape Top: 5.08 Bottom: 5.08
Plate Loader	Display Options
	Show page breaks
	The above parameters will be applied as the default page settings for new experiment.
	OK Cancel

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

Options		23
Tube	Plate Default Setting	
Plot	Sampling Sequence	
Gate	$\textcircled{\tiny{}} \textcircled{\tiny{}} \rightrightarrows \textcircled{} \bigcirc \overrightarrow{\rightrightarrows} \textcircled{} \bigcirc \operatornamewithlimits{} \biguplus \fbox \textcircled{} \bigcirc \operatornamewithlimits{} \o \textcircled{} \bigcirc \operatornamewithlimits{} \blacksquare \r{} \bigcirc \operatornamewithlimits{} \blacksquare \r{} \bigcirc \operatornamewithlimits{} \blacksquare \r{} \blacksquare \r{} \bigcirc \operatornamewithlimits{} \blacksquare \r{} \blacksquare \r{ \blacksquare \r \r{\scriptsize} \blacksquare \r{\scriptsize} \blacksquare \r{} \blacksquare \r{} \blacksquare \r{ \blacksquare \r{\scriptsize} \blacksquare \r{\scriptsize} $	
Page Setup	Backflush 6 sec	
		-
	OK Canc	al

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.

Language Setting		Σ	3
English		-	
This setting will be effective after res	tarting the sof	tware.	
	ОК	Cancel]

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.

Using the CytExpert Software Software Settings

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
- Turning On the Instrument
- Logging Into the Software
- Initializing the Instrument

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]

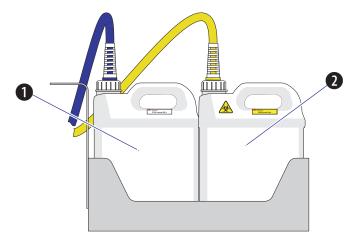




Risk of instrument damage. Do not use a saline-based sheath fluid on the CytoFLEX instrument. Saline-based sheath fluid could damage instrument components. Beckman Coulter recommends using CytoFLEX Sheath Fluid or a similar nonionic sheath fluid to ensure system performance.

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

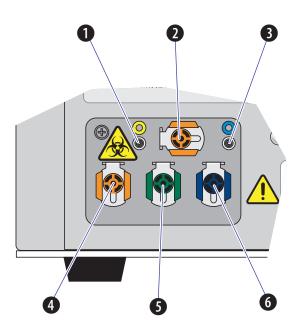
Risk of instrument damage. Remove the sheath fluid container from the Fluid Container holder before filling the sheath fluid container to avoid damage to instrument electronics.

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:



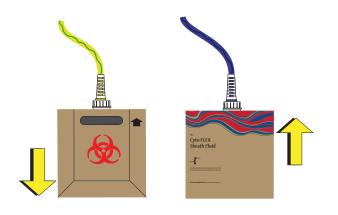
- 1. Waste level sensor connector. Connects to the waste liquid sensor cable.
- 2. Flow cell waste out. Connects to the flow cell waste tubing.
- 3. Sheath fluid level sensor connector. Connects to the sheath fluid sensor cable.
- 4. Waste out. Connects to the waste liquid tubing.
- 5. Sheath return.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

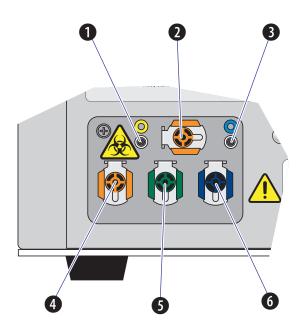
Risk of instrument damage. Do not use a saline-based sheath fluid on the CytoFLEX Series instrument. Saline-based sheath fluid could damage instrument components. Beckman Coulter recommends using CytoFLEX Sheath Fluid or a similar nonionic sheath fluid to ensure system performance.

- 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

- 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 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.

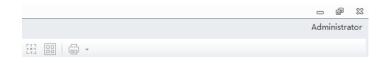


NOTE The default software shortcut appears on the desktop. If you do not see the icon, the default installation path is under C:/Program Files/CytExpert. Double-click CytExpert.exe to run the software.

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]

CytExpert 22	
New Experiment Open Experiment Exit	
New Experiment from Template Open Compensation	
Experiment Template Compensation	
	LA E - M & D & H - I P O D + + Y A. O & Q O O O O A & A A A B E 4 F A E 4 F D E E D E E D O A B A A A A A A A A A A A A A A A A A

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.

兽 Disconnected 🛛 兽 Erro	🙁 🚫 [2015-01-19 10:57:27] 00001: Cytometer disconnected.
-------------------------	--

• 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.

ystem Log			Σ
8 [2015-01-19	09:52:25] 00001: Cytometer disconnected.		
[2015-01-19]	09:57:32] Cytometer connected.		
1 [2015-01-19	09:57:35] Ready to standby		
[2015-01-19]	09:57:35] Cytometer standby.		
1 [2015-01-19	09:57:52] Initializing		
 [2015-01-19] 	09:57:53] Completed Initialize.		
1 [2015-01-19	09:57:55] Ready to standby		
[2015-01-19]	09:57:55] Cytometer standby.		
1 [2015-01-19	09:57:55] Priming		=
[2015-01-19]	09:59:00] Completed Prime.		
1 [2015-01-19	09:59:00] Initializing		
[2015-01-19]	09:59:01] Completed Initialize.		
1 [2015-01-19	10:12:06] Ready to standby		
[2015-01-19]	10:12:06] Cytometer standby.		
🔇 [2015-01-19	10:57:27] 00001: Cytometer disconnected.		
			Ŧ
		Clear Close	

B49006AL

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 .

			đ	23
		Adm	inistra	tor
\cap	Adminis	trato	r	
38	Username:	Admir	n	
		Lo	g out	ŧ

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.

Cyto	ometer	Settings	QC/Stand	ardiza	tion	Advance	ed	Hel	р
×	Acq. Setting						2		
	Detect	tor Config	uration		R	estart			
(å_{0})	Backflu	ush			I E	Boost			
Û	Boost								
ø	Initializ	ze			F				
Ο	Stand	by							
	Prime.								
	Deep	Clean							
	Calibra	ate Sample	e Flow Rate			Events			
	System Startup Program				Events				
	Daily C	lean				-			
	Sampl	e Injectior	n Mode	•		Manual			
	Sampl	er Reset			\checkmark	Semi Au	tor	natio	:
	Turn C	n				Plate Loa	ade	er	
	Turn C	Off			ast				
	Acq. S	etting Cat	alog		1	1 1 1 1			
	Cytom	neter Conf	iguration		-				
	Cytom	eter Infor	mation						

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*.

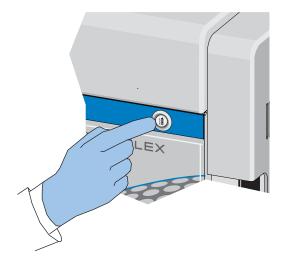


2 Select Initialize. The sample tube holder swings out from the standby position to the sample loading position (see Figure 1.13) so that you can load the sample tube.

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.

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- μ L sample volume. Running samples with a cap attached to the sample tube or with volumes exceeding 300 μ L 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*.

🧈 Manual Sampler 🛛 😁 Sheath 😁 Waste

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.

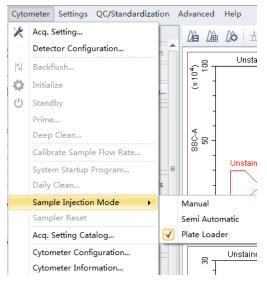
The sample runs at the designated flow rate and the Cytometer begins to acquire data.

- **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

- 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 **ΟΚ**.

Warning	23
	Sample loading mode has been reset, please restart the cytometer, connect the appropriate probe and follow the cleaning procedure.

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.

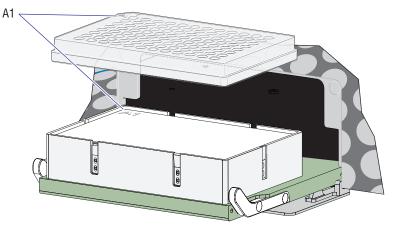
The sampler status icon located in the bottom right side of the screen changes to display *Plate Loader*.



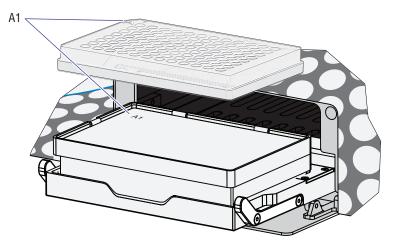
NOTE If you have a CytoFLEX LX instrument, you can turn the Cytometer's power on by selecting Cytometer > Turn On.

- 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)]

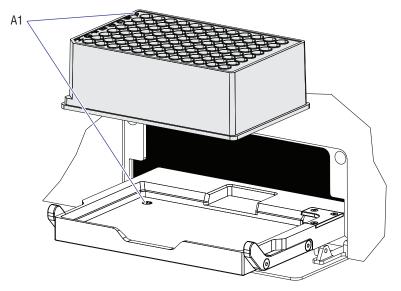


I



[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.

I

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.

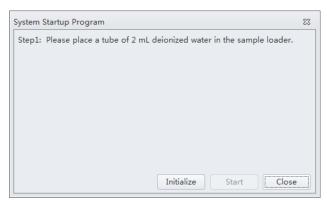
2 Select System Startup Program in the Cytometer menu.

[CytoFLEX LX Shown]

Cyto	meter Settings QC/Standardizati				
×	Acq. Setting				
	Detector Configuration				
$\left \begin{smallmatrix} b \\ b \end{smallmatrix} \right $	Backflush				
Û	Boost				
ø	Initialize				
\bigcirc	Standby				
	Prime				
	Deep Clean				
	Calibrate Sample Flow Rate				
	System Startup Program				
	Daily Clean				
	Sample Injection Mode				
	Sampler Reset				
	Turn On				
	Turn Off				
	Acq. Setting Catalog				
	Cytometer Configuration				
	Cytometer Information				

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

System	Startup Program	23
Step1:	Please place a tube with 2 mL of deionized water in the sample load and move the sample loader to the sample acquisition position.	er,
	Initialize Start Close	

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.

System Startup Program	
Step2: Priming	
	Remaining Time: 7 min 50 sec
	Initialize Stop Close

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

System Startup Program	
Step3: Starting to acquire deionized water.	
Remaining Time: 6 min 45 sec	
Initialize Stop Close	

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

System Startup Program	
Step4: Warm up	
	Remaining Time: 3 min 26 sec
	Initialize Stop Close

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

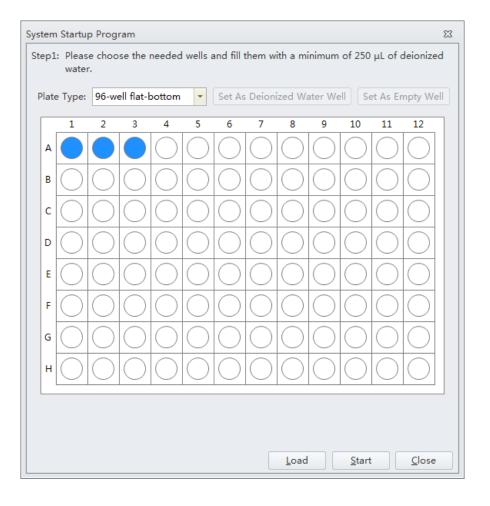
Close

Running the System Startup Program [With Plate Loader]

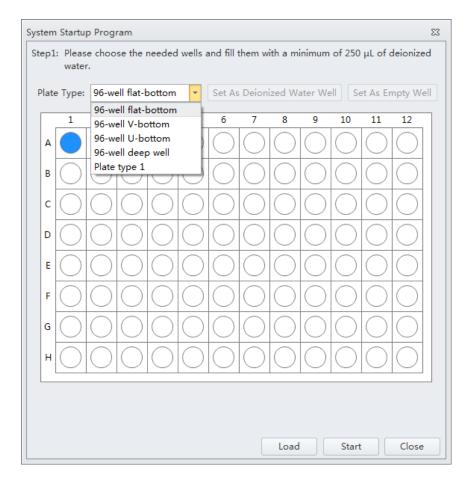
The system startup program takes approximately 10 minutes.

- 1 Select Initialize.
- **2** Select **System Startup Program** from the Cytometer menu to open the System Startup Program window.
 - [CytoFLEX LX Shown]

Cyto	meter Settings QC/Standardizati
Х	Acq. Setting
	Detector Configuration
⁶ .	Backflush
Û	Boost
Ф	Initialize
\bigcirc	Standby
	Prime
	Deep Clean
	Calibrate Sample Flow Rate
System Startup Program	
	Daily Clean
	Sample Injection Mode
	Sampler Reset
	Turn On
	Turn Off
	Acq. Setting Catalog
	Cytometer Configuration
	Cytometer Information



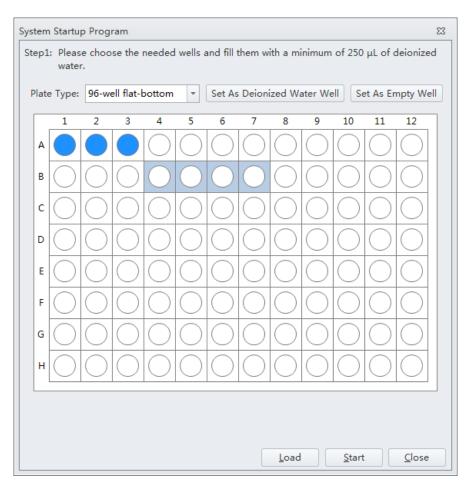
The plate loader automatically ejects the plate holder stage and the System Startup Program window appears.



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.

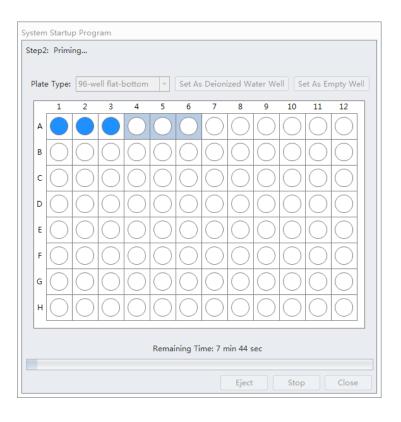
4 Follow the on screen software prompts and select the desired wells and select **Set As Deionized** Water Well.



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

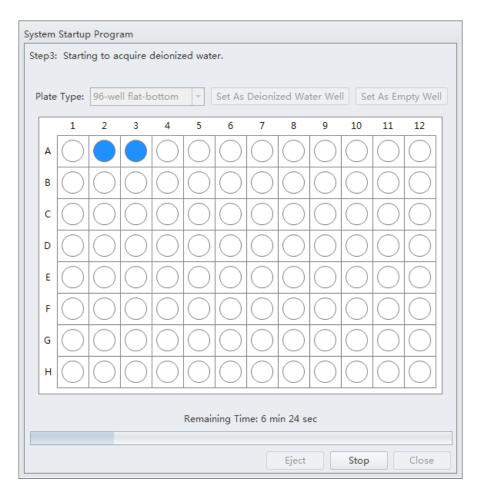
NOTE Prepare two to six sample wells with deionized water.

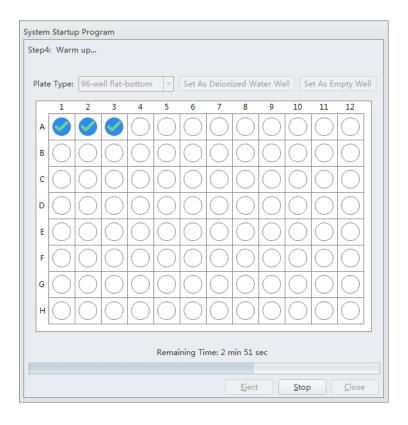
- **5** Select **Load** to load the plate.
- **6** Select **Start** to start the program. The message *Please confirm that the correct plate is placed properly and press OK* appears. Select **OK**.



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

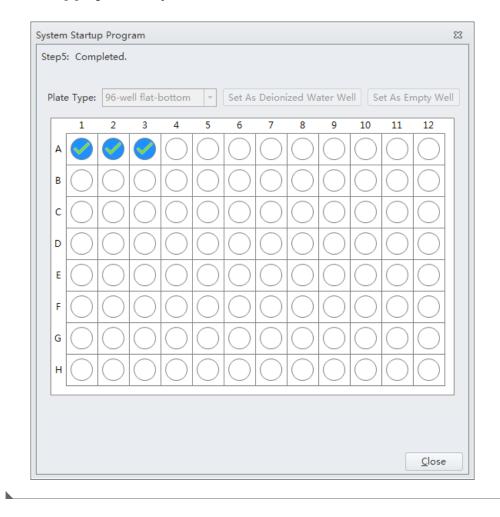
After a self check, the system initializes again. The sample is loaded automatically. This process takes about 1 minute per well.





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.

🛟 Initialize	Record	O Restart
也 Standby	* Backflush	1 Boost
•∭ Next Tube	🔀 Acq. Set	ting

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.

Run	Record	C	Restart
() Standby	Backflush	Û	Boost
•∭ Next Tube	Acq. Set	ting	

Daily Startup Initializing the Instrument

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 μ s from the previous setting. The software automatically changes the laser delay setting.

OR

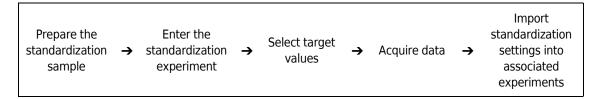
Notifies you if laser delay is >5 µs from the previous setting. Manual laser delay adjustments are required. Refer to Setting Laser Delay in CHAPTER 11, Replacement/Adjustment Procedures.

- **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:



Standardization Workflow:



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 t	ube.
--	------

- **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 $200 \,\mu L$ 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.
IM	PORTANT 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.

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.

.ot No.	Expires	Lot No.: 6	5347					
5347	2015-06-26	Expires: 2	2015-06	-26				
		Threshold						
		Channel		Mode	Value			
		FSC(Heig	ht)	Manual	10	00000		
		Signal Laser	Filte	er	Gain	Median	Median Tolerance(rCV(%)
			FSC	;	142	237500.0	5.00	
		Blue	488	/8	59	570000.0	5.00	5.(
		Blue	525	/40	159	3400000.0	5.00	5.0 =
		Blue	585	/42	119	1335000.0	5.00	5.(
		Blue	690	/50	381	2080000.0	5.00	5.(
		Blue	780		633			5.0
		Red	660		436			5.(
		Red	712		388			5.(
		Red	780	/60	440		5.00	
								Þ

- **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.

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dt View Favorites Tools Help							
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				To access My Technical Docum	ients, please login or register.		
	Technical Documents	B My Technical Documents	Safety Data Sheets (S	DS/MSDS) Software Download	*Indicates required fields		
	Technical Documents		Safety Data Sheets (S	DS/MSDS) Software Download	*Indicates required fields		
			Safety Data Sheets (S Product Series	DS/HSDS) Software Download	*Indicates required fields		
	Search By Prod	uct Product Line*		Product			
	Search By Prode	Product Line* Please select one Language	Product Series	Product	Software Name		
	Search By Prode Market Segment* Please select one Lot Number Al	Product Line* Please select one Language	Product Series	Product	Software Name		
	Search By Prode Market Segment* Please select one Lot Number	Product Line* Pease select one Language	Product Series	Product	Software Name		
	Search By Prod Market Segment* Pesse select one Lot Number Al Search	Vect Product Line* Please select one Language English	Product Series	Product	Software Name		
	Search By Prod Market Segment* Pesses select one Lot Number At Search Search By Softw	Vert Product Line* Product Line* Product Line* Language English Varé Namé	Product Series	Product	Software Name		
	Search By Prod Market Segment* Pesse select one Lot Number Al Search	Vert Product Line* Product Line* Product Line* Language English Varé Namé	Product Series	Product	Software Name		
	Search By Prod Market Segment* Pesses select one Lot Number At Search Search By Softw	uct ProductLine* ProductLine* ProductLine Product_Ine	Product Series	Product	Software Name		
	Search By Prod Market Segment* Pesse select one Lot Number At Search Search By Softw Search By Item/	uct ProductLine* ProductLine* ProductLine Product_Ine	Product Series	Product	Software Name		
	Search By Prod Market Segment* Pesse select one Lot Number At Search Search By Softw Search By Item/	uct ProductLine* ProductLine* ProductLine Product_Ine	Product Series	Product	Software Name		
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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]

Technical Documents	My Technical Documents	Safety Data Sheets (SDS/MSDS) Software Download	* Indicates required fields
Search By Product				
Market Segment* Research & Discovery V Lot Number All V Search	Product Line* Flow Cytometry Language English		Product CytoFLEX	Software Name CytoFLEX QC Fluorospheres T
Search By Software	e Name			
Search By Item/REF	Number			
Search By Lot Num	ber			

[CytoFLEX IR QC Fluorospheres Target]

Technical Documents	My Technical Documents	Safety Data Sheets (SDS/MSDS)	Software Download	*Indicates required fields
Search By Prod	uct			
Market Segment* Research & Discovery Lot Number All Search	Language		Product CytoFLEX	Software Name CytoFLEX Daily IR GC Fluoro
Search By Softw	ware Name			
Search By Item	REF Number			
Search By Lot N	Number			

- **5** Select **Search**.
- **6** The search results appear below the Search By Lot Number tab.

[CytoFLEX QC Fluorospheres Target]

Technical Documents	My Technical Docum	nents	Safety Data Sheets (SDS/N	ISDS) Software Do	ownload		*Indicates required fiel
Search By Prod	uct				121		
larket Segment*	Product Line*		Product Series	Product		Software Name	
Research & Discovery	Flow Cytometry	*	Instruments	CytoFLEX	*	CytoFLEX QC Fluc	prospheres T 💌
.ot Number	Language						
All	English	v					
Search By Item	REF Number						
Search By Lot N	lumber						
Search By Lot N ftware Download Search F rst Prev 1 Next Last	Results						View 25 per page
ftware Download Search F	Results	Lot	lo. <u>Version</u>	<u>item/REF NO</u>	2 8	lelease Date	View 25 per page

[CytoFLEX IR QC Fluorospheres Target]

/larket Segment* Product Line*		Product S	eries	Product		Software Name	
Research & Discovery	Flow Cytometry	▼ Instrumen	ts 🗸 🗸	CytoFLEX	~	CytoFLEX Daily IR	QC Fluoro
ot Number	Language						
All	English	~					
Search							
Search By Soft	ware wane						
Search By Item	I/REF Number						
	Number						
 Search By Lot 	N umber Results						View 25 per page
Search By Lot	N umber Results	Lot No.	Version	Item/REF NO,	R	elease Date	View 25 per page
Search By Lot ftware Download Search I rst Prev 1 Next Las	Number Results t	<u>Lot No.</u> 699001F	Version	Item/REF NO, C06147		elease Date 06/23/2017	

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

		🛎 Login I Contact Us I	Careers 📜 Cart (0) 📑 Quote (0)
COMPANY SUPPORT	RESEARCH & DISCOVERY DIAGNOSTICS INDUSTRIAL		nter product, item number or keyword Q
	CytoFLEX QC Fluorospheres Target Values		
	Description		
	The files contain assay values to program into your instrument using the CytoFLEX Fluorospheres. Please print out this page and follow instructions below carefully.		
	Requirements You must have a CytoFLEX instrument and suitable CytoFLEX reagents.		
•	Instructions Download the file. Open the file and enter the values from the file as instructed in your user manuals.		
	Download Files		
	BAH03.tot # BAH03 Download		
	Back		

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.

BEC	CKMAN TER	💻 Login I C	ontact Us I Careers	Cart (0) Guote (0)
COMPANY SUPPO	RT RESEARCH & DISCOVERY DIAGNOSTICS INDUSTRIAL		Enter produ	ict, Item number or keyword Q
	CytoFLEX QC Fluorospheres Target Values			
	Description			
	The files contain assay values to program into your instrument using the CytoFLEX Fluorospheres.			
	Please print out this page and follow instructions below carefully.			
	Requirements You must have a CytoFLEX instrument and suitable CytoFLEX reagents.			
	Instructions = Download the file. = Open the file and enter the values from the file as instructed in your user manuals.			
	Download Files			
	BAH03 tot # BAH03 Download			
	Back			
Copyright/Tradei	Do you want to open or save BAH03.tgt (13.2 KB) from beckmancoulter.com? Op	en Save	▼ Cance	X II rights reserved.

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.

QC/Standardization Setting	23
FCS Files	
✓ Automatically save acquired data to FCS file.	
Path: C:\Users\Public\Documents\CytExpert QC Data	
PDF Files	
Automatically export QC result to PDF file.	
Path: C:\Users\Public\Documents\CytExpert QC Data	
ОК	Cancel

1 Double-click ito start the CytExpert software.

a. Ensure that the **Connected** icon on the Status Bar near the bottom-left side of the display is green.

Connected Standby ✓ [2015-01-19 10:12:06] Cytometer standby.

- **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.

xpert(Cyto-DX) rtometer Settings Advanced Help											
o seange seanced halp	e de d	•									
Fask: QC Standardization											
				Q	C Report						
🏠 Initialize	Bead Lot No.:		102 SE								
🖒 Standby	Bead Expires:		9-07-20				OC Date:		2016-11-0	2 15:07	
Events/Sec: 0.0	Cytometer Na						Cytomete				
Abort(%): 0.00	Detector Conf	guration: Cyt	p-LX								
Events: 0	Loader Type:	Ser	ni Automatic								
Time: 00:00:00	Threshold										
	Channel: FSC(He	iaht) Mode	Manual Vali	ie: 50000							
Lot No.:	Laser										
	Laser	Delay(µs)	Default De	law(uc) Diff	erence Delay(µs)	Power(mW)	Tarr	et Powe	r(m)M)	Result	
Current Detector Configuration:	NUV	72,		72.96	-0.16		60	jerrone	50-70	Ø	
Cyto-LX	Blue	0.		0.00	0.00		49		40-60	ø	
Report	Red	36.	16	36.32	-0.16		49		40-60	0	
epon	Yellow	-35.	58	-35.84	0.16		30		20-40	0	
Detector Configuration:	Violet	-70.	24	-70.40	0.16		88		70-120	0	
Cyto-LX *	Signal Value										
Date: 2016-10-03 × ~ 2016-11-02 ×	Parameter G		%Difference Target Gain	Median		%Difference Target Median	rCV(%)	Target rCV(%)	Width	Result	
rocess Date Lot No. Result	FSC	252 252	0.00			-1.33			802.0		
016-11-02 15:02 BAH02 😵 🗠	SSC	81 81	0.00			3.17			883.2		
016-11-02 15:05 BAH02_SE 🔇	PB450	56 56 34 34	0.00			-1.05	2.13				
16-11-02 15:07 BAH02_SE	KO525 Violet610	34 34 202 202	0.00			0.43	2.03				
16-11-02 15:17 BAH02 🥥	Violet60	185 185	0.00			-0.55	2.23				
	Violet780	62 62	0.00			0.43	2.03				
	DAPI	69 69	0.00			-0.61	0.80				
	NUV525	21 21	0.00			1.53	0.78				
	HoechstRed	160 160	0.00	1364065	.0 1361715.0	0.17	0.87	7.00	820.7		
	APC	341 341	0.00	326824	.1 328142.5	-0.40	3.55	5.00	1063.2	0	
	APC-A700	329 329	0.00	5459777	.0 5340872.0	2.23	3.74	5.00	1067.4		
Ψ	APC-A750	44 44	0.00	666992	6 649742.0	2.65	3.78	5.00	1070.0	0	

[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.

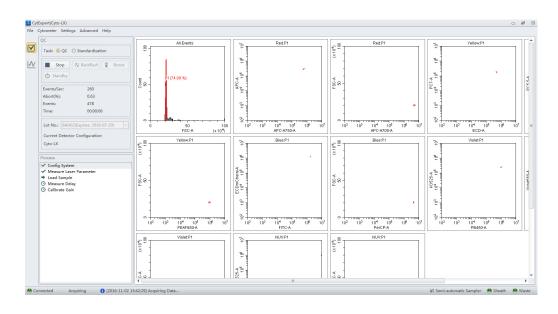
5 Select Initialize.

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.

- Config System
- Measure Laser Parameter
- Load Sample
- Measure Delay
- 🕒 Calibrate Gain



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.

QC/Standardization Setting	23
FCS Files	
Automatically save acquired data to FCS file.	
Path: C:\Users\Public\Documents\CytExpert QC Data	
PDF Files	
Automatically export QC result to PDF file.	
Path: C:\Users\Public\Documents\CytExpert QC Data	
OK Can	cel

- **1** Double-click **i** 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.

(♠ Connected ♠ Standby ✔ [2015-01-19 10:12:06] Cytometer standby.

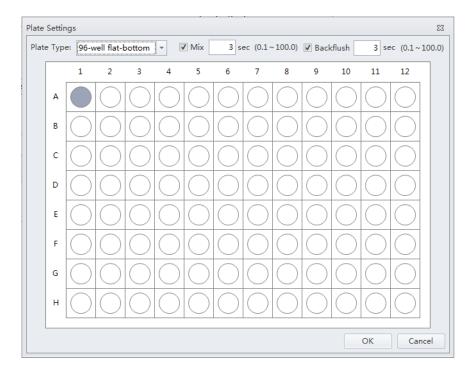
- **b.** If the icon is not green, ensure that the Cytometer USB is securely connected to the Workstation and restart the Workstation.
- **2** Verify the detector configuration. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, Data Acquisition and Sample Analysis.
 - **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.
- **4** Select **Start QC/Standardization** in the QC/Standardization menu to access the QC experiment.

pert(Cyto-DX)														
ometer Settings	Advanced Help													
2C			F A B 6	в.										
				<i>w</i>										
Task: 🧿 QC 🛛	Standardization						~							
	the second states of						Q	C Report						
🗘 Initialize 🏻 *	🖁 Backflush 👔 Bo													
() Standby			Bead Lot N	lo:	BAHO									
			Bead Expire	esi	2019-	07-20				C Date:		16-11-1	B 15:30	1
Events/Sec:	0		Cytometer I						c	ytometer	SN:			
Abort(%):	0.00		Detector Co											
Events:	0		Loader Typ	ie:	Semi J	Automatic								
Time:	00:00:00		Threshold											
			Channel: FSCI	(Height)	Moder M	arread Valu	w 50000							
Lot No.		-	Laser	(meigini)	moue.m	anuar van								
Current Detector C	Configuration:		Laser	Del	ay(µs)	Default De	lay(µs) Diffe	erence Delay(µs)	Power(mW)	Targe	et Power(e	mW) I	Result	
Cyto-LX			NUV		80.80		80.80	0.00		65		50-70	۲	
			Blue		0.00		0.00	0.00		51		40-60	۲	
leport			Red		34.72		34.72	0.00		50		40-60	9	
Detector Configura	tion		Yellow		-46.24		-46.24	0.00		30		20-40	9	
			Violet		-89.12		-89.12	0.00		90	7	70-120	۲	
Cyto-LX		*	Signal Value											
Date: 2016-10-19	· ~ 2016-11-18	-				-14								
		_	Parameter	Gain	Target % Gain Ta	Difference arget Gain	Median	Target Median	%Difference Target Median		Target rCV(%)	Nidth	Result	t
Process Date	Lot No. Result		FSC	149		0.00	256788.		-0.12			961.7	0	
016-11-15 15:53	JD-AH01	-	SSC	321		0.00			-1.27			1086.5		
016-11-16 09:13	45180 😵		PB450	35		0.00			0.73	1.97	5.00	1376.7		
016-11-16 09:16	45180		KO525	86		0.00			0.73	2.15	5.00	1377.9		
2016-11-16 09:23	JD-AH01 🥥		Violet610	220		0.00			-0.19	2.34	5.00	1380.4		
2016-11-16 16:26	JD-AH01 🥝		Violet660	223		0.00			1.49	2.43	5.00	1381.6		
016-11-16 16:32	45180		Violet780	270		0.00			-0.04	2.67	5.00	1407.8		
2016-11-17 08:36	45180		DAPI	43		0.00			3.26	1.90	7.00	1137.6		
016-11-17 08:51	JG-AH03-SF 🥝		NUV525	114		0.00			0.19	1.82	7.00	921.7		
016-11-17 13:32	BAH02		HoechstRed			0.00			-0.27	1.71	7.00	942.5		
016-11-17 13:34	BAH02		APC	72		0.00			-0.38	2.19	5.00	1403.0		
2016-11-17 13:45	BAH02 Ø		APC-A700	109		0.00			-0.48	2.35	5.00	1415.8		
2016-11-17 16:12	BAH02 Ø BAH02 Ø		APC-A750	64		0.00			-1.38	2.34	5.00	1414.0		
2016-11-17 16:15	BAH02 S		PE	72		0.00			0.36	2,79	5.00	1325.2		
2016-11-18 08:36	JG-AH03-SF		ECD	95		0.00			-0.40	2,79	5.00	1323.0		
016-11-18 09:04	45180	=	PC5.5	162		0.00			-3.02	3.18	5.00	1324.6		
2016-11-18 11:18	45180		PEAF680	122		0.00			0.02	3.41	5.00	1330.6		
016-11-18 15:16	BAH02-SF		PC7	153		0.00			-0.93	3.02	5.00	1331.7		
016-11-18 15:18	BAH02-SF		FITC	107		0.00			-1.21	1.11	5.00	1086.1		
	BAH02-SF		ECDmChe	158		0.00			-0.59	1.24	5.00	1078.6		
016-11-18 15:19														
	BAH02-SF		PerCP	85	85	0.00	1084768	0 1106620.0	-1.97	1.65	5.00	1092.7	0	

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.

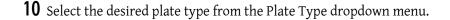
[CytoFLEX LX Shown]

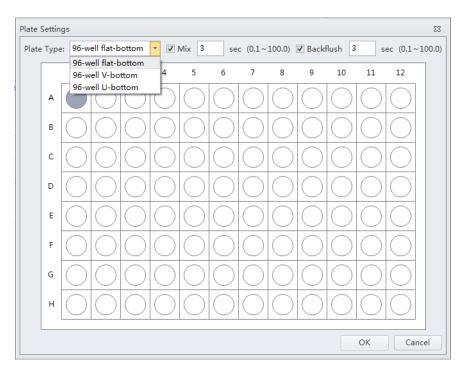
- 5 Select Initialize.
- 6 Select Eject.
- 7 Insert the prepared QC well plate (see Preparation Process CytoFLEX Daily QC Fluorospheres) into the plate holder.



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

9 Select the appropriate QC well.





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.

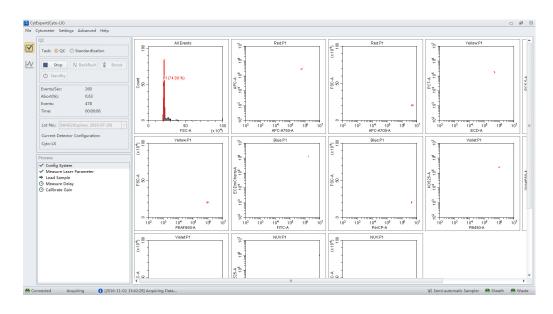
11 Select the Mix and Backflush settings in the top of the Plate Settings window.

12 Select ок.

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.

- Config System
- Measure Laser Parameter
- 🕒 Load Sample
- 🕒 Measure Delay
- 🕒 Calibrate Gain



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.

Detec	tor Configuration:	
Defau	ult-Configuration	-
Date:	2016-10-23 💌 ~ 2016-11-22 🔻	

NOTE At least one date range must be specified.

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

Process Date	Lot No.	Result	
2016-11-02 15:02	BAH02	8	-
2016-11-02 15:05	BAH02_SE	8	
2016-11-02 15:07	BAH02_SE		
2016-11-02 15:17	BAH02		
2016-11-02 15:41	BAH02	0	
			-

NOTE The results column indicates a passing QC result with a \bigcirc and a failed QC result with \bigotimes .

QC results must meet the following criteria to pass:

- The gain differences must be $\leq 20\%$ from the target gain.
- The median fluorescence intensity (MFI) differences must be \leq 5% from the target MFI.
- The rCV must be within the range.
 - **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 \leq 5%.
 - The rCV for channels of the 355 nm, 375 nm, 808 nm lasers must be \leq 7%.

The report area on the right displays detailed experiment results, including laser power, delay, testing conditions, and signal results. The same \checkmark and \bigotimes 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 Re	port
-------	------

Bead Lot No.:	A555		
Bead Expires:	2015-09-30	QC Date:	2015-01-20 10:23
Cytometer Name:	CytoFLEX	Cytometer SN:	AR38012
Detector Configuration	on: 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	0
Red laser	-37.12	-39.36	2.24	53	40-60	0

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	8
SSC	14	96	-85.42	608237.2	601799.5	1.07	-	-	1187.8	8
FITC	19	426	-95.54	3279233.0	3313982.0	-1.05	2.27	5.00	1188.6	8
PE	26	71	-63.38	1117258.0	1145402.0	-2.46	2.22	5.00	1194.0	8
APC	3000	472	535.59	29386.1	483724.0	-93.93	11.13	5.00	1700.8	8
APC-A750	3000	495	506.06	20604.3	689351.1	-97.01	17.46	5.00	1433.5	8

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 in the CSV format) or 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 **W** 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 in the top of the LJ Chart screen. The LJ Chart Settings screen appears.

L	U Charts Setting									
	Detector C	onfigura	ation:	Default-Configu	ration	-				
	Lot No. Laser Channel Alarm Boundary and Scale Range									
		Lot	t No.	Start Date	End Date					
	0	6347		2014-08-08	2014-08-08					
	L									
					ОК	Cancel	Apply			

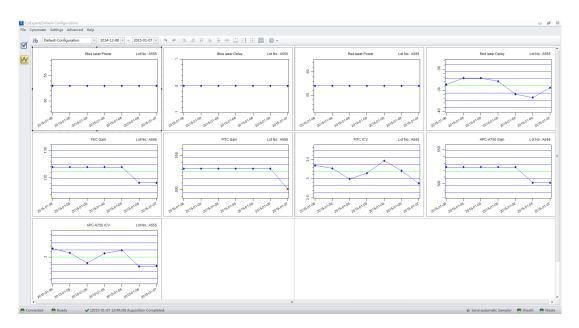
4 Select the **Laser** tab, and select the power and/or delay checkboxes for each laser as needed.

LJ Charts Setting										
Detector Configuration: 1223										
Lot No.	Lot No. Laser Channel Alarm Boundary and Scale Range									
	Lase									
	2000		Power	Delay						
	Blue	laser								
	Red									
	Viole	t laser								
Select All	Cle	ear All								
				ОК	Cance	I Apply				

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

Lot No.	Laser Char	nnel Alarm	Boundary and	d Scale Range	
	Channel	Gain	rCV		
	FSC		-		
	SSC		-		
	FITC				
	PE				
	ECD				
	PC5.5				
	PC7				
	APC				
	APC-A700				
	APC-A750				
	PB450				
	KO525				
	Violet610				
	Violet660				
	Violet780				

- 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.

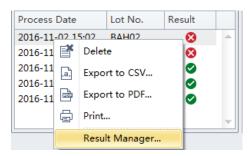


9 Select **Close QC/Standardization** in the File menu to exit the QC screen.

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.



)etector	Configuration: Default-C	onfiguration 🔹		
ot No.:	•	Date: 2016-10-23 - 201	16-11-22 🔻	
	Process Date	Detector Configuration	Lot No.	Result
1	2016-11-18 17:50:00	Default-Configuration	Moni	S
1	2016-11-18 17:52:00	Default-Configuration	123	✓
1	2016-11-18 17:55:00	Default-Configuration	4Laser	8
	2016-11-18 17:58:00	Default-Configuration	Moni	✓
	2016-11-18 18:01:00	Default-Configuration	123	8
	2016-11-18 18:05:00	Default-Configuration	123	8
	2016-11-18 18:07:00	Default-Configuration	123	8
	2016-11-18 18:09:00	Default-Configuration	Moni	S
	2016-11-18 18:11:00	Default-Configuration	Moni	✓
7 Select	+ All			
Select	t All			

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.

Standardization	Target Library					83
Item	Lot No.	Expires	Lot No.: Expires: Threshold			
			Туре	Channel	Mode	Value
			Signal			
			Channel	Median		Median Tolerance(%)
			Add	Delete Edit D	uplicate Import	Export Close

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

Adding a New Standardization Item

Item:		✓ Lot No.:		▼ Expire: 2016-11-22	-	
Thre	eshold					
Sta	andardization Test (Setting	Acquisition Settin	g		
ESC	Manual:	10000 (>0)	Channel: FSC	-		
						-
	Automatic		Manual	10000 (>0) 💿 Heigh	t 🔘 Area	
			O Automatic			
V V	se the same thresh	old setting for acquisition	on setting			
	1			1		
	Channel	Median		Median Tolerance(%)		
1	FSC		0.00		5.00	ć
	SSC		0.00		5.00	
	Fitc		0.00		5.00	
	mCherry		0.00		5.00	
	PerCP		0.00		5.00	
	APC		0.00		5.00	
	APC-A700		0.00		5.00	-
	APC-A750		0.00		5.00	
	PE		0.00		5.00	
	ECD		0.00		5.00	
	PC5.5		0.00		5.00	
	PE-AF680		0.00		5.00	
	PC7		0.00		5.00	
	PB450		0.00		5.00	
	KO525		0.00		5.00	
	Violet610		0.00		5.00	
	Violet660		0.00		5.00	
	V. 1. (700				F 00	

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.

tem	Lot No.	Expires	Lot No.: 123456			
SC STD.	123456	2016-12-01	Expires: 2016-12-01			
			Threshold			
			Туре	Channel	Mode	Value
			Standardization Test Setting	FSC(Height)	Manual	50000
			Acquisition Setting	FSC(Height)	Manual	50000
				1 Se(neight)	Walluar	50000
			Signal			
			Channel	Median		Median Tolerance(%)
			FSC		1000000.00	5.00
			SSC V450-PB		1000000.00 50000.00	5.00
			V450-PB V525-KrO		956025.00	5.00
			R660-APC		300000.00	5.0
			R763-APCA750		652000.00	5.00
			Y585-PE		80000.00	5.0
			B525-FITC		100000.00	5.0

6 Select **Close** to exit the Standardization Target Library window.

Editing Standardization Item Parameters

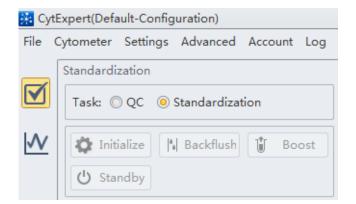
1 Select an item from the Item column on the Standardization Target Library window and select Edit...

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.
Im	porting 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.
De	leting Standardization Items
Sel	ect 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.

Standardization	
Task: 🔘 QC (Standardization
🔯 Initialize	H Backflush Eject
🖒 Standby	Plate Settings
Plate Type: 96-v	vell flat-bottom
Well: A1	
Mix: 3.0 sec	
Backflush: 3.0 s	ec
Events/Sec:	0
Abort(%):	0.00
Events:	0
Time:	00:00:00
Lot No.: moni(Expires: 2016-08-31) 🔹
Item: 🗹 mo	ni
Current Detector	r Configuration:
Default-Configur	ation

4 Select Start

The Process section of the screen displays the process details.

Process
✓ Config System
➡ Load Sample
🕒 Calibrate Blue Gain

Once the process is complete, the Standardization Report displays.

Standardization	* B B G -						
Task: O QC (9) Standardization		-	Stand	lardization R	eport		-
🗘 Initialize 🕅 Backfush 🔺 Eject							
Image: Constraint of the setting standard standar	Task Item Bead Lot No.: Cytometer Nam Detector Config Loader Type:	wration: Del			Date: Bead Explores: Cytometer SN:		-58 17:20 -58
Mix 3.0 sec Backflush: 3.0 sec Eventa/Sec: 0	Threshold Channel: FSCHeig Signal Value	ht) Mode	Manual Value 1000	0			
Abort(%): 0.00	Parameter	Gain	Median	Target Median	%Difference Target Median		Result
Events: 0							
Time: 00:00:00	FSC	\$293		65000.0		-1.04	0
Lot No.: moni(Expires: 2016-08-31)	SSC Fitz	1293		65000.0		-0.80	8
Lot IVo.: moni(Expires: 2010-08-31)	mCherry	1303		65000.0		-0.61	ő
Item: 🗵 moni	PerCP	1297		65000.0		-2.08	ő
	APC	1280		65000.0		-2.40	ő
	APC-A700	1 300		65000.0		-0.90	ŏ
	APC-A750	1286		65000.0		-1.92	0
	PE	1335		65000.0		4.27	ö
Current Detector Configuration:	ECD	1315	66570.2	65000.0		2.42	0
Default-Configuration	PC5.5	1288	62975.3	65000.0		-3.11	0
	PE-AF680	1281	61897.8	65000.0		-4.77	•
laport	PC7	1285		65000.0		-3.10	•
Detector Configuration:	P8450	1312		65000.0		0.99	0
Default-Configuration	K0525	1329		65000.0		2.29	•
	Wolet630	1302		65000.0		-0.01	۲
Date: 2016-04-18 v - 2016-05-18 v	Violett60	1324		65000.0		2.89	0
	Wolet780	1312		65000.0		-0.08	•
Process Date Lot No. Task Item Result	EAPE	1305		65000.0		-0.55	٢
2026-05-18 17-20 Target Blue 👩 -	NUV525	1305		65000.0		-0.76	٠
	HoschstRed	1292		65000.0		-1.36	
	AF790	1320		65000.0		1.56	•
	PF840	1299	63957.9	65000.0		-1.60	۲

- **5** Verify the gain settings.
 - **a.** Select **Acq. Setting Catalog** from the Cytometer menu. The Acq. Setting Catalog window appears.

	Item	Date Created	Detector Configurati	Gain	Threshold	Width	1		
	Default Sett	2016-05-05 12:2		Channe	el Name		Gain		Г
S	BAH-02 stan	2016-11-10 15:4	Cyto-LX	FSC				500	
				SSC				500	Γ
				Fitc				500	0 0 0 0 0
				mCher	ry			500 500 500 ≡	
				PerCP					j.
				APC				500	
				APC-A	700			500	i.
				APC-A	750			500	
				PE				500	
				ECD				500	-
				PC5.5				500	00 00 00 00 00 00 00 00 00 00 00 00 00
				PE-AF6	680			500	
				PC7				500	
				PB450				500	
				KO525				500	

NOTE <u>S</u> 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:



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

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

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.

Options	8
Experiment	Default Path
Tube	C:\Users\KMCABRERA\Documents\CytExpert Data
Plot	Default
Gate	
Page Setup	
Plate Loader	
	Cancel

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

Creating an Experiment [With Plate Loader]



OK.

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]

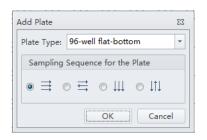
P	late												2	23
	•+	- 0.20	0	Ŧ		~	~		U di		A Q			
F														_
					0 1 10						0.10.1			
		lame			Sample ID		Events	Group		Mix	Backflush	lime		

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

[CytoFLEX LX Shown]

Pla	ate										23
	t,	• X . 0 =		-	-	11	W	П	491 (A) 🖄 🕓	
	::	Add Plate									
		Add Plate from Temp	late								
	3	Add Plate from Layou	t								
	00	Duplicate Current Plat	e without Data								
1											
	N	ame	Sample ID	Event	ts Gro	μp			Mix	Backflush	Time

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:

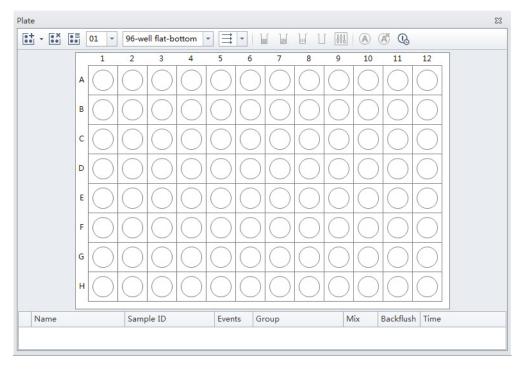
- 1. In the first row, enter the following column titles WellLabel, TubeName, SampleID, Group in sequence as shown in Figure 5.1. The table header cannot be customized.
- 2. In the WellLabel column, a maximum of 96 wells can be defined, beginning with A1-A12, B1-B12, and so on.

Figure 5.1 CSV template

	А	В	С	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.

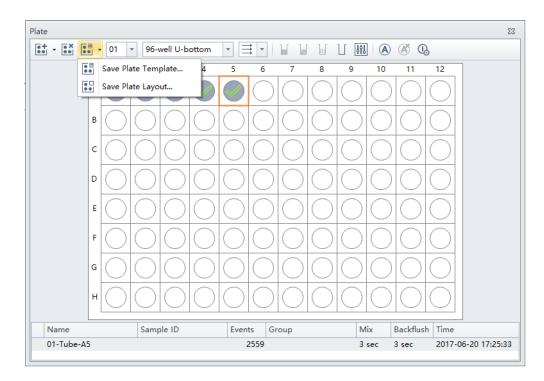
5 Select the direction of the workflow from the sampling sequence from the plate section of the window. The Plate window appears.



[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 (\bigcirc) indicates autoshutdown is disabled. A yellow background (\bigcirc) 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.

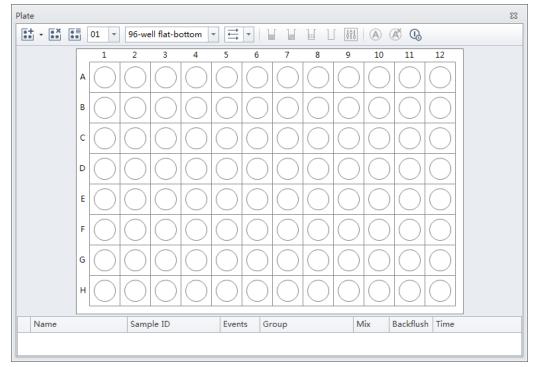


7 Select OK.

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]



\bigcirc	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
\checkmark	A blue check mark means the data is acquired, not recorded.
\checkmark	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.

t As Sample Wells		5
Naming Rules	Mix/Backflush Gro	up
Prefix: Tube Example: 01-Tube-A1	Mix 3 sec (0.1-100) Nan Backflush 3 sec (0.1-100) Colo	
Acq.Setting Channel Compensation Matrix Sto	oping Rules Custom Metadata	
Gain	Threshold	Width
FSC 81 + (1~3000)	Primary Threshold (Trigger Level)	Channel: FSC +
SSC 59 + (1~3000)	Channel: FSC -	
IR840-A790 328 🗘 🕨 (1~3000)	Manual 10000 (>0) Height Area	
IR885 232 🗘 🕨 (1~3000)	 Automatic 	
V450-PB 37 ↓ (1~3000)	Logic Operator:	
V525-KrO 88 2 + (1~3000)	Secondary Threshold (Trigger Level)	
V610 232 2 V610 (1~3000)	Channel:	
V660 206 206 1~3000)	◯ Manual 1 (>0) ◯ Height ◯ Area	
V763 261 + (1~3000)	Automatic	
Default	Defau	lt Default
		Import from File Import from Catalog
		Import Acquisition Conditions from FCS File
		OK Cancel

NOTE Select U 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.

As Sample Wells		
Naming Rules	Mix/Backflush Group	
Prefix: Tube	Image: Mix 3 sec (0.1-100) Name:	Apply to Sample
Example: 01-Tube-A1	Backflush 3 sec (0.1-100) Color:	*
Acq.Setting Channel Compensation Matrix St	opping Rules Custom Metadata	
Gain	Threshold	Width
FSC 81 📜 + (1~3000)	Primary Threshold (Trigger Level)	Channel: FSC 👻
SSC 59 + (1~3000)	Channel: FSC *	
IR840-A790 328 + (1~3000)	■	
IR885 232 2 + (1~3000)	Automatic	
V450-PB 37 🗘 🕨 (1~3000)	Logic Operator:	
V525-KrO 88 2 (1~3000)	Secondary Threshold (Trigger Level)	
V610 232 ÷ + (1~3000)	Channel:	
V660 206 206 1~3000)	O Manual 1 (>0) O Height Area	
V763 261 ÷ (1~3000)	Automatic	
Default	ded Default	Default
		Import from File Import from Catalog
		Import Acquisition Conditions from FCS File.
		OK Cance

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.

✓ Backflush	3 sec (0.1-100) 3 sec (0.1-100) tom Metadata Label	
rix Stopping Rules Cust		
	Label	

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

Naming R	ules				Mix,	'Backflush	i i i			Gro	Group							
refix:	Tube				V N	lix	3	sec	(0.1-100)	Nar	Name: 🖉 Apply to					ly to Sam	Sample ID	
xample:	01-Tube-A1				▼ B	ackflush	3	sec	(0.1-100)	Col	or:		T					
Acq.Settir	ng Channel	Compe	ensation N	1atrix St	opping Ri	ules Cus	tom Meta	data										
Use	Show Aut	ofluoresce	ence										🗌 Area	and Heigl	nt in Sync	Area	-	
Autofl.	Channel	-IR840 -A790%	-IR885	-V450- PB%	-V525- KrO%	-V610%	- V66 0%	-V763%	-NUV4 50%	-NUV5 25%	-NUV6 75%	-R660- APC%	-R712- APCA7 00%	-R763- APCA7 50%	-Y585- PE%	-Y610- ECD%	-Y6 PC	
0.00 ‡	IR840-A7		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
0.00	IR885	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
0.00	V450-PB	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
0.00	V525-KrO	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	=	
0.00	V610	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
0.00	V660	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
0.00	V763	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
0.00	NUV450	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00		
0.00	NUV525	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00		
0.00	NUV675	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00		
0.00	R660-APC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00		
4																		
													Imp	ort from	Library	Impor	t	
												In	nport Acq	uisition Co	onditions	rom FCS I	File.	

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

s Sample Wells		
aming Rules	Mix/Backflush	Group
efix: Tube	✓ Mix 3 sec (0.1-100)	Name: Apply to Sample
ample: 01-Tube-A1	☑ Backflush 3 sec (0.1-100)	Color:
cq.Setting Channel Compensation Matrix St	opping Rules Custom Metadata	
Events to Record: 10,000 Events		
in All Events 💌		
Time to Record: 600 sec		
Volume to Record: 10 µL		
		Import Acquisition Conditions from FCS File
		OK Cance

- **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.

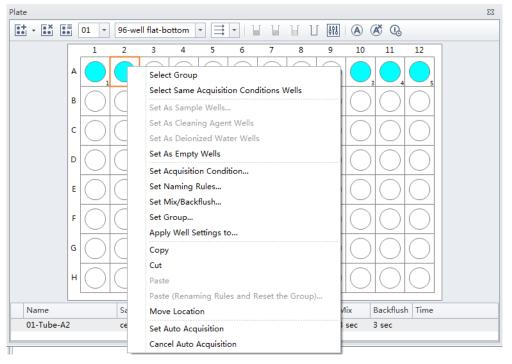
Naming Rule	5			Mix/B	ackflush		Group				
Prefix: Tube				🗹 Mix	✓ Mix 3 sec (0.1-100)				Name:		
Example: 01	-Tube-A1		🗹 Bac	kflush	3	sec (0.1	100)	Color:	~		
Acq.Setting Channel Compensation Matrix Stoppin					Custor	n Metadata					
Name Value Add Custom Metadata Name: Patient 1 Value: female											
					Canc	ES el					
						Add	Delete				
							Impor	t Acquisiti	ion Conditions	from FCS File	

12 Select ок.

Modifying Well Settings

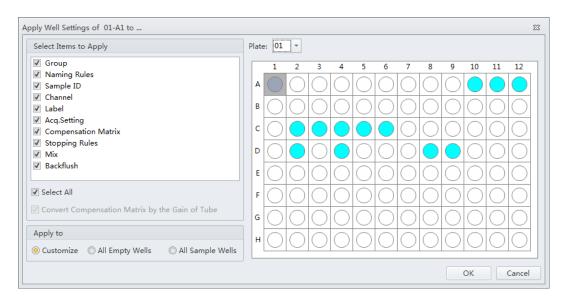
If any well settings require modification, right-click the sample well and select the setting to change.

[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.

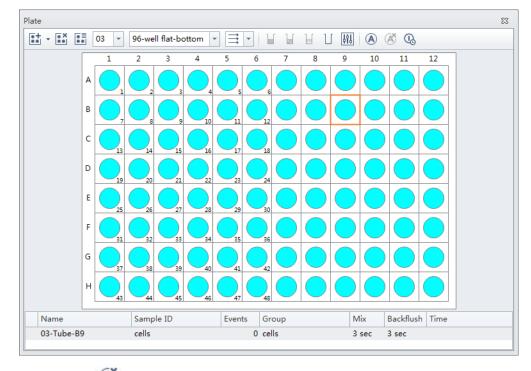
- **1** To run a single well:
 - **a.** Select the well in the Plate window.
 - **b.** Select **Run** to prompt the system to begin sample aspiration.

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]

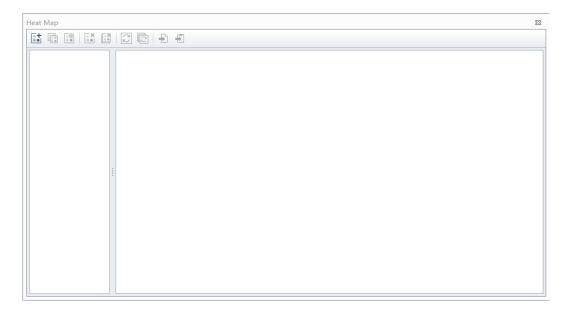


NOTE Select *intersection* to remove the auto record setting from the selected wells.

2	Select Auto Record	to begin sample acquisition.
	NOTE Select Skip to s	skip the current well and move to the next well for acquisition.
	NOTE Select Pause acquisition and pauses w while the sample acquisit	to pause acquisition. The software completes the current sample well hen the current well is complete. The plate can be loaded and unloaded ion is paused.
	NOTE Select Resume	1

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.



ame:											🗌 Dis	play N	Name	Plate	01	
olor N	Mode:	Same setting f	for all parameters	\bigcirc	Diffe	rent sett	ings pe	er pai	ramete	r						
Para	meter															
No.	Expres	sion	Label			Use Cu		ange	Min		Max		Actua	l Rang	e	
1																
Dis Dis	splay Va	lue											Add		D	elete
0.1	6 m²	CALL D			/ell											
	r	of All Parameters		V	/ell											
Base	Color:	Bands:	5 1 (2-10)													
⊙ Pe	ercentile	Fixed Range	•			1 2	3	4	5	6	7	8	9	10	11	12
No.	o Maxim	um Limit 🔲 No	Minimum Limit		^ [
		100 🗘 %			в											
					c											
-[80 ‡ %			┍┝											
					_ -											
-[60 ‡ %			E											
					F											
		40 ‡ %			G											
					н											
		20 1 %										I				
		0 1 %									In	clude			Exclu	de

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.

Name:	Heat Map 1	Display Name	Plate:	01 -	1
					Г., - с.,

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

4 Select the desired color mode.

Color Mode: (a) Same setting for all parameters (b) Different settings per parameter

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.

lo.	Expression	Label	Use Custom Range	Min	Max	Actual Range

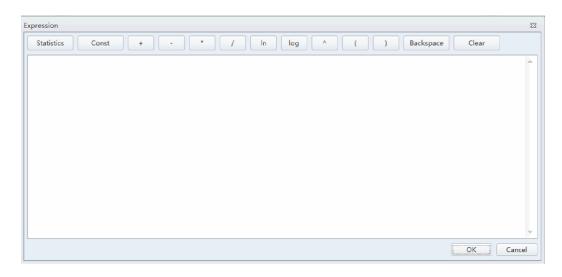
NOTE A maximum of 6 parameters can be added to a single heat map.

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

to delete a parameter. Select $\ensuremath{\textbf{Yes}}$ when the

Confirm		
?	Are you sure you want to delete the selected heat map parameter?	
	Yes	

- **6** Change the Parameter elements in the Parameter section of the New Heat Map window.
 - **a.** Change the Label name if needed.
 - **b.** Select in from the Statistic Expression section of the Parameter list. The Expression window appears.

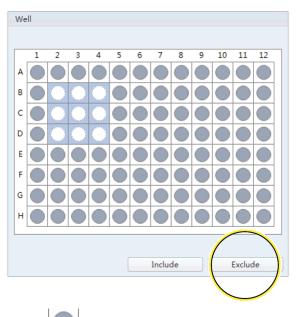


- **c.** Enter the desired expression for the selected parameter then select **OK**. The Actual Range displays in the Parameter section of the Heat Map window.
- **d.** Select the **Use Custom Ranges** checkbox if needed and enter the Min and Max ranges.

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

 $\textbf{7} \quad \text{Select any wells that should be excluded from the heat map in the Wells section of the New Heat}$

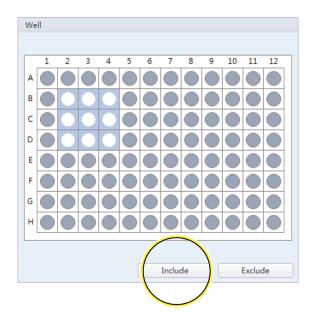
Map window then select	Exclude
------------------------	---------





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.

Color
Base Color: Bands: 5 + (2-10)
Percentile Fixed Range
No Maxin imit No Minimum Limit
80 1 %
40 1 %
20 🗘 %
0 0 %

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

Color
Base Color: Bands: 4 + (2-10)
● Percentile ○ Fixed Range
🗆 No Maximum Limit 🔲 No Minimum Limit
100 2 %
- 75 ‡ %
50 ‡ %
- 25 + %
0 * %

NOTE You can choose between 2 and 10 color bands.

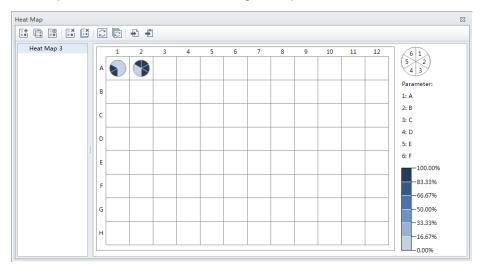
- c. Select **Percentile** to assign colors based on a percentage range.
 - **NOTE** If Use **Custom Range** is selected, the percentile is calculated according to "Min" and "Max". If **Use Custom Range** is not selected, the percentile is calculated according to "Actual Range".

Or

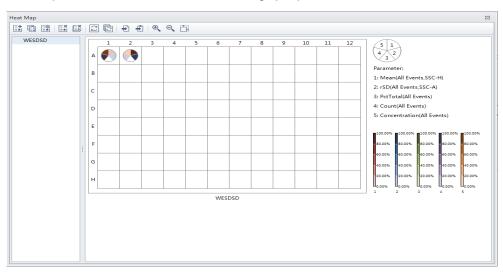
Select **Fixed Range** to assign colors based on a fixed range specified by the user. The heat map is created directly based on the result of the expression. The color of the heat map displays according to the legend range.

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.

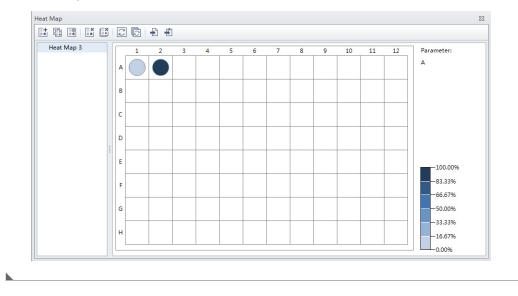


Heat Map with 6 Parameters [Same setting for all parameters]



Heat Map with 6 Parameters [Different settings per parameter]

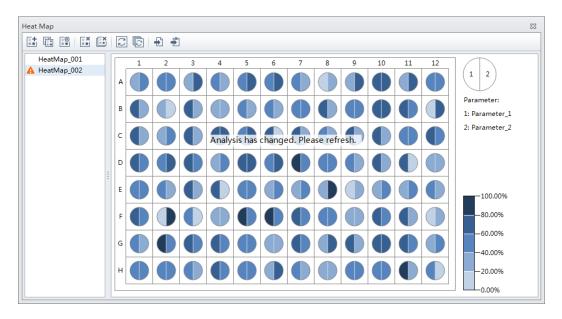
NOTE When viewing more than one parameter, the parameter location relative to the pie chart is visible in the top, right corner of the Heat Map screen.



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 *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.

ame:	sss							🔲 Displa	ay Nam	e Plate	e: 01	
olor Mode:	Same setting for	r all parameters	O Diff	erent sett	ings per p	aramete	ər					
Parameter												
No. Expres	sion	Label		Use Cus	tom Rang	e Min		Max	Act	ual Ran	ge	
1 rCV(P1	,B525-FITC-H) …								0.0	5-1.46		
🔲 Display Va	lue							[Ad	d	D	elete
Color Setting	of All Parameters		Well									
Base Color:	■ ▼ Bands:	5 (2-10)										
Percentile	O Fixed Range			1 2	3 4	5	6	7	8 9	10	11	12
	num Limit 🔲 No M	Minimum Limit	A									
	100 ‡ %		в									
			с			_						
	80 ‡ %					_				_		
			D			_						
	60 ‡ %		E									
			F									
_	40 1 %		G									
			н									
	20 1 %											
	0 🗘 %							Inclu	de		Exclu	ıde

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 is from the heat map toolbar. The Delete Multiple Heat Maps window appears.

Delete Multiple Heat Map		23
Heat Map 1		
Heat Map 2		
Select All	OK Can	cel

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

	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

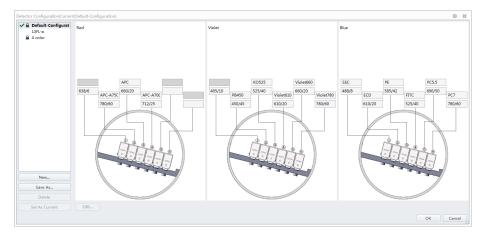
AUTION

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.

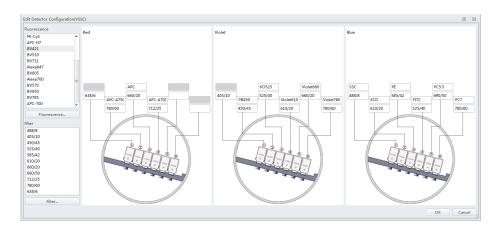
4 If a saved configuration requires changes, edit that configuration.

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.

New	
Save As	
Delete	
Set As Current	Edit

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.

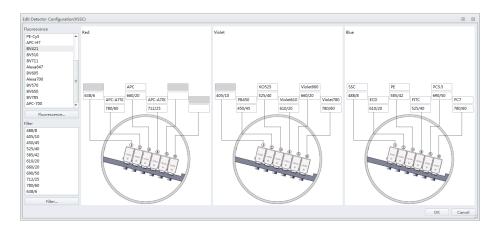


- **c.** Continue to Step 6.
- **5** If an appropriate configuration is not saved, create a new configuration.
 - **a.** Select **Detector Configuration**... in the Cytometer menu.
 - **b.** Select **New**... and name your configuration.

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.

PacBlu	
KrO	
Violet610	
Violet660	
Violet780	
SSC488	
APC-A700	
APC-A750	_
PacificBlue	
KromeOrange	≡
PB450	
KO525	-
Fluorescence	
Filter	
Filter 488/8	
Filter 488/8 405/10	
Filter 488/8 405/10 450/45	
Filter 488/8 405/10 450/45 525/40	
Filter 488/8 405/10 450/45 525/40 585/42	
Filter 488/8 405/10 450/45 525/40 585/42 610/20	
Filter 488/8 405/10 450/45 525/40 585/42 610/20 660/20	
Filter 488/8 405/10 450/45 525/40 585/42 610/20 660/20 690/50	
Filter 488/8 405/10 450/45 525/40 585/42 610/20 660/20 660/20 690/50 712/25	
Filter 488/8 405/10 450/45 525/40 585/42 610/20 660/20 660/20 690/50 712/25 780/60	
Filter 488/8 405/10 450/45 525/40 585/42 610/20 660/20 660/20 690/50 712/25	

- **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 **ок**.

12 To delete a configuration created in error, select **Delete**. The following confirmation message appears. Select **OK**.

?	The following data affiliated with this configuration will be deleted Compensation library QC results LeveyJennings setting Are you sure to delete the configuration?	
	OK Cancel	

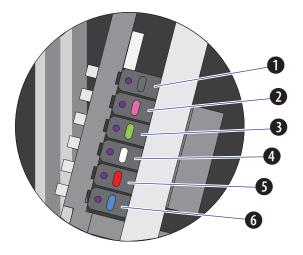
Setting Up Violet Side Scatter (VSSC) Channel

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.

Risk of erroneous results. Beckman Coulter recommends using the VSSC channel to detect side scatter signals for particles smaller than 0.5 μ m. VSSC could be too sensitive when large particles are being acquired. Switch back to the original detector configuration for particles larger than 5 μ m. For particles larger than 5 μ m, set the gain of the VSSC to 1 to increase the threshold and decrease the collection of sample noise.

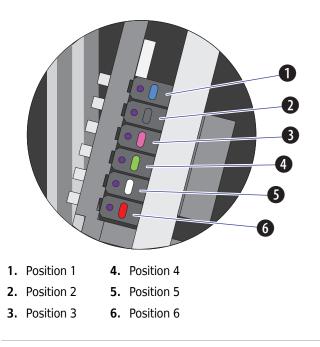
1 Open the Violet WDM lid (see Replacing the Optical Filter in CHAPTER 11, Replacement/ Adjustment Procedures) and remove the 405-nm filter, the 450-nm filter, and a third filter not required for the test, for example, the 780-nm filter.



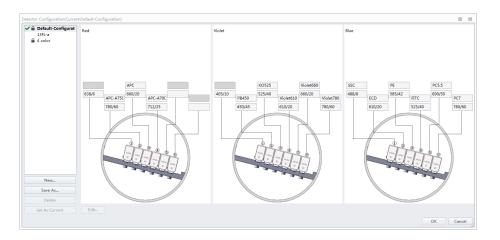
NOTE Refer to Table 5.1 to identify the WDM filter mount color codes.

- **1.** Position 1 **4.** Position 4
- **2.** Position 2 **5.** Position 5
- **3.** Position 3 **6.** Position 6
 - **NOTE** The orientation for position 1 through 6 starts with position 1 located closest to the fiber coming into the WDM, and position 6 located on the side furthest from the fiber coming into the WDM.

- **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.



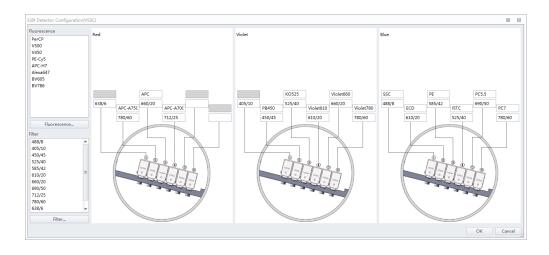
- **3** Start the CytExpert software. Refer to Logging Into the Software in CHAPTER 3, Daily Startup.
- **4** Select **Detector Configuration** from the Cytometer menu. The Detector Configuration window appears.



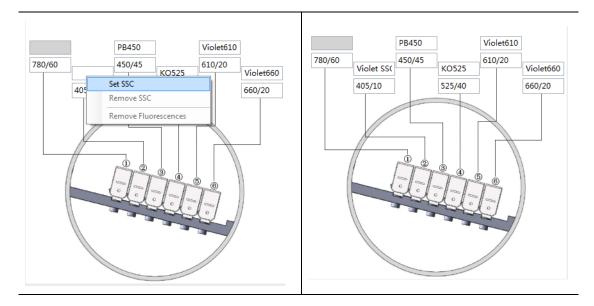
5 Select the Default Configuration and select **Save As**. The Configuration Save As window appears.

The Confi	guration Save As	23
Name:	VSSC	
	OK Cano	el

- **6** Name the new configuration VSSC and select **Οκ**.
- 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 to Importing and Exporting Compensation in CHAPTER 6, Compensation.

1 Select if from the Test Tube screen to create the new sample tube.

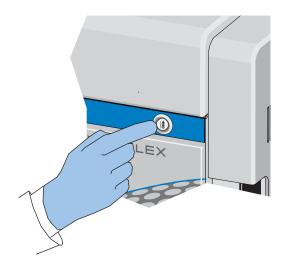
NOTE The first sample tube is already created by default.

- **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- μ L sample volume. Running samples with a cap attached to the sample tube or with volumes exceeding 300- μ L 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 \bigcirc in the test tube section of the screen, the following message appears:

Confirm	23
The current tube already contains data. Would you like to: Create a new tube Overwrite the data 	
ОК	Cancel

- 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:

Confirm	23
The current tube already contains recorde	d data. Would you like to:
O Append data to existing file	
	OK Cancel

• **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 \bigcirc in the test tube section of the screen, the following message appears:

Confirm			23
The current tube already cont	ains data. Wo	ould you like to:	
◯ Create a new tube			
Overwrite the data			
Append data to existing			
V Events to Record:	10000	Events	
in	All Events	-	
☑ Time to Record:	600	Sec	
Volume to Record:	10	μί	
		OK Cancel	

- **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.

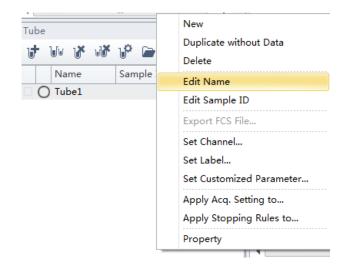
13 Repeat steps 1-12 until all sample tube data required for testing has been collected.

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

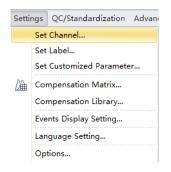
Changing the Tube Name

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.

Use	Channel	Label	
1	B525-FITC		
1	B610-ECD		
1	B690-PC5.5		
1	Y585-PE		
1	Y610-mCHERRY		
1	Y675-PC5		
1	Y710-PC5.5		
1	Y763-PC7		
1	R660-APC		
1	R712-APCA700		
1	R763-APCA750		
1	V450-PB		
1	V525-KrO		
1	V610		
	V660		

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

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

Apply C	hannel Setting of Tu	be3 to		23
	Name		Sample ID	
1	O Tube4			
1	O Tube5			
~	O Tube6			
1	O Tube7			
✓ Sele	ect All		OK	Cancel

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.

Channel	Label	
FITC		
PE		
ECD		
PC5.5		
PC7		
APC		
APC-A700		
APC-A750		
PB450		
KO525		
Violet610		
Violet660		
Violet780		

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

	Name	Sample ID	
1	🔵 Tube1		
V	O Tube2		
1	O Tube4		
1	O Tube5		
\checkmark	O Tube6		
\checkmark	O Tube7		

f. Select the tubes to apply the label settings to and select $\ensuremath{\mathsf{OK}}$.

h

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.

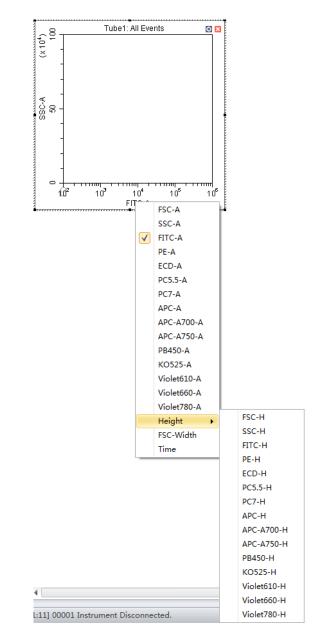
Use the *icons* to generate histograms, dot plots, density plots, pseudo color plots, and contour plot.

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

Tube1: All Events S Tube1: All Events S Tube1: All Events	Tube1: All Events
۵ 	
880-A 890-A 10-1 11-1 11-1 11-1 11-1 11-1 11-1 1	1 dr APC-A

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.

Acq. Setting		23
Gain Thresho	old Width	
FSC	1100 1 > (1~3000)	
SSC	500 1~3000)	
FITC	1000 1 (1~3000)	
PE	1000 1 (1~3000)	
ECD	1000 1 (1~3000)	
PC5.5	477 🗼 🕨 (1~3000)	
PC7	1013 🗼 🕨 (1~3000)	
APC	1100 1 > (1~3000)	
APC-A700	634 🗼 🕨 (1~3000)	
APC-A750	527 📜 🕨 (1~3000)	
PB450	106 1 > (1~3000)	
КО525	71 🗼 🕨 (1~3000)	
Violet610	377 📜 🕨 (1~3000)	
Violet660	1096 📜 🕨 (1~3000)	
Violet780	1107 📜 🕨 (1~3000)	
Set As Default	Default Recommended	
Import from F	ile Import from Catalog	
Export to File.	Export to Catalog Close	

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.

Acq. Setting	23
Gain Threshold Width	
Channel: FSC 🔹	
Default	
Import From File Import From Catalog	
Export To File Export To Catalog Close	

- **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.

≈ X Axis	
Label: <u>FITC-A</u>	X Axis Defau
Min: 100	
Max: 1000000	Log-Linear Coefficient
Fit With Sample	Recommended 1
Auto	Custom 1
≈ Y Axis	
Label: <u>PE-A</u>	Y Axis Defau
	● Log ○ Linear
Min: 100	
Min: 100 Max: 1000000	Log-Linear Coefficient
	Log-Linear Coefficient Recommended 1

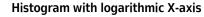
- 2) Select whether to display the axes in logarithmic or linear format for both the X-axis and Y-axis. Enter a value for log-linear coefficient if the log-linear view is desired.
- 3) Select Close.

Or

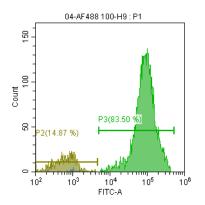
Select the logarithmic axis on the plot. The slider appears. Drag the slider along the axis to change the log-linear coefficient and view events that are not shown, including events with negative values.

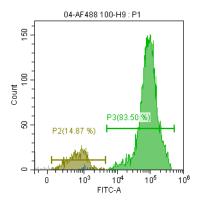
NOTE The log-linear slider is also available during data acquisition.

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.



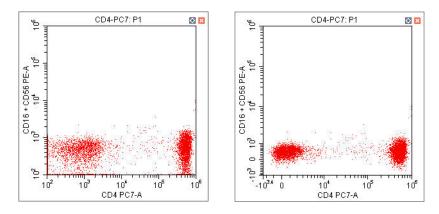
s Histogram with log-linear X-axis





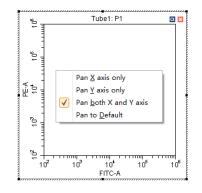


Dot plot with log-linear X-axis



- **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 <u>Select</u> 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.

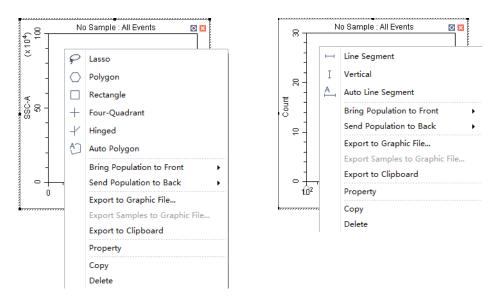


Single side pan: Modifies the axis display range dimensions when panning one axis.

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 $\vdash \square P \bigcirc \square + \square \bigtriangleup$ 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.

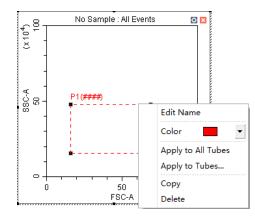


NOTE To add a vertex to a polygon gate:

- 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.

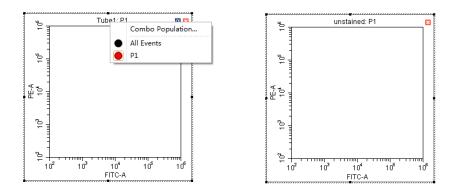
The position of the same gate in different sample tubes may vary. To change the position of a gate and apply the change to all sample tubes accordingly, you can right-click the gate and select **Apply to All Tubes**.

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

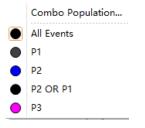
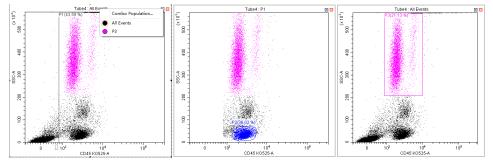


Figure 5.4 Circular Gating Logic - Example Experiment



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.

Combo Po	pulation				23
Name :	P1 OR P2				-
Logic :	P1 OR P2				
Populatio	on	Operator			
✓ P1		NOT	OR	O AND	
✓ P2		NOT	○ OR	O AND	
Clear A	.11		0	K	Cancel

- "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.

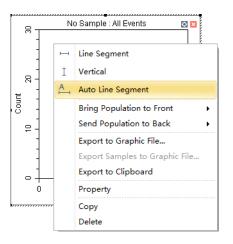
The Population Hierarchy function allows you to view how gates rank in relation to one another. To change the display color, double-click the default color and select the desired color from the drop-down color palette. To change the name of each gate, double-click the name of the desired gate. By hovering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

Populati	on	Events	% Total	% Parer
~ 🔴 Al	l Events	0	####	###
	P1	0	####	###
) P2	0	####	###
	P1 OR P2	0	####	###

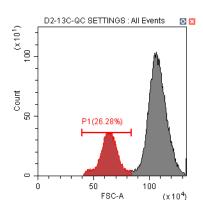
Creating and Adjusting Auto Gates

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

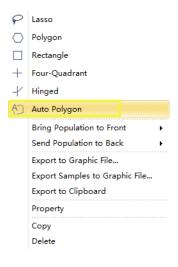
To create an auto line segment gate, select A from the toolbar or right-click on the histogram and select **Auto Line Segment** from the drop down menu.



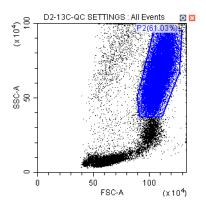
Select the population you want to gate in the histogram to automatically gate that population.



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.

	Edit Name
	Color 📃 🔻
	Apply to All Tubes
\checkmark	Auto Recalculate
	Movement:
	Extent:
	Сору
	Delete

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.



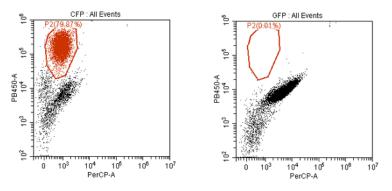
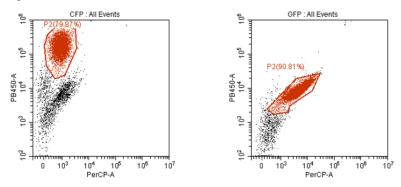


Figure 5.6 Movement - Max Setting



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.

		Edit Name
		Color 🗾 🔻
		Apply to All Tubes
	\checkmark	Auto Recalculate
		Movement:
Γ		Extent:
		Сору
		Delete

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.

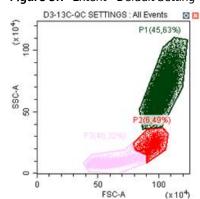
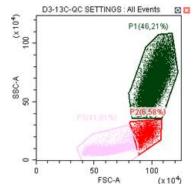


Figure 5.7 Extent - Default Setting

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.

Laser Setting				
Laser	Enable/Disable	Target Power(mW)		Actual Power(mW)
NUV	🧿 Enable 🔘 Disable	0 🌲 Set	Read Power	-
Blue	🔘 Enable 🧿 Disable	0 🗘 Set	Read Power	-
Red	🖲 Enable 🔘 Disable	0 🌲 Set	Read Power	-
YG	🧿 Enable 🔘 Disable	0 🗘 Set	Read Power	-
Violet	🧿 Enable 🔘 Disable	0 🌲 Set	Read Power	-
IR	🖲 Enable 🔘 Disable	0 🌲 Set	Read Power	-
			0	K Cancel

- 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.

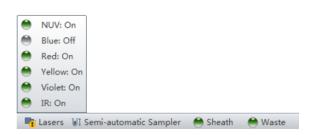
4. Read Power : Reads the current laser power before the flow cell assembly and displays the current laser power in the Actual Power (mW) column of the Laser Setting window. Refer to Table 5.1 for a list of laser ranges.

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

Table 5.1 Target Power Ranges in the Laser Setting Screen

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:

Warning	23
	The lasers listed below are disabled. The related channels of the current detector configuration cannot acquire data. Blue

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.

Acq. Sett	ting				23		
Gain	Thresho	ld Width					
FSC	[50	÷. ▼ ►	-)00)			
SSC	[200	÷	000)			
FITC	[223	÷	000)			
PE	[162	•)00)			
PerCP	-Cy5.5	336	÷)00)			
APC	[391	÷				
Set As	Default	Default	R	Recommen	ded		
Import From File Import From Catalog							
Expor	Export To File Export To Catalog Close						

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 $\times Acq. Setting...}$ on the left side of the screen.
- **3** Select the **Threshold** tab in the Acq. Setting window.

Acq. Setting	23
Gain Threshold Width	_
Primary Threshold (Trigger Level)	
Channel: FSC -	
Manual 5000 (>0)	
O Automatic	
Logic Operator:	
Secondary Threshold (Trigger Level)	
Channel:	
⊙ Manual 1 (>0)	
O Automatic	
	1
Default	
Import From File Import From Catalog	
Export To File Export To Catalog Close	

- **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 A 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.

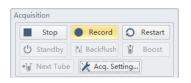
Three stop count collection conditions are available for sample recording:

- 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.

Events to Display:	500000	Events
Events to Record:	3000	Events
in	P1	~
🗷 Time to Record:	600	Sec
Volume to Record:	10	μ

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.

Tube	New
17 10 18 18 18	Duplicate without Data
	Delete
Name Sa	Edit Name
O Tube1	Edit Sample ID
	Export FCS File
	Set Channel
	Set Label
	Set Customized Parameter
	Apply Acq. Setting to
	Apply Stopping Rules to
	Property

Setting Plot Display Conditions

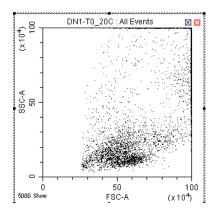
Select Events Display Setting in the Settings menu. The Events Display Setting window appears.

Events	Display Setting	23					
When	When displaying plots:						
i 🧿 Di	splay all events						
O Di	splay first 5000 🗘 events acquired	ł					
O Di	splay 20 🗘 percent of events acquire	d					
	OK Cancel Apply						

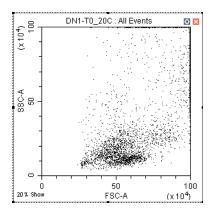
Three display options are available:

- 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

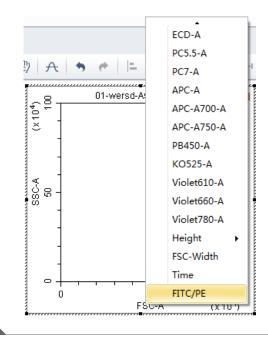
Set custom parameter to create fluorescence calculations.

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.

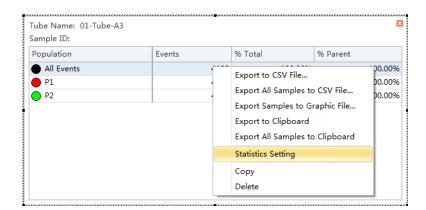
The new parameter name is displayed in the list of parameters and statistic items.



Setting Custom Statistics

Set custom statistics to create calculations based on populations of interest.

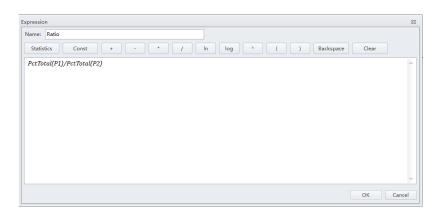
1 Right-click the statistics table and select **Statistics Setting**. The Statistics Setting window appears.



Statistics Setting	23
Header Statistics Population	
Experiment Name	
✓ Tube Name	
Sample ID	
Record Time	
Volume	
Abort(%)	
Expression Edit	
Name:	
Equation:	
Select All Clear All	
Apply to:	
O Current Tube ○ All Tubes Set As Default OK OK	Cancel

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.

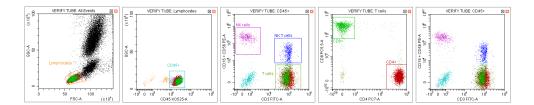
Statistics Setting			23		
Header Statistics Population					
Experiment Name					
🗹 Tube Name					
✓ Sample ID					
C Record Time					
🗌 Volume					
Abort(%)					
Expression Edit					
Name: Ratio					
Equation: PctTotal(P1)/PctTotal(P2)					
Comment					
	×				
Operator					
Select All Clear All					
Select All Clear All					
Apply to:					
Ourrent Tube ○ All Tubes	🔲 Set As Default	OK Cancel			
			_		

Population	Events		% Total	% Parent
All Events		7803	100.00 %	100.00
P 1		6799	87.13 %	87.13
🔵 P2		7370	94.45 %	94.45
P3		6904	88.48 %	88.48
P5		0904	00,40 70	00,4

Analyzing and Exporting Data

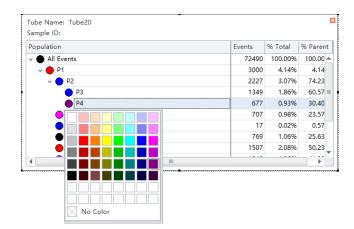
1 Select the sample tube to be analyzed.

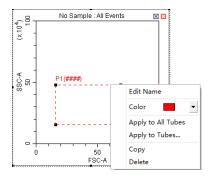
2 Establish new gates or adjust the position of existing gates. Refer to Creating Plots and Gates.



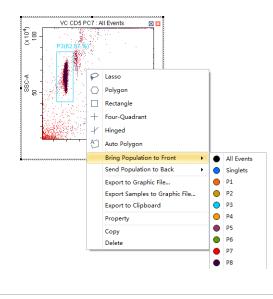
NOTE Changing a gate's position does not affect the positions of other gates already established on a given sample tube. Each test tube individually records the positions of its associated gates. If you need to make a change that concerns all the tubes, you must select the gate, then right-click the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

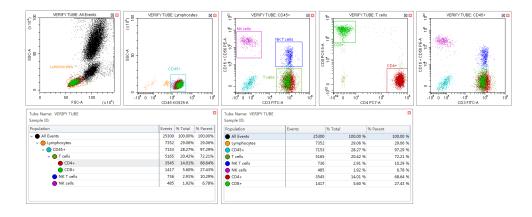
All Francis			
All Events		Export All Samples to CSV File Export Samples to Graphic File Export to Clipboard	##
P1			##
Q1-UR			##
Q1-UL		Export All Samples to Clipboard	##
Q1-LL		Statistics Setting	##
Q1-LR		Сору	##
		Delete	1

Statistics Setting 23	Statistics Setting
Header Statistics Population	Header Statistics Population
□ Experiment Name	Parent Population V Events V % Total V % Parent Events/µL(V)
✓ Sample ID	Events/µL(B) Beads Population: Select
Record Time	Beads Count: 0 Sample Volume: 0.00 µL
Volume	
Abort(%)	Parameter Mean Median rCV rSD CV SD
Edit	FSC-A C C C C C C C C C C C C C C C C C C
Name:	SSC-A
Equation:	FITC-A
	PE-A D D D D D
Comment	ECD-A
	PC5.5-A
	PC7-A
	APC-A
	APC-A700
	APC-A750
	Select All Clear All @ Area
Operator	Preview
	Events % Total % Parent
Select All Clear All	
Apply to:	Apply to:
Current Tube OK Cancel OK Cancel	Current Tube All Tubes Set As Default OK Cancel

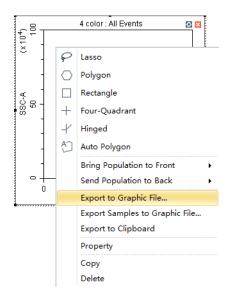
The Statistics Setting window allows you to change the display of the header, statistical elements and cell populations included.

tatistics Setting	23
Header Statistics Population	n
All Events	
V 🔴 P1	
🔽 🛑 Q1-UR	
🔲 😑 Q1-UL	
🔲 😑 Q1-LL	
V 🕘 Q1-LR	
🔲 🔵 Q2-UR	
🔲 🔴 Q2-UL	
🔲 🔴 Q2-LL	
🔲 🔴 Q2-LR	
🔲 🛑 Q3-UR	
🔽 😑 Q3-UL	
🔲 🔵 Q3-LL	
🔽 🔵 Q3-LR	
Select All Clear All	
Apply to:	
	Set As Default OK Cancel

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.



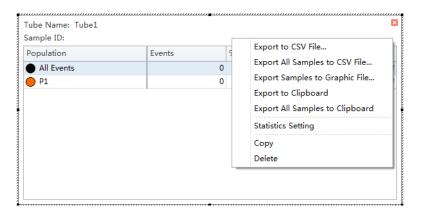
• **Export to Clipboard** copies the plot to the clipboard, allowing you to paste it directly into documents in common file formats.

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.

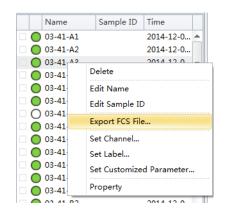
10 Export the FCS file if necessary. Refer to Exporting FCS Files.

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.



Population: All Events	*		
File Version: 🔘 FCS 2.0 🔘 FC	S 3.0 🔘 FCS 3.0 (High aut	o-fluorescence)	
Channel	Parameter Typ	e	
FSC	🔘 Linear	🔘 Log	
SSC	O Linear	🔘 Log	
FITC	🔘 Linear	🔘 Log	
PE	Linear	🔘 Log	
ECD	🔘 Linear	🔘 Log	=
PC5.5	🔘 Linear	🔘 Log	1
PC7	Linear	🔘 Log	
APC	🔘 Linear	🔘 Log	
APC-A700	🔘 Linear	🔘 Log	
APC-A750	🔘 Linear	🔘 Log	
PB450	🔘 Linear	🔘 Log	
KO525	Linear	🔘 Log	
Violet610	🔘 Linear	O Log	-
🗸 Area 📝 Height			
		(market)	Cancel

- 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.

Population: All Events	-		
File Version: 🧿 FCS 2.0 🔘	FCS 3.0 🔘 FCS 3.0 (High aut	to-fluorescence)	
Channel	Parameter Typ	be	
FSC	O Linear	🔘 Log	-
SSC	O Linear	🔘 Log	
FITC	🔘 Linear	🧿 Log	
PE	🔘 Linear	O Log	
ECD	🔘 Linear	🧿 Log	=
PC5.5	🔘 Linear	🧿 Log	-
PC7	🔘 Linear	O Log	
APC	🔘 Linear	🧿 Log	
APC-A700	🔘 Linear	🧿 Log	
APC-A750	🔘 Linear	O Log	
PB450	🔘 Linear	🧿 Log	
KO525	🔘 Linear	Eog	
Violet610	🔘 Linear	O Log	
🗸 Area 🛛 🗹 Height			
		ОК	Cancel

- **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.

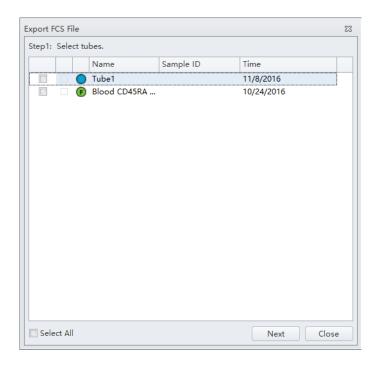
Exporting Multiple FCS Files

Step1:	Sele	ct tu	bes.			
			Name	Sample ID	Time	Τ
		0	01-11-A1		2014-12-08 10:20:04	
		\bigcirc	01-11-A2		2014-12-08 10:20:14	
		\bigcirc	01-11-A3		2014-12-08 10:20:25	
		\bigcirc	01-11-A5		2014-12-08 10:20:47	
		\bigcirc	01-11-A8		2014-12-08 12:07:56	
		\bigcirc	01-11-A9		2014-12-08 12:08:07	
		\bigcirc	01-11-A10		2014-12-08 12:08:17	
		\bigcirc	01-11-A11		2014-12-08 12:08:28	
		\bigcirc	01-11-A12		2014-12-08 12:08:40	
		\bigcirc	01-11-B1		2014-12-08 12:08:52	
		\bigcirc	01-11-B2		2014-12-08 12:09:03	
		\bigcirc	01-11-B3		2014-12-08 12:09:14	
		\bigcirc	01-11-B4		2014-12-08 12:09:24	
		\bigcirc	01-11-B5		2014-12-08 12:09:35	
		\bigcirc	01-11-B6		2014-12-08 12:09:46	
		\bigcirc	01-11-B7		2014-12-08 12:09:56	
		\bigcirc	01-11-В8 2014-12		2014-12-08 12:10:07	
		\bigcirc	01-11-B9		2014-12-08 12:10:18	
		\bigcirc	01-11-B11		2014-12-08 12:10:39	
[2003]						

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 \bigwedge 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 \times 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.

Acq. Setting		23							
Gain Threshold	Width								
FSC	500 ÷ + (1~3000)								
SSC	500 ÷ + (1~3000)								
IR840-AF790	71 + (1~3000)								
IR885	55 ÷ + (1~3000)								
V450-PB	57 ÷ + (1~3000)								
V525-KrO	159 2 (1~3000)		Setting Catalog						
V610	394 2 (1~3000)	Acq.							
			Item Default Sett	Date Created	Detector Configurati	Gain	Threshold	Width	
V660	241 🗘 🕨 (1~3000)					Chann	el Name	Gain	
V763	51 + (1~3000)		2016-11-07	2016-11-07 15:1		FSC			500 🔺
						SSC			500
NUV450	69 ↓ ▶ (1~3000)					Fitc			500
NUV525	500 ÷ + (1~3000)					mCher	ry		500
						PerCP			500
NUV675	145 ↓ ▶ (1~3000)					APC			500
R660-APC	288 📜 🕨 (1~3000)					APC-A			500
						APC-A	750		500
R712-APCA700	282 282 (1~3000)					PE			500
R763-APCA750	276 🗘 🕨 (1~3000)					ECD PC5.5			500
Y585-PE	40C 1 (1 2000)					PE-AF			500
1080-PE	406 0 1~3000)					PC7	000		500
Y610-mCherry	158 🗘 🕨 (1~3000)					PB450			500
Y675-PC5	507 ÷ + (1~3000)					KO525	i		500
Y710-PC5.5	344 🕻 🕨 (1~3000)					1.	Im	port [Delete Close
Y763-PC7	416 . + (1~3000)								
B525-FITC	180 2 + (1~3000)								
B610-ECD	130 2 + (1~3000)								
B690-PerCP	1103 . + (1~3000)								
Set As Default	Default Recommended								
Import from File	Import from Catalog								
Export to File	Export to Catalog Close	e							

- **3** Select Close.

Exporting Instrument Settings

1 Select the desired sample tube to export. Then select \Join 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:

ē	•
6	Print Preview
ò	Page Setup
D	Batch Print
	Batch Export to PDF File

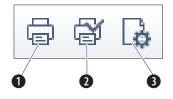
• **Print Preview.** Used to access the Preview screen.

$ \begin{bmatrix} \frac{1}{2} & \frac$
(1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2

- Select **i** to select the required format of the file to be exported and to save the file in that format.

Ģ	Ş	Q	Em	Θ,	100%	-	€	\triangleleft	\triangleright	\triangleright	G	•
											√	PDF File CSV File

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.

atch Pi	rint				Σ
		Name	Sample ID	Time	T
	0	38012-TF 6359	1	2015-01-18 13:52:34	
	\bigcirc	38012-Aquios B		2015-01-18 13:53:59	
	\bigcirc	38012-B28479		2015-01-18 13:57:05	
	\circ	38012-test-30-2		2015-01-19 15:44:47	
C Sele	ect Al	1		OK Can	1

- **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.

Name	Sample ID	Time
O 38012-TF 635	9	2015-01-18 13:52:34
38012-Aquios	В	2015-01-18 13:53:59
38012-B28479	9	2015-01-18 13:57:05
38012-test-30	-2	2015-01-19 15:44:47
 t All		OK Can

- **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.

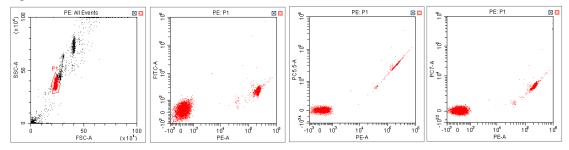
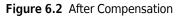
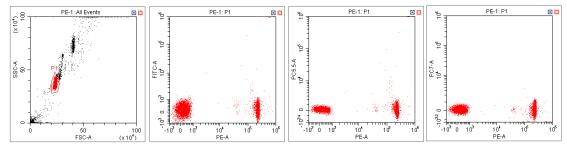


Figure 6.1 Before Compensation





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
- Creating a Compensation Experiment [With Plate Loader]
- Creating the Compensation Matrix from Previously Acquired Data
- Adjusting Compensation

Creating a Compensation Experiment

Before creating a compensation experiment, you must verify the instrument's detector configuration settings (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.

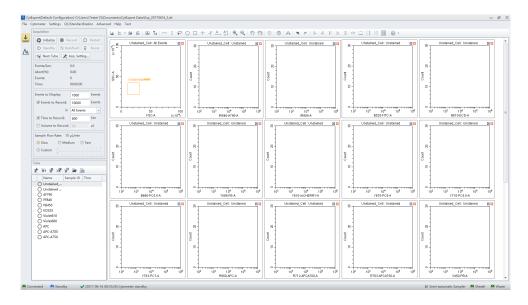
If a negative population is not present in each single color tube, then an unstained control tube is recommended.

- **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.

Use	Tube	Label	Lot No.	Sample Type
V	Unstained_Cell	Cabor	Lot Hor	© Cell © Bead
v	Unstained_Bead			O Cell O Beac
1	FITC			O Cell ○ Bead
1	PE			O Cell ○ Beac
1	ECD			O Cell ○ Bead
1	PC5.5			O Cell ○ Bead
1	PC7			O Cell ○ Bead
1	APC			O Cell ○ Beac
1	APC-A700			O Cell ○ Beac
1	APC-A750			🖲 Cell 🔘 Bead
1	PB450			🖲 Cell 🔘 Bead
1	KO525			🖲 Cell 🔘 Bead
1	Violet610			🔘 Cell 🔘 Bead
1	Violet660			🧿 Cell 🔘 Bead
1	Violet780			O Cell O Beac

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.

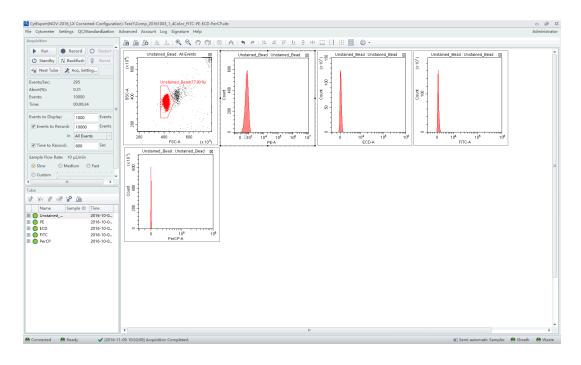
Using Control Samples to Generate the Compensation Matrix

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.

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.

Events to Display:	500000	Events
Events to Record:	3000	Events
in	P1	-
Time to Record:	600	Sec
Volume to Record:	10	μL

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.

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.

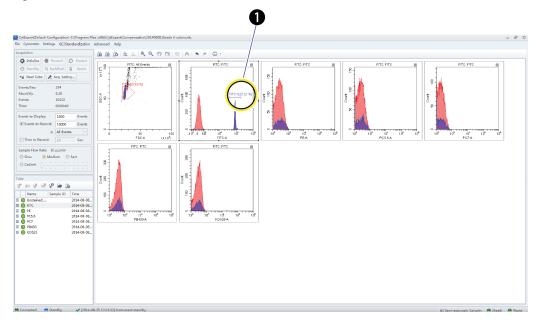


Figure 6.3 Positive Population Selected from the Single-Stained Sample

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 A or select Compensation Calculation in the Compensation menu to calculate the compensation values.

Setti	ngs	QC/Standardization	Advan							
Do.	Cor	Compensation Setup								
<i>D</i> ≙	Cor	Compensation Calculation								
Dà	Compensation Matrix									
	Eve	Events Display Setting								
	Lan	guage Setting								
	Op	tions								

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	0.0
PE	35.08		18.23	1.13	2.39	0.00	0.10	0.00	0.00	1.14	4.10	0.07	0.0
ECD	12.83	41.79		0.55	1.75	0.00	0.09	0.00	0.00	0.80	9.47	0.34	0.0
PC5.5	4.96	18.15	65.09		1.64	0.52	0.88	0.03	0.00	0.49	9.15	2.04	0.0
PC7	1.36	4.32	19.07	71.65		0.17	1.04	1.39	0.00	0.05	3.28	0.58	7.2
APC	0.02	0.03	1.12	2.67	0.22		9.25	6.07	0.00	0.16	1.10	75.01	0.0
APC-A700	0.03	0.15	0.45	43.95	0.00	28.94		2.48	0.00	0.20	0.50	36.76	0.3
APC-A750	0.10	0.21	0.20	21.48	13.85	13.34	56.58		0.00	0.13	0.23	14.52	23.0
PB450	0.10	0.22	0.00	0.04	0.00	0.00	0.15	0.00		8.26	8.62	6.12	2.9
KO525	1.89	0.15	0.00	0.03	0.33	0.00	0.06	0.00	7.67		0.55	0.35	0.1
Violet610	0.47	3.11	6.79	0.02	1.14	0.01	0.05	0.00	0.58	81.36		13.97	0.0
Violet660	0.26	1.48	3.56	0.22	0.00	2.03	0.19	0.10	0.00	45.01	92.28		0.1
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 **Οκ**.

Save To Compensation Libr	ary	23
Keywords:		
Compensation Library		
Keywords	Calculation Date	
TEST1	2014-03-25 16:19:19	
	OK Cance	el 🛛

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.

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.

Compensa	ation Setup					23
Compen	sation on:					Plate Type: 96-well flat-bottom 🔹 Sampling Sequence: 🗮 🔹
Area	🔘 Height					Set As Sample Well Set the Sample Well As Empty
Use	Tube	Label	Lot No.	Sample Type	Location	Set Tube FITC to Well
	Unstained_Cell			🔘 Cell 🔘 Bead		1 2 3 4 5 6 7 8 9 10 11 12
	Unstained_Bead			O Cell O Bead		1 2 3 4 5 6 7 8 9 10 11 12
1	FITC			🔘 Cell 🧿 Bead		
1	PE			🔘 Cell 💿 Bead		
1	APC			🔘 Cell 🧿 Bead		
	APC-A750			⊙ Cell ◎ Bead		
						Mix 3 sec (0.1~100.0) Backflush 3 sec (0.1~100.0)
						OK Cancel

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

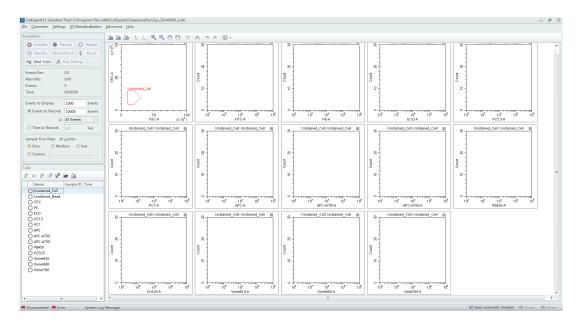
B49006AL

- **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

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 ^(A) or select **Compensation Calculation** in the Settings menu to calculate the compensation values.

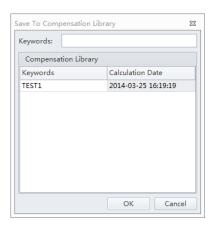
The Compensation Matrix window appears, displaying the calculated compensation values.

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	0.0
PE	35.08		18.23	1.13	2.39	0.00	0.10	0.00	0.00	1.14	4.10	0.07	0.
ECD	12.83	41.79		0.55	1.75	0.00	0.09	0.00	0.00	0.80	9.47	0.34	0.
PC5.5	4.96	18.15	65.09		1.64	0.52	0.88	0.03	0.00	0.49	9.15	2.04	0.0
PC7	1.36	4.32	19.07	71.65		0.17	1.04	1.39	0.00	0.05	3.28	0.58	7.3
APC	0.02	0.03	1.12	2.67	0.22		9.25	6.07	0.00	0.16	1.10	75.01	0.
APC-A700	0.03	0.15	0.45	43.95	0.00	28.94		2.48	0.00	0.20	0.50	36.76	0.3
APC-A750	0.10	0.21	0.20	21.48	13.85	13.34	56.58		0.00	0.13	0.23	14.52	23.0
PB450	0.10	0.22	0.00	0.04	0.00	0.00	0.15	0.00		8.26	8.62	6.12	2.9
KO525	1.89	0.15	0.00	0.03	0.33	0.00	0.06	0.00	7.67		0.55	0.35	0.1
Violet610	0.47	3.11	6.79	0.02	1.14	0.01	0.05	0.00	0.58	81.36		13.97	0.
Violet660	0.26	1.48	3.56	0.22	0.00	2.03	0.19	0.10	0.00	45.01	92.28		0.
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.

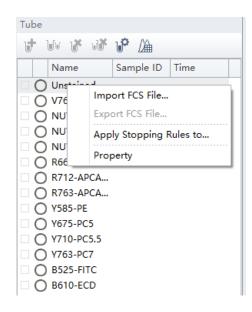
6 Select Close.

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.

Tube			
t I	ulu 🔥 við 📌 🕯	≥ / <u>A</u>	
	Name	Sample ID Time	
0	Unstained_Cell		
- F	FITC]	
	PE		
	PC5.5		
	PC7		
	KO525		

- **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 ⁽¹⁾/₍₂₎ 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.

Import Compensation	23
O Import compensation matrix and convert based on the current ga	ain.
Import compensation matrix only.	
 Import compensation matrix and gain. 	
OK Cance	

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]

Use	Show Aut																					ea
Autofl.	Channel	-IR840 -AF79 0%	-IR885	-V450- PB%	-V525- KrO%	-V610%	-V660%	-V763%	-NUV4 50%	-NUV5 25%	-NUV6 75%	-R660- APC%	-R712- APCA7 00%	-R763- APCA7 50%	-Y585- PE%	-Y610- mCher ry%	-Y675- PC5%	-Y710- PC5.5%	-Y763- PC7%	-B525- FITC%	-B610- ECD%	-B690- PerCP- PC5.5%
0.00	IR840-AF		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	IR885	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	V450-PB	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	V525-KrO	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	V610	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	V660	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	V763	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	NUV450	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	NUV525	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	NUV675	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	R660-APC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	R712-AP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	R763-AP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	Y585-PE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	Y610-mC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.0
0.00	Y675-PC5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.0
0.00	Y710-PC5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.0
0.00	Y763-PC7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.0
0.00	B525-FITC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.0
0.00	B610-ECD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.0
0.00	B690-Per	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

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.

Ke	ywords List		Select fluorencens	e	
~	20140805-Comp1		Channel	Label	Keywords
	FITC 20140805-Comp1		FITC		20140805-Comp2 2014-08-0 🔻
	PE 20140805-Comp1 20		PE		
	PC5.5 20140805-Comp1		ECD		20140805-Comp1 2014-08-05 12:21:41
	PC7 20140805-Comp1 2		PC5.5		20140805-Comp2 2014-08-05 12:40:17
	PB450 20140805-Comp		PC7		CD3 20140805-Comp3 2014-08-05 12:58
	KO525 20140805-Comp		APC		CD3 20140805-Comp4 2014-08-05 14:09
>	20140805-Comp2	>	APC-A700		
>	20140805-Comp3		APC-A750		
>	20140805-Comp4		PB450		20140805-Comp2 2014-08-05 1
			KO525		20140805-Comp2 2014-08-05 1
			Violet610		
			Violet660		
			Violet780		
			Sample	e Type: 💿 Cell	O Bead OK Cancel

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]

Use	Show Aut	tofluoresco	ence																Area and	Height in	Sync Ar	ea
utofl.	Channel	-AF79 0%	-PF840	-PB45 0%	-KO52 5%	-Violet 610%	-Violet 660%	-Violet 780%	-DAPI%	-NUV5 25%	-Hoec hstRe	-APC%	-APC- A700%	-APC- A750%	-PE%	-ECD%	-PC5.5%	-PEAF 680%	-PC7%	-FITC%	-ECDm Cherry	-PerCP.
0.00 0	AF790		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	PF840	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	PB450	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	KO525	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	Violet610	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	Violet660	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	Violet780	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	DAPI	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.00	NUV525	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.
0.00	HoechstR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.
0.00	APC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.
0.00	APC-A700	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.
0.00	APC-A750	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.
0.00	PE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.
0.00	ECD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.
0.00	PC5.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.
0.00	PEAF680	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.
0.00	PC7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.
0.00	FITC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.
0.00	ECDmCh	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.
0.00	PerCP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

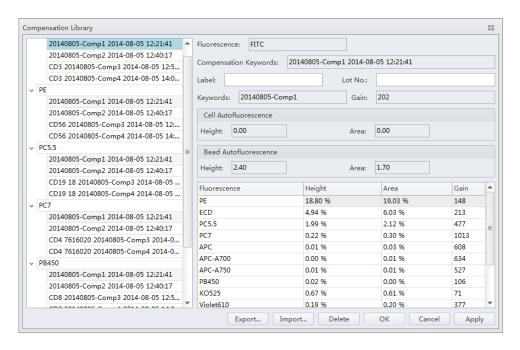
4 Select Save.

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.

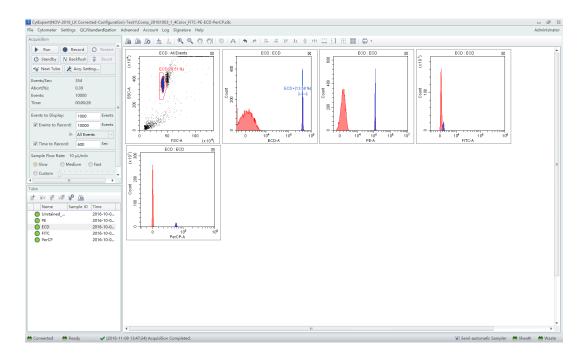
Adding Channels for Compensation

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.

Compensation Adjusting Compensation

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 \rightarrow Plot and configure statistics \rightarrow Export results

This chapter contains information on:

- Copying Experiments and Importing Data
- Setting the Plots and Statistics
- Calculating Sample Volume and Concentration
- Adjusting Compensation Settings
- Exporting Results

Copying Experiments and Importing Data

Copying a Previously Acquired Experiment

Experiments acquired by other CytoFLEX instruments using CytExpert software can be imported to your computer for analysis, provided your computer also uses CytExpert 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.

🔣 C	ytExpert(Default-Config	uration)-C:\Users
<u>F</u> ile	<u>Cytometer</u> <u>S</u> ettings	<u>Q</u> C/Standardiza
	<u>N</u> ew Experiment	Ctrl+N
	New Experiment from	Template
	New Compensation	
	Open Experiment	Ctrl+O
	Open Compensation	
	<u>S</u> ave	Ctrl+S
	Save <u>A</u> s	
	Save As <u>T</u> emplate	
	Import FCS File	
	Export FCS File	
	<u>R</u> ecent	F
	Recent Temp <u>l</u> ate	• • [
	Recent Compensation	•
	<u>C</u> lose Experiment	
	Exit	

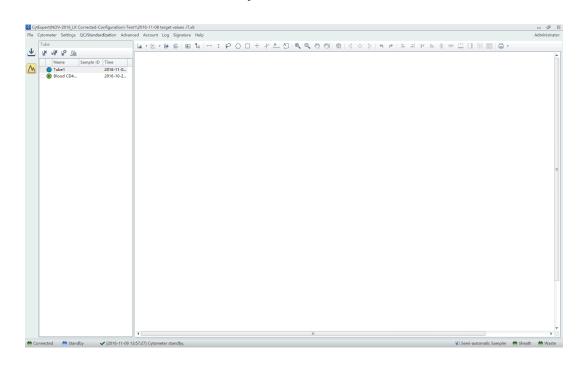
Imported data files appear in the Tube screen.

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 \boxed{M} on the left to enter the Analysis screen.

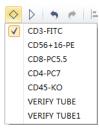


- **2** Copy plots obtained during data acquisition.
 - **a.** If you need original plots used during data acquisition, select \checkmark to access the Acquisition screen.
 - **b.** Select the appropriate plots.
 - **c.** Right-click the selected plots and select **Copy** from the drop-down menu or press 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

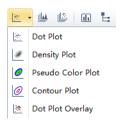
The CytExpert software supports histogram and dot plot data overlay functionality, allowing you to combine data from differing sources onto the same histogram or dot plot.

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

 Image: Image

Or

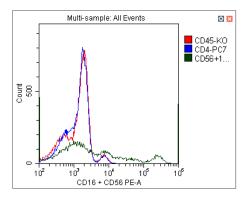
Select **Dot Plot Overlay** under the dot plot icon drop-down list to create a new dot plot overlay.



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.

\diamond	> • / =
	CD3-FITC
	CD56+16-PE
\checkmark	CD8-PC5.5
\checkmark	CD4-PC7
\checkmark	CD45-KO
	VERIFY TUBE
	VERIFY TUBE1



To remove a sample, select in 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.

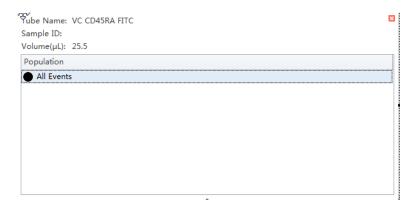
[CytoFLEX LX Shown]

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]

atistics Setting			Σ3
Header Statistics Population			
Experiment Name			
✓ Tube Name			
▼ Sample ID			
Record Time			
Volume			
Abort(%)			
Expression Edit			
Name:			
Equation:			
Comment			
	<u>^</u>		
Operator			
Select All Clear All			
Apply to:			
Current Tube	Set As Default	OK Can	

Statistics Setting 23 Header Statistics Population Parent Population Events % Total % Parent Events/µL(V) Events/µL(B) Select... Beads Population: Beads Count: 1 Sample Volume: 1.00 μL Parameter Mean Median rCV rSD CV SD FSC-A SSC-A CD8 IR84... VIABILITY... CD45 V52... CD5 V610... CD34 V66... CD3 V763... CD20 NU... Image: CD20 NU... CD4 NUV... Image: CD20 NUV... CD38 NU... b. Select All Clear All ◎ Area ○ Height ○ Area + Height Preview Apply to: ○ Current Tube ○ All Tubes Set As Default ОК Cancel



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.

Data Review Exporting Results

CHAPTER 8 Daily Shutdown

Overview

This chapter provides procedures for shutting down the CytoFLEX instrument.

Workflow:

Prepare the cleaning solution \rightarrow Clean the instrument \rightarrow 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

- 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.

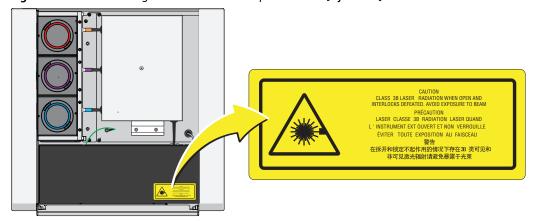


Figure 9.1 Laser Warning Label on the Laser Optical Bench [CytoFLEX]

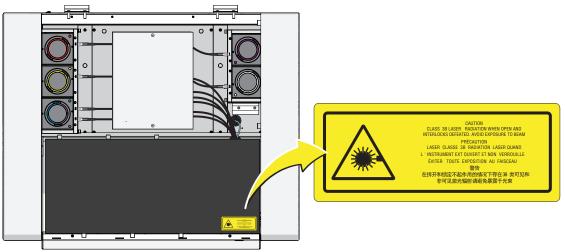


Figure 9.2 Laser Warning Label on the Laser Optical Bench [CytoFLEX LX]



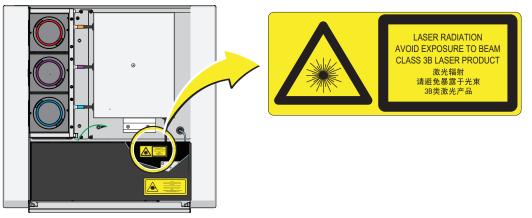
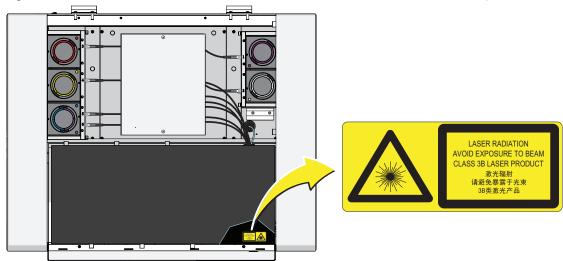


Figure 9.4 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX LX]



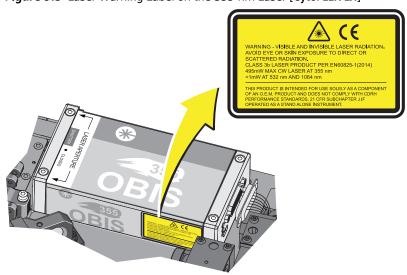
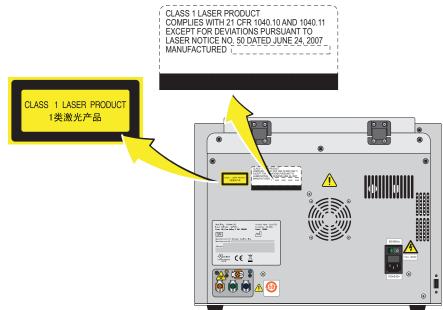


Figure 9.5 Laser Warning Label on the 355-nm Laser [CytoFLEX LX]

Figure 9.6 Laser Warning Labels on the Cytometer Back Cover [CytoFLEX]



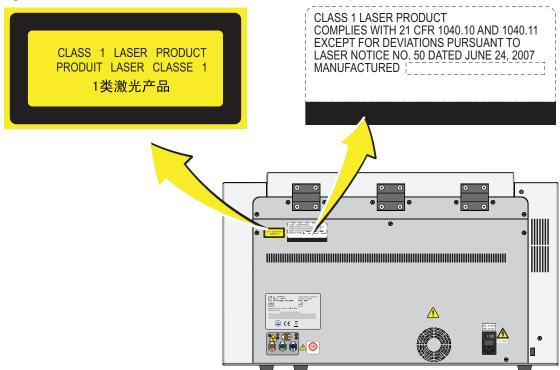


Figure 9.7 Laser Warning Labels on the Cytometer Back Cover [CytoFLEX LX]

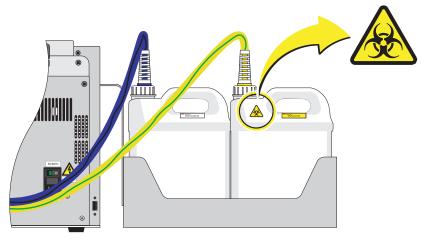
Hazard Labels and Locations

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



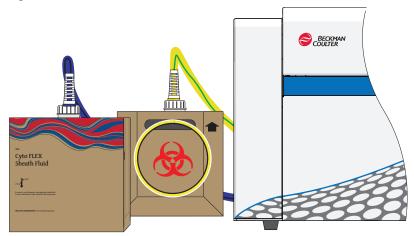
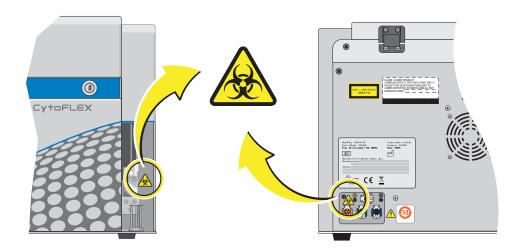


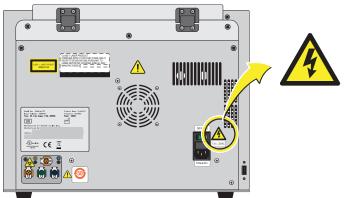
Figure 9.9 Biohazard Label on the 10 L Fluid Cubitainers

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]

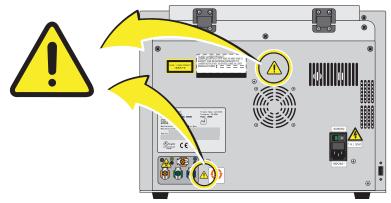
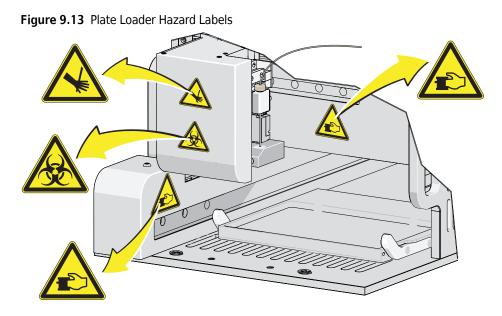


Plate Loader Hazard Labels and Location



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.

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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."

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. 	 Ensure the power switch is in the on position on the back of the Cytometer. [CytoFLEX LX] Select Turn On in the Cytometer menu. [CytoFLEX LX] Ensure that the power cable is securely connected to the back of the Cytometer. Replace the fuse. Refer to CHAPTER 11, Replacing the Fuse in CHAPTER 11, Replacement/Adjustment Procedures. 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.	 Ensure that the power cable is securely connected to the back of the Cytometer. Unplug the power cable. Wait 10 seconds, then plug the power cable back in. Then, restart the computer. If the problem persists, contact us.
The connection indicator light in the lower left corner of the software screen is red and displays <i>Disconnected</i> and <i>Error</i> .	 Data connection error The Cytometer is not turned on. The Cytometer's power cable is disconnected. 	 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. Restart the software. Restart the Workstation.Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. Turn on the Cytometer using the power switch on the back of the instrument. Verify that the power cable is securely connected to the back of the Cytometer. If the problem persists, contact us.

Table 9.1 Troubleshooting

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. 	 Ensure the sheath fluid/waste harnesses are secured to the correct container. 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. Restart the Workstation. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. Restart the software. MARNING 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. Verify that the float of the sensor in the sheath fluid/waste container moves freely. Replace the sheath fluid/waste harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. If the problem persists, contact us.

Table 9.1 Troubleshooting (Continued)

Problem	Probable Cause	Corrective Action
The fluid status information displays red	Instrument data connection error.	1. Ensure the sheath fluid/waste harnesses are secured to the correct container.
for <i>Sheath</i> and/or <i>Waste</i> even though the sheath fluid container is full and the waste container is	 The sensor connection is not working properly. The sensor does not work properly. 	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.
empty.	The sheath fluid/waste	3. Restart the software.
	harnesses have been secured on the wrong container.	 Ensure the sheath fluid harness and/or the waste harness are properly connected.
		 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.	 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. 	 Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures.
	 There are air bubbles in the flow cell. 	2. Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures.
	 The sample peristaltic pump tubing is aged. The sample peristaltic pump tubing is not properly connected. 	3. Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures.
		 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.
		7. If the problem persists, contact us.

Table 9.1	Troubleshooting	(Continued)
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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. 	 Use the manual threshold setting to increase the threshold. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. 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. Dilute the sample and adjust the concentration to approximately 10⁶/mL. Filter the sample using an appropriately sized mesh aperture filter. Restain the sample. Replace the Sheath Fluid Filter. Refer to Replacing the Sheath Fluid Filter in CHAPTER 11, Replacement/Adjustment Procedures. If the problem persists, contact us.
Laser power is low.	Communication error	 Reinitialize. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. Restart the Cytometer. 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. 	 Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Ensure that the sheath fluid harness and/ or waste harness is not kinked. Ensure that the sheath fluid harness and/ or waste harness is securely connected. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Verify that the float of the sensor in the sheath fluid container moves freely. Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. 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. 	 Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Run the Deep Clean procedure. Refer to Deep Clean Procedure in CHAPTER 10, Cleaning Procedures. Verify that the float of the sensor in the sheath fluid container moves freely. Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. 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. 	 Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Ensure that the sheath fluid harness and/ or waste harness is not kinked. Ensure that the sheath fluid harness and/ or waste harness is securely connected. Verify that the float of the sensor in the sheath fluid container moves freely. Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. If the problem persists, contact us.

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. 	 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. Ensure that the sheath fluid harness and/ or waste harness is not kinked. Ensure that the sheath fluid harness and/ or waste harness is not kinked. Ensure that the sheath fluid harness and/ or waste harness is securely connected. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Reinitialize. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. Restart the Cytometer. Verify that your sample does not have excessive debris. If it does: Filter the sample using an appropriately sized mesh aperture filter. Restain the sample. Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures. 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. 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.	 Ensure that the laser delay is set correctly. Refer to Setting Laser Delay in CHAPTER 11, Replacement/Adjustment Procedures. If the problem persists, contact us

Table 9.1	Troubleshooting	(Continued)
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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. 	 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. 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. Ensure that the optical filter is installed correctly. Refer to Replacing the Optical Filter in CHAPTER 11, Replacement/ Adjustment Procedures. Follow the QC procedure. Refer to CHAPTER 4, Instrument Quality Control and Standardization. Review the gain and threshold settings. Refer to Adjusting the Gain and Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. Review the display ranges. Refer to Creating Plots and Gates in CHAPTER 5, Data Acquisition and Sample Analysis. 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)
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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. 	 Ensure that the corresponding negative control tube and the individual positive tube acquired are from the same sample type. Ensure that the single colors collected correspond to the correct tube name. Ensure that the gate in the FSC/SSC plot encloses the correct sample population. Ensure that the positive gate in each tube is correctly placed. Modify the events to record to ensure that enough events are collected for the data population. 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. 	 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. Ensure that the parent gate is gated correctly. Change the display color. 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. 	 Ensure that the pipette used in sample processing is calibrated. Verify that the concentration of the sample is between 2x10⁴-10⁷ events/mL. 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. Ensure that the sample flow rate does not exceed 10,000 events/second. Adjust the threshold to remove sample debris. Ensure that the sample volume analyzed exceeds 10 μL. 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)
The sample probe is too low.	The sample probe is not securely connected.	 hierarchy are correct. Ensure that the sample probe is securely connected to the sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Presistaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. Ensure the sample pump cover is securely fastened. 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. 	 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. Ensure the sample pump cover is securely fastened. If the problem persists, contact us.

Table 9.1	Troubleshooting	(Continued)
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Problem	Probable Cause	Corrective Action
The mixer is not functioning.	 Sample mixing is disabled in the software. The mixer motor is defective. 	 Ensure sample mixing is enabled in the software. Refer to Changing Sample Mixing and Backflush Settings in CHAPTER 11, Replacement/Adjustment Procedures. 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. 	 Select Initialize. Ensure that the power switch to the Cytometer is turned on. Restart the software. Restart the Workstation. 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. 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. 	 Add 1 drop of CytoFLEX Daily QC Fluorospheres to the QC solution. Then, rerun QC. Reload the target value file. Refer to Importing Lot-Specific Target Values in CHAPTER 4, Instrument Quality Control and Standardization. Then, rerun QC. Prepare a new sample of the CytoFLEX Daily QC Fluorospheres. Then, rerun QC. Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures. Then, rerun QC. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Then, rerun QC. Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures. Then, rerun QC. 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. If problem persists, contact us.

Table 9.1	Troubleshooting	(Continued)
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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. 	 Ensure the sheath fluid container and the waster container are on the same level as the Cytometer. Rerun QC. Refer to CHAPTER 4, Instrument Quality Control and Standardization. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/ Adjustment Procedures. Then, rerun QC. Prime the sheath fluid filter with sheath fluid as follows, then rerun QC. Remove the vent cap from the sheath fluid filter. Ensure that the instrument is in Standby. 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. Wait until the sheath fluid approaches the vent port in the sheath fluid filter, then immediately reinstall the vent cap to avoid overflow. Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures. Then, rerun QC. Run Deep Clean. Refer to Deep Clean Procedure in CHAPTER 10, Cleaning Procedures. Then, rerun QC. If the problem persists, contact us.

Table 9.1 Troubleshooting (Continued)

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. 	 Verify that the correct plate type is selected in the Plate window. 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. 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. 	 Verify that the correct plate type is selected in the Plate window. 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. 	 Verify that the correct plate type is selected in the Plate window. Verify the sample mixing duration in the Plate window. 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.	 Verify the correct configuration file is installed. 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.	 Select Forgot password. 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.

Table 9.2	Troubleshooting [With Plate Loader]
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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.

Backup		
Select backup direct	ory	
Backup Directory:	F:\Backup CytFLEX	Next > Close

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:

W	arning	
	1	It is not recommended to store data on the same drive as the operating system due to the potential data loss risk. Are you sure you want to continue?
		Yes No

4 Select **Next**. The Prechecking window appears.

Backup	
Prechecking	
Prechecking the backup data, please wait a while.	

The backup starts automatically after the prechecking.

Backup				
Backup is in prog	gress			
Backing up				
Total Size:	240.66 KB			
Size Remaining:	868 bytes			
Time Remaining:	About 0 Second(s)			
		< Back	Next >	Cancel

Backup	
Backup succeeded	
The backup of the CytExpert data succeeded.	
Backup Directory: F:\Backup CytFLEX\20170602-111241	
	Finish

- 5 Select Finish.

Restore

Restore Welcome ▲ It is strongly recommended to backup to avoid possible data loss before the restoration.

1 Select **Backup/Restore > Restore**. The Restore window appears.

NOTE Ensure the data is backed up before the restoration.

- 2 Select Next.
- $\textbf{3} \quad \text{Select} \quad \underline{-} \quad \text{and browse to the desired directory to restore.}$

alast the vestere sein					
elect the restore poir	it			⊙⇔∟	
Backup Directory: D:\spring backup					
Restore Points					
Backup Time		CytExpert Version		Size	
2017-06-02 11:22:22		2.1.0.66		240.66 KB	
2017-06-02 13:08:16		2.1.0.66		240.66 KB	
			< Back	Next > Close	

4 Select **Next**. The Warning window appears.

Warning	9
<u> </u>	It may take some time to restore, and you can not turn off your computer during the restoration. Are you sure you want to restore? Yes No

IMPORTANT Do not turn off your computer during the restoration.

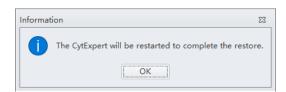
5 Select **Yes**. The Preparing window appears.

Restore		
Preparing		⊙⇒
Restore preparing		

The restoration starts automatically after finishing the preparation.

Restore	
Restore is in progress	
	⊙⇒
Restoring the CytExpert data	
Total Size: 240.66 KB	
Size Remaining: 24.66 KB	
Time Remaining: Calculating	
	Next > Cancel
1	
Restore	
Restore succeeded	⊙⇒
	070
The CytExpert data has been restored successfully.	
	Finish

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.

Log Clean-up	
Set clean-up condition	
Clean up experiment operation log and system operation log b 2017-07-11 00:00:00 🔻	Defore: Next > Close

Close the experiment and try again if the operation system prompts the following message:

Informa	ation 8	3
1	Please close the experiment and try again.	
	OK	

3 Select the desired date and time.

Log Clean-up	
Set clean-up condition	
Clean up experiment operation log and system operation log b 2017-07-11 00:00:00 ▼	Defore: Next > Close

4 Select **Next**. The Confirm window appears.

Confirm	
?	Are you sure you want to clean up the logs older than 2017-07-11 00:00:00?
	Yes

5 Select **Yes**. The Clean-up succeeded window appears.

Log Clean-up	
Clean-up succeeded	
The clean-up of the logs older than 2017-07-11 00:00:00 comp	oleted successfully.

6 Select Finish.

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 [With Plate Loader]
 - Cleaning the Sample Station
 - Deep Clean Procedure
 - Cleaning the 4 L Sheath Fluid Container
 - Cleaning the 4 L Waste Container
- Nonscheduled Cleaning
 - Surface Cleaning and Disinfection
 - Preparing the Instrument for Transport or Storage

Routine Cleaning

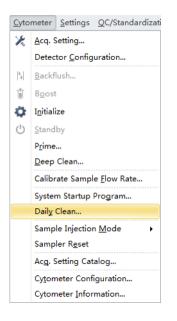
Daily Clean

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.

Daily Clean	23
Step1: Please load a tube with cleaning liquid.	
Duration:	
	Run

Step1:	Please load a tube with cleaning liquid.	
	Duration: 3 , minutes (1~100)	
	Remaining Time: 2 min 48 sec	
		Stop

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.

Daily Cl	ean	23
Step2:	Please load a tube with deionized water.	
	Duration: ninutes (1~100)	
	Run	
Daily Cl	ean	
Step2:	Please load a tube with deionized water.	
	Duration: 5 ; minutes (1~100)	
	Remaining Time: 4 min 29 sec	

8 After the process has been completed, remove the sample tube and close the Daily Clean window.

Stop

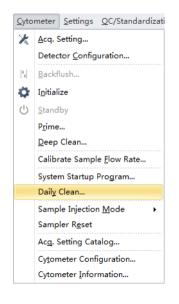
Daily Clean	
Step3: Finished.	
	Close

Daily Clean [With Plate Loader]

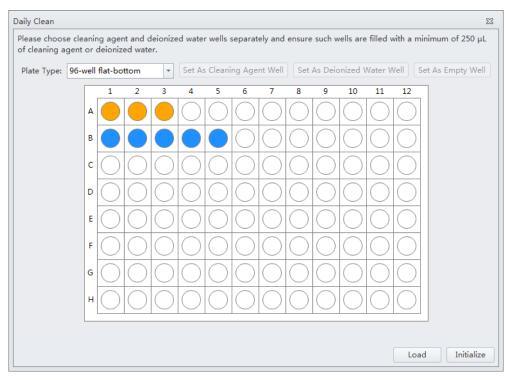
Daily Clean should be performed during instrument startup and instrument shutdown to clean the sample line.

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



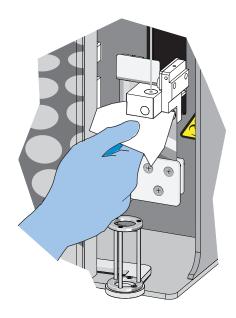
Carry out semi-automatic cleaning for the sample injection device once a week.

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.

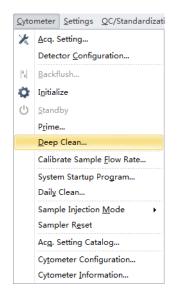
Deep Clean Procedure

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.

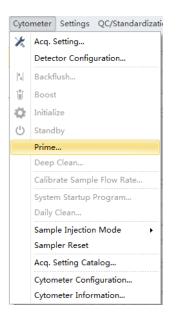


5 The status bar prompts that a deep clean is under way. Wait for the Deep Clean process to finish. The following software message appears:

Informati	ion X	3
1	Deep Clean has been done, please wait for at least 30 minutes to start Prime, and then you may start your next step.	
	ΟΚ	

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.

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.

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.

<u>/</u> 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.

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.

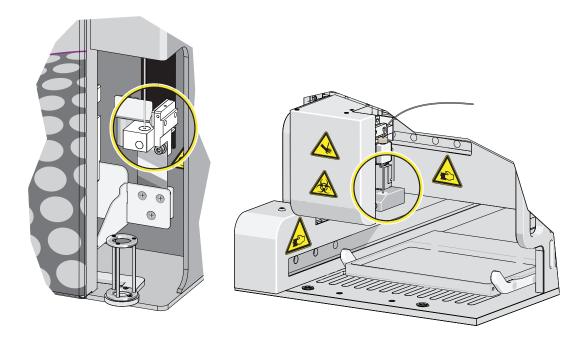
- **8** Dispose of the sodium hypochlorite solution in accordance with your local regulations and acceptable laboratory procedures.
- **9** Use deionized water to rinse the waste container and the waste harness. Ensure that there is no sodium hypochlorite residue.

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.

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.

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.

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

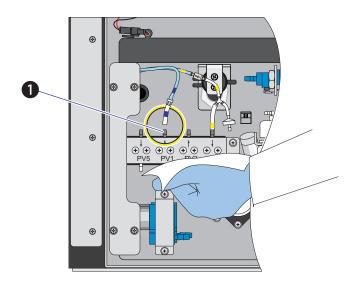
Risk of biohazardous contamination if you have skin contact with the waste container, its contents, and its associated tubing. The waste container and its associated tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of 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.

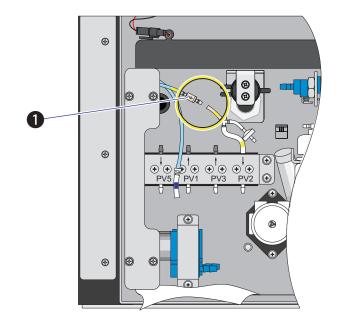
Risk of instrument damage. The Cytometer can suffer irreparable damage if it is exposed to subfreezing temperatures while it still contains liquid. Always drain the flow cell after cleaning the Cytometer if the Cytometer will be stored or transported in subfreezing temperatures.

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.

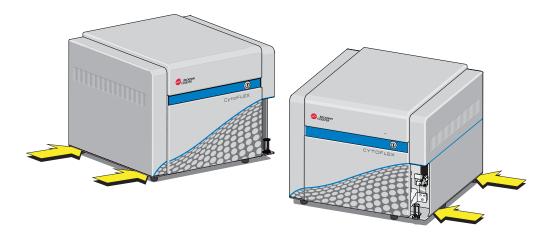
 $\ensuremath{\textbf{NOTE}}$ The flow cell is empty when liquid stops dripping from the blue-labeled tubing.

- **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.

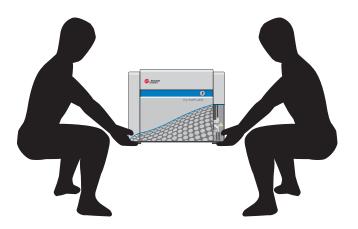
20 If the instrument is to be transported or stored, put the instrument and the Plate Loader module (if applicable) into the Beckman Coulter supplied packing and comply with the requirements in Instrument Transportation and Storage in APPENDIX A, Instrument Installation, regarding correct placement during 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.

Cleaning Procedures Nonscheduled Cleaning

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
 - Right-Side Cover Removal and Reinstallation
 - Filling the 4 L Sheath Fluid Container [CytoFLEX]
 - Replacing the 10 L Sheath Fluid Cubitainer
 - Emptying the 4 L Waste Container [CytoFLEX]
 - Emptying the 10 L Waste Cubitainer
 - Managing the Maintenance Reminder
 - Adding the Deep Clean Solution
 - Replacing the Sheath Fluid Filter
 - 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]
 - Inspecting the Liquid Flow Path for Leaks
 - Priming the Flow Cell
 - Replacing the Plate Holder [With Plate Loader]
 - Plate Loader Module Removal and Reinstallation [With Plate Loader]
 - Changing the Event Rate Setting
- Nonscheduled Replacement/Adjustment
 - Calibrating the Sample Flow Rate

- Calibrating the Sample Flow Rate [With Plate Loader]
- Setting Laser Delay
- Replacing the Optical Filter
- Replacing the Fuse
- Replacing the Sheath Fluid Harness and/or Waste Harness
- Changing Sample Mixing and Backflush Settings
- Calibrating the Plate Position [With Plate Loader]

Routine Replacement/Adjustment

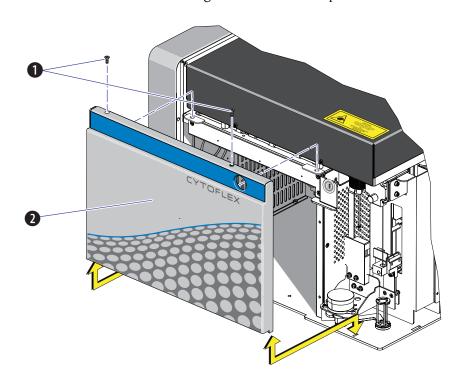
Front Cover Removal and Reinstallation

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.

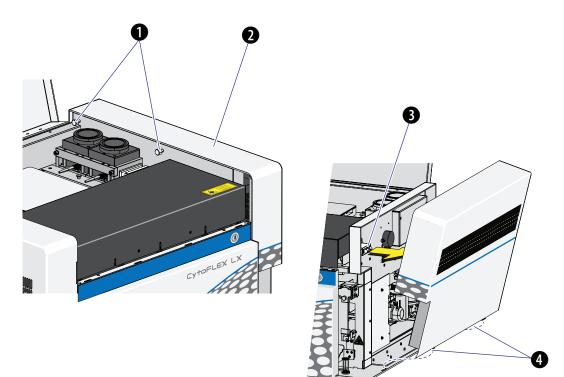
- 1. Securing screws
- 2. Front cover
- **5** Lift the front cover up and out of the slots in the frame.

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

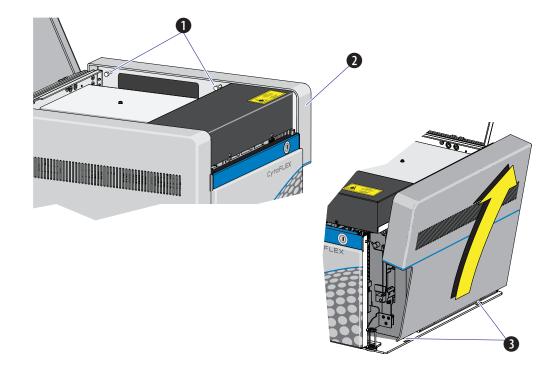
IMPORTANT The CytoFLEX LX flow cytometer only requires you to unfasten the thumbscrews the first time. There is a retaining clip that is used to secure the right-side cover without the need for the thumbscrews, if desired. Once the thumbscrews are unfastened, the right-side cover can simply be pulled 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.

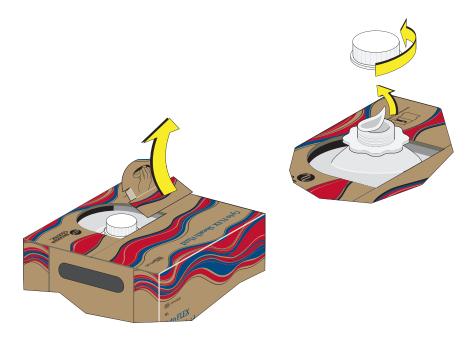
<u>A</u>CAUTION

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.



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.

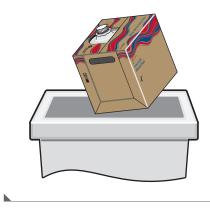


4 Inspect the pickup tube assembly and replace it if necessary.

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.

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



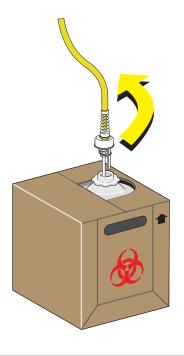
1 Confirm that the instrument is turned off or is in the standby state.

 ${\bf 2} \quad {\rm Lift \ the \ waste \ cubitainer \ and \ swirl \ it \ before \ removing \ the \ cap.}$



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.

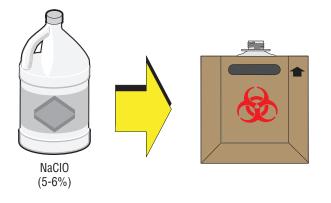


- **4** Empty the waste cubitainer according to your laboratory's procedures.
 - **NOTE** Take proper precautions to avoid spills if you are emptying the waste cubitainer into a sink, drain, or larger container. When moving the waste cubitainer to dispose of its contents, be sure the cap is secure to avoid spills.

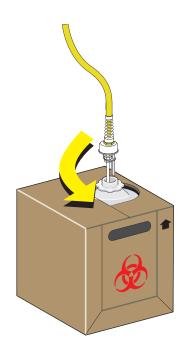
🕂 WARNING

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 Maintenance Message VI Semi-automatic Sampler Sheath Waste appears in the right side of the status bar.

1 Select the Maintenance Message icon <u>Maintenance Message</u> from the status bar to access the

Maintenance window. The expired item appears with a warning triangle | **A** 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	A	to the left of the item listed
is displayed with a warning triangle		to the feft of the field listen.

aintenance		Σ
Item	Last Maintenance Date	
🛕 Refill Deep Clean Solution Bottle	2014-01-05	
🛕 Replace Sheath Fluid Filter	2014-01-05	
🛕 Replace Peristaltic Pump Tubing	2013-10-06	
	Detail Close	

- **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.

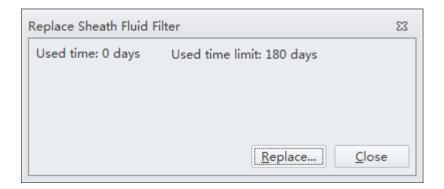
3 Select **Detail**. The Refill Deep Clean Solution Bottle window appears.

Refill Deep Clean Solution Bottle		
	Used time: 0 days Used time limit: 60 days	
Estimated remaining volume: 60 ml		
	Estimated remaining times of deep clean: 9 times	
	Refill Close	

Select **Refill**. A pop-up window appears to reset the maintenance date as the current date that the maintenance was performed.

Refill Deep Clean Solution Bottle 🛛 🕅			
Maintenance Date:	2017-10-12 -		
Use Time Limit:	60	days	
Refilled Volume:	60	ml	
	ок	Cancel	

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.

Replace Sheath Fluid Filter 🛛 🕅			
Maintenance Date:	2014-08-28 🔻		
Use time limit:	180	days	
	<u>o</u> ĸ	<u>C</u> ancel	

5 Select **Detail**. The Replace Peristaltic Pump Tubing window appears.

Replace Peristaltic Pump Tubing ຂ		
Used time: 0 days U	sed time limit: 180 days	
	Replace Close	

Select **Replace**. A pop-up window appears to reset the maintenance date as the current date that the maintenance was performed.

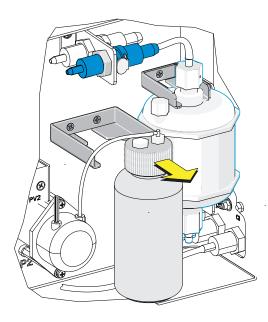
Replace Peristaltic Pump Tubing		
Maintenance Date:	2014-08-28 🔻	
Use time limit:	180	days
	<u>O</u> K	<u>C</u> ancel

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.

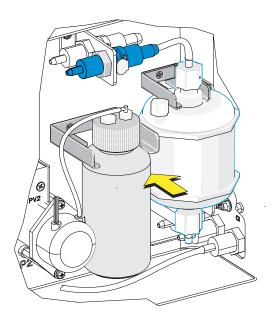
Risk of chemical injury from Contrad® 70 reagent. To avoid contact with the Contrad® 70 reagent, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

- **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.



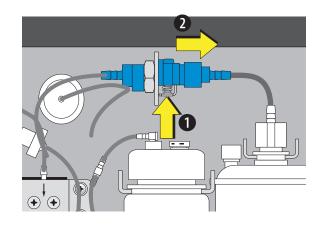
- 7 Reinstall the right-side cover (see Right-Side Cover Removal and Reinstallation), and fasten the thumbscrews.
- **8** Reset the maintenance reminder tracker. Refer to Managing the Maintenance Reminder.

Replacing the Sheath Fluid Filter

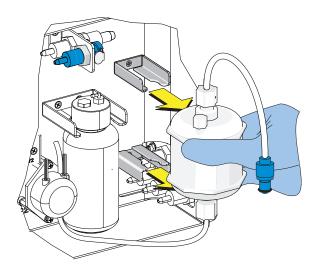
It is recommended to replace the sheath fluid filter every six months when the maintenance reminder prompts you to do so. The life of the filter is related to the quality of the sheath fluid used. If it is found that there are impurities in the light scatter pattern, replace the sheath fluid filter.

- 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.

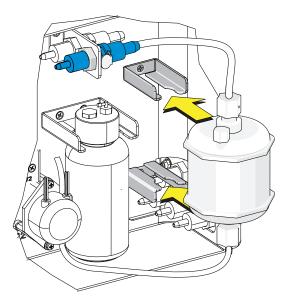


- 4 Repeat Step 3 for the quick connector behind the quick connector removed in the previous step.NOTE The spring is on the opposite side.
- **5** Remove the sheath fluid filter from the bracket.

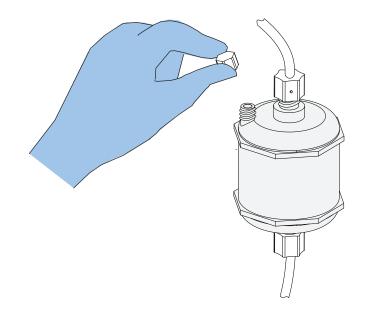


6 Connect the new, unused filter using the quick connector springs.

7 Attach the filter to the filter bracket.



8 Remove the vent cap and set it aside.

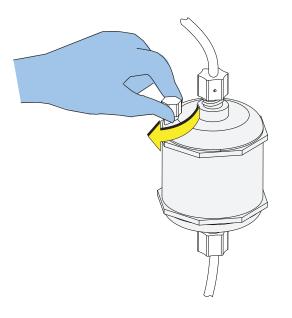


- **9** Turn on the Cytometer and open the software.
- $10 \hspace{0.1 cm} \text{Select Prime in the Cytometer menu.}$

<u>A</u> 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



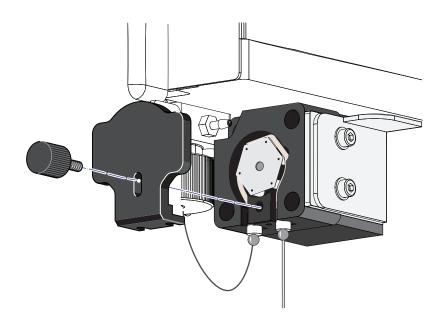


Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample 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.

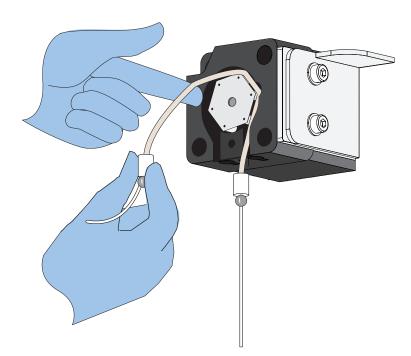
It is recommended to replace the tubing of the sample peristaltic pump every six months, as tubing used for an excessively long time can cause degradation of the stability of the sample flow and increase of the CV detected.

- **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.



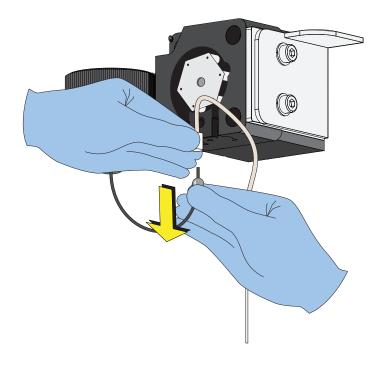
4 Remove the sample pump cover thumbscrew and the sample pump cover.

5 Take out the sample peristaltic pump tubing.

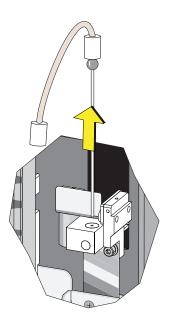


Risk of optical misalignment. Removing the PEEK tubing from the bottom of the flow cell could cause misalignment of the optical components. Do not remove the PEEK tubing from the bottom of the flow cell.

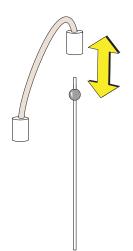
6 Remove the sample PEEK tubing from the sample peristaltic pump tubing.



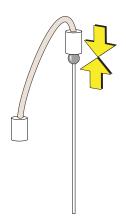
7 Lift the sample probe out of the wash station.



8 Remove the sample peristaltic pump tubing from the sample probe.

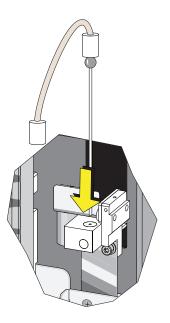


- **9** Dispose of the old sample probe and/or sample peristaltic pump tubing in accordance with your local regulations and acceptable laboratory procedures.
- **10** Connect the sample peristaltic pump tubing to the sample probe.

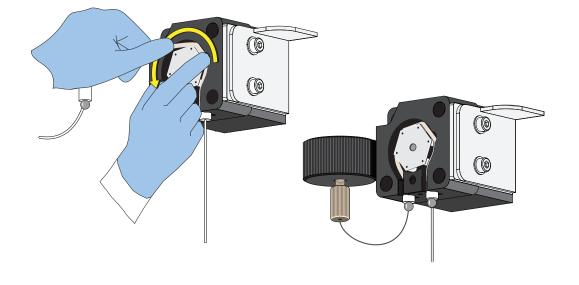


- <image>
- **11** Connect the sample PEEK tubing to the sample peristaltic pump tubing.

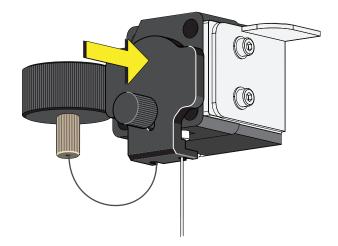
 $12\,$ Slide the sample probe into the wash station.



- **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.

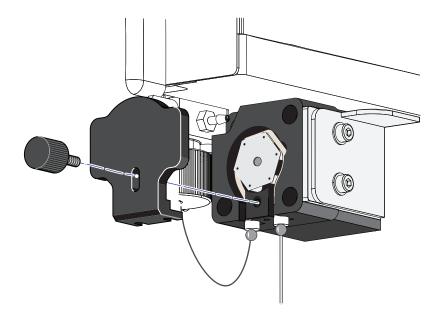
Replacing the Sample Probe Assembly [With Plate Loader]



IMPORTANT If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, contact us to replace the sample probe assembly.

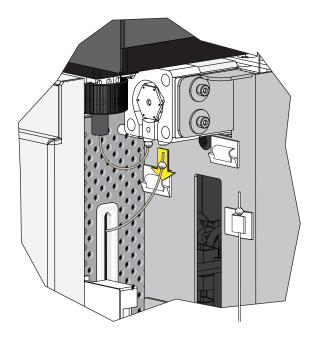
Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample 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.



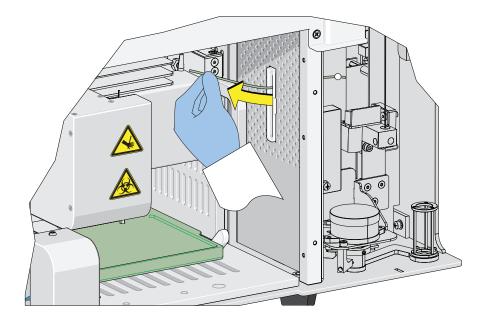
Risk of optical misalignment. Removing the PEEK tubing from the bottom of the flow cell could cause misalignment of the optical components. Do not remove the PEEK tubing from the bottom of the flow cell.

5 Remove the plate loader PEEK tubing from the sample peristaltic pump tubing.

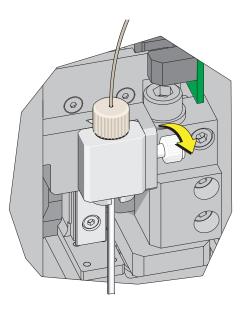


The plate loader PEEK tubing can be deformed, which could affect sample flow. When routing the plate loader PEEK tubing to and from the Sample Station, be careful not to pinch, crimp, stretch, or break the tubing.

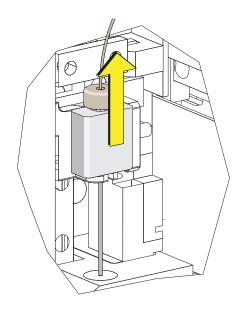
6 Pull the plate loader PEEK tubing through the slot so the tubing sits inside the instrument.



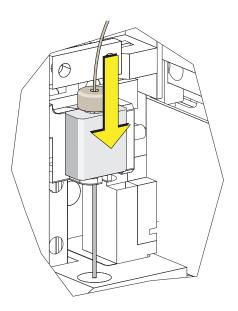
7 Loosen the white plastic thumbscrew behind the plate loader sample probe.



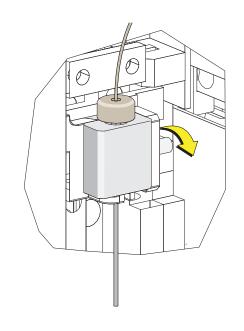
8 Lift the plate loader sample probe assembly straight up to remove it from the probe holder.



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.



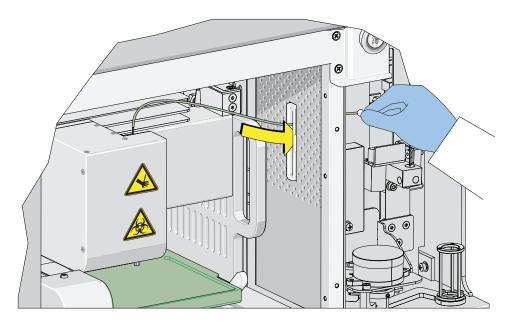
10 Tighten the white plastic thumbscrew.

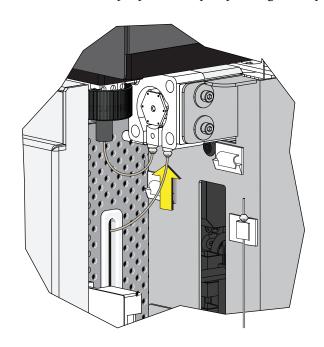




The plate loader PEEK tubing can be deformed which could affect sample flow. When routing the plate loader PEEK tubing to and from the Sample Station, be careful not to pinch, crimp, stretch, or break the tubing.

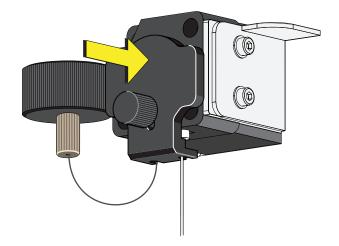
11 Slide the new plate loader PEEK tubing through the slot into the single tube sample station area.





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.

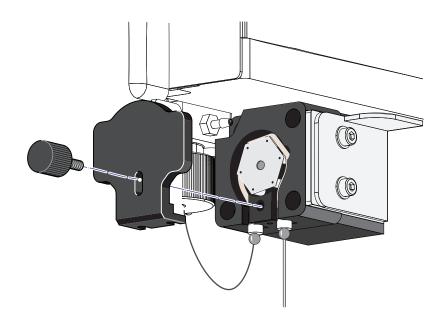
Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [CytoFLEX With Plate Loader]



🕂 WARNING

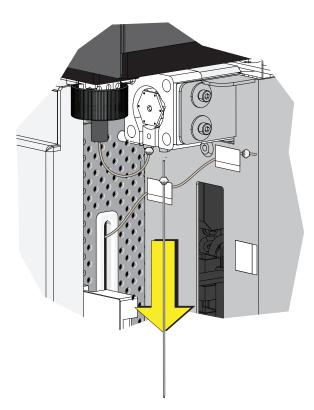
Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately.

- **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.

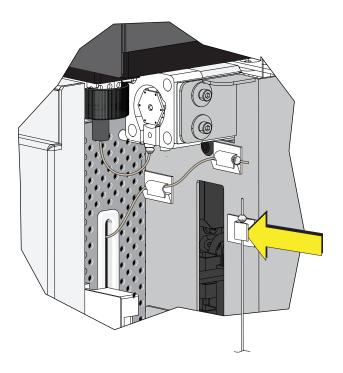


5 Remove the sample pump cover thumbscrew and the sample pump cover.

6 Remove the sample probe from the single tube sample station.



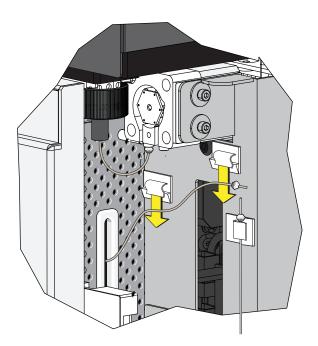
7 Place the sample probe in the white clip located on the right side of the sample station.



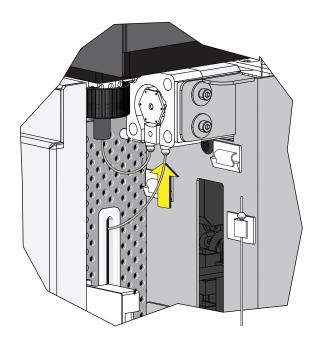
<u>A</u> CAUTION

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.

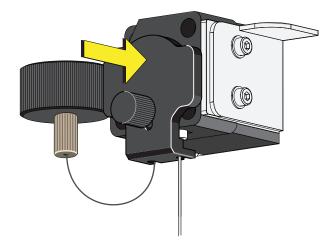
8 Remove the plate loader PEEK tubing from the two white clips located at the top of the sample station.



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]



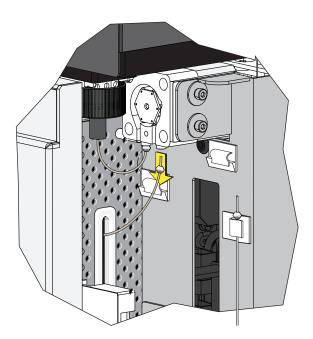
🕂 WARNING

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.

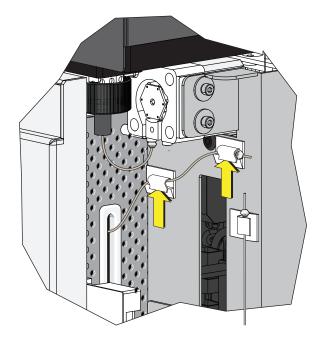
- **5** Remove the sample pump cover thumbscrew and the sample pump cover.

6 Remove the plate loader PEEK tubing from the sample peristaltic pump tubing.

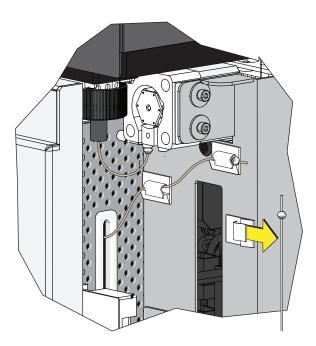


The plate loader PEEK tubing can be deformed, which could affect sample flow. When placing the plate loader PEEK tubing in the white clips, be careful not to pinch, crimp, stretch, or break the tubing.

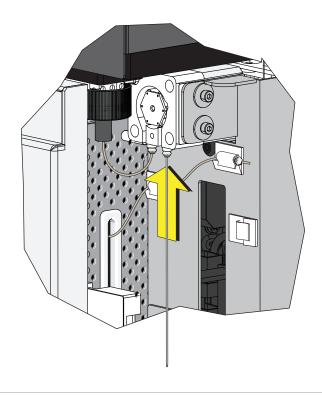
7 Place the plate loader PEEK tubing in the two white clips located at the top of the sample station.



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.

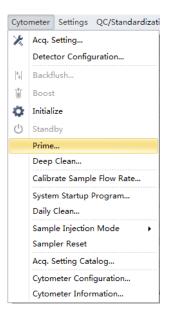
Priming the Flow Cell

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.

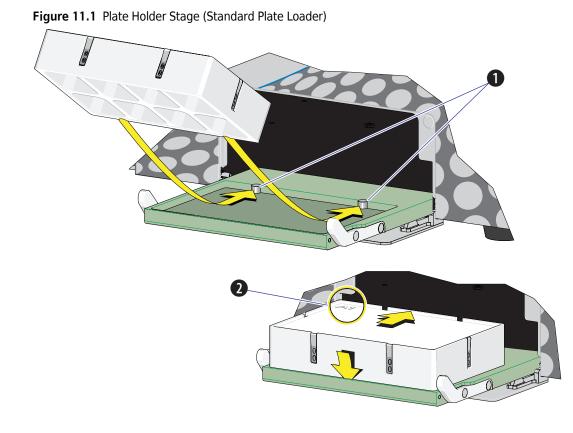


Otherwise, look for the status bar to display that priming has completed.



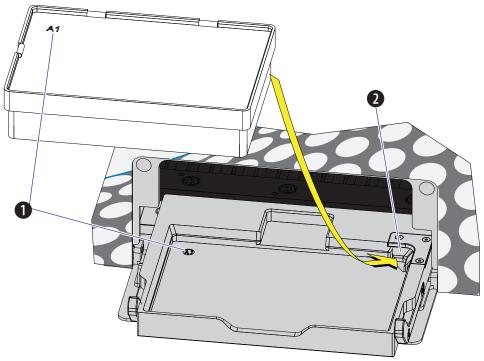
3 Run Daily Clean to clean the sample line. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures

Replacing the Plate Holder [With Plate Loader]



- 1. Pins
- 2. Position A1

Figure 11.2 Plate Holder Stage (Plate Loader DW)



- 1. Position A1
- 2. Spring lock

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]



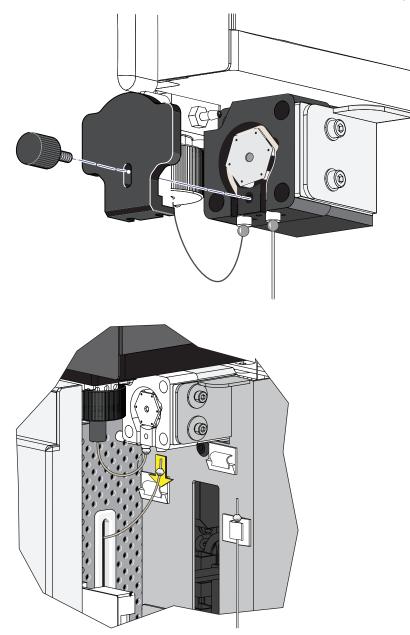
🕂 WARNING

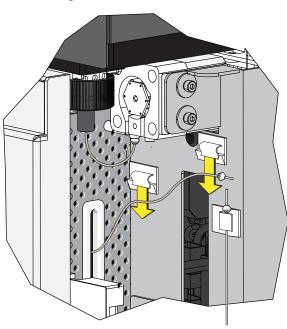
Risk of contamination from biohazardous material. Always wear PPE when performing this procedure as you can contact components with blood residue.

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.



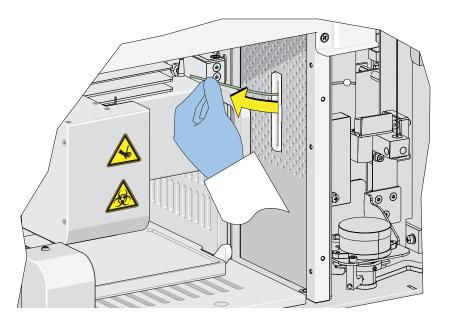


• If the plate loader PEEK tubing is in the plate loader PEEK tubing clips, remove the tubing from the clips.

AUTION

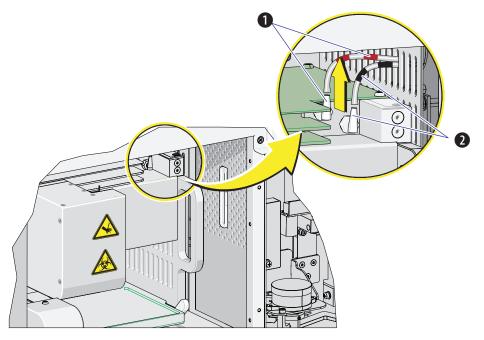
The PEEK tubing can be deformed which could affect sample flow. When routing the Plate Loader PEEK tubing to and from the Sampling Station, be careful not to pinch, crimp, stretch, or break the tubing.

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.

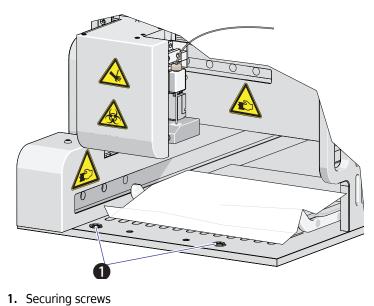
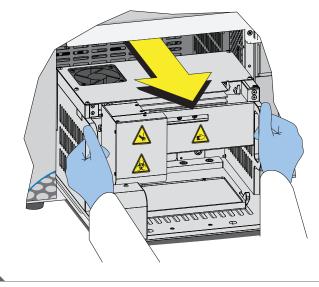


Figure 11.4 Plate Loader Module Securing Screws

8 Slide the Plate Loader module out of the Cytometer. Refer to Figure 11.5.



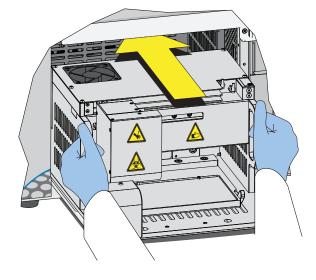


Installation

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.

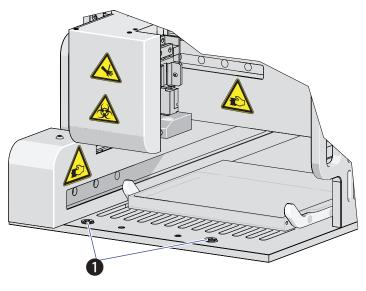
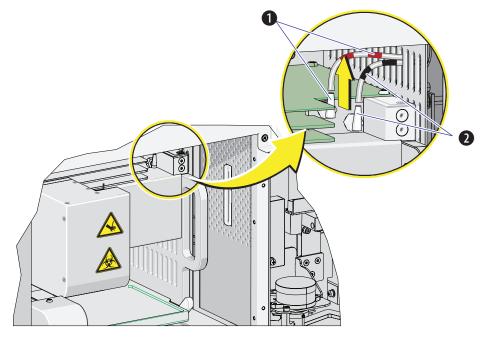


Figure 11.7 Plate Loader Module Securing Screws

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

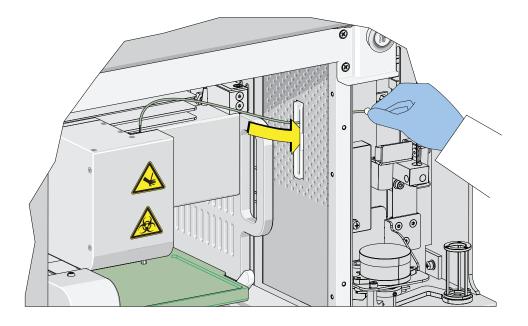


1. Red

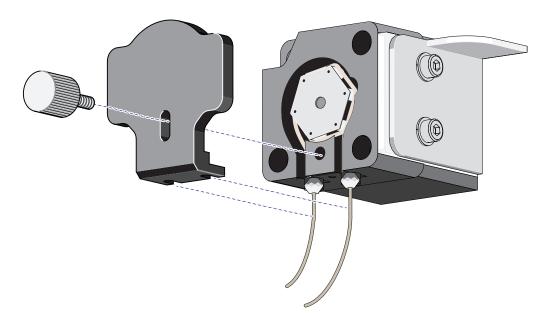
2. Black

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.



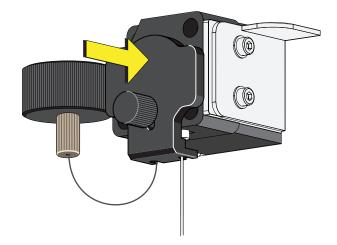
7 Remove the sample pump cover thumbscrew and the sample pump cover.



Connect the plate loader PEEK tubing to the sample peristaltic pump tubing.

9 Reinstall the sample pump cover.

8



- **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.

Event Rate Setting	23
When the event rate and abort rate are high (event rate > 10,000 events/s set to High mode. Otherwise, please choose Default mode.	second) , please
 Default 	
© High	
ОК	Cancel

2 Select **High** if the event rate is >10,000 events/second.

Or

Select **Default** if the event rate is <10,000 events/second.

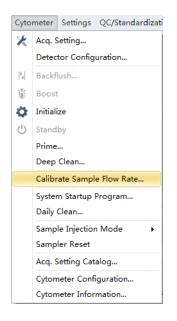
З Select ок.

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.

Calibrate S	ample Flow Rate	23
Flow Rate		
Fast:	60 µL/min	Calibrate
Medium:	30 µL/min	Calibrate
Slow:	10 µL/min	Calibrate
	ant to calibrate all flow rate at o n is recommended.)	one time, fast

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.

Calibrate Sample Flow Rate(Fast)
Step1: Please prepare at least 1mL of deionized water,
and enter the weight g.
Acquisition Duration(minutes):
<u>N</u> ext Cancel

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.

Calibrate Sample Flow Rate(Fast)
Step2: Start acquiring sample.
Acquisition Duration(minutes): 3
Previous Initialize Cancel
Previous Initialize Cancel

7 Select **Run** to begin acquiring the sample.

Calibrate Sample Flow Rate(Fast)	
Step2: Start acquiring sample.	
Acquisition Duration(minutes): 3	
Previous Run Can	cel 📗

Calibrate Sample Flow Rat	te(Fast)
Step2: Start acquiring sa	mple.
Acquisition Durati	on(minutes): 3
	Remaining Time: 2 min 9 sec
	Previous Run Cancel

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.

	ample Flow F				
Step3: Ple	ase enter the	e weight of the r	emaining dei	onized water	
		g.			
				Next	Cancel

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.

Calibrate Sample Flow Rate(Fast)	
The weight before acquisition: 3.5000 g. Acquisition duration: 3 minute(s). The weight after acquisition: 3.3200 g.	
The speed is correct!	
	Previous OK

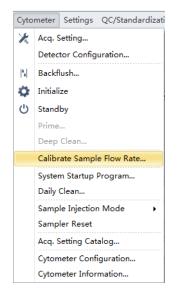
If deviation occurs, the setting is automatically corrected.

The weight before acquisit	
Acquisition duration: 3 min	
The weight after acquisitio	n: 3.3500 g.
The calibration result is ou	ut of error limit!
Click on "OK" button to se	et as calibrated flow rate.
According to this value	calibrate other flow rate
	Previous OK Cancel

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.



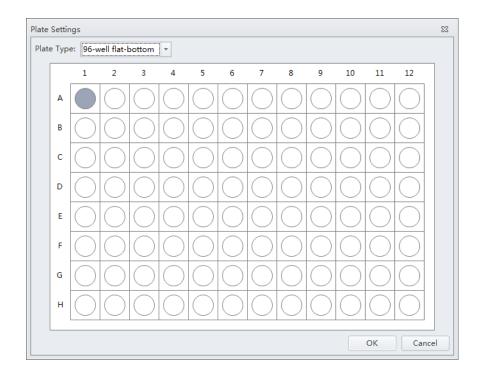
3 Select the sampling speed that needs to be calibrated and select **Calibrate**. The Calibrate Sample Flow Rate window appears.

Calibrate 9	Sample Flow Rate	Σ	3	
Flow Rate	e:			
Fast:	60 µL/min	Calibrate		
Medium:	30 µL/min	Calibrate		
Slow:	10 µL/min	Calibrate		
		ne time, fast		
Calibrate S	Sample Flow Rate(Fast)			
Step1: Pl	ease prepare at least 250µL of o	leionized water,		
ar	nd enter the weight 47.0000	g.		
Ad	equisition Duration(minutes):	3 ‡		
Pla	ate Type: 96-well flat-bottom			
w	ell: A1			
	Plate Settings			
			Next	Cancel
	Flow Rate Fast: Medium: Slow: (If you w calibrate Step1: Pl- ar Ac Pl. W	Calibrate Sample Flow Rate(Fast)	Flow Rate: Fast: 60 μL/min Medium: 30 μL/min Calibrate Slow: 10 μL/min Calibrate (If you want to calibrate all flow rate at one time, fast calibration is recommended.) Calibrate Sample Flow Rate(Fast) Step1: Please prepare at least 250μL of deionized water, and enter the weight 47.0000 g. Acquisition Duration(minutes): 3 ‡ Plate Type: 96-well flat-bottom Well: A1 Plate Settings	Flow Rate: Fast: 60 μL/min Calibrate Medium: 30 μL/min Calibrate Slow: 10 μL/min Calibrate (If you want to calibrate all flow rate at one time, fast calibration is recommended.) Calibrate Sample Flow Rate(Fast) Step1: Please prepare at least 250μL of deionized water, and enter the weight 47.0000 g. Acquisition Duration(minutes): 3 ‡ Plate Type: 96-well flat-bottom Well: A1

- **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.

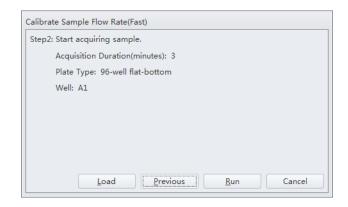
NOTE Do not exceed three minutes when using the fast sampling rate.

Calibrate Sample Flow Rate(Fast)
Step1: Please prepare at least 250µL of deionized water,
and enter the weight 47,0000 g.
Acquisition Duration(minutes):
Plate Type: 96-well flat-bottom
Well: A1
Plate Settings
<u>N</u> ext Cancel



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.

Calibrate Sample Flow Rate(Fast)
Step2: Start acquiring sample.
Acquisition Duration(minutes): 3
Plate Type: 96-well flat-bottom
Well: A1
Remaining Time: 2 min 40 sec
Eject Previous Run Cancel

- **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.

Calibrate Sample Flow Ra	te(Fast)	
Step3: Please enter the v	veight of the remaining deionized water	
	g.	
	Next Cancel	

14 Select ок.

The weight before acquisition: 47.0000 g. Acquisition duration: 3 minute(s). The weight after acquisition: 46.8200 g.	
The speed is correct!	
	Previous

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.

Error	8	
	The difference between the actual delay below and default delay is greater than 5µs, please adjust to the actual delay value and set it as default, try again, if the problem persists, please check fluid system. Laser: Red, Actual Delay: -16, Default Delay: -40	
	QK	

1 Select **Delay Setting** from the Advanced menu. The Delay Setting window appears.

Delay Setting				23
Detector	Current(µs)	Calc(µs)	Set Calc to Cu	urrent
Red	-45.12 📜	-	Set	
Blue	0.00 ‡	-	Set	
Violet	38.08 ‡	-	Set	
			Set All	
Set As Defa	ult <u>D</u> efau	lt	Clo	se

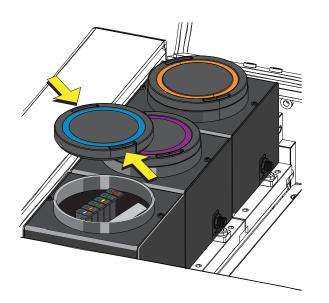
- **2** Set the current to the actual delay for the specified detector stated in the error message received.
- **3** Select **Set as Default**.

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.

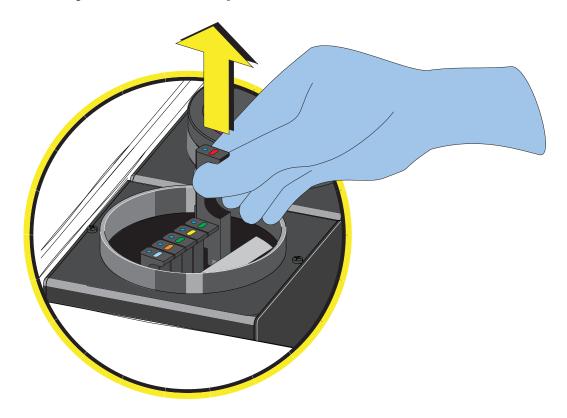
- 1 Confirm that the instrument is in the standby state or that the instrument is turned off.
- **2** Confirm the laser corresponds to the channel in which the optical filter is to be replaced.
- **3** Open the top cover of the instrument.
- **4** Press the spring piece of the WDM cap corresponding to the laser, and open the WDM cap.



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.

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.



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.

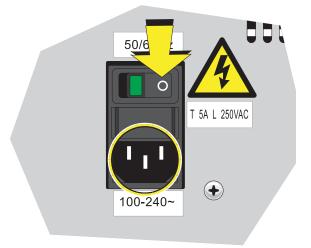
Replacing the Fuse

Use a 5 A, time delay, T 5 AL, 250 VAC fuse.

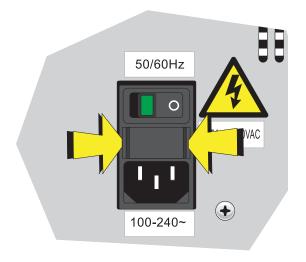


Risk of personal injury. A shock hazard exists if the power cable is connected. Turn off the Cytometer and disconnect the primary power cable before performing these procedures.

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.



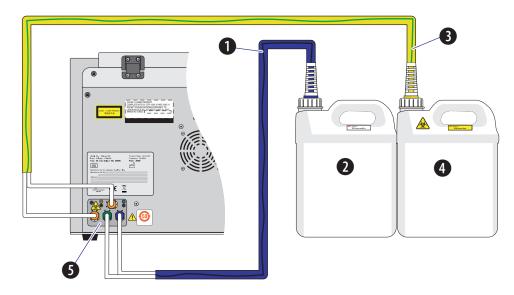
4 Insert the fuse holder.

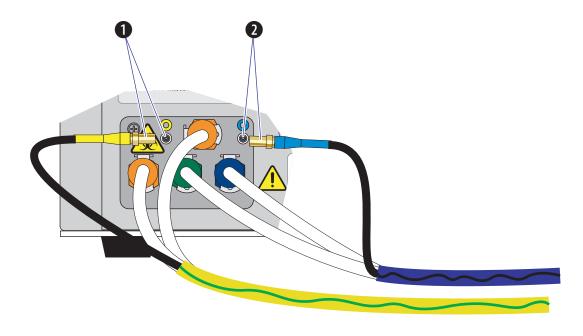
5 Reconnect the power cable.

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.

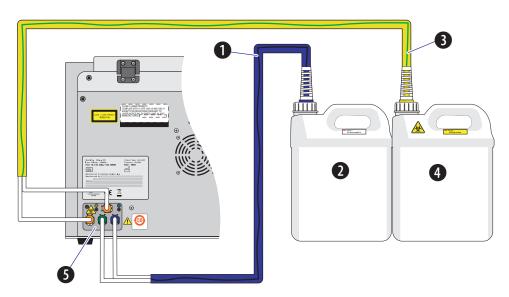
- 1 Confirm that the instrument is turned off or is in the standby state.
- **2** Remove sheath and/or waste pickup tubing from the appropriate container.
- 3 Disconnect the blue harness (1) from the sheath fluid container (2) and/or the yellow harness (3) from the waste container (4) from the fluid connector panel (5) on the back right corner of the instrument according to the color code.

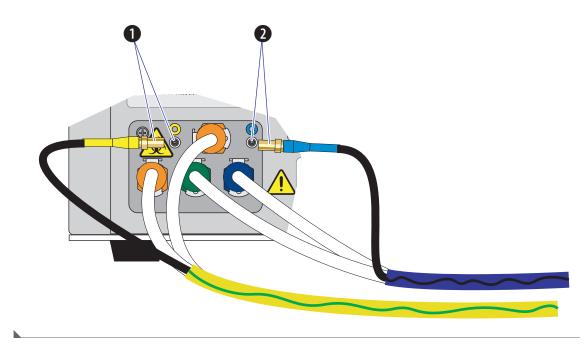




Disconnect the waste (1) and/or sheath (2) level sensors.

- **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- μ L 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- μ L 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.

Cytometer Configuration	23		
Backflush Duration			
Plate Loader: 3 sec (1 ~ 600)			
Semi Automatic			
Duration: 1.0 sec (0.1 ~ 5.0)			
Default OK Cance	el		

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.



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.

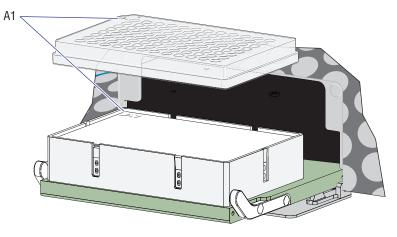
Active	Name	Mixing Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard	-	0
\checkmark	96-well V-bottom	Standard	-	0
1	96-well U-bottom	Standard	-	0
1	96-well deep well	Deep Well	-	0

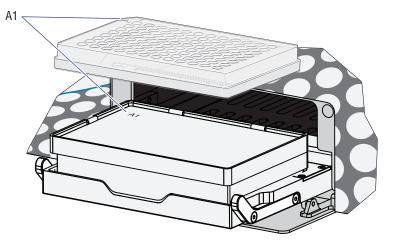
NOTE The 96-well deep well plate is only available for use if the Plate Loader DW is installed.

[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)]

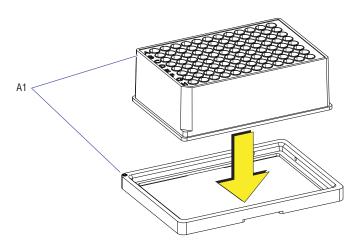




[Standard 96-Well Plate in the Plate Holder (Plate Loader DW -With groove)]

NOTE Ensure plate well A1 aligns with position A1.

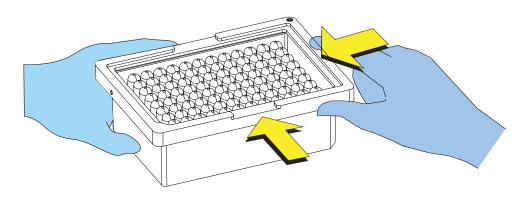
- **5 [96-Well Deep Well Plate]**: Select the 96-Well Deep Well plate type.
- **6 [96-Well Deep Well Plate]**: Place a 96-well deep well plate into the calibration frame.



NOTE Ensure plate well A1 aligns with position A1.

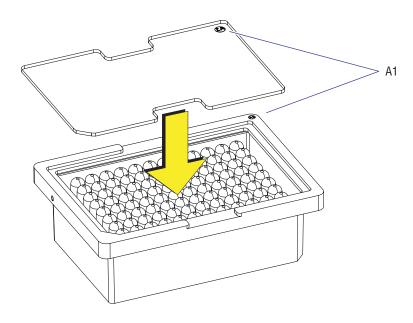
NOTE The calibration frame and the transparent plate are used to assist the calibration in X-axis and Y-axis. They are delivered together with the Plate Loader DW.

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.



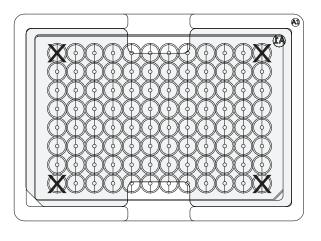
NOTE Ensure the deep well plate is correctly placed into the calibration frame.

8 [96-Well Deep Well Plate]: Place the transparent plate on the frame.

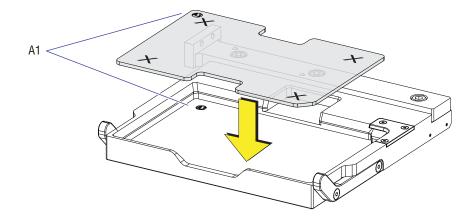


NOTE Ensure the A1 of the transparent plate aligns with position well A1.

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]

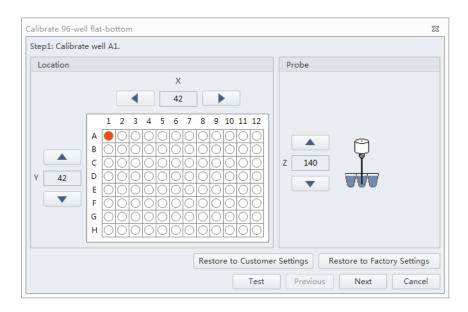
Edit Plate Type			53
Name:	96-well V-bot	tom	
Mixing Mode:	Standard	~	
Mix:	4	sec	
Remark:			
			-
			_
Last Calibration Time	e: -		
Calibrate		ОК	Cancel

[96-Well Deep Well Plate]

Edit Plate Type	X
Name:	96-well deep well
Mixing Mode:	Deep Well
Mix:	10 sec
Remarks:	
	Υ
Last Calibration Time:	-
Calibrate	OK Cancel

- **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.

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 **A** and **v** or **A** and **b** 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.

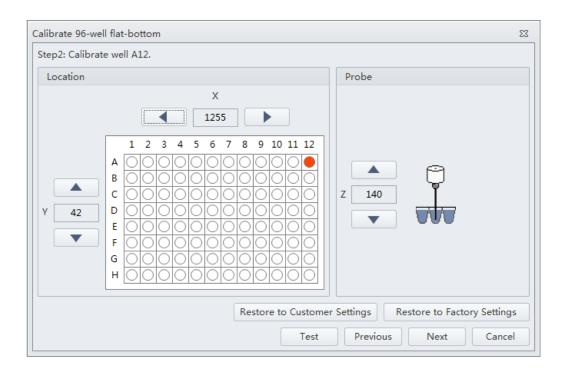
16 Select **Test** to verify the sample probe position.

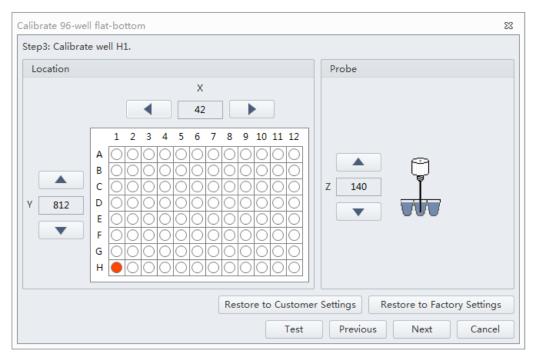
Listen for the click to ensure the probe has made contact with the bottom of the well. Readjust the sample probe position by moving the probe 3 steps up. This is the correct position for the Z-axis of the probe.

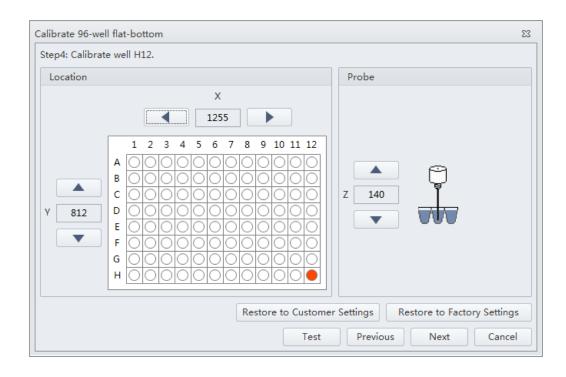
NOTE [Transparent Plate] The sample probe cannot come in contact with the surface of the transparent plate in the Z-axis. This is normal. Do not adjust the sample probe positions in the X-axis. The probe position in the Z-axis will be calibrated using a deep well plate.

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.

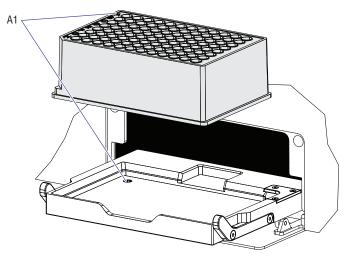
alibrate Plate 1	23
Step 5: Calibration completed.	
Click on the "Finish" button to save the data and exit, or click on the "Cancel" button to discard the data.	
Previous Finish Cance	el l

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.

Edit Plate Type		23
Name:	96-well deep well	
Mixing Mode:	Deep Well	
Mix:	10 sec	
Remarks:		
Last Calibration Time:	-	
Calibrate	OK Cance	

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.

ep1: Calibrate w	ell A1.	
Location		Probe
A B C 42 D E F G H		Z 140
	Restore to Custome	er Settings Restore to Factory Settings

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.

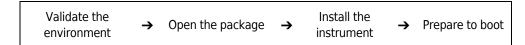
Listen for the click to ensure the probe has made contact with the bottom of the well. Readjust the sample probe position by moving the probe 3 steps upper. This is the correct position for the Z-axis of the probe.

- **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.



[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
- Installation Environment Validation
- Unpacking the Instrument and Inspecting the Materials for Defects or Omissions [CytoFLEX]
- CytExpert Software Installation Options
- Installing the Software [CytoFLEX]
- Upgrading the CytExpert Software
- Reinstalling the CytExpert Software

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

ACAUTION

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.

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

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.

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.

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.

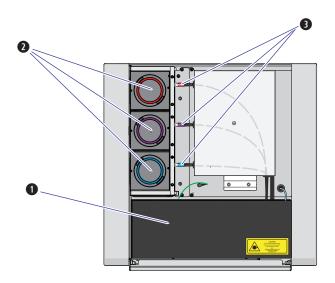
Reach under the base of the instrument to lift the Cytometer out of the package. Beckman Coulter recommends that two people lift the Cytometer out together.

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.

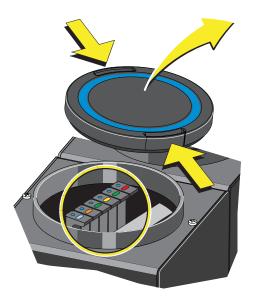
3 After removing the monitor and attaching the base, place them on the worktable with the computer.

[CytoFLEX Without Plate Loader Shown]

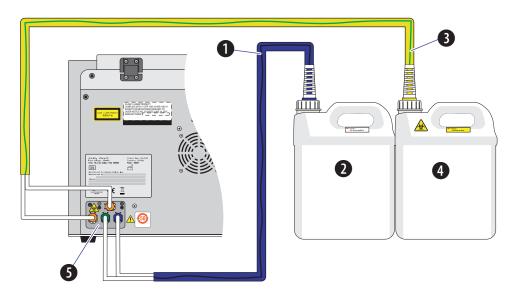
- 1. Fluid Containers. Place on the left side of the Cytometer.
- 2. Cytometer. Place between the Fluid Containers and the Workstation.
- 3. Workstation. Place on the right side of the Cytometer.
- 4 Open the top cover of the Cytometer. Check inside to verify that the optical bench cover (1) is tightly 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.

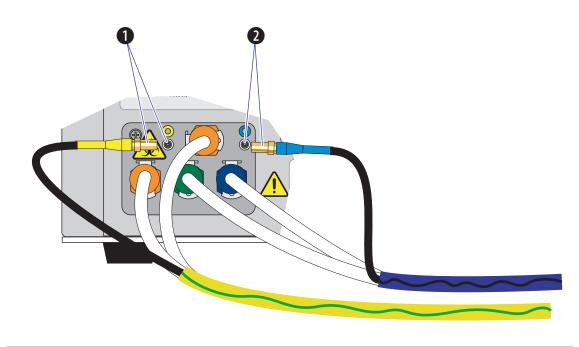


- **6** Insert sheath and waste pickup tubing into the appropriate container.
- 7 Connect the blue harness (1) from the sheath fluid container (2) and the yellow harness (3) from the waste container (4) to the fluid connector panel (5) on the back right corner of the Cytometer according to the color code.

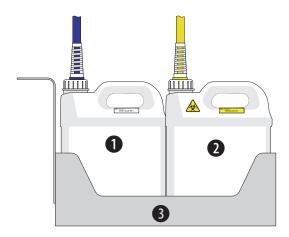


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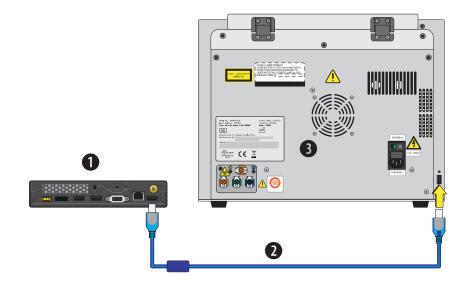
8 Connect the waste (1) and sheath (2) level sensors.



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.



 $11 \ \ {\rm Plug} \ {\rm the} \ {\rm Cytometer} \ {\rm power} \ {\rm cable} \ {\rm in} \ {\rm to} \ {\rm the} \ {\rm back} \ {\rm of} \ {\rm the} \ {\rm Cytometer}.$

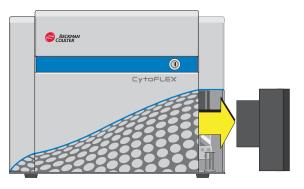


12 Connect the computer keyboard, mouse, and monitor.

A

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.

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**.

Confi	m	
(QC failed, do you want to save the adjusted gain as bead gain for the current batch and recommended gain?	
	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 Setup
Advanced installe
CytExpert Installation Options
Please select the CytExpert installation option:
 Default
🔘 User Management
Electronic Record Management
Please refer to the CytoFLEX Series Instructions for Use manual for details regarding each installation option. Download the Instructions for Use manual at: <u>www.beckmancoulter.com/ifu</u>
< Back Next >
< DACK NEXT >

- 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:



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.

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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.

😌 Use	r Account Control	
٢	Do you want to allow the following program from an unknown publisher to make changes to this computer?	
	Program name: Publisher: File origin:	CytExpert_1.2.11.0_Setup.exe Unknown Hard drive on this computer
🕑 s	how details	Yes No
		Change when these notifications appear

3 Select **Yes**. The CytExpert Setup Welcome window appears.

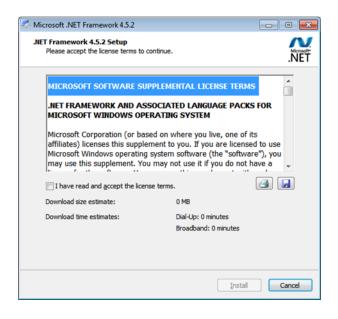


- 4 Select Next.
- **5** Select both support program checkboxes in the CytExpert Setup Prerequisites window.

💀 CytExpert Setup		×
Prerequisites These programs are needed for the ap next to a prerequisite to select it for in		x
Name	Version	Action
 Microsoft .NET Framework 4.5.2 Microsoft Visual C++ 2013 Redistri 	Required: 4.5.51209.34209 or h Required: 12.0.21005.1 or high	Install Install
Press the Next button to install the prerequ	uisites. Back Next Finit	ih Cancel

А

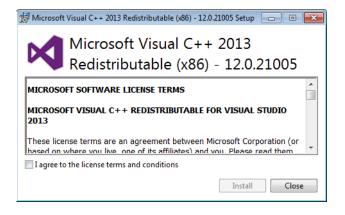
6 Select **Next**. The Microsoft .NET Framework window appears.



- 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.

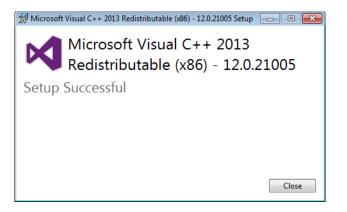


9 Select **Finish**. The Microsoft Visual C++ 2010 Redistributable Setup window appears.



10 Select the *I* have read and accept the license terms checkbox.

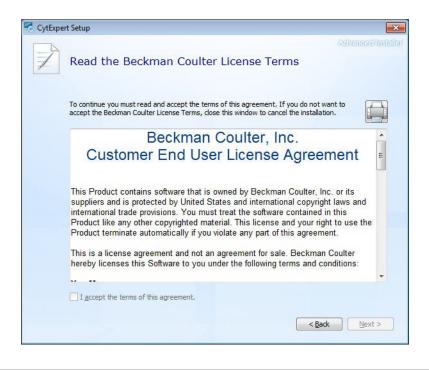
11 Select **Install**. The Setup Successful window appears when installation has finished.



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.

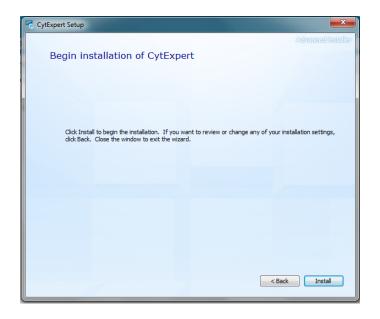
🗟 CytExpe	rt Setup		- • -
	Choose a file location	n	Advensed (Installer
	To install in this folder, dick "Next". T	To install to a different folder, enter it below or	dick "Browse".
	C:\Program Files\CytExpert\		Browse
	Total space required on drive: Space available on drive:	83 MB 47 G8	
	Remaining free space on drive:	47 G8	
		< Ba	k Next >

 $17 \hspace{0.1 cm} \text{Select Next. The Installation Options window appears.}$

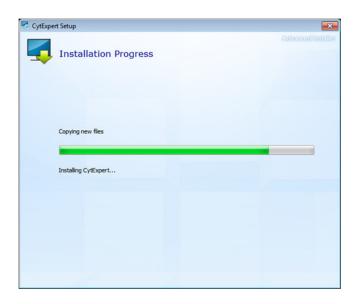


А

- **18** Select the desired installation option. Refer to CytExpert Software Installation Options.
- **19** Select **Next**. The Begin installation of CytExpert window appears.



 $20 \ \ {\rm Select} \ {\rm Install} \ {\rm to} \ {\rm begin} \ {\rm installing} \ {\rm the} \ {\rm software}. \ {\rm The} \ {\rm Installation} \ {\rm Progress} \ {\rm window} \ {\rm appears}.$



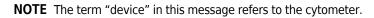
NOTE The software will install into the default file path provided unless otherwise specified.

21 If a Windows Security window appears. Select *Install this driver software anyway* to install the USB drive.

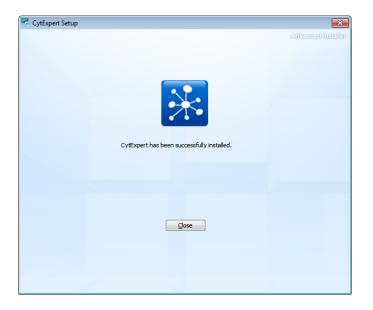


 ${\bf 22}\,$ The following software prompt appears. Select ${\bf 0}{\bf K}.$





 $\label{eq:23} \textbf{W} ait for the software to finish installing. The install complete window appears.$



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

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.

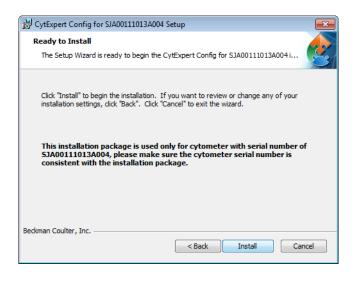
NOTE XXXX refers to the serial number of the instrument.

😌 User	Account Control	
۲	Do you want to allow the following program from an unknown publisher to make changes to this computer?	
	Program name: Publisher: File origin:	CytExpert_1.2.11.0_Setup.exe Unknown Hard drive on this computer
🕑 si	now details	Yes No
		Change when these notifications appear

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.

А

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.

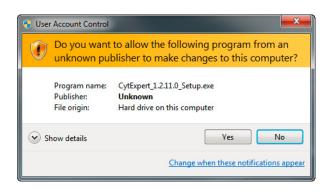
Risk of data loss. Reinstalling the CytExpert Software could overwrite your database. Ensure you backup your database prior to software reinstallation.

1 Insert the software USB into the computer.

NOTE If the Autoplay window appears, select Open folder to view files.

🖡 AutoPlay 📃 🖃 🗮 🗙
Removable Disk (D:)
Mixed content options
Play using Windows Media Player
Import pictures and videos using Windows
General options
Open folder to view files using Windows Explorer
Use this drive for backup using Windows Backup
Speed up my system using Windows ReadyBoost
View more AutoPlay options in Control Panel

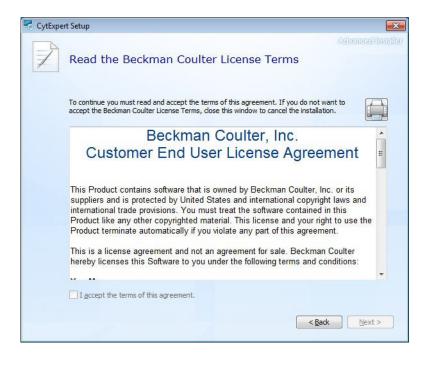
2 Select CytExpert_X.X_Setup.exe. The User Account Control window appears.



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.

А

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.

🗟 CytExpe	t Setup	—
4	Installation Progress	Advanced India'er
	Copying new files	
	Instaling CytExpert	

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.

dytExpert Setup		
		Advanced Installer
	*	
	CytExpert has been successfully installed.	
	Close	
		Second Co.

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.

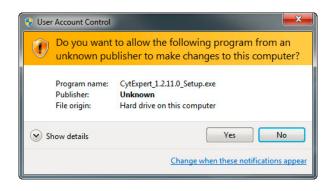
Risk of data loss. Reinstalling the CytExpert Software could overwrite your database. Ensure you backup your database prior to software reinstallation.

- **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.



3 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.



А

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.

CytExpert Setup	×
Begin remove of CytExpert	Advancadilinsiallar
Click Remove to remove CytExpert from your computer. If you want to review or ch installation settings, click Back. Close the window to exit the wizard.	
< Back	: Remove

- **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.

Α

8 Select **Remove**. When software removal is complete, the software displays the message *CytExpert has been successfully configured*.



9 Select Close.

 ${\bf 10}\,$ Navigate back to the software USB folder.

11 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.

😚 User Account Control		
	Do you want to allow the following program from an unknown publisher to make changes to this computer?	
	Program name: Publisher: File origin:	CytExpert_1.2.11.0_Setup.exe Unknown Hard drive on this computer
Show details		Yes No
		Change when these notifications appear

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.

А

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.

🗟 CytExpe	t Setup		- • -
	Choose a file location		Advanced installer
	To install in this folder, click "Next". To ins	tall to a different folder, enter it below or	dick "Browse".
	C:\Program Files\CytExpert\		Browse
	Total space required on drive: Space available on drive: Remaining free space on drive:	83 MB 47 G8 47 G8	
	1	< <u>B</u> ac	k Next >

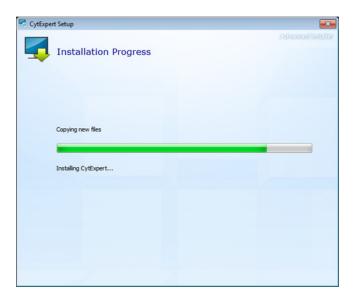
17 Select **Next**. The Installation Options window appears.



- **18** Select the desired installation option. Refer to CytExpert Software Installation Options.
- $19 \hspace{0.1 cm} \text{Select Next. The Begin installation of CytExpert window appears.}$



 $20 \ \ {\rm Select} \ {\rm Install} \ {\rm to} \ {\rm begin} \ {\rm installing} \ {\rm the} \ {\rm software}. \ {\rm The} \ {\rm Installation} \ {\rm Progress} \ {\rm window} \ {\rm appears}.$



Α

21 If the following software prompt appears, select **Ο***κ*.



NOTE The term "device" in this message refers to the cytometer.

 ${\bf 22}\,$ Wait for the software to finish installing. The install complete window appears.

🗟 CytExpert Setup		
	*	
	CytExpert has been successfully installed.	
	Glose	

 $\label{eq:23} \textbf{Select Close to finish the CytExpert software installation.}$

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

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.

Experiment Explorer					• **
WorkSpace			Enter	text to se	arch P
Open New Folder	Delete Rename Import E	xperiment.	Export Ex	operiment	
VorkSpace (110.96 GB	Name	Author	Date created	Modif	Date modified
v 퉬 Mouse 10Color	퉬 Mouse 10Color				
🐌 Experiment	🔢 Exp_20161121_1.xit	Admin	2016-11-21	Admin	2016-11-22 1
🐌 Templates	M Comp_20161121_1.xitc	Admin	2016-11-21	Admin	2016-11-21 1
Compensation	M Comp_20161122_1.xitc	Admin	2016-11-22	Admin	2016-11-22 1
v 🗄 TechSupport (15.55 GB	Exp_20161122_1.xit	Admin	2016-11-22	Admin	2016-11-22 1
🐌 Rat 6color	Exp_20161122_2.xit	Admin	2016-11-22	Admin	2016-11-22 1
	im template1.xitm	Admin	2016-11-24	Admin	2016-11-24 1
]

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.

Set Experi	mer	nt Directory	_	5	۰	23
Nan	ie	Path	Free Sp	bace	е	
		Add Rename Dele	te	(Close	

2 Select **Add**. The Add New Experiment Directory window appears.

Add New	Experiment Directory	23
Name:		
Path:		
	OK	cel

- **3** Enter a name for the experiment directory.
- **4** Select _____ and browse to the desired Windows file system folder.

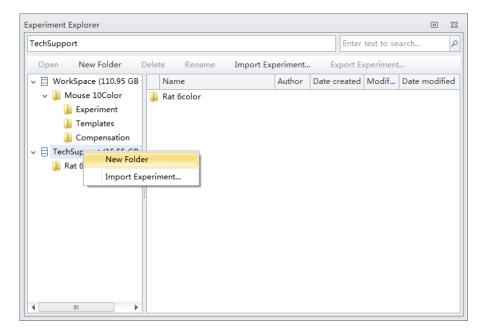
5 Select **OK**. The specified folder appears in the Set Experiment Directory window.

Set E	xperiment Director	у	_ 0 %
	Name	Path	Free Space
E	Kelly	C:\CytoFLEX-S_AS24001_Data	411.68 GB free
B	TechSupport	D:\CytoFlex Data	12.04 GB free
		Add	Delete Close

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.

/orkSpace\Mouse 10Color\E	(periment		Enter	text to se	arch
Open New Folder I	Delete Rename Impo	rt Experiment	. Export Ex	periment	
WorkSpace (110.97 GB	Name	Author	Date created	Modif	Date modified
🗸 퉬 Mouse 10Color	Exp_20161124_1.xit	Admin	2016-11-24	Admin	2016-11-24 1
) Experiment	Exp_20161124_2.xit	Admin	2016-11-24	Admin	2016-11-24 1.
) Templates					
퉬 Compensation					
TechSupport (15.55 GB					

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.

Exp	periment Explorer							۵	23
N	VorkSpace Enter text to search P Open New Folder Delete Rename Import Experiment Export Experiment Image: Specific content of the								
	Open New Folder [Dele	te Rename Import Exp	periment	Ex	port E	periment		
 	WorkSpace (110.97 GB		Name	Author	Date c	reated	Modif	Date modifi	ied
	🗸 퉬 Mouse 10Color		Mouse 10Color						
) Experiment		Exp_20161121_1.xit	Admin	2016-1	1-21	Admin	2016-11-22	1
	퉬 Templates		Comp_20161121_1.xitc	Admin	2016-1	1-21	Admin	2016-11-21	1
	퉬 Compensation		Comp_20161122_1.xitc	Admin	2016-1	1-22	Admin	2016-11-22	1
>	E TechSupport (15.55 GB		Exp_20161122_1.xit	Admin	2016-1	1-22	Admin	2016-11-22	1
			Exp_20161122_2.xit	Admin	2016-1	1-22	Admin	2016-11-22	1
		Ŵ	template1.xitm	Admin	2016-1	1-24	Admin	2016-11-24	1
		-							

2 Select Import Experiment from the right-click drop down menu or from the Experiment Explorer toolbar.

WorkSpace\Mouse 10Color\Experiment Enter text to search P Open New Folder Delete Rename Import Experiment Export Experiment WorkSpace (110.97 GB) Name Author Date created Modif Date modified Import Experiment Reparation Reperiment Reperiment Rev Folder Delete Rename Delete Rename Admin 2016-11-24 Admin 2016-11-24 1 Templa Compe Rename Delete Rename Import Experiment Import Experiment Import Experiment Import Experiment Import Experiment 	Experiment Explorer						23
 ✓	WorkSpace\Mouse						
✓ Mouse 10Color Image: Exp_20161124_1.xit Admin 2016-11-24 Admin 2016-11-24 1 Image: Experiment in the experiment in th	Open New Fo	older Delete Rename Impo	rt Experiment	Export Ex	periment		
WorkSpace\Mouse 10Color\Experiment Enter text to search Open New Folder Delete Rename Import Experiment Export Experiment V WorkSpace (110.97 GB Name Author Date created Modif Date modified V Mouse 10Color Exp_20161124_1.xit Admin 2016-11-24 Admin 2016-11-24 1 Experiment Experiment 2.xit Admin 2016-11-24 Admin 2016-11-24 1 Templa Delete Rename Delete Rename							
Image: Templa New Folder Image: Delete Delete > ☐ TechSupport Rename	v 퉬 Mouse 100	Color Exp_20161124_1.xit	Admin	2016-11-24	Admin	2016-11-24	1
	🍌 Templa	New Folder Delete Rename	Admin	2016-11-24	Admin	2016-11-24	1

3 Browse to the desired file path to import and select **Open**.

Open				×
Computer 🕨 🔾	DSDisk (C:) ► Files	► - <i>4</i> 9	Search Files	٩
Organize 🔻 New folder			= -	
쑦 Favorites	<u></u>	Name	Date modified	Туре
🧮 Desktop		Comp_20161115_1	11/24/2016 11:10	File folder
🐌 Downloads		Exp_20161115_3	11/24/2016 11:11	File folder
🕎 Recent Places		Exp_20161115_4	11/24/2016 11:10	File folder
Organize ▼ New folder				
🥽 Libraries	=	Comp_20161115_1.xitc	11/15/2016 10:28	XITC File
Organize New folder Pesktop Desktop Downloads Comp_20161115_1 11/24/2016 11:10 File name: Name Date modified Type Name Documents Music Pictures Subversion Pictures Open <				
	11/15/2016 11:45	XIT File		
Pictures		Exp_20161118_1.xit	11/18/2016 7:24 PM	XIT File
📄 Subversion		Template2.xitm	9/12/2014 1:00 PM	XITM File
📑 Videos				
🖳 Computer				
DSDisk (C:)				•
File name:	"Comp_20161115_1.			Cancel

A progress bar appears when importing files.

CytExpert	
Importing Exp_20161115_4.xit	40% Finished

Once the import is complete, the imported files display in the Experiment Explorer.

WorkSpace\Mouse 10Color\E	xperiment		Enter	text to se	arch	1
Open New Folder I	Delete <mark>Rename</mark> Im	port Experiment	. Export Ex	operiment		
v 🗄 WorkSpace (110.77 GB	Name	Author	Date created	Modif	Date modifi	ec
🗸 퉬 Mouse 10Color	Exp_20161124_1.xit	Admin	2016-11-24	Admin	2016-11-24	1.
퉬 Experiment	Exp_20161124_2.xit	Admin	2016-11-24	Admin	2016-11-24	1
鷆 Templates	Komp_20161115_1.xite	Admin	2016-11-24	Admin	2016-11-24	1
)) Compensation	🔢 Exp_20161115_3.xit	Admin	2016-11-24	Admin	2016-11-24	1
> 📋 TechSupport (15.55 GB	🔢 Exp_20161115_4.xit	Admin	2016-11-24	Admin	2016-11-24	1
	🔢 Exp_20161118_1.xit	Admin	2016-11-24	Admin	2016-11-24	1
	🔟 Template2.xitm	Admin	2016-11-24	Admin	2016-11-24	1
▲						

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.

Workspace\Experiment	s				ırch			
Open New Folde	er Delete	Rename	Import Experi	ment	Export Exp	eriment		
v 🗄 Workspace (270.4	46 GB free)	Name		Author	Date crea	Modi	Date modi	fi.
) Experiments		Exp_2016	1122_1.xit	Admin	2016-11-2	Admin	2016-11-22	2
Compensation	15	Exp_2016	1122_2.xit	Admin	2016-11-2	Admin	2016-11-22	2
🌗 Templates		Exp_20160	0616_1.xit	Admin	2016-11-2	Admin	2016-11-22	2
		Exp_20160	0613_1.xit	Admin	2016-11-2	Admin	2016-11-22	2
		Exp_14370	0902_2.xit	Admin	2016-11-2	Admin	2016-11-22	2
		Exp_25590	0607_1.xit	Admin	2016-11-2	Admin	2016-11-22	2
		Exp_14370	0902_1.xit	Admin	2016-11-2	Admin	2016-11-22	2
		Exp_20160	0531_1.xit	Admin	2016-11-2	Admin	2016-11-22	2
		Exp_2016)527_1.xit	Admin	2016-11-2	Admin	2016-11-22	2
		Comp_20:	160331_1.xitc	Admin	2016-11-2	Admin	2016-11-22	2
		Template.	xitm	Admin	2016-11-2	Admin	2016-11-22	2

2 To export a single experiment:

a. Select **Export Experiment** from the right-click drop down menu or from the Experiment Explorer toolbar.

CytoFLEX S AS24001	
Open New Folder Delete Renar	me Import Experiment Export Experiment
> 📋 CytoFLEX S AS24001 (405.39 GB free)	Name
🛃 CORE LAB (Unavailable)	B Mouse 10Color
	🔋 Lymph Phenotyping
	Exp_20161024_1.xit
	Exp_20161025_1 Open
	2010-10-17_KF_f Delete
	2010-10-17_KF_f
	2010-10-17_fitc-
	Exp_20161004_1 Export Experiment

b. Browse to the desired file path for export and select **Save**.

Save As		
○ ◯ ⊂ 📜 « OSDisk (C:) → Users → jzhao03 → De	sktop 👻 🍫 Search Desktop	٩
Organize 🔻 New folder		= • 0
★ Favorites ■ Desktop ③ Downloads ③ Libraries ③ Documents ④ Music ■ Pictures ⑤ Subversion ▼ Videos	Date modified Typ	ie
File name: Exp_20161019_1.wit Save as type: Experiment (*.wit)		•
Hide Folders	Save	Cancel

A progress bar appears when exporting files.

	62% Finished

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.

				ch	
Workspace\Experiments		Ente	Enter text to search		
Open New Folder Delete	e Rename Import Experin	ment Export	Experiment		
y	Name	Author Date cre	a Modi	Date modifi.	
🐌 Experiments	Exp_20161122_1.xit	Admin 2016-11	2 Admin	2016-11-22	
鷆 Compensations	🔢 Exp_20161122_2.xit	Admin 2016-11-	2 Admin	2016-11-22	
鷆 Templates	🔢 Exp_20160616_1.xit	Admin 2016-11-	2 Admin	2016-11-22	
	Exp_20160613_1.xit	Admin 2016-11-	2 Admin	2016-11-22	
	Exp_14370902_2.xit	Admin 2016-11-	2 Admin	2016-11-22	
	Exp_25590607_1.xit	Admin 2016-11-	2 Admin	2016-11-22	
	Exp_14370902_1.xit	Admin 2016-11-	2 Admin	2016-11-22	
	🔢 Exp_20160531_1.xit	Admin 2016-11-	2 Admin	2016-11-22	
	Exp_20160527_1.xit	Admin 2016-11-	2 Admin	2016-11-22	
	M Comp_20160331_1.xitc	Admin 2016-11-	2 Admin	2016-11-22	
	Template.xitm		2 Admin	2016-11-22	
		Open			
		Delete			
		Rename			
		Export Experime	nt		

b. Browse to the desired file path for export and select **OK**.

Browse For Folder	×
	_
E Desktop	<u>^</u>
Libraries	
Description: Parallel Description: Parall	E
4 🖳 Computer	-
a 🚰 OSDisk (C:)	
BitLocker	
Intel	
D 🏭 MININT	
D MSOCache	
PerfLogs	
Program Files	
Program Files (x86)	
ProgramData	
Jan Temp	
Users	
Windows	
▲ Workspace	
0 Work	
Make New Folder OK Cance	e
	.d

A progress bar appears when exporting files.

Importing Exp_20160926_1.xit	

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

Experi	ment Opera	tion Log					2			
Que	ery Criteria									
Exp	eriment:	Workspace\Exper	iments\Exp_20161122_1.xit			Current Experiment Select	1			
Exp	eriment ID:	D: BACBF084B198455CA734FEDA3FD4A39E								
User:		(ALL)								
Time	e Range:	All time								
		Customized 2	016-10-22 00:00:00 +	~ 2016-11-22 23:59:	59 -					
		- L								
- Q	Juery					Print & Exp	ort			
	Operati	ion	Username	User Full Name	Timestamp	Record				
	1 Add pla	ite	Admin	Administrator	2016-11-22 15:32:49	Add a new plate 01.	4			
	2 Add Tu	be	Admin	Administrator	2016-11-22 15:35:14	Add a new tube 01-Tube-A1.				
	3 Modify	Well Type	Admin	Administrator	2016-11-22 15:35:53	Modify plate 01 well A10 type from empty well to sample well.				
	4 Modify	Well Type	Admin	Administrator	2016-11-22 15:35:53	Modify plate 01 well A11 type from empty well to sample well.				
	5 Modify	Well Type	Admin	Administrator	2016-11-22 15:35:53	Modify plate 01 well A12 type from empty well to sample well.				
	6 Add Tu	be	Admin	Administrator	2016-11-22 15:35:53	Add a new tube 01-Tube-A10.				
	7 Add Tu	be	Admin	Administrator	2016-11-22 15:35:53	Add a new tube 01-Tube-A11.				
	8 Add Tu	be	Admin	Administrator	2016-11-22 15:35:53	Add a new tube 01-Tube-A12.				
	9 Modify	Well Type	Admin	Administrator	2016-11-22 15:36:02	Modify plate 01 well C2 type from empty well to sample well.				
	10 Modify	Well Type	Admin	Administrator	2016-11-22 15:36:02	Modify plate 01 well C3 type from empty well to sample well.				
	11 Modify	Well Type	Admin	Administrator	2016-11-22 15:36:02	Modify plate 01 well C4 type from empty well to sample well.				

- 1. Experiment: Used to specify the experiment file 6. Query display: Displays the queried results. 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.
- Query : Runs the query on the specified 5. query criteria.

- Print & Export... : Displays the print and export 7. preview dialog. Refer to Figure B.3.
- 8. Select... : Selects an experiment from the Select Experiment Profile window. Refer to Figure B.2.
- Current Experiment : Selects the experiment 9. that is currently open.
 - **NOTE** An experiment must be open for the button to be selectable.

В

lect Experiment Profile									8
Enter text to search	* Clear								
xperiment ID	Name	Location	Creator Userna	Creator Full Na	Creation Time	Last Modifier Usern	Last Modifier Full N	Last Modification T	Deleted
BCA319BDA9E4735AD90FA052EE8	Exp_20161024_1.xit	Kelly	Admin	Administrator	2016-10-24 10:3.	Admin	Administrator	2016-10-27 14:23:37	No
53185B327944189BE7B7ECCD774	Exp_20161025_1.xit	Kelly	TechSupport	TechSupport	2016-10-25 12:3.	TechSupport	TechSupport	2016-10-25 12:36:05	No
42C64DBF9048AF983AA1C3EF62	2010-10-17_KF_fite-ecd-pc7 stdzn setup.sit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:38	No
LDDB2C8DF5C4478960699E4DF3B	2010-10-17_KF_fitc-ecd-pc7.xit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:16:07	No
500E44E983A4FB6B75B26454F1FA	2010-10-17_fite-ecd-pc7 setup.xit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:38	No
8589F05D2BF4ED9BE00787D9816A	Exp_20161004_1_Test Flow Plate.xit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:41	No
4D10415C8464688926514F2E4676	fitc-pe-ecd-pc7-apc-apca700_Exp_2016092	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:41	No
A5B627FBD67469B8C89C1F58EB3F	FITC-PE-ECD-PC7KO_Exp_20160928_1.nit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:42	No
933340035E34E0BA16240337289D	QC Bead_Exp_20160928_1.wit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:42	No
58C708D0372423AA8D5684608E4	KF_FITC_PE_ECD_PC7_KO_Exp_20160927_2.sit		Admin	Administrator	2016-10-27 08:1	TechSupport	TechSupport	2016-10-27 15:13:11	Vec
B2D80023D9E4538B17B47955F005	laser delay_Exp_20160927_1.xit		Admin	Administrator	2016-10-27 11:0	Admin	Administrator	2016-10-27 11:05:00	Yes
785C03539C84778AE9B56630A3F6	template1.xitm	Kelly	Admin	Administrator	2016-10-27 11:1	Admin	Administrator	2016-10-27 11:18:51	No
49EC18229EA458B8A20907799A33	temp1.xit	Kelly	Admin	Administrator	2016-10-27 11:2	Admin	Administrator	2016-10-27 11:22:33	No
F2F7C1938A54EBAB315C5CF7ECA1	Comp_20161027_1.xite	Kelly	Admin	Administrator	2016-10-27 11:2	Admin	Administrator	2016-10-27 11:23:25	No
CFB2D4C3A7A45358668320CDDD3	Comp_20161027_2.xitc	Kelly	Admin	Administrator	2016-10-27 14:2	Admin	Administrator	2016-10-27 14:27:17	No
D64C01D042148E98A451A20AD83	Danxitm		Admin	Administrator	2016-10-27 14:3	TechSupport	TechSupport	2016-10-27 15:13:12	Yes
882E814A9864D90ADOF9DE8D1AB	Exp_20161027_1.xlt	Kelly/Mouse 10Color/Experiment	Kelly	Kelly	2016-10-27 15:2	TechSupport	TechSupport	2016-10-27 17:06:04	No
A94B69A71B446FDA2CBE2CF1FCD	Mouse 10Cwitm	Kelly\Mouse 10Color\Templates	Kelly	Kelly	2016-10-27 15:2	Kelly	Kelly	2016-10-27 15:24:31	No
D683D73C654446580F6EE97C1EC5		Kelly\Mouse 10Color\Experiment		Kelly	2016-10-27 15/2	Kelly	Kelly	2016-10-27 15:25:27	
FEE499EC40D4C649EE273CFA7285	Comp_20161027_1.xitc	Kelly\Mouse 10Color\Compens		Kelly	2016-10-27 15:2	Kelly	Kelly	2016-10-27 15:27:06	
D531C63FB994973901626465A70B		TechSupport\Rat 6color	Kelly	Kelly	2016-10-27 15:2.	Kelly	Kelly	2016-10-27 15:29:18	
312EAE81D4F42A3B3FCAC83D5A9	Comp_20161027_2.xitc	TechSupport\Rat 6color	Kelly	Kelly			Kely	2016-10-27 15:30:08	
904336A439343ABB490C347D18A	Exp_20161027_1.xit	TechSupport\Rat 6color	TechSupport	TechSupport	2016-10-27 15:3	Kelly	Kelly	2016-10-27 15:50:02	No

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

0	100%	• 🔍	. K d	D D B G	/ •							
					Operation Lo ated Date: 2016-10-23		4	Operation	Username	User Full Name	Timestamp	Record
_	Jery Crit						1	L Open Experiment	TechSupport	TechSupport	2016-10-27 15:39:55	Open experiment Exp_20161027_1, experiment path:TechSupport\Rat 6color\Exp_20161027_1.xit
Ex	periment:	Tech		color\Exp_20161027 8B490C347D18A907			1	Add Gate	TechSupport	TechSupport	2016-10-27 15:40:01	Add a new Line segment gate P1 at worksheet Collection mode.
Us Tir		All U All Ti	sers me	n Time (US & Canada		8	1	Save Experiment	TechSupport	TechSupport	2016-10-27 15:40:08	Save experiment Exp_20161027_1, experiment path:TechSupport\Rat 6color\Exp_20161027_1.nit
	uery Res		-03:00) Easter	n nine (Os & Canada	9	•	2	Close Experiment	TechSupport	TechSupport	2016-10-27 15:40:14	Close experiment Exp_20161027_1, experiment path: TechSupport\Rat 6color\Exp_20161027_1.xit
H		ation	Username	User Full Name	Timestamp	Record	1:	5 Open Experiment	Kelly	Kelly	2016-10-27 15:49:50	Open experiment Exp_20161027_1, experiment path: TechSupport\Rat 6color\Exp_20161027_1.xit
	1 New Experi	ment	TechSupport	TechSupport	2016-10-27 15:37:53	Create new experiment Exp_20161027_1, experiment path: TechSupport\Rat Gcolor(Exp_20161027_1.sit.	- 1	5 Add Gate	Kelly	Kelly	2016-10-27 15:49:57	Add a new Line segment gate P2 at worksheet Collection mode.
	2 Add		TechSupport	TaskConstant	2016 10 27 15 29 09	Add a new Histogram x I SSC-A, y : Count at	1	Save Experiment	Kelly	Kelly	2016-10-27 15:50:01	Save experiment Exp_20151027_1, experiment path:TechSupport\Rat 6color\Exp_20161027_1.sit
	Histor	iram	recosupport	Techsupport	2016-10-27 15/38/08	worksheet Collection mode.	11	B Close	Kelly	Kelly	2016-10-27 15:51:20	Close experiment Exp_20161027_1, experiment
	3 Add Histog	Iram	TechSupport	TechSupport	2016-10-27 15:38:08	Add a new Histogram x : FITC-A, y : Count at worksheet Collection mode.		Experiment				path: TechSupport\Rat 6color\Exp_20161027_1.xit.
	4 Add Histog	Iram	TechSupport	TechSupport	2016-10-27 15:38:09	Add a new Histogram x : PerCP-A, y : Count at worksheet Collection mode.						
	s Add Histog	jram	TechSupport	TechSupport	2016-10-27 15:38:09	Add a new Histogram x : FSC-A, y : Count at worksheet Collection mode.						
	6 Add Histog	jram	TechSupport	TechSupport	2016-10-27 15:38:09	Add a new Histogram x : APC-A, y : Count at worksheet Collection mode.						
	7 Edit Tu Name	ibe	TechSupport	TechSupport	2016-10-27 15:38:38	Change Tubel name from Tubel to Tech Support Tubel.						
	8 Click R Buttor Acquir Tube	to	TechSupport	TechSupport	2016-10-27 15:39:05	Click Record button, Tech Support Tube1 record 731 events-						
	9 Save	ment	TechSupport	TechSupport	2016-10-27 15:39:06	Save experiment Exp_20161027_1, experiment path: TechSupport\Rat 6color\Exp_20161027_1.xit.	1					

- 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.

nt	R
9	D

Figure B.4	System	Operation	Log	Window
------------	--------	-----------	-----	--------

	Operation Log				
Query	y Criteria				
User:	(ALL)				
lime F	Range: 2016-09-28 00:00:00	· ~ 2016-10-28	23:59:59		
Que	ry				Print & Export
¥	Operation	Username	User Full Name	Timestamp	Record
1	L 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	2 Modify Laser Settings	TechSupport	TechSupport	2016-10-24 11:19:15	Modified laser settings.
з	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	5 Import QC Target File	Admin	Administrator	2016-10-24 15:28:55	Import QC target file D:\BAH02.tgt, lot No.: BAH02.
e	5 Run QC	Admin	Administrator	2016-10-24 15:31:36	Run QC, lot No.: BAH02.
7	7 Run QC	Admin	Administrator	2016-10-24 15:32:29	Run QC, lot No.: BAH02.
8	3 Run QC	Admin	Administrator	2016-10-24 15:33:35	Run QC, lot No.: BAH02.
g	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	L Run QC	Admin	Administrator	2016-10-24 15:44:10	Run QC, lot No.: BAH02.
12	2 Run QC	Admin	Administrator	2016-10-24 16:10:50	Run QC, lot No.: BAH02.
13	3 Delete QC Result	Admin	Administrator	2016-10-24 16:12:44	Delete QC result, lot No.: BAH02, date: 2016-10-24.
14	4 Run QC	Admin	Administrator	2016-10-25 08:28:52	Run QC, lot No.: BAH02.
15	5 Run QC	Admin	Administrator	2016-10-27 08:57:23	Run QC, lot No.: BAH02.
16	5 Run QC	Admin	Administrator	2016-10-27 09:00:44	Run QC, lot No.: BAH02.
17	7 Run QC	Admin	Administrator	2016-10-27 09:10:54	Run QC, lot No.: BAH02.
18	3 Run QC	Admin	Administrator	2016-10-27 09:18:44	Run QC, lot No.: BAH02.
10	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.

ser Management Oper	ration Log			E	9 3
Query Criteria					
User: (ALL)					
Time Range: 2016-10	0-09.00:00:00 - ~ 2016-11-	09 23:59:59 -			
Time Range: 2010-10	0-09 00:00:00 - 2016-11-	19 22:29:29 T			
Query				Print & Exp	oort.
# Operation	Username	User Full Name	Timestamp	Record	
1 Login	Admin	Administrator	2016-11-04 16:18:49	Login successfully.	
2 Change passv	vord 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 in	nformation Admin	Administrator	2016-11-09 07:39:32	Modify user usability (Username: Sarah) from [Yes] to [No].	
19 Modify user in	nformation Admin	Administrator	2016-11-09 07:39:39	Modify user usability (Username: Sarah) from [No] to [Yes].	
20 Reset passwo	rd 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	

Figure B.5	User Management Operation Log Window
------------	--------------------------------------

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.

Sign		23
Username:	Admin	
Password:		
Comment:		
		-
	OK Cancel	

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.

<u>File Cytometer Settings QC/Standardization Advanced Account Log Signature Help</u>

(i) This experiment is signed. Editing and saving the experiment will retract the electronic signature.

5 Select **Signature > Electronic Signature Details** to view the electronic signature details.

Username:	Admin	
Date Time:	2016-12-07 16:39:58	
Comment:		
This experi	ment is signed.	
	saving the experiment will retract the	

Retracting an Experiment Signature

1 Select Signature > Electronic Signature Details to view the electronic signature details.

Electronic Signature Details	23
Username: Admin	
Date Time: 2016-12-07 16:39:58	
Comment:	
	-
This experiment is signed.	
Editing and saving the experiment will retract the electronic signature.	
<u>R</u> etract <u>C</u> los	e

- 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.

GR	100 (x 10 ⁴)		(22.38%)	01-L1 50 FSC-A	R(58.90 %) 100 (x10 ⁴)
Tube Name: 01-N1G-A3					
Sample ID:					
Population			Events	% Total	% Parent
✓ ● All Events			10064		100.00 %
			1691 193		16.80 % 1.92 %
⊗ Q1-UL ⊗ Q1-LL			2252		22.38 %
⊘Q1-LL ⊗Q1-LR			5928		58.90 %
⊗ V1L			2687	26.70 %	26.70 %
⊗ V1R			7377		73.30 %
Tube Name: 01-N1G-A3 Sample ID:					
Sample ID: Population	Events	% Total		% Parent	
Sample ID: Population ● All Events	10064	% Total	100.00 %	% Parent	100.00 %
Sample ID: Population All Events &Q1-UR	10064 1691	% Total	16.80 %	% Parent	16.80 %
Sample ID: Population All Events Q Q1-UR Q Q1-UL	10064 1691 193	% Total	16.80 % 1.92 %	% Parent	16.80 % 1.92 %
Sample ID: Population All Events Q1-UR Q1-UL Q1-UL	10064 1691 193 2252	% Total	16.80 % 1.92 % 22.38 %	% Parent	16.80 % 1.92 % 22.38 %
Sample ID: Population All Events Q Q1-UR Q Q1-UL	10064 1691 193	% Total	16.80 % 1.92 %	% Parent	16.80 % 1.92 %

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]
- Sample Injection Mode Control Kit Components
- Switching the Sample Probe from the Single Tube Sample Station to the Plate Loader [With Sample Injection Mode Control Knob]
- Switching the Sample Probe from the Plate Loader to the Single Tube Sample Station [With Sample Injection Mode Control Knob]

Performance Characteristics [With the Sample Injection Mode Control Knob]

Performance [CytoFLEX With Standard Plate Loader]				
Throughput [With	10 second acquisition without mixing or backflush: <35 min.			
Standard Plate Loader] ^a	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	10 second acquisition without mixing or backflush: <38 min.			
Standard Plate Loader] ^a	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.				

 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

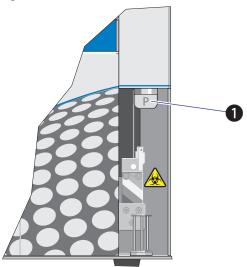


Figure C.1 Sample Injection Mode Control Knob

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]



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.

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.

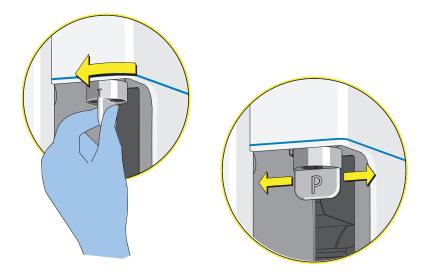
Risk of instrument damage. The sample probe can easily become damaged or deformed. To avoid damage to the sample probe, turn the switch module knob carefully and avoid contacting the sample probe.

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.

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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 **I** 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]



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.

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.

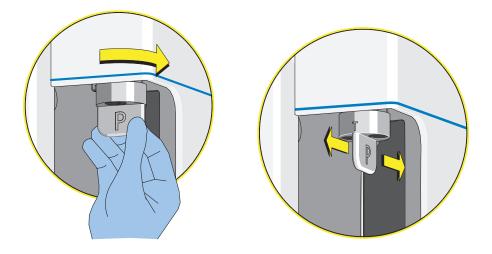
Risk of instrument damage. The sample probe can easily become damage or deformed. To avoid damage to the sample probe, turn the switch module knob carefully and avoid contacting the sample probe.

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.

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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 **I** 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]
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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 \geq 5.8 mm.
- The plate height is \leq 45.5 mm.

Deep Well Plate Specimen Collection Plate Specifications

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

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						
		有毒有害物质或元素 Hazardous Substances Name					
Component 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	Х	0	0	0	0	0	
电源组件 Power Supplies	0	0	0	0	0	0	
计算机 Computer	0	0	0	0	0	0	
功率调节器 Power Conditioner	0	0	0	0	0	0	
光量传感器 Optical Sensors	0	0	0	0	0	0	
激光 Laser	0	0	0	0	0	0	
发动机/泵/阀门/ Motors/Pumps/Valves	0	0	0	0	0	0	
电线 Cables	Х	0	0	0	0	0	
管路及橡胶 Tubing & Rubber	0	0	0	0	0	0	
塑料部件 Plastic	0	0	0	0	0	0	
连接部件 Hardware	0	0	0	0	0	0	
包装材料 Packing Material	0	0	0	0	0	0	

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]

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.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]
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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	Х	0	0	0	0	0	
电源组件 Power Supplies	0	0	0	0	0	0	
计算机 Computer	0	0	0	0	0	0	
功率调节器 Power Conditioner	0	0	0	0	0	0	
光量传感器 Optical Sensors	0	0	0	0	0	0	
激光 Laser	Х	0	0	0	0	0	
发动机/泵/阀门/ Motors/Pumps/Valves	0	0	0	0	0	0	
电线 Cables	Х	0	0	0	0	0	
管路及橡胶 Tubing & Rubber	0	0	0	0	0	0	
塑料部件 Plastic	0	0	0	0	0	0	
连接部件 Hardware	Х	0	0	0	0	0	
包装材料 Packing Material	0	0	0	0	0	0	

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.

电子电气产品号码 EEP Part Number: C06779							
部件名称		有毒有害物质或元素 Hazardous Substances Name					
Component 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	Х	0	0	0	0	0	
电源组件 Power Supplies	0	0	0	0	0	0	
计算机 Computer	0	0	0	0	0	0	
功率调节器 Power Conditioner	0	0	0	0	0	0	
光量传感器 Optical Sensors	0	0	0	0	0	0	
激光 Laser	Х	0	0	0	0	0	
发动机/泵/阀门/ Motors/Pumps/Valves	0	0	0	0	0	0	
电线 Cables	Х	0	0	0	0	0	
管路及橡胶 Tubing & Rubber	0	0	0	0	0	0	
塑料部件 Plastic	0	0	0	0	0	0	
连接部件 Hardware	Х	0	0	0	0	0	
包装材料 Packing Material	0	0	0	0	0	0	

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]

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.

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	BP — band-pass filter
" — inch	CDRH — Center for Devices and Radiological Health
%— percent	CFSE — carboxyfluorescein succinmidyl ester
° C — degrees Celsius	cm — centimeters
° F — degrees Fahrenheit	CSV - comma separated value
± — plus or minus	CV - coefficient of variation
< — less than	
> — greater than	DNA — deoxyribonucleic acid
\leq — less than or equal to	DW — deep well
μ — micron	ECD — Energy Coupled Dye
μL — microliters	EFUP — Environmentally friendly Use Period
μm — micrometer	EMF — enhanced metafile format
A — ampere	EMR — electromagnetic radiation
AC — alternating current	FAPD — Fiber Array Photo Detector
APC — Allophycocyanin	FCS — flow cytometry standard
APC-A700 — Allophycocyanin-Alexa	FITC — Fluorescein isothiocyanate
Fluor™ 700 tandem dye	FSC — forward scatter
APC-A7500 — Allophycocyanin-Alexa	GB — gigabyte
Fluor™ 750	GHz — gigahertz
APC-Cy7 — Allophycocyanin-Cyanin 7	Gr Wt — gross weight
API — Application Programming Interface	H — humidity
Acq. — Acquisition	Hz — hertz
BCI — Beckman Coulter Incorporated	IEC — International Electrotechnical
BMP — bitmap	Commission
	IR — infrared

kg — kilograms **RAM** — random access memory **rCV** — robust coefficient of variation **KO** — Krome Orange **LED** — light emitting diode **RH** — relative humidity L — liter **RoHS** — Restriction of Hazardous Substances Directive LJ — Levey-Jennings **RPTM** — real-time messaging protocol **LWH** — length, width, height **S/N** — serial number **m** — meter **SNR** — signal to noise ratio MB — megabyte **SSC** — side scatter **MFI** — median fluorescence intensity **USB** — universal serial bus MHz — megahertz **UV** – ultraviolet min — minute V – volts **mL** — milliliter **VA** — volt-ampere **mm** — millimeter **VAC** — voltage alternating current **mW** – milliwatt **VSSC** — violet side scatter **NA** — numerical aperture **WDM** — wavelength division multiplexer NaCIO — sodium hypochlorite solution W – watts NaN₃ — 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

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