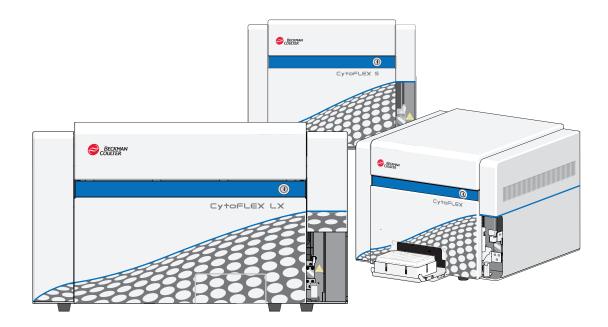


CytoFLEX Platform

CytoFLEX, CytoFLEX S, and CytoFLEX LX Flow Cytometers For Research Use Only. Not for use in diagnostic procedures.





B49006AS December 2021



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

Original Instructions

CytoFLEX Platform Flow Cytometer PN B49006AS (December 2021)

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

May be covered by one or more pat. - see www.beckman.com/patents

Revision History

Initial Issue AA, 09/2014 Software Version 1.0

Issue AB, 12/2014 Software Version 1.0

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

Issue AC, 02/2015 Software Version 1.1

Updates were made to the following sections:

Safety Notices

Symbol Explanations

CHAPTER 1, System Overview

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

CHAPTER 2, Using the CytExpert Software

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

CHAPTER 3, Daily Startup

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

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

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

Creating Plots and Gates Sampling and Collecting Data Setting the Channel and Label Setting Collection Conditions

Laser Settings

Analyzing and Exporting Data

Printing Graphics

CHAPTER 6, Compensation

Creating a Compensation Experiment Manually Adjusting Compensation

CHAPTER 8, Daily Shutdown

Shutting Down the Instrument

CHAPTER 9, Troubleshooting

Laser Beam Hazards

Laser Warning Labels

Backup and Restore

CHAPTER 11, Replacement/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

Updates were made to the following sections:

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

Issue AM, 07/2018 Software Version 2.3 Updates were made to the following sections: Safety Notices Safety Precautions Symbol Explanations CHAPTER 1, System Overview Wavelength Division Multiplexer (WDM) Fluidics Module Consumables and Supplies **Instrument Specifications Performance Characteristics** CHAPTER 2, Using the CytExpert Software Start Page Software Menu Settings Menu Signature Menu **Role Management** Account Policies User Management Operation Log Plate Type Library CHAPTER 3, Daily Startup Logging into the Software Logging Out of the Software Locking the Account 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 Overview Importing Lot-Specific Target Values **Confirming Results** CHAPTER 5, Data Acquisition and Sample Analysis Verifying, Selecting, Editing, and Creating Detector Configuration Creating Plots and Gates **Printing Graphics** CHAPTER 10, Cleaning Procedures Preparing the Instrument for Transport or Storage

CHAPTER 11, Replacement/Adjustment Procedures Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing Calibrating the Plate Position [With Plate Loader] CHAPTER A, Instrument Installation Installing the Instrument and Connecting the Equipment [CytoFLEX] Installing the CytExpert Software APPENDIX B, CytExpert Electronic Record Management **Experiment Management Electronic Signature** User Management **APPENDIX E, Custom Optical Filters** Overview Installing a Custom Optical Filter Issue AN, 07/2019 Software Version 2.3 Updates were made to the following sections: CHAPTER 1, System Overview Main Components Plate Holder Components Sample Station System Configuration Instrument Specifications CHAPTER 5, Data Acquisition and Sample Analysis Creating Plots and Gates Creating and Adjusting Auto Gates Analyzing and Exporting Data CHAPTER 6, Compensation Creating a Compensation Experiment Creating a Compensation Experiment [With Plate Loader] CHAPTER 9, Troubleshooting Biohazard Label and Location CHAPTER 10, Cleaning Procedures Daily Clean Daily Clean [With Plate Loader] Cleaning the Sample Station CHAPTER 11, Replacement/Adjustment Procedures Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing

CHAPTER A, Instrument Installation
Instrument Transportation and Storage
Installing the Software [CytoFLEX Platform]
APPENDIX E, Custom Optical Filters
Installing a Custom Optical Filter
Issue AP, 11/2019
Software Version 2.4
Updates were made to the following sections:
CHAPTER 1, System Overview
Wavelength Division Multiplexer (WDM)
Plate Holder Components
Cytometer
Reagent Limitations
CHAPTER 2, Using the CytExpert Software
Start Page
Software Menu
CHAPTER 3, Operation Principles
Overview
Sample Flow
Laser Beam Shaping
Cell Illumination
Light Collection, Separation and Measurement
Signal Processing
Data Storage
Automated Software Features
Parameters
Plot Display
Statistics
CHAPTER 4, Daily Startup
Logging into the Software
CHAPTER 5, Instrument Quality Control and Standardization
Overview
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

Preparing the Standardization Sample Generating Target Median Values Adding a New Standardization Item Performing the Standardization Applying the Standardized Acquisition Settings Standardization Target Library CHAPTER 6, Data Acquisition and Sample Analysis Creating an Experiment Adjusting the Threshold Verifying, Selecting, Editing, and Creating Detector Configuration Sampling and Collecting Data [Without Plate Loader] Sampling and Collecting Data [With Plate Loader] Exporting Plots or the Statistics Table of Multiple Tubes as Picture Files CHAPTER 7, Compensation Creating a Compensation Experiment CHAPTER 12, Replacement/Adjustment Procedures Replacing the Plate Holder [With Plate Loader] CHAPTER A, Instrument Installation Installing the Instrument and Connecting the Equipment [CytoFLEX] APPENDIX F, WDM Beam Splitter Overview Modifying Detector Configuration [With WDM Beam Splitter] APPENDIX G, Cyber Security Good Practices for Cyber Security Issue AR 08/2021 Software Version 2.4 Updates were made to the following sections: CHAPTER 1, System Overview Consumables and Supplies **Performance Characteristics** CHAPTER 5, Instrument Quality Control and Standardization Overview 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]

CHAPTER 10, Troubleshooting Hazard Labels and Locations Troubleshooting Table CHAPTER 12, Replacement/Adjustment Procedures Changing Sample Mixing and Backflush Settings APPENDIX C, Sample Injection Mode Control Kit Performance Characteristics [With the Sample Injection Mode Control Knob] Issue AS 12/2021 Software Version 2.5 Updates were made to the following sections: CHAPTER 1, System Overview Cytometer CHAPTER 2, Using the CytExpert Software Start Page Software Menu CHAPTER 4, Daily Startup Logging into the Software CHAPTER 5, Instrument Quality Control and Standardization Collecting QC Data **Confirming Results** Applying the Standardized Acquisition Settings CHAPTER 6, Data Acquisition and Sample Analysis **Creating Plots and Gates** Verifying, Selecting, Editing, and Creating Detector Configuration Importing and Exporting Instrument Settings APPENDIX A, Instrument Installation Installing the CytExpert Software APPENDIX G, Good Practices for Cyber Security **Drive Encryption** Protection from Malware Software **Operating System Updates** System Hardening

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

Revision History

Safety Notices

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate the 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.

MARNING

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.
- Installation of software that is not authorized by Beckman Coulter onto your CytoFLEX workstation. Only operate the CytoFLEX with software authorized by Beckman Coulter.
- Installation of 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.

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

🕂 WARNING

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

Symbol Explanations

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

Symbol	Warning Condition	Action
	Recycling Symbol WEEE Wheeled Bin Symbol	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:
		1. that the device was put on the European Market after August 13, 2005 and
		2. that the device is not to be disposed via the municipal waste collection system of any member state of the European Union.
		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.
		For the Japan market:
		This system is considered an industrial waste, subject to special controls for infectious waste. Before disposal of the system, refer to the Waste Disposal and Public Cleaning Law for compliance procedures.
	Laser Radiation	The laser radiation symbol indicates that there can be laser light radiation in the area. Take precautions to prevent exposure.
50	RoHS Caution Symbol	This label indicates that the electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.
CLASS 1 LASER PRODUCT PRODUIT LASER CLASSE 1 1类激光产品	Laser Class I	This label indicates that this product is a Class I Laser product. Take precautions to prevent exposure.

Symbol	Warning Condition	Action
Info for USA only: California Proposition 65 WARNING Cancer & Reproductive Harm www.P65Wamings.ca.gov	California Proposition 65 Labeling	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.
	Consider all materials (specimens, controls, monoclonal antibodies, and so forth) as being potentially infectious.	Wear standard laboratory attire and follow safe laboratory procedures when handling any material in the laboratory.

Safety Notices Symbol Explanations

Contents

Revision History, iii

Safety Notices, xvii

Alerts for Danger, Warning, and Caution, xvii Safety Precautions, xviii Symbol Explanations, xix

Introduction, xxxix

Overview, xxxix How to Use Your Manual, xxxix

About this Manual, xxxix

Conventions Used, xli

Graphics, xlii

CHAPTER 1: System Overview, 1-1

Overview, 1-1

Product Description, 1-1

Main Components, 1-2

Optical Components, 1-4 Wavelength Division Multiplexer (WDM), 1-5 Optical Fiber, 1-16

Fluidics System, 1-17 Fluid Containers/Cubitainers, 1-18 Fluidics Module, 1-19

Sample Station, 1-21 Sample Tube Holder Positions, 1-22

Plate Loader Components, 1-23 Plate Holder Components, 1-27

System Configuration, 1-31 System Configuration [CytoFLEX and CytoFLEX S], 1-31 System Configuration [CytoFLEX LX], 1-33

Consumables and Supplies, 1-35 Reagents, 1-35 Material Safety Data Sheets (SDS/MSDS), 1-35 Ordering Information, 1-35 Instrument Specifications, 1-36 Dimensions [CytoFLEX and CytoFLEX S], 1-36 Dimensions [CytoFLEX LX], 1-37 Installation Category, 1-37 Pollution Degree, 1-38 Acoustic Noise Level, 1-38 Electrical Ratings, 1-38 Cytometer, 1-38

Performance Characteristics, 1-41 Performance Characteristics [CytoFLEX and CytoFLEX S], 1-41 Performance Characteristics [CytoFLEX LX], 1-43

Reagent Limitations, 1-44

CHAPTER 2: Using the CytExpert Software, 2-1

Overview, 2-1

Launching the Software, 2-1

Main Software Screen, 2-1 Start Page, 2-2 Acquisition Screen, 2-3 Acquisition Screen Navigation, 2-4 Collection, 2-5 Collection [With Plate Loader], 2-6 Test Tubes, 2-7 Plot area, 2-8 Status Bar, 2-9 Analysis Screen, 2-9 Compensation Experiment Screen, 2-11 QC Experiment Screen, 2-12 QC Report Screen, 2-12 QC Experiment Screen, 2-13 QC Screen Navigation, 2-13 Software Menu, 2-14 Acquisition and Analysis Screen Menu, 2-15

User Management, 2-23 Creating, Deleting, and Modifying Users in User Manager, 2-25 Unlocking a User Account, 2-27 Resetting a User Passwords, 2-27 Changing a User Password, 2-27

Role Management, 2-27 Creating, Deleting, and Modifying User Roles in Role Manager, 2-29

Account Policies, 2-31

User Management Operation Log, 2-33

Graphic and Gating Styles, 2-34 Plots, 2-34 Gates, 2-35 Plate Type Library, 2-36 Adding a Plate Type, 2-37 Editing a Plate Type, 2-41 Duplicating a Plate Type, 2-42 Deleting a Plate Type, 2-44

Software Settings, 2-45 Language Settings, 2-48 Setting Up CytExpert Application Programming Interface (API) Test Client, 2-48

CHAPTER 3: Operation Principles, 3-1

Overview, 3-1

Sample Flow, 3-1 Sample Loading, 3-1 Hydrodynamic Focusing, 3-2

Laser Beam Shaping, 3-3

Cell Illumination, 3-4 Forward Scatter, 3-4 Side Scatter and Fluorescent Light, 3-4

Light Collection, Separation and Measurement, 3-4 Forward Scatter Collection, 3-4 Side Scatter and Fluorescent Light Collection, 3-5 Side Scatter, 3-5 Fluorescent Light, 3-5

Signal Processing, 3-6

Data Storage, 3-6

Automated Software Features, 3-6

Parameters, 3-7 TIME Parameter, 3-7

Plot Display, 3-7

Statistics, 3-8

CHAPTER 4: Daily Startup, 4-1

Overview, 4-1

Pre-Startup Inspection, 4-1

Check Waste and Reagent Levels [4 L Fluid Containers], 4-2 Check Waste and Reagent Levels [10 L Fluid Cubitainers], 4-4 Power Source Inspection, 4-5 Workstation Connections Inspection, 4-5

Turning On the Instrument, 4-6

Logging into the Software, 4-6 Logging Out of the Software, 4-9 Locking the Account, 4-9 Selecting the Proper Sample Injection Mode, 4-9 Selecting the Plate Loader Sample Injection Mode [With Plate Loader], 4-12 Running the System Startup Program [with the Single Tube Loader], 4-16 Running the System Startup Program [With Plate Loader], 4-19 Selecting Experiments from the Start Page, 4-27 Initializing the Instrument, 4-28

CHAPTER 5: Instrument Quality Control and Standardization, 5-1

Overview, 5-1

Quality Control, 5-2 Preparing the QC Sample, 5-3 Required Materials, 5-3 CytoFLEX Daily QC Fluorospheres Preparation Process, 5-3 CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process, 5-4 CytoFLEX Daily IR QC Fluorospheres Preparation Process, 5-4 Preparing the QC Sample [With Plate Loader], 5-5 **Required Materials**, 5-5 Preparation Process CytoFLEX Daily QC Fluorospheres, 5-5 CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process, 5-6 CytoFLEX Daily IR QC Fluorospheres Preparation Process, 5-6 Importing Lot-Specific Target Values, 5-7 Collecting QC Data, 5-12 Collecting QC Data [With Plate Loader], 5-15 Confirming Results, 5-20

QC Result Manager, 5-27

Standardization, 5-28 Preparing the Standardization Sample, 5-28 Required Materials, 5-29 Preparation Process, 5-29 Generating Target Median Values, 5-29 Adding a New Standardization Item, 5-36 Performing the Standardization, 5-40 Applying the Standardized Acquisition Settings, 5-43 Standardization Target Library, 5-46

CHAPTER 6: Data Acquisition and Sample Analysis, 6-1

Overview, 6-1

Creating an Experiment, 6-1 Creating an Experiment [Without Plate Loader], 6-1 Creating an Experiment [With Plate Loader], 6-3 Setting Sample Wells, 6-9 Modifying Well Settings, 6-14 Setting the Channel and Label, 6-16 Creating Plots and Gates, 6-19 Creating and Adjusting Auto Gates, 6-28 Turning Auto Recalculate On/Off, 6-30 Adjusting Autogate Movement and Extent, 6-31 Setting Customized Parameters, 6-33 Setting Custom Statistics, 6-34

Configuring Acquisition Settings, 6-38 Changing the Tube Name, 6-38 Laser Settings, 6-38 Setting Laser Target Power Settings [CytoFLEX LX Only], 6-40 Adjusting the Gain, 6-41 Adjusting the Threshold, 6-43 Setting Collection Conditions, 6-44 Setting Plot Display Conditions, 6-46

Load Sample and Record Data, 6-47 Before Running Samples, 6-47 Verifying, Selecting, Editing, and Creating Detector Configuration, 6-48 Setting Up Violet Side Scatter (VSSC) Channel, 6-52 Sampling and Collecting Data [Without Plate Loader], 6-55 Sampling and Collecting Data [With Plate Loader], 6-58 Creating a Heat Map [with Plate Loader], 6-60 Refreshing a Heat Map, 6-67 Modifying Existing Heat Map Settings, 6-68 Deleting an Existing Heat Map, 6-69 Exporting a Heat Map, 6-69

Analyzing and Exporting Data, 6-70 Exporting FCS Files, 6-75 Exporting Plots or the Statistics Table of Multiple Tubes as Picture Files, 6-77 Importing and Exporting Instrument Settings, 6-78 Importing and Exporting Compensation Settings, 6-80 Printing Graphics, 6-80

Saving the Experiment, 6-83 Concluding the Experiment, 6-83

CHAPTER 7: Compensation, 7-1

Overview, 7-1

Creating a Compensation Experiment, 7-2 Preparing the Compensation Sample, 7-4 Using Control Samples to Generate the Compensation Matrix, 7-4 Calculating Compensation Values, 7-8

Creating a Compensation Experiment [With Plate Loader], 7-10 Preparing the Compensation Sample, 7-13 Using Control Samples to Generate the Compensation Matrix, 7-13 Calculating Compensation Values, 7-13

Creating the Compensation Matrix from Previously Acquired Data, 7-14

Adjusting Compensation, 7-16 Manually Adjusting Compensation, 7-16 Importing and Exporting Compensation, 7-16 Importing Compensation Settings from Compensation Matrix Files, 7-16 Importing Compensation Settings from the Compensation Library, 7-18 Exporting Compensation Settings, 7-19 Managing the Compensation Library, 7-21 Adding Channels for Compensation, 7-22

CHAPTER 8: Data Review, 8-1

Overview, 8-1

Copying Experiments and Importing Data, 8-1 Copying a Previously Acquired Experiment, 8-1 Importing Previously Acquired Data, 8-1

Setting the Plots and Statistics, 8-3 Creating Histogram and Dot Plot Overlays, 8-5

Calculating Sample Volume and Concentration, 8-7

Adjusting Compensation Settings, 8-8

Exporting Results, 8-9

CHAPTER 9: Daily Shutdown, 9-1

Overview, 9-1

Preparing the Cleaning Solution, 9-1

Shutting Down the Instrument, 9-1

Auto Shutdown [CytoFLEX LX Only], 9-2

CHAPTER 10: Troubleshooting, 10-1

Overview, 10-1

Precautions/Hazards, 10-1 Laser Related Hazards, 10-1 Laser Beam Hazards, 10-2 Laser Warning Labels, 10-3

Hazard Labels and Locations, 10-6 Biohazard Label and Location, 10-6 Electrical Shock Hazard Label and Location, 10-7 Caution Labels and Location, 10-8 Plate Loader Hazard Labels and Location, 10-8

Disposal Precaution, 10-8

Troubleshooting Table, 10-9

Backup and Restore, 10-22 Backup, 10-22 Restore, 10-25 Log Cleanup, 10-28

CHAPTER 11: Cleaning Procedures, 11-1

Overview, 11-1

Routine Cleaning, 11-1 Daily Clean, 11-1 Daily Clean [With Plate Loader], 11-5 Cleaning the Sample Station, 11-7 Cleaning the Sample Probe, 11-8 Deep Clean Procedure, 11-8 Cleaning the 4 L Sheath Fluid Container, 11-10 Cleaning the 4 L Waste Container, 11-11

Nonscheduled Cleaning, 11-13 Surface Cleaning and Disinfection, 11-13 Preparing the Instrument for Transport or Storage, 11-14

CHAPTER 12: Replacement/Adjustment Procedures, 12-1

Overview, 12-1

Routine Replacement/Adjustment, 12-2 Front Cover Removal and Reinstallation, 12-2 Removal, 12-2 Reinstallation, 12-3 Right-Side Cover Removal and Reinstallation, 12-4 Removal, 12-4 Reinstallation, 12-5 Filling the 4 L Sheath Fluid Container, 12-6 Replacing the 10 L Sheath Fluid Cubitainer, 12-7 Emptying the 4 L Waste Container, 12-9 Emptying the 10 L Waste Cubitainer, 12-10 Managing the Maintenance Reminder, 12-14 Adding the Deep Clean Solution, 12-17 Replacing the Sheath Fluid Filter, 12-18 Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing, 12-22 Replacing the Sample Probe Assembly [With Plate Loader], 12-32 Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [CytoFLEX With Plate Loader], 12-38 Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [CytoFLEX With Plate Loader], 12-43 Inspecting the Liquid Flow Path for Leaks, 12-47 Priming the Flow Cell, 12-48 Replacing the Plate Holder [With Plate Loader], 12-49 Plate Loader Module Removal and Reinstallation [With Plate

Loader], 12-51 Removal, 12-51 Installation, 12-55 Changing the Event Rate Setting, 12-60

Nonscheduled Replacement/Adjustment, 12-61 Calibrating the Sample Flow Rate, 12-61 Calibrating the Sample Flow Rate [With Plate Loader], 12-65 Setting Laser Delay, 12-69 Replacing the Optical Filter, 12-70 Replacing the Fuse, 12-72 Replacing the Sheath Fluid Harness and/or Waste Harness, 12-74 Changing Sample Mixing and Backflush Settings, 12-76 Calibrating the Plate Position [With Plate Loader], 12-78

APPENDIX A: Instrument Installation, A-1

Overview, A-1

Instrument Transportation and Storage, A-1

Installation Environment Validation, A-2 Worktable, A-2 Ventilation and Cleaning, A-2 Power Source, A-3 Temperature and Humidity, A-4 Waste Disposal, A-4

Unpacking the Instrument and Inspecting the Materials for Defects or Omissions [CytoFLEX], A-4 Installing the Instrument and Connecting the Equipment [CytoFLEX], A-5 Installing the Instrument and Connecting the Equipment [CytoFLEX LX], A-11

CytExpert Software Installation Options, A-12

Installing the Software [CytoFLEX Platform], A-12 Required Materials, A-13 Installing the CytExpert Software, A-13 Installing the Instrument Configuration File, A-22 Starting the Software, A-24

Upgrading the CytExpert Software, A-24

Reinstalling the CytExpert Software, A-29

APPENDIX B: CytExpert Electronic Record Management, B-1

Overview, B-1

Software Menu, B-1

Experiment Management, B-2 Closed File System, B-2 Experiment Directory Management, B-3

	Folder Hierarchy Management, B-4 Experiment Related Operations, B-5 Importing an Experiment/Template, B-6 Exporting an Experiment/Template, B-9 Log, B-12
	Experiment Operation Log, B-12 System Operation Log, B-15 User Management Operation Log, B-16
	Electronic Signature, B-17 Signing Experiments, B-17 Rejecting Experiment, B-20 Setting the Signature Retention Period, B-21 Signature Setting, B-22 Printing an Experiment Signature, B-25
	User Management, B-26 User Administration, B-26 Logging In and Out of the Software, B-26 Locking the Account, B-26 Role Management, B-26 User Management, B-26 Account Policies, B-26
APPENDIX C:	Sample Injection Mode Control Kit, C-1
	Overview, C-1
	Performance Characteristics [With the Sample Injection Mode Control Knob], C-1
	Sample Injection Mode Control Kit Components, C-2
	Switching the Sample Probe from the Single Tube Sample Station to the Plate Loader [With Sample Injection Mode Control Knob], C-3
	Switching the Sample Probe from the Plate Loader to the Single Tube Sample Station [With Sample Injection Mode Control Knob], C-5
APPENDIX D:	Deep Well Plate, D-1
	Specimen Collection Plate Specifications, D-1
APPENDIX E:	Custom Optical Filters, E-1
	Overview, E-1
	Installing a Custom Optical Filter, E-1
APPENDIX F:	WDM Beam Splitter, F-1
	Overview, F-1
	Modifying Detector Configuration [With WDM Beam Splitter], F-4

APPENDIX G: Good Practices for Cyber Security, G-1

Overview, G-1

Drive Encryption, G-2 BitLocker Keys, G-2 Changing Encryption Strength Level for BitLocker, G-2 Enabling BitLocker, G-6 Backing Up Your Recovery Key, G-13 BitLocker Decryption, G-17 Suspending BitLocker Drive Encryption, G-21 Recovering Your BitLocker Key, G-23

Protection from Malware Software, G-25 Installing McAfee Application Control, G-26 Enabling McAfee Application Control, G-31 Exporting the Certificate, G-35 Importing BEC Certificate, G-45 Switching McAfee Application Control to Update Mode, G-47 Disabling McAfee Application Control, G-49

Operating System Updates, G-53 Enabling System Protection, G-53 Creating a Restore Point, G-56 Downloading Operating System Updates, G-57 Notification Options, G-58 Installing the Operating System Updates, G-59 Recovering from Failed Operating System Updates, G-61

System Hardening, G-66

Enabling Automatic Log-Off, G-66 Setting Account Lockout Policy, G-75 Setting Password Policy, G-77 Disabling Remote Access, G-80 Disabling Auto Play, G-82 Disabling Unnecessary Services, G-84 Disabling Unauthorized Applications, G-89 Enabling SMB Digitally Sign Communications, G-92 Enabling Installation Restriction, G-95 Enabling Firewall Defender, G-97 Enabling Network Time Protocol, G-99

APPENDIX H: Table of Hazardous Substances, H-1

Table of Hazardous Substances, H-1

Abbreviations

Beckman Coulter, Inc. SOFTWARE END-USER LICENSE AGREEMENT

Related Documents

Illustrations

1.1	Main Components [CytoFLEX Without Plate Loader], 1-2
1.2	Main Components [CytoFLEX LX], 1-3
1.3	Optical Filter Mounts, 1-5
1.4	Optical Filter Mount with Optical Filter, 1-5
1.5	Optical Filter Mount Labeled with the Band-Pass Information, 1-6
1.6	Optical Filter Mount (Top), 1-6
1.7	Fluid Containers [CytoFLEX and CytoFLEX S], 1-17
1.8	Fluid Cubitainers [CytoFLEX LX], 1-18
1.9	Fluidics Module View, 1-19
1.10	Fluidic Connections, 1-20
1.11	Sample Station with Tube Holder, 1-21
1.12	Sample Tube Holder Positions, 1-22
1.13	Standard Plate Loader [CytoFLEX Shown], 1-23
1.14	Plate Loader DW [CytoFLEX Shown], 1-24
1.15	Standard Plate Loader (Front Cover Removed), 1-25
1.16	Plate Loader DW (Front Cover Removed), 1-26
1.17	Standard Plate Holder without Groove (Standard Plate Loader), 1-27
1.18	Plate Holder with Groove (Plate Loader DW), 1-28
1.19	Plate Holder Stage (Standard Plate Loader), 1-29
1.20	Plate Holder Stage (Plate Loader DW), 1-29
1.21	Calibration Tool for 96-Well Deep Well Plate, 1-30
1.22	System Connections, 1-31
1.23	Back Cover Connections, 1-32
1.24	Front of Cytometer [CytoFLEX Without Plate Loader Shown], 1-32
1.25	System Connections, 1-33
1.26	Back Cover Connections, 1-34
1.27	Front of Cytometer, 1-34
1.28	Plate Loader DW Dimensions [CytoFLEX and CytoFLEX S], 1-36
1.29	Plate Loader DW Dimensions [CytoFLEX LX], 1-37
2.1	Drawing Controls Toolbar (Top of Screen), 2-10
2.2	QC Report Screen, 2-12

2.3	Software Menu Tree*, 2-14
2.4	QC Software Menu Tree, 2-15
2.5	User Manager (Card View), 2-23
2.6	User Manager (Grid View), 2-24
2.7	Role Manager, 2-28
2.8	Account Policies - Password Policy, 2-31
2.9	Account Policies - Account Lockout Policy, 2-32
2.10	Account Policies - Application Inactivity Policies, 2-32
2.11	Plate Type Library, 2-36
3.1	Flow Cell, 3-3
3.2	Laser Beam Shaping, 3-4
3.3	Light Path through the WDM with a Single Port, 3-5
3.4	Time vs Fluorescence plot, 3-7
6.1	CSV template, 6-6
6.2	Plate Window [CytoFLEX LX Shown], 6-9
6.3	All Gates - Example Experiment, 6-26
6.4	Circular Gating Logic - Example Experiment, 6-26
6.5	Movement - Default Setting, 6-31
6.6	Movement - Max Setting, 6-32
6.7	Extent - Default Setting, 6-32
6.8	Extent - Maximum Setting, 6-33
6.9	Laser Setting Window [CytoFLEX LX shown], 6-39
7.1	Before Compensation, 7-1
7.2	After Compensation, 7-1
7.3	Positive Population Selected from the Single-Stained Sample, 7-7
7.4	Positive and Negative Populations Without an Unstained Sample, 7-8
10.1	Laser Warning Label on the Laser Optical Bench [CytoFLEX], 10-3
10.2	Laser Warning Label on the Laser Optical Bench [CytoFLEX LX], 10-4
10.3	Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX and CytoFLEX S], 10-4
10.4	Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX LX], 10-4
10.5	Laser Warning Label on the 355-nm Laser [CytoFLEX LX], 10-5
10.6	Laser Warning Labels on the Cytometer Back Cover [CytoFLEX and CytoFLEX S], 10-5
10.7	Laser Warning Labels on the Cytometer Back Cover [CytoFLEX

	LX], 10-6
10.8	Biohazard Label on the 4 L Fluid Containers, 10-6
10.9	Biohazard Label on the 10 L Fluid Cubitainers, 10-7
10.10	Biohazard Label Located in the Sample Station and on the Back of the Cytometer [CytoFLEX Shown], 10-7
10.11	Electrical Shock Hazard Label by the Power Switch [CytoFLEX or CytoFLEX S], 10-7
10.12	Caution Labels [CytoFLEX or CytoFLEX S], 10-8
10.13	Plate Loader Hazard Labels, 10-8
12.1	Plate Holder Stage (Standard Plate Loader), 12-49
12.2	Plate Holder Stage (Plate Loader DW), 12-50
12.3	Removing the Tubings from the Fluidics Module to the Plate Loader, 12-54
12.4	Plate Loader Module Securing Screws, 12-55
12.5	Removing the Plate Loader from the Cytometer, 12-55
12.6	Installing the Plate Loader into the Cytometer, 12-56
12.7	Plate Loader Module Securing Screws, 12-56
12.8	Connecting the Tubings from the Fluidics Module to the Plate Loader, 12-57
B.1	Experiment Operation Log Window, B-13
B.2	Select Experiment Profile Window, B-14
B.3	Print and Export Preview Window, B-15
B.4	System Operation Log Window, B-16
B.5	User Management Operation Log Window, B-17
C.1	Sample Injection Mode Control Knob, C-2
E.1	Optical Filter Mounting Fixture and Optical Filter, E-2
F.1	WDM Beam Splitter, F-2
F.2	CytoFLEX LX with the WDM Beam Splitter, F-4
G.1	Choosing Drive Encryption Method and Cipher Strength, G-4
G.2	Choosing the Encryption Level, G-5
G.3	Checking PC Configurations, G-8
G.4	BitLocker Encrypting the Drive, G-12
G.5	BitLocker Successfully On, G-12
G.6	Security Update Setup Installing, G-60
G.7	Security Update Setup Successfully Installed, G-60
G.8	Windows Shutdown, G-60
G.9	Working on Completing Security Updates, G-61

Illustrations

Tables

1.1	WDM Optical Filter Mount Color Codes [CytoFLEX], 1-7
1.2	WDM Optical Filter Mount Color Codes [CytoFLEX S_Violet-Blue-Yellow-Red], 1-7
1.3	WDM Optical Filter Mount Color Codes [CytoFLEX S_NUV-Violet-Blue-Red], 1-9
1.4	WDM Optical Filter Mount Color Codes [CytoFLEX S_NUV-Violet-Blue-Yellow], 1-9
1.5	WDM Optical Filter Mount Color Codes [CytoFLEX S_Violet-Blue-Red-IR], 1-11
1.6	WDM Optical Filter Mount Color Codes [CytoFLEX LX without WDM Beam splitter], 1-12
1.7	WDM Optical Filter Mount Color Codes [CytoFLEX LX with WDM Beam Splitter], 1-14
6.1	Target Power Ranges in the Laser Setting Screen, 6-39
10.1	Troubleshooting, 10-9
10.2	Troubleshooting [With Plate Loader], 10-21
D.1	Deep Well Plate [with Plate Loader DW], D-1
E.1	CytoFLEX Platform Optical Filter Specifications, E-1
F.1	Configuration Comparison, F-3
F.2	Optical Fibers with Indicator, F-4
G.1	List of Unnecessary Services, G-84
H.1	Table of Hazardous Substances Name and Concentration [CytoFLEX], H-2
H.2	Table of Hazardous Substances Name and Concentration [CytoFLEX S], H-3
H.3	Table of Hazardous Substances Name and Concentration [CytoFLEX LX], H-4

Tables

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, customizing detector configuration, 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 guiding operation. It also contains procedures for cleaning and maintenance.
- 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 series. The CytoFLEX S Special Configuration Specifications package insert should be used along with the Instructions for Use manual.

About this Manual

The information in the 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, Operation Principles

Describes how the Cytometer measures scattered light and fluorescence as cells pass through the laser beam.

CHAPTER 4, Daily Startup

Provides instructions for starting your CytoFLEX flow cytometer and navigating to the sample testing standby state.

CHAPTER 5, 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 6, 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 7, 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 8, Data Review

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

CHAPTER 9, Daily Shutdown

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

CHAPTER 10, Troubleshooting

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

CHAPTER 11, Cleaning Procedures

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

CHAPTER 12, 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, Custom Optical Filters

Describes how to install the custom optical filter.

APPENDIX F, WDM Beam Splitter Provides the instructions for using the WDM beam splitter.

APPENDIX G, Good Practices for Cyber Security Provides instructions for cyber security.

APPENDIX H, 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 platform, it is marked either [CytoFLEX], [CytoFLEX S] 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 e of the section title.
- The CytExpert Default software installation screens are shown in all instances unless otherwise specified.

- **IMPORTANT** IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the IMPORTANT adds benefit to the performance of a piece of equipment or to a process.
- **NOTE** NOTE is used to call attention to notable information that should be followed during use, or maintenance of this equipment.

Graphics

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

CHAPTER 1 System Overview

Overview

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

This chapter contains information on:

- Product Description
- 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.

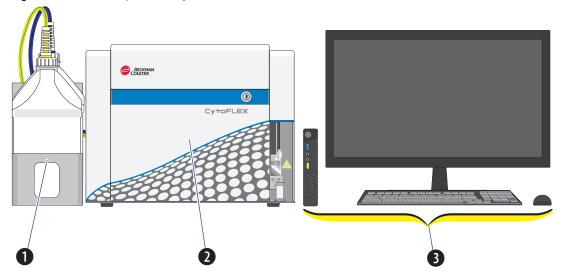
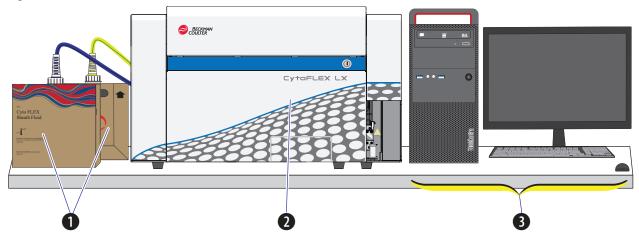


Figure 1.1 Main Components [CytoFLEX Without Plate Loader]

- 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.

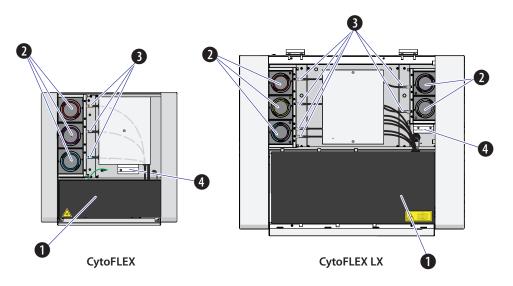
- 2. Cytometer. Provides signal generation and collection.
- **3.** Workstation. Displays and manipulates the contents of the Workstation and displays data generated by the Cytometer.

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.

AUTION

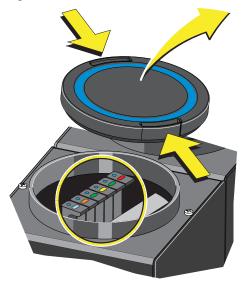
Risk of instrument damage. Do not place sample tubes in the optical filter holder. Liquid spills can damage instrument components. Use a tube rack to hold any sample tubes.

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

Wavelength Division Multiplexer (WDM)

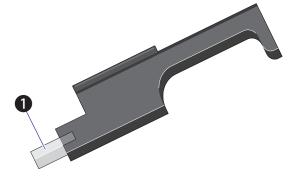
Each WDM corresponds to a different laser, or in some cases two lasers. The color of the ring on each cap corresponds to the color of the respective laser. Pressing the two release buttons on opposite edges of the cap allows you to open the WDM and replace the filters inside. See Figure 1.3. All optical filters are designed to be interchangeable. Refer to Replacing the Optical Filter in CHAPTER 12, Replacement/Adjustment Procedures to replace an optical filter.

Figure 1.3 Optical Filter Mounts



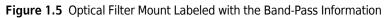
Each optical filter mount has an optical filter glass piece. See Figure 1.4.

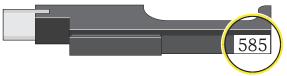
Figure 1.4 Optical Filter Mount with Optical Filter



1. Optical filter glass piece

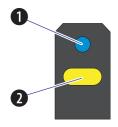
Each optical filter mount is labeled with the corresponding laser and band-pass information. See Figure 1.5.





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 range 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
405 nm	450/45 BP	PB450	Pacific Blue™dye, V450, eFluor™ 450, BV421
	525/40 BP	КО525	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
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™

Table 1.2 WDM Optical Filter Mount Color Codes [CytoFLEX S_Violet-Blu	ue-Yellow-Red]
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Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
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

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
488 nm	525/40 BP	FITC	FITC, Alexa Fluor™ 488, CFSE, Fluo-3
	690/50 BP	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI, DRAQ7™
561 nm	610/20 BP	ECD/mCherry	ECD, mCherry, PE-CF594
	585/42 BP	PE	PE, DsRed
	690/50 BP	PC5.5	PC5.5, PC5, 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™

 Table 1.2
 WDM Optical Filter Mount Color Codes [CytoFLEX S_Violet-Blue-Yellow-Red] (Continued)

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
375 nm	450/45 BP	NUV450	BUV395, DAPI
	675/30 BP	NUV675	Hoescht Red, BUV661
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
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, Pl
	690/50 BP	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI, DRAQ7™
	780/60 BP	PC7	PC7, DRAQ7™
638 nm	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™
	660/10 BP	APC	APC, Alexa Fluor™ 647, eFluor™ 660, Cy5

Table 1.3	WDM Or	otical Filter	Mount Color	Codes [C	vtoFLEX S	NUV-Violet-Blue-Red]
				0000010	,	

 Table 1.4
 WDM Optical Filter Mount Color Codes [CytoFLEX S_NUV-Violet-Blue-Yellow]

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
375 nm	450/45 BP	NUV450	BUV395, DAPI
	675/30 BP	NUV675	Hoescht Red, BUV661

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
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
488 nm	525/40 BP	FITC	FITC, Alexa Fluor™ 488, CFSE, Fluo-3
	690/50 BP	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI, DRAQ7™
561 nm	610/20 BP	ECD/mCherry	ECD, mCherry, PE-CF594
	585/42 BP	PE	PE, DsRed
	690/50 BP	PC5.5	PC5.5, PC5, PI, DRAQ7™
	780/60 BP	PC7	PC7, DRAQ7™

 Table 1.4
 WDM Optical Filter Mount Color Codes [CytoFLEX S_NUV-Violet-Blue-Yellow] (Continued)

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
405 nm	450/45 BP	PB450	Pacific Blue™dye, V450, eFluor™ 450, BV421
	525/40 BP	КО525	Krome Orange, AmCyan, V500, BV510
	610/20 BP	Violet610	BV605, Qdot® 605
	660/10 BP	Violet660	BV650, Qdot® 655
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™
808 nm	840/20 BP	IR840-A790	Alexa Fluor®790
	885/40 BP	IR885	PromoFluor-840, IR fixable viability dye

Table 1.5 WDM Optical Filter Mount Color Codes [CytoFLEX S_Violet-Blue-Red-IR]

NOTE The CytoFLEX and CytoFLEX S have the following additional BP filters supplied within the respective WDM:

- 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	DAPI
375 nm	450/45 BP	UV450	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 1.6 WDM Optical Filter Mount Color Codes [CytoFLEX LX without WDM Beam splitter] (Continued)

NOTE The CytoFLEX LX has the following additional BP filters supplied within the respective WDM:

- 405/10 BP
- 638/6 BP
- 561/6 BP
- 488/8 BP
- 450/30 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	DAPI
	N/A 740/35 BP	U(S)740	BV711
	N/A 819/44 BP	U(S)819	BUV805
375 nm	450/45 BP	UV450	BUV395, DAPI
	525/40 BP	NUV525	BUV496
	675/30 BP	NUV675	Hoescht Red, BUV661
	N/A 740/35 BP	N(S)740	BUV737
	N/A 819/44 BP	N(S)819	BUV805
405 nm	405/10 BP	VSSC	N/A
	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	V(S)763	BV785, Qdot® 800
	712/25 BP	V(S)712	BV711
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™

 Table 1.7
 WDM Optical Filter Mount Color Codes [CytoFLEX LX with WDM Beam Splitter]

Laser	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
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
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

Table 1.7 WDM Optical Filter Mount Color Codes [CytoFLEX LX with WDM Beam Splitter] (Continued)

NOTE The following are additional channels created when the WDM beam splitter is engaged:

- U(S)740 and U(S)819
- N(S)740 and N(S)819
- V(S)763 and V(S)712

Refer to APPENDIX F, WDM Beam Splitter.

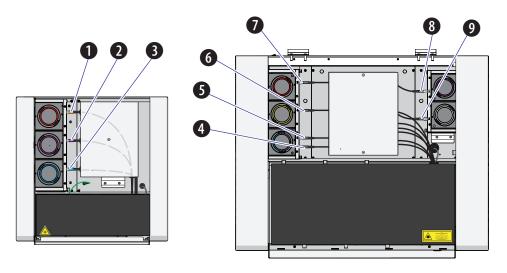
Optical Fiber

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 12, 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.

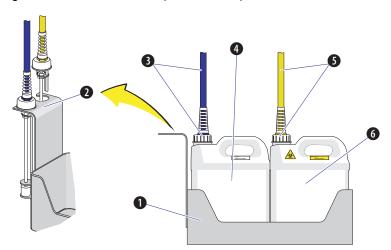


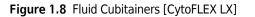
Figure 1.7 Fluid Containers [CytoFLEX and CytoFLEX S]

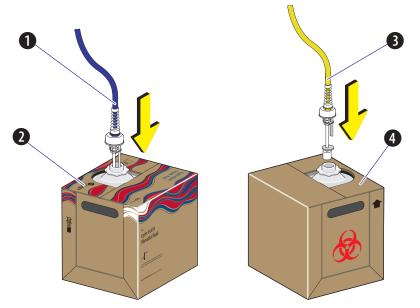
- 1. Fluid Container holder. Holds the Fluid Containers.
- 2. Fluid sensor holder cutout. Holds the sheath fluid harness and the waste harness when removed from their respective containers to protect the sensors from damage and/or contamination.
- **3. Sheath fluid harness.** Connects to the sheath fluid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

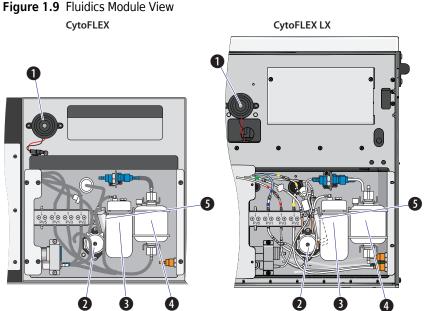
NOTE The waste cubitainer must be on the same level as the Cytometer.

Fluid Containers/Cubitainers

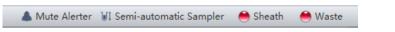
On the CytoFLEX and CytoFLEX S, two Fluid Containers are placed in the Fluid Container holder: a sheath fluid container and a waste container. See Figure 1.7. On the CytoFLEX LX, two 10-L Fluid Cubitainers are placed to the left side of the instrument: a sheath fluid cubitainer and a waste cubitainer. 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 pressurization. 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 12, 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 12, Replacement/ Adjustment Procedures) or to add Deep Clean solution (see Adding the Deep Clean Solution in CHAPTER 12, Replacement/Adjustment Procedures).

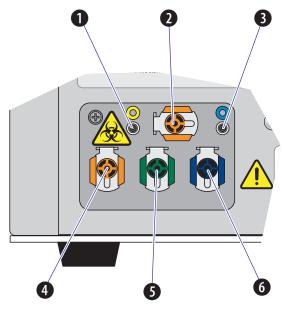


- 1. Alarm. Emits a warning sound when there is a problem with the Fluid Container/Cubitainer capacity or with the performance of certain operations.
 - **NOTE** When the alarm sounds, the Mute Alerter icon appears in the status bar. The alarm continues for about 30 seconds. To mute the alarm temporarily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

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

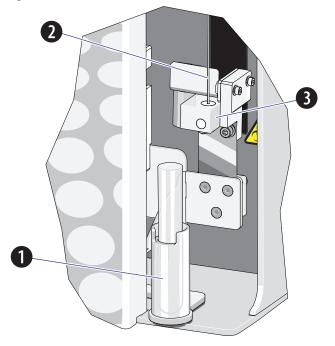
Sample Station





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 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 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 1.12: sample loading position (1), standby position (2), and sample acquisition position (3). You can only distinguish the mixing position from the sample acquisition position by 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 1.12 Sample Tube Holder Positions

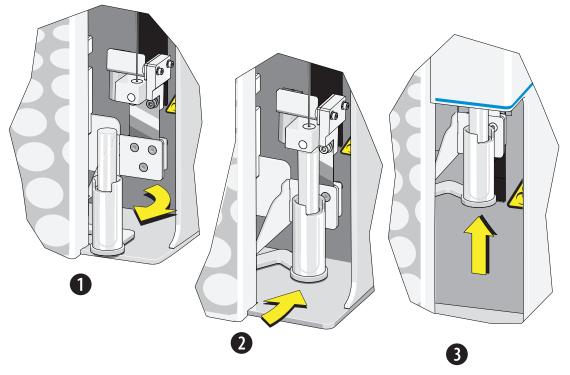


Plate Loader Components

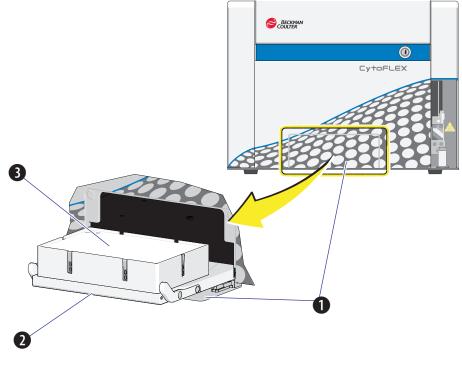
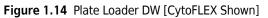
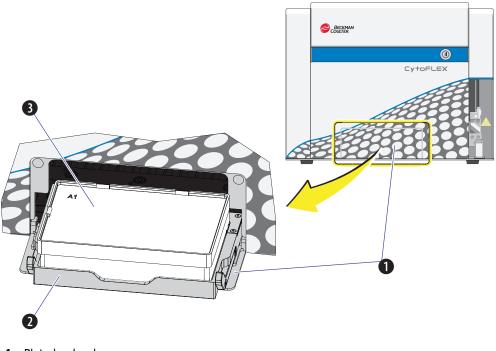


Figure 1.13 Standard Plate Loader [CytoFLEX Shown]

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





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

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

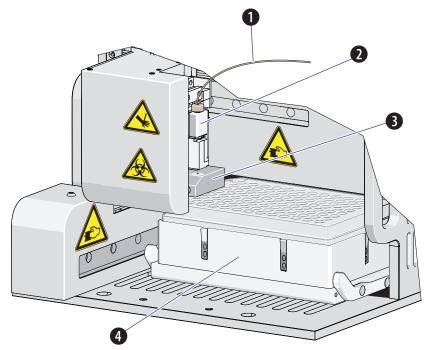


Figure 1.15 Standard Plate Loader (Front Cover Removed)

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

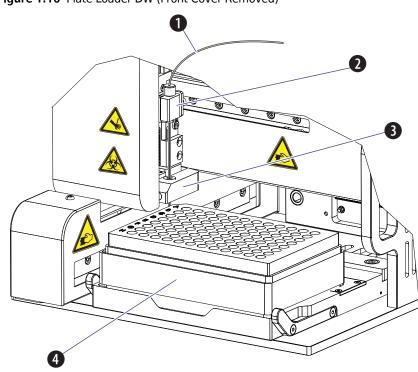


Figure 1.16 Plate Loader DW (Front Cover Removed)

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

Plate Holder Components

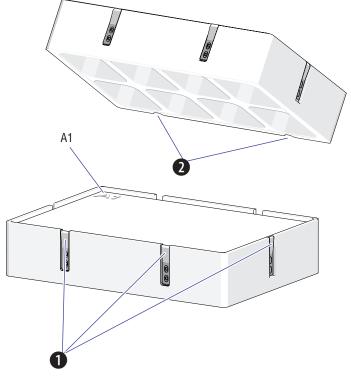


Figure 1.17 Standard Plate Holder without Groove (Standard Plate Loader)

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

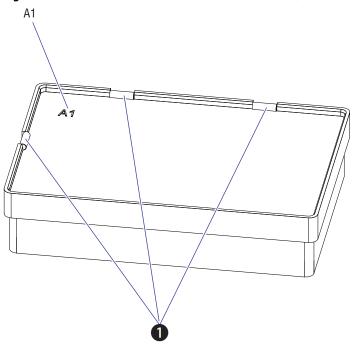


Figure 1.18 Plate Holder with Groove (Plate Loader DW)

- 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 12, Replacement/Adjustment Procedures.

The plate holder is only used to hold standard 96-well plates. Note that 96-well deep well plates do not use a plate holder.

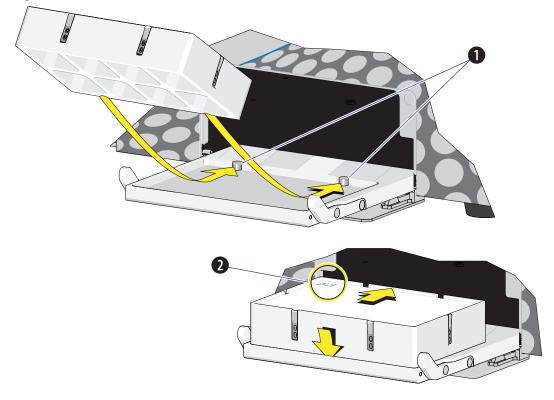
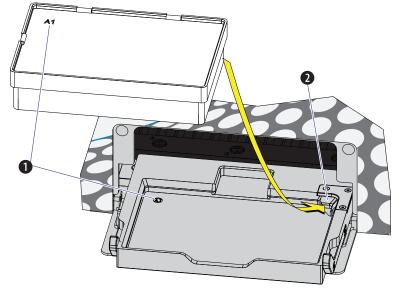


Figure 1.19 Plate Holder Stage (Standard Plate Loader)

- 1. Pins
- 2. Position A1

Figure 1.20 Plate Holder Stage (Plate Loader DW)



- 1. Position A1
- 2. Spring Lock

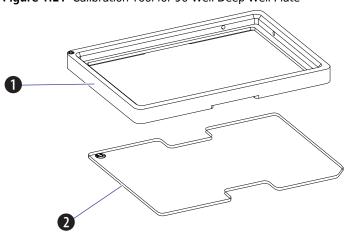


Figure 1.21 Calibration Tool for 96-Well Deep Well Plate

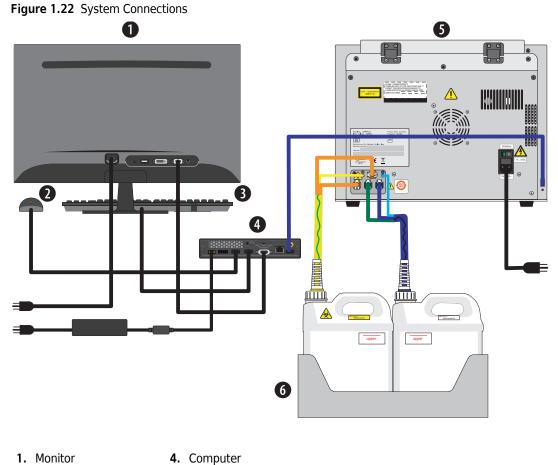
- 1. Calibration frame
- 2. Transparent plate

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.

System Configuration

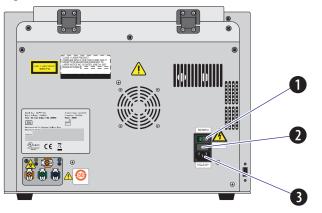
Risk of data loss and/or instrument damage. Never shut off the power or disconnect a data cable while the Cytometer is in the process of performing a task. This could cause data loss or damage to the system.

System Configuration [CytoFLEX and CytoFLEX S]



- 2. Mouse
- 5. Cytometer
- 3. Keyboard
- 6. Fluid Container holder

Figure 1.23 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.

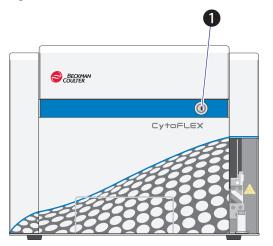


Figure 1.24 Front of Cytometer [CytoFLEX Without Plate Loader Shown]

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 1.25 System Connections

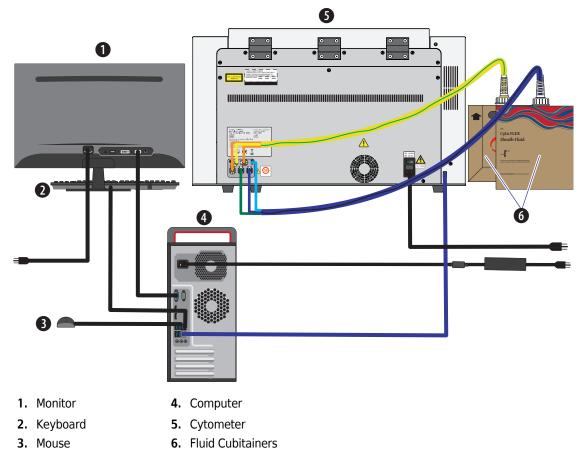
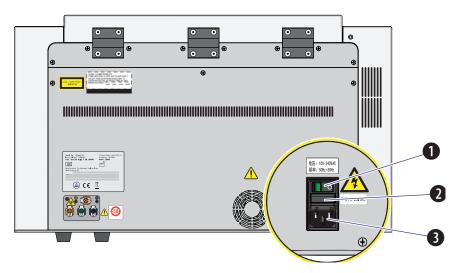
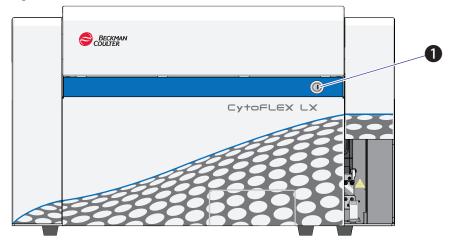


Figure 1.26 Back Cover Connections



- 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 1.27 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, CytoFLEX S and CytoFLEX LX instrument:

CytoFLEX Ready to Use Daily QC Fluorospheres

The CytoFLEX Ready to Use Daily QC Fluorospheres are a ready to use formulation of fluorospheres that are used for QC standardization on the CytoFLEX Platform.

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 IR laser QC.

CytoFLEX Sheath Fluid

A nonionic, non-fluorescent, anti-microbial 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 during Daily Clean procedure for cleaning the sample lines.

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 Platform reagents used on the CytoFLEX Platform systems:

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

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/supplies/cytoflex-platform-upgrades

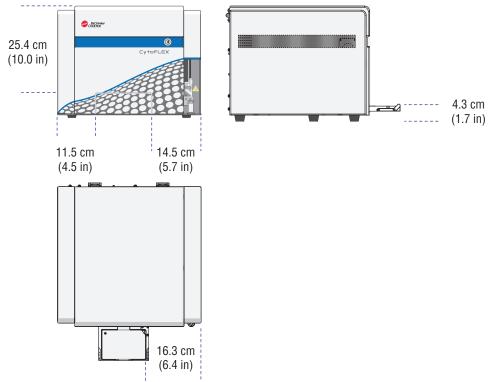
Otherwise, contact us.

Instrument Specifications

Dimensions [CytoFLEX and CytoFLEX S]

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

Refer to Figure 1.28 for the [CytoFLEX and CytoFLEX S] Plate Loader DW dimensions.





Dimensions [CytoFLEX LX]

	Dimensions		
Instrument dimensions (Length x Width x Height)	Cytometer [With or Without Plate Loader]	73.3 cm x 60.5 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	

Refer to Figure 1.29 for the [CytoFLEX LX] Plate Loader DW dimensions.

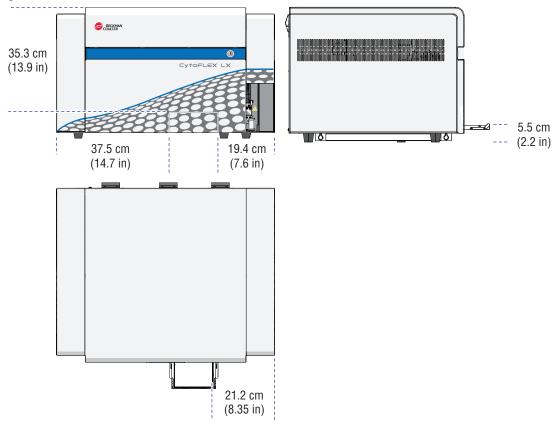


Figure 1.29 Plate Loader DW Dimensions [CytoFLEX LX]

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 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.
	CytoFLEX: The system can be configured with up to three spatially-separated lasers.
	CytoFLEX S : The system can be configured with up to four spatially-separated lasers.
	CytoFLEX LX : The system can be configured with up to six spatially-separated lasers.
Emission Optics	Patent-pending alignment-free integrated optics quartz flow cell design with >1.3 NA.
	Flow Cell dimensions: 430-µm x 180-µm internal diameter.

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	Optics		
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	
	, -	Ultraviolet (UV) laser	
		Wavelength: 355 nm, 20 mW	
		 Beam spot size: 5 μm x 80 μm 	
		Near Ultraviolet (NUV) laser	
		Wavelength: 375 nm, 60 mW	
		 Beam spot size: 5 μm x 80 μm 	
		Infrared (IR) laser	
		• Wavelength: 808 nm, 60 mW	
		 Beam spot size: 5 μm x 80 μm 	
Forward scatter detection	Silicon photodiode with built-in 488/8 band-pass filter.		
Fluorescence and side scatter detection	Fluorescence and side scatter light collected by the objective lens is delivered by fiber optics to a patent-pending design with high performance, solid-state, high efficiency, low-noise detector array.		
	Reflective optics with a si	ngle transmission band-pass filter in front of each detector.	
Violet side scatter configuration (VSSC)	The system offers the ability to configure the violet laser WDM to collect side scatter to better resolve nanoparticles from noise.		

Fluidics System		
Sample flow rates	Fixed Flow Rates: 10 μL/min, 30 μL/min, 60 μL/min	
	Custom Flow Rates: 10 - 240 µL/min in 1 µL increments	
Fluid capacity	CytoFLEX and CytoFLEX S: 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	Initialize, system startup program, sample mix, backflush, prime, Daily Clean, Deep Clean	

Fluidics System		
Sample input formats	Single Tube Loader format5 mL (12 x 75 mm) polystyrene and polypropylene sample tubes	
		1.5 mL and 2 mL micro-centrifuge sample tubes
Dead Volume	10 μL	
Minimum Sample Volume	100 μL	

Fluidics System [With Standard Plate Loader]		
Sample input formats	Plate 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	45 μL/well	
Maximum Sample Volume	250 μL/well	

Fluidics System [With Plate Loader DW] ^a		
Sample input formats	Plate format	96-well deep well plate;
		Flat/U/V bottom standard 96-well plate
Dead Volume	96-well deep well plate	20 μL
	Flat bottom standard 96-well plate	20 μL
	U/V bottom standard 96-well plate	10 μL
Minimum Sample	Standard 96-well Plate: 50 µL/well	
Volume	lume Deep Well Plate: 200 μL/well ^b	
Maximum Sample Volume	Refer to the related specification of the deep well plates.	

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 manufactured by Beckman Coulter. Refer to APPENDIX D, Deep Well Plate.

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

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

Data Management			
Software	CytExpert software	CytExpert software	
Language	English and Chinese		
FCS format	FCS 3.0		
Minimum	Operating system	Windows7, or 10 Professional 64-bit	
Workstation/ computer	Processor	7th Gen Intel Core i3 (3MB Cache, 3.4 GHz)	
requirements	Memory	8 GB RAM	
[CytoFLEX/ CytoFLEX S]	Storage	500 GB	
CytorLex Sj	Ethernet	Ethernet LAN 10/100/1000 or equivalent	
	USB	4 USB 2.0 and above ports	
Minimum	Operating system	Windows 10 Professional 64-bit	
Workstation/ computer	Processor	4th Generation Intel Core i7 (8MB cache 4.0 GHz)	
requirements	Memory	8 GB RAM	
[CytoFLEX LX]	Storage	500 GB	
	Ethernet	Integrated 10M/100M/1000M Gigabyte	
	USB	4 USB 2.0 and above ports	
Compensation	Full matrix compensat	tion, manual and automatic.	
	-	Novel Compensation Library for storage of spillover values of dyes to easily determine the correct compensation matrix with new gain settings.	

Performance Characteristics

Performance Characteristics [CytoFLEX and CytoFLEX S]

Performance		
Sensitivity	MESF	FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE)
		APC: <25 molecules of equivalent soluble fluorochrome (MESF-APC)
Fluorescence	rCV <3%	
resolution	The CytoFLEX Flow cytometer is capable of achieving <3% rCV. Using CytoFLEX Daily QC Fluorospheres, CytoFLEX Ready to Use Daily QC Fluorospheres or 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, and IR lasers.	
Blue Side scatter resolution	<300 nm	

Performance		
Violet Side scatter resolution	80 nm relative to polystyrene particles	
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	≤1.0%			
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 cytometer. Refer to APPENDIX C, Sample Injection Mode Control Kit.

Performance [With Plate Loader DW]						
Carryover	Plate Loader format	≤1.0%				
Throughput [With Plate Loader DW] ^a	Standard 96-well plate, 10 sec min.	ond acquisition without mixing or backflush: <36				
	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 secon backflush: <54 min.					
	Deep-well 96-well plate, 10 second acquisition with 10 second mixing and 4 second backflush: <64 min.					

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

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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)			
		APC: <25 molecules of equivalent soluble fluorochrome (MESF-APC)			
Fluorescence resolution	rCV <3%				
	The CytoFLEX LX Flow cytometer is capable of achieving <3% rCV. Using CytoFLEX Daily QC Fluorospheres, CytoFLEX Ready to Use Daily QC Fluorospheres, or 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	80 nm relative to	o polystyrene particles			
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	≤1.0%			
Throughput [With	10 second acquisition without mixing or backflush: <34 min.				
Standard Plate Loader]a10 second acquisition with 3 second mixing and 3 second backflush: < -					

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≤1.0%						
Throughput [With Plate Loader DW] ^a	 Standard 96-well plate, 10 second acquisition without mixing or backflush: <39 min. Deep-well 96-well plate, 10 second acquisition without mixing or backflush: <40 min 						
	Standard 96-well plate, 10 second acquisition with 5 second mixing and 6 second backflush: <60 min. Deep-well 96-well plate, 10 second acquisition with 10 second mixing and 6 second backflush: <69 min.						

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

Using the CytExpert Software

Overview

The CytExpert software is a full-feature software package that controls the instrument operation, collection of experiment data, and analysis of the results. This chapter will explain the software 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 4, 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.

CytE:	xpert(Default-Configuration)			0 9	522
File C	ytometer Settings QC/Standardization Advance	d Help 🚯 Cytobank			
	Acquisition	<u>はた・## 回社 -IPOI++A.ひ ��� の の の の ろ ちゃ 下ヨアエチ+111118000-</u>			
	ytometer Settings QC/Standardization Advance				
Con	nexted A Standby 2021-11-19 14	17481 Colometer connected	💷 Plate Loader 🛛 🚔 Shr	eath 🚔 Was	-

The following operations can be selected from the start page:

New Cor	eriment from mpensation	m Template	Open Experiment Open Compensation	
xperiment	Template	Compensation		
<u>C:\Users\r</u>	nhan03\Doc	uments\CytExpe	rt Data\2.5 Experiment\Exp	<u>20211117_1.xit</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 creating a new compensation 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.

F Cytometer Settings QC/Standardization Advances		- I P O I + -		[개] ③ ③ 슈 ㅎ @	비비리 ㅠ	L + ++ □] : : : 0	•	
Time: 0000000 Events to Display: 1000 Events Versets to Record: 1000 Events Wither to Record: 000 Events Wither to Record: 100 Fvents Sample flow Rate: 10 µ/lmin © Store Medium Fast	0 0 0 0 0 0 0 0 0 0 0 0 0 0	to BC C 100	50 t T FSC-A (x157 55 Parent		Events	% Total 0 0	% Parent		
da ∦ W ¥ ¥ ¥ ₩ ₽ <u>a</u> () 1de1					6				
F Connected Standby V[2017-06-16 08:1	0.55] Cytometer standby.							₩1 Semi-automatic Sample	r 🖨 Sheath 🚔 Waste

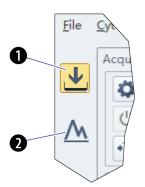
- 1. Navigation. Gives the option of accessing the Acquisition screen or Analysis screen.
- 2. Menu. Allows you to configure settings for sample acquisition, instrument operation, and software options.
- **3.** Instrument Operation Controls. Controls sample loading/unloading and data acquisition and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

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

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

Acquisition Screen Navigation

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



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

Collection

Standby state			Initialized 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]

Acquisition		Acquisition
🔯 Initialize 🔵 Recor	rd 🛛 🕄 Restart	Run Record Restart
🖒 Standby 🕅 Backflu	ush 🔺 Eject	🕐 🕐 Standby 🕅 Backflush 🔺 Eject
Auto Record	🔀 Acq. Setting	🙆 Auto Record 🔀 Acq. Setting
Events/Sec: 0.0		Events/Sec: 0.0
Abort(%): 0.00	0	Abort(%): 0.00
Events: 0		Events: 0
Time: 00:0	00:00	Time: 00:00:00
Events to Display: 10	000 Events	Events to Display: 1000 Events
✓ Events to Record: 10	0000 Events	Events to Record: 10000 Events
in All	Events 💌	in All Events -
☑ Time to Record: 60	00 Sec	☑ Time to Record: 600 Sec
□ Volume to Record: 10) µL	□ Volume to Record: 10 µL
Sample Flow Rate: 10 µL/r	min	Sample Flow Rate: 10 µL/min
Slow ○ Medium	Fast	● Slow ○ Medium ○ Fast

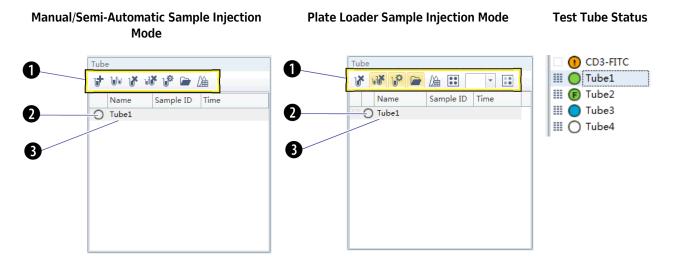
Initialized state

Standby 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.
 - 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** 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 HI I 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.

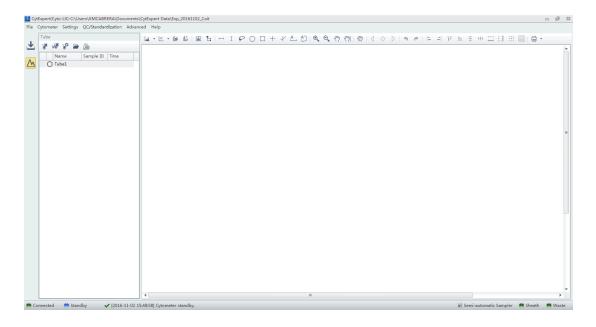
4. Sampler status. Displays the sample injection mode state. There are two sample injection modes: Semi-automatic sample injection mode and manual sample injection mode.

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

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

Analysis Screen

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



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

Tube Management

Tube								
J.	Մա	v	uð	ı,		ĹÆ	Ì	
	Na	me		Sam	ple ID)	Time	
0	Tuk	be1						

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)

🔟 🖄 🝷 🛄 🎼			1 (A 🤊 🕈	
Plots	La 🖄 🖌 🔐	Zoom	⊕, ⊝,	Threshold	A
Statistics	da .	Scale	KM3 KM3	Undo/ Redo	h e
Hierarchy	"	Gain	80)		
Gates	$\vdash I $	Compensation	<27		

Compensation Experiment Screen

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



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

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

Compensation Controls

The control 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.

	. 🔍 🔍 🖑	M	• e ⊨	4 11	њ Ŧ	ΗI		8 -
Compensation Calculation	<i>D</i> è	Single Side Pan	<m></m>					
Compensation Matrix		Adjust Gain	< T					
Compensation	Λα.	Undo/Redo	• , e					
Setup	<i>∐</i> ¢-							
Add/Delete Negative Gate	九九	Alignment	= =	<u>т</u> <u>ь</u>	Ŧ	++ :	[+] + = + L + J	



QC Experiment Screen

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

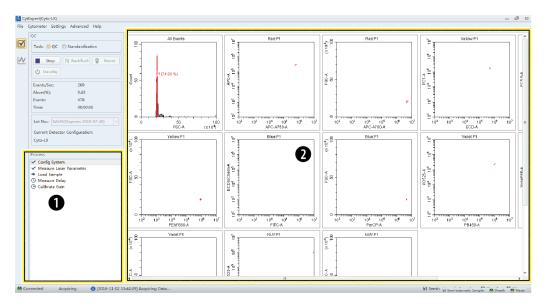
QC Report Screen

Before starting the QC routine, a Settings screen appears.

ure 2.2 QC Repor	t Screen										
n	A										
V G											
xper (Cvto-LX)											0 6
Cytometer Settings Advance, Help											
QC	*										
Task: QC Stande dization					D						
😋 Initialize 🔢 Backflush 👔 Boost				QC	Report						
Sacciusi Boos	Bead Lot No.:										
🖒 Standby	Bead Lot INO.: Bead Expires:		02_SE -07-20				DC Date:		016-11-0	2 15:07	
	Cytometer Name		-07-20				Cytomete		.010-11-0.	2 13/07	
Events/Sec: 0.0	Detector Configu		-ux			_	,				
Abort(%): 0.00	Loader Type:		Automatic			5					
Events: 0	Threshold										
Time: 00:00:00	Channel: FSC(Heig	المليمة الم	Manual Malu	50000							
Lot No.:	Laser	ing model	vianual valu	6. 30000							
LOTINO.											
Current Detector Configuration:		Delay(µs)	Default De		ence Delay(µs)			pet Power		Result	
Cyto-LX	NUV	72.8		72.96	-0.1		60 49		50-70	0	
	Blue Red	0.0 36.1		0.00	0.0		49		40-60 40-60	0	
Report	Yellow	-35.6		-35.84	-0.1		30		20-40	0	
Detector Configuration:	Violet	-70.2		-70.40	0.1		88		70-120	ŏ	
Cyto-LX +	Signal Value										
		Turnet	%Difference		Torrest	%Difference		T			
Date: 2016-10-03 × ~ 2016-11-02 ×	Parameter Gair		Target Gain	Median	Target Median	Target Median		Target rCV(%)	Width	Result	
Process Date Lot No. Result		52 252	0.00	206408.8	209181.4	-1.33	-		802.0		
2016-11-02 15:02 BAH02 😒 🦳		81 81	0.00	604528.9		3.17	-		883.2		
2016-11-02 15:05 BAH02_SE 🔇		56 56	0.00	910944.4		-1.05	2.13		1316.2		
2016-11-02 15:07 BAH02_SE		34 34	0.00	234576.1		0.43	2.03	5.00	1315.0		
2016-11-02 15:17 BAH02 Ø		02 202 85 185	0.00	413936.1 108776.3	416223.8	-0.55	2.23	5.00	1318.8		
		62 62	0.00	63120.5		0.43	2.02	5.00	1294.6		
U		69 69	0.00	8750280.0		-0.61	0.80		1604.2	-	
		21 21	0.00	951872.7	937573.0	1.53	0.78		802.9		
		60 160	0.00	1364065.0		0.17	0.87	7.00	820.7		
	APC 3	41 341	0.00	326824.1	328142.5	-0.40	3.55	5.00	1063.2		
		29 329	0.00	5459777.0		2.23	3.74		1067.4		
~ ·	APC-0750	44 44	0.00	666992.6	649742.0	2.65	3.78	5.00	1070.0	0	

- 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

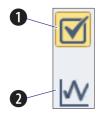


When acquiring QC samples, the software opens the QC screen.

- 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 5, 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			
New Experiment	Acq.Setting		Set Channel		QC/Standardization	Delay Setting	User Manager##	Operation Log***	Sign***	Backuptt	View H
New Experiment						· · ·		System	-		
From Template	Detector Configur	ation	Set Label			Laser Setting	Role Managertt	Operation Log***	Reject***	Restore ^{††}	About
New Compensation					1	_		User Management	Signature		
	Backflush		Set Customized Parameter			Maintenance	Account Policies††	Operation Log ⁺⁺	Records***	Log Cleanup††	
Open Experiment									Signature		-
	Boost†		Set Retentio	n Period***		Event Rate Setting	Change Passwordtt		Setting***		
Open Compensation	Initialize		Retention P	eriod Options***	1	Plate Type Library		-		-	
Save	Standby		Compensati	on Matrix	1		•				
Save As	Prime		Compensati	on Library							
Save As Template	Deep Clean		Events Disp	lay Setting							
Import FCS File	Calibrate Sample	Flow Rate	Language Se	tting							
Export FCS File	System Startup Pr	ogram	Set Experim	ent Directory***							
Recent	Daily Clean		Options	Experiment##							
Recent Template	Sample Injection	Manual		Tube							
Pecent Compensation	Mode	Semi Automatic		Plot							
Close Experiment	Wouc	Plate Loader**		Gate							
Experiment Explorer***	Sampler Reset			Page Setup							
Exit	Turn On†††			Plate Loader							
	Turn Off###										
	Acq.Setting Catalo	og									
	Cytometer Config	uration									
	Cytometer Inform	ation									

* 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.

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

Figure 2.4 QC Software Menu Tree

File	Cytometer		Settings		Advanced	Account##	Log#	Signature**	Backup/Restore##	Help
							Experiment			
New Experiment	Detector Config	uration	Retention P	eriod Options**	Delay Setting	User Manager‡‡	Operation Log**	Sign***	Backup‡‡	View Help Fil
New Experiment							System			
From Template	Backflush		QC/Standar	dization Setting	Laser Setting	Role Manager‡‡	Operation Log**	Reject***	Restore##	About
							User Management	Signature		
New Compensation	Boost*		Target Libra	ry	Maintenance	Account Policies##	Operation Log##	Records***	Log Cleanup‡‡	
Open Experiment								Signature		
	Initialize		Standardiza	tion Target Library	-	Change Password‡‡		Setting***		
Open Compensatior	Standby		Set Experim	ent Directory**	Plate Type Library					
Recent	Prime		Language Se	1 ⁻						
Recent Template	Deep Clean			Experiment††						
Recent Compensatio	Calibrate Sampl	e Flow Rate		Tube						
Close	System Startup									
QC/Standardization			Options	Plot						
Experiment	Daily Clean		l '							
Explorer**		h		Gate						
Exit	Sample	Manual Constitutementie		Page Setup						
	Injection Mode	Semi Automatic		Plate Loader‡	l					
		Plate Loader								
	Sampier Reset Turn On***	Sampler Reset								
Turn Off***		las								
	Acq.Setting Cata									
	Cytometer Conf	•								
	Cytometer Infor	mation								

* 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

<u>File Cytometer Settings QC/Standardization Advanced Help</u>

CytExpert User Management Software Option

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

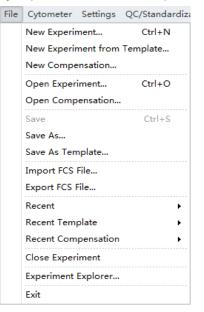
CytExpert Electronic Record Management Software Option

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.

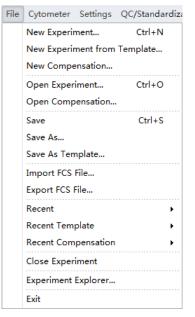
CytExpert Default Software Option



CytExpert User Management Software Option

File Cytometer Settings QC/Standardiza New Experiment... Ctrl+N 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/Standardizat
×	Acq. §	Setting	
	Detec	tor Config	juration
(*)	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 (
	Turn Off		
	Acq. Setting Catalog		
	Cyton	neter Conf	figuration
	Cyton	neter Info	rmation

CytExpert User Management Software Option -Standby state

CytExpert User Management Software Option -Initialized state

ŕ	Acq. Setting	*	Acq. Setting			
~	Detector Configuration	*	Detector Configuration			
6	Backflush	 *	Backflush			
j	Boost	4	Initialize			
¥.	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 Sampler Reset			
	Sample Injection Mode					
	Sampler Reset		Turn On			
	Turn On		Turn Off			
Turn Off Acq. Setting Catalog			Acq. Setting Catalog			
			Cytometer Configuration			
	Cytometer Configuration		Cytometer Information			

CytExpert Electronic Record Management Software Option - Standby state

CytExpert Electronic Record Management Software Option - Initialized state

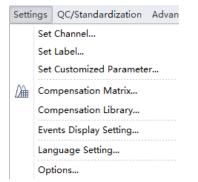
Cyto	meter Settings QC/Standardizat	Cyte	ometer Settings QC/Standardizat
×	Acq. Setting	×	Acq. Setting
	Detector Configuration		Detector Configuration
⁰	Backflush	**)	Backflush
Û	Boost	Û.	Boost
•	Initialize	φ.	Initialize
Ċ	Standby		Standby
	Prime		Prime
	Deep Clean		Deep Clean
	Calibrate Sample Flow 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.

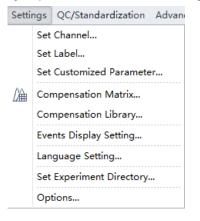
CytExpert Default Software Option



CytExpert User Management Software Option

Settings		QC/Standardization	Advan				
	Set	Channel					
	Set	Label					
	Set Customized Parameter						
<i>∐</i> `	Compensation Matrix						
	Compensation Library						
	Events Display Setting						
	Lan	guage Setting					
	Opt	tions					

CytExpert Electronic Record Management Software Option



QC/Standardization Menu

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

QC/Standardization Advanced A Start QC/Standardization

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.

2

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

Adv	anced	Help
	Delay	Setting
	Laser	Setting
	Mainte	enance
	Event	Rate Setting

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

Advance	d Account Log
De	ay Setting
Las	er Setting
Ma	intenance
Eve	nt Rate Setting

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

Advanced	Account	Log	S				
Delay Setting							
Laser	Laser Setting						
Maint	Maintenance						
Event Rate Setting							

CytExpert Default Software Option - Plate Loader Sample Injection Mode

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

CytExpert User Management Software Option -Plate Loader Sample Injection Mode

Adv	anced	Account	Log
	Delay Setting		
	Laser Setting		
	Maintenance		
	Event Rate Setting		
	Plate Type Library		

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

Adva	nced	Account	Log
	Delay	Setting	
	Laser Setting		
	Maintenance		
	Event Rate Setting		
	Plate Type Library		

Account Menu

Used to for user account management settings.

CytExpert User Management Software Option

Acc	ount	Log	Backup/Res
	User	Mana	iger
	Role Manager		
	Account Policies		
	Chan	ige Pa	ssword

CytExpert Electronic Record Management Software Option

Acc	ount	Log	Signature	В
	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

```
Log Signature Backup/Restore Help
Experiment Operation Log...
System Operation Log...
User Management Operation Log...
```

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

Signature Menu

Used to sign experiment and view signature details.

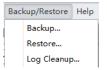
CytExpert Electronic Record Management Software Option

```
Signature Backup/Restore
Sign...
Reject...
Signature Records...
Signature Setting...
```

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

Backup/Restore Menu

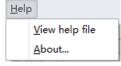
Used to backup/restore databases. Refer to Backup and Restore in CHAPTER 10, Troubleshooting.



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

Help Menu

For displaying software version information and system Instructions for Use.



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

User Management

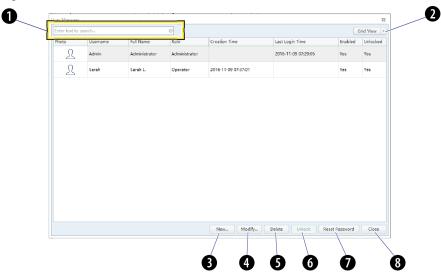
IMPORTANT Only an Administrator can manage users. You must have either the CytExpert User Management or CytExpert Electronic Record Management software option installed to use this feature. Refer to CytExpert Software Installation Options in APPENDIX A, Instrument Installation.

User Management is used to create and manage user accounts.

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

Figure 2.5 User Manager (Card View)





- 1. Search text box: Filters users by username and 6. Unlock: Used to unlock an existing account that 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.
- 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.

New		23
	Username:	
	Full Name:	
25	Password:	password
	Role:	Operator 🔹
	Enabled:	
		OK Cancel

- **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 Cance	

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.

З Select ок.

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.

lame	Description	Permission
dministrator	Administrator	V V Advanced
perator	Operator	✓ Delay setting
		✓ Modify maintenance date
		✓ Modify event rates setting
		✓ Modify plate type library
		✓ 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
		✓ Set retention period
		✓ Set retention period options
		✓ ✓ Account
		✓ Manage role
		✓ Manage user
		✓ Access account policies
		v √ Log
		✓ View operation logs
		V V QC
		✓ Delete QC result
		✓ ✓ Signature
		✓ Sign/Reject
		✓ Set signatures
		✓ ✓ Backup/Restore
		✓ Data backup and restore
		✓ Log clean-up
		✓ ✓ Experiment
		✓ Delete experiment
		✓ Delete acquired data
		New. Modify Delete Clore

- 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.

Name:	
Name:	
Description:	
Permission	
V Advanced	
 Delay setting Modify maintenance date 	
Modify maintenance date	
Modify event rates setting Modify plate type library	
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	
Set retention period	
Set retention period options	
✓ □ Account	
Manage role	
Manage user	
Access account policies	
✓ □ Log	L
View operation logs	
~ 🔲 QC	
Delete QC result	
🗸 🔲 Signature	
Sign/Reject	
ОК	Cancel

- **2** Fill in the new role information.
 - **a.** Enter the role name.
 - **b.** Enter the role description.
 - **c.** Select the permissions applicable to the new role.
- **3** Select OK. The new role displays in the role list.
- 4 Select Close

Deleting User Roles in Role Manager

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

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

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

Modifying User Roles in the Role Window

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

lodify	(Σ
Name	e:	User 1	
Desci	ription:	Reviewer	
Pern	nission		Т
~ V	Advan	nced	
	🔽 De	lay setting	1
	✓ Mo	odify maintenance date	
	V Mo	odify event rates setting	
	✓ Mo	odify plate type library	
	✓ Mo	odify laser setting	
~ V	Cytom	neter	=
	V Ne	w, edit and delete detector configuration	
	_	librate sample flow rate	
		cess cytometer configuration	
¥	Setting	-	
		w, edit and delete standardization target va	
		lete QC target value	
		t experiment directory	
		t retention period	
_	Accou	t retention period options	
× 🖿	-		
		anage role anage user	
	_	cess account policies	
	AC	Less account policies	
		OK Cance	

2 Modify the role information as necessary.

3 Select OK

4 Select Close

Account Policies

IMPORTANT Only an Administrator or an account has the Manage user permission 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 define the default properties for the password policy, account lockout policy, and application inactivity policy.

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

Figure 2.8 Account Policies - Password Policy

A	ccount Policies			23
	Password Policy	Account Lockout Policy	Application Inactivity Policy	
	Password must	meet complexity requirer	nents <u>Explain</u>	
	✓ Enforce passwo	ord history		
	Number of pas	swords remembered:	12 ‡	
	I Enforce minimu	m password length		
	Minimum passv	word length: 8 ‡	characters	
	🗷 Enforce minimu	im password age		
	Minimum passv	vord age: 1 📜 da	iys	
	✓ Enforce passwo	ord expiration		
	Maximum pass	word age: 90 🗘 d	ays	
	Remind before	expiration: 15 📫 a	lays	
			OK	1

NOTE The allowable range for each entry is as follows:

- Password History: 12-24 times
- Password Length: 8-14 characters
- Minimum Age for Password: 0-998 days
- Password Expiration: 1-999 days
- Reminder for Expiration: 1-90 days

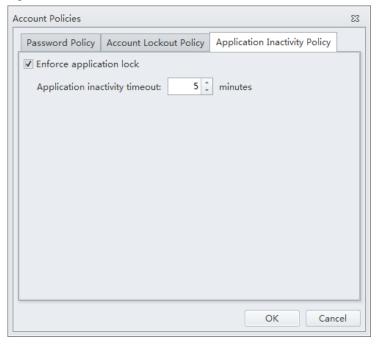
count Policies		-	ε
Password Policy	Account Lockout Policy	Application Inactivity Policy	
Enforce account	t lockout threshold		
Invalid login at	tempts: 3 🗘 times	5	
Enforce acco	ount lockout duration		
Lockout dur	ation: 30 🗘 minute	25	
			_
		OK Cancel	I

Figure 2.9 Account Policies - Account Lockout Policy

NOTE The allowable range for each entry is as follows:

- Invalid Login Attempts: 3-10 times
- Lockout Duration: 15-1440 minutes

Figure 2.10 Account Policies - Application Inactivity Policies



NOTE The allowable range for each entry is as follows:

• Inactivity Duration: 1-60 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.

er Ma	nagement Operation Log					9
Quen	y Criteria					
Jser:	(ALL)	⊘ -				
ime l	Range: 2016-10-09 00:00:00	- 2016-11-0	9 23:59:59 👻			
Que	201				Print & Exp	
ŧ	Operation	Username	User Full Name	Timestamp	Record	
	I 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	5 Login	Admin	Administrator	2016-11-04 16:41:13	Login successfully.	
	5 Logout	Admin	Administrator	2016-11-04 16:43:42	Logout.	
7	7 Login	Admin	Administrator	2016-11-04 16:44:36	Login successfully.	
8	3 Login	Admin	Administrator	2016-11-04 16:48:40	Login successfully.	
9	9 Logout	Admin	Administrator	2016-11-04 17:07:29	Logout.	
10) Login	Admin	Administrator	2016-11-07 09:35:10	Login successfully.	
11	l Logout	Admin	Administrator	2016-11-08 17:08:07	Logout.	
12	2 Login			2016-11-08 17:10:11	Login failed (Username: Admin).	
13	3 Login	Admin	Administrator	2016-11-08 17:10:14	Login successfully.	
14	4 Logout	Admin	Administrator	2016-11-08 18:01:52	Logout.	
15	5 Login			2016-11-09 07:28:53	Login failed (Username: Admin).	
16	5 Login	Admin	Administrator	2016-11-09 07:29:05	Login successfully.	
17	7 Create user	Admin	Administrator	2016-11-09 07:37:02	Create new user: [Username=Sarah, Full Name=Sarah L., Role=Operator, Enabled=Yes].	
18	8 Modify user information	Admin	Administrator	2016-11-09 07:39:32	Modify user usability (Username: Sarah) from [Yes] to [No].	
19	9 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).	
2	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...** .

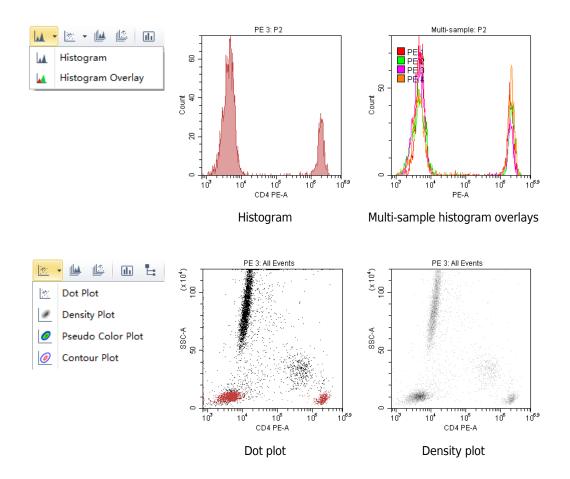
NOTE User logs are exported as a .pdf or .csv file.

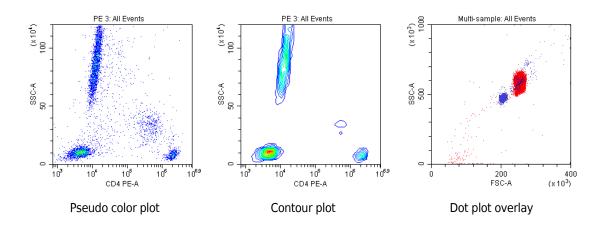
Graphic and Gating Styles

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.



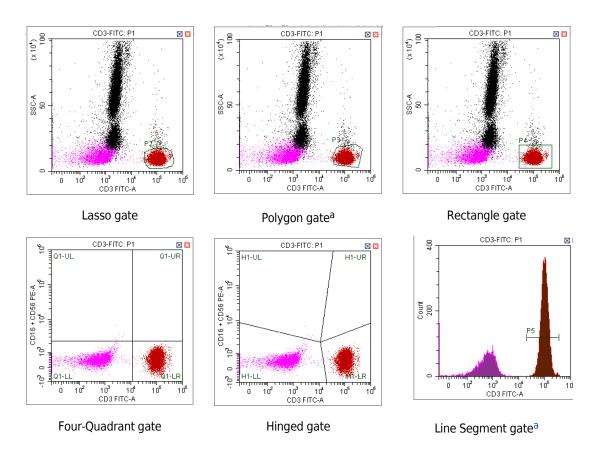


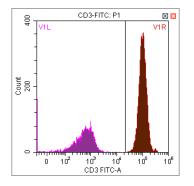
Gates

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 6, 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 12, Replacement/Adjustment Procedures.

Select Advanced > Plate Type Library to access the Plate Type Library. Refer to Figure 2.11.

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

Figure 2.11 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	Plate Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard	-	0
1	96-well V-bottom	Standard	-	\bigcirc
1	96-well U-bottom	Standard	-	0
\checkmark	96-well deep well	Deep Well	-	0

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

Add Plate Type				23
Name:				
Plate Mode:	Standard		Ŧ	
Mix:	5	sec		
Remarks:				
				A
				v
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	
Plate Mode:	Standard	-
V Mix:	5 sec	
Remarks:		
Last Calibration Time:		
	-	
Calibrate		OK Cancel

5 Select the Plate Mode.

Add Plate Type		23
Name:	Plate type 1	
Plate Mode:	Standard	•
Mix:	Standard Deep Well	
Remarks:		-
		*
		Y
	22.01	
Last Calibration T	inte	

6 Enter the Mix time.

Add Plate Type		23
Name:	Plate type 1	
Plate Mode:	Standard	Ŧ
Mix:	5 sec	
Remarks:		
		*
		
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 12, 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	Plate 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
\checkmark	Plate type 1	Standard	2018-05-07 15:50:25	0

10 Select ок.

Editing a Plate Type

Active	Name	Plate Mode	Last Calibration Time	Default
1	96-well flat-bottom	Standard	-	0
1	96-well V-bottom	Standard	-	0
\checkmark	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 be edited, and select **Edit**. The Edit Plate Type window appears.

Edit Plate Type		Σ
Name:	96-well V-bottom	
Plate Mode:	Standard 👻	
Mix:	5 sec	
Remarks:		
		-
		A
		A
		~
Last Calibration Ti	me: -	

- **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 12, Replacement/Adjustment Procedures
 The Calibration can be performed at any time.

6 Select OK.

Duplicating a Plate Type

96-well flat-bottom Standard - Image: Constraint of the standard of th	ctive	Name	Plate Mode	Last Calibration Time	Default
Image: Second standard - O Image: Second standard - O Image: Second standard - O	1	96-well flat-bottom	Standard	-	0
	1			-	0
✓ 96-well deep well	1	96-well U-bottom	Standard	-	0
	\checkmark	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	om 96-well V-bottom	23
Name:	96-well V-bottom-Copy	
Plate Mode:	Standard -	
I Mix:	5 sec	
Remarks:		
		^
Last Calibration Time:		
Last Calibration Time:	-	
Calibrate		DK Cancel

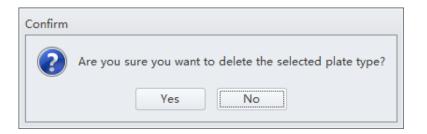
- **3** Enter the Mix setting.
- **4 Optional:** Enter any remarks.
- 5 Optional: Select Calibrate to calibrate the plate position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 12, Replacement/Adjustment Procedures The Calibration can be performed at any time. The duplication retains the calibration information if the original plate was already calibrated.
- 6 Select OK.

Deleting a Plate Type

Active	Name	Plate 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	96-well V-bottom-Copy	Standard	-	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		23
Experiment	Default Path	
Tube	C:\Users\KMCABRERA\Documents\CytExpert Data	
Plot		Default
Gate		
Page Setup		
Plate Loader		
	OK	Cancel

Options		23
Experiment Tube Plot Gate Page Setup Plate Loader	Select Columns Select the columns that will appear on the tube list. Sample ID Time Events	
	Preview Sample ID Time	
	OK Cance	*

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: 🔘 Border 🛛 Fill 💿 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 10	000000				
	Time: Min 0 Max 3	120000				
	Width: Min 0 Max	4096				
	ОК	Cancel				

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

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

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	23				
Experiment	Paper				
Tube	Size: A4: 210mm X 297mm -				
Plot	Orientation Margins(millimeters)				
Gate	Portrait Left: 5.08 Right: 5.08				
Page Setup	◯ 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	$\textcircled{\tiny{\textcircled{\tiny{\bullet}}}} \begin{array}{[]{c}c} \blacksquare \end{array} \bigcirc \overrightarrow{=} \end{array} \bigcirc \overrightarrow{=} \\ \bigcirc \end{array} \bigcirc \overrightarrow{=} \\ \bigcirc \\ \bigcirc \\ \bigcirc \\ \rule{[]{c}c} \blacksquare \end{array} \bigcirc \\ \bigcirc \\ \rule{[]{c}c} \blacksquare \\ \bigcirc \\ \rule{[]{c}c} \blacksquare \\ \bigcirc \\ \rule{[]{c}c} \blacksquare \rule{[]{c}c} \blacksquare \\ \rule{[]{c}c} \blacksquare [$	
Page Setup Plate Loader	☑ Backflush 6 sec	
	OK Cance	el

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		23	
English		-	
This setting will be effective after restarting the software.			
	ОК	Cancel	

Setting Up CytExpert Application Programming Interface (API) Test Client

The CytExpert API is available for external software to control CytoFLEX Platform. 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.

Operation Principles

Overview

This chapter explains how the Cytometer measures scattered light and fluorescence as cells pass through the laser beam.

The illustrations in this chapter are not exact representations of the inside of the Cytometer. They are for explanatory purposes only.

This chapter contains information on:

- Sample Flow
- Laser Beam Shaping
- Cell Illumination
- Light Collection, Separation and Measurement
- Signal Processing
- Data Storage
- Automated Software Features
- Parameters
- Plot Display
- Statistics

Sample Flow

CAUTION

Possible flow cell damage. To avoid clogging the sample probe, sample tubing or flow cell, ensure that 12×75 mm test tubes are free of debris before you use them.

Sample Loading

The system supports two ways to load sample, by sampler module (for single tube) or by plate loader (for 96-well plates).

The sampler module includes three stepper motors. A 12 VDC stepper motor (sample tube loader motor) at the bottom of the module moves (swings) the sample tube holder in/out. A 12 VDC stepper motor (sample tube lifter motor) at the top of the module moves the sample tube holder up/down, using a toothed belt. An additional 12 VDC stepper motor at the top of the module

(sample peristaltic pump motor) moves the sample peristaltic pump to achieve liquid flow. Refer to Figure 1.12. The sampler module handles a sample tube as follows:

- It swings the tube holder out for loading.
- It swings the tube holder in and lifts the tube holder.
- The sample probe rates to mix the sample.
- The sample peristaltic pump starts to aspirate the sample. Sample flow begins.

The plate loader module is installed in the inner compartment of the Cytometer, as shown in Figure 1.13 and Figure 1.14. The plate module includes three movement modules.

- The Y-Axis movement module ejects or retracts the plate holder stage.
- The X-Axis movement module moves the probe from left to right across the plate to position the plate loader sample probe over all of the possible columns in the well plate.
- The vertical movement module supports and moves the plate loader wash station assembly up and down as needed to mix a sample in a well, aspirate a sample from a well, and clean the plate sample probe.

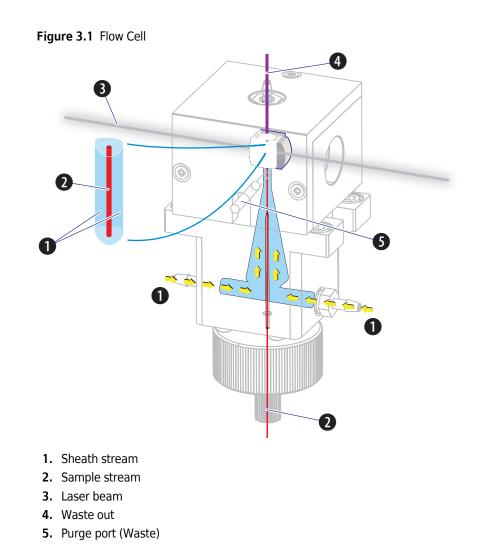
The sample probe is cleaned automatically when sample flow ends.

Hydrodynamic Focusing

The instrument uses a process called hydrodynamic focusing to ensure that the cells move through the laser beam one at a time, along the same path through the flow cell.

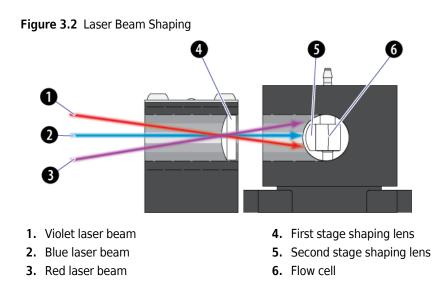
The flow cell (Figure 3.1) contains a rectangular channel. A pressurized stream of sheath fluid enters the channel at the lower end and flows upward. The sensing area of the flow cell is at the center of the channel.

While the sheath stream is flowing through the channel, a stream of sample is injected into the middle of the sheath stream. As shown in Figure 3.1, the sheath stream surrounds, but does not mix with, the sample stream. The pressure of the sheath stream focuses the sample stream so that the cells flow through the laser beam single file. If the cells were to move through the laser beam in different ways during sample flow, sample analysis could be distorted.



Laser Beam Shaping

Before the laser beam reaches the sample stream, lenses focus the beam (see Figure 3.2). Focusing keeps the beam perpendicular to the sample stream flow while making the beam small enough to illuminate only one cell at a time.



Cell Illumination

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

Forward Scatter

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

Side Scatter and Fluorescent Light

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

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

Light Collection, Separation and Measurement

Forward Scatter Collection

The FALS (Forward Angle Light Scatter) detector collects scattered light from a particle that intersects with a laser and delivers information roughly proportional to the size of the particle. The

forward angle light is filtered with a 488 nm band pass before it reaches the FS sensor which generates voltage pulse signals. These signals are proportional to the amount of light the sensor receives.

Side Scatter and Fluorescent Light Collection

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

Side Scatter

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

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

Fluorescent Light

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

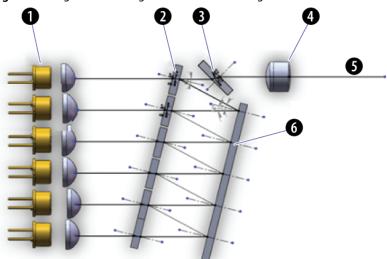


Figure 3.3 Light Path through the WDM with a Single Port

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

Signal Processing

The CytoFLEX is a fully digital system with an active range of 7 logarithmic decades, signal collection speed of 30,000 events/second (includes 15 parameters).

Data Storage

The instrument Workstation is vulnerable to malware, viruses, data corruption, and unauthorized instrument setting changes, or privacy breaches if unauthorized access is gained by malicious personnel. To reduce the risk of such events, only allow authorized laboratory personnel access to the instrument Workstation. Contact your institutional security department for assistance.

The instrument Workstation is vulnerable to malware and viruses from physical media such as CDs, DVDs, or USB drives. To reduce the risk of data corruption or unauthorized setting changes, only use physical media such as CDs, DVDs, or USB drives that are known to be free from viruses or malware. Contact your IT professional for assistance.

The instrument Workstation is vulnerable to malware, viruses, and unauthorized access if connected to a network without using the PROService RMS hardware available from Beckman Coulter. To reduce the risk of data corruption, unauthorized instrument setting changes, or privacy breaches, only connect the instrument Workstation to a network via the PROService RMS hardware. Contact us to obtain PROService.

Sample results can be printed out, saved to removable media, saved to a local hard drive or saved to a network drive. You can store sample results in Flow Cytometery Standard (FCS 3.0) files.

Automated Software Features

CytExpert for CytoFLEX software contains the following automated software features.

- Auto Delay. The system reads and automatically adjusts laser delay during QC. You can also read the laser delay value while acquiring samples.
- **Auto Threshold**. Easily find target populations. No need to worry about the threshold setting while adjusting gains when auto threshold is enabled.

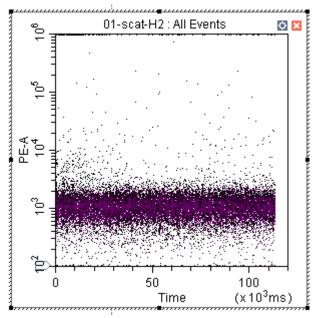
• Auto Gating. There are two types of autogates available in the CytExpert for CytoFLEX software: auto line segment and auto polygon.

Parameters

TIME Parameter

The TIME parameter is the amount of time, in seconds, the instrument acquires data. It is displayed on the plot. The axis labels vary, depending on plot resolution and stop time (duration) if **Fit with sample** is selected.





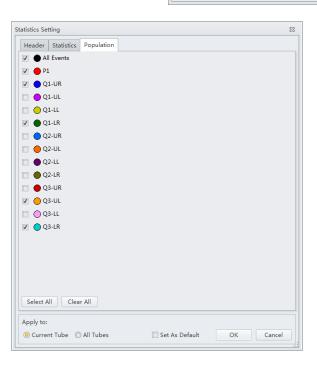
Plot Display

The results of sample analysis appear on the Workstation screen as graphs called plots. Refer to Graphic and Gating Styles in CHAPTER 2, Using the CytExpert Software.

Statistics

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

Statistics Setting 🛛	Statistics Setting
Header Statistics Population	Header Statistics Population
Experiment Name	□ Parent Population
✓ Tube Name	Events/µL(B)
▼ Sample ID	Beads Population: Select
	Beads Count: 0 Sample Volume: 0.00 µL
Record Time	ample volume. 0.00 pc
Volume	Parameter
Abort(%)	Mean GeoMean Median rCV rSD CV
Expression Edit	FSC-A 🔽 🔽 🗌
	SSC-A
Name:	FITC-A
Equation:	PE-A
Comment	ECD-A
	PC7-A
	PC-A C C C APC-A C C C C
	APC-A750
	PB450-A
© Operator	Select All Clear All @ Area
	Preview
Select All Clear All	Events % Total % Parent GeoMean
Apply to:	Apply to:
Current Tube All Tubes Set As Default OK Cancel	Ocurrent Tube All Tubes Set As Default OK Cancel



CHAPTER 4 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 \rightarrow 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 platform flow cytometer, perform the following system checks.

Check Waste and Reagent Levels [4 L Fluid Containers]

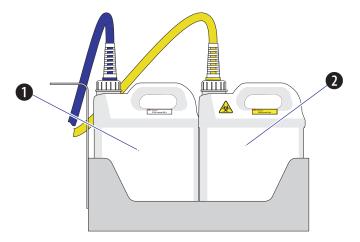




Risk of instrument damage. Do not use a saline-based sheath fluid on the CytoFLEX instrument. Saline-based sheath fluid could damage instrument components. Beckman Coulter recommends using CytoFLEX Sheath Fluid or a similar nonionic sheath fluid to ensure system performance.

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

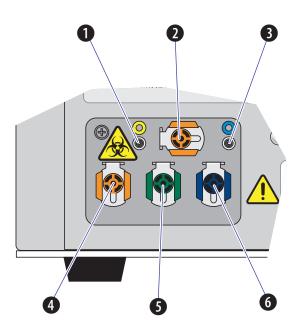
Risk of instrument damage. Remove the sheath fluid container from the Fluid Container holder before filling the sheath fluid container to avoid damage to instrument electronics.

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 in CHAPTER 12, Replacement/Adjustment Procedures.

🕂 WARNING

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

- **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 in CHAPTER 12, Replacement/Adjustment 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.

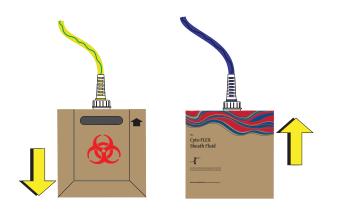


Check Waste and Reagent Levels [10 L Fluid Cubitainers]

<u>A</u> CAUTION

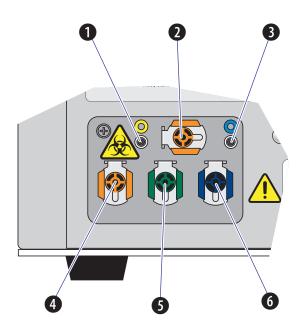
Risk of instrument damage. Do not use a saline-based sheath fluid on the CytoFLEX Platform instrument. Saline-based sheath fluid could damage instrument components. Beckman Coulter recommends using CytoFLEX Sheath Fluid or a similar nonionic sheath fluid to ensure system performance.

- 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 12, Replacement/Adjustment Procedures.
- **4** If necessary, empty all waste liquid from the waste container. Refer to Emptying the 10 L Waste Cubitainer in CHAPTER 12, Replacement/Adjustment 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 1.22.

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.

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	Adm	inistra	ator
# III 🖶 🔹			

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

[CytExpert Electronic Record Management Software Option Shown]

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



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



NOTE

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

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



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

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	

B49006AS

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**.

			ē	23
		Adm	inistra	ator
\cap	Adminis	trato	r	
25	Username:	Admir	ו	
	Lock	Lc	g out	:

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

Locking the Account

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 Lock.

			ē	23
		Adm	inistra	ator
\cap	Adminis		-	
25	Username:	Admir	ו	
	Lock	Lc	g out	

The account locks automatically if it remains inactive for a specified duration. Refer to Account Policies - Application Inactivity Policies in CHAPTER 2, Using 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/Standa	ardiza	tion	Advanc	ed	Hel	р
×	Acq. Setting								2
	Detect	tor Config	uration		R	estart			
(å_{0})	Backflu	ush			T E	Boost			
Û	Boost								
ø	Initialia	ze							
Ċ	Stand	by							
	Prime.								
	Deep	Clean							
	Calibra	ate Sample	e Flow Rate.			Events			
	System	n Startup I	^o rogram			Events			
	Daily C	lean			,	Ŧ			
	Sampl	e Injection	Mode	•		Manual			
	Sampl	er Reset			\checkmark	Semi Au	itor	matic	;
	Turn C	n				Plate Lo	ade	er	
	Turn C	Off			ast				
	Acq. S	etting Cat	alog		1	1 1 1			
	Cytom	neter Confi	guration		-				
	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.



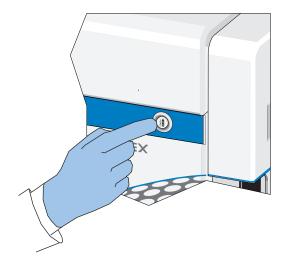
2 Select Initialize. The sample tube holder swings out from the standby position to the sample loading position (see Figure 1.12) so that you can load the sample tube.

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 1.12), 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 1.12) and the Cytometer backflushes the sample probe.

🕂 WARNING

Risk of biohazardous contamination. When using 1.5-mL and/or 2-mL sample tubes, always cut the cap off and do not exceed 300- 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 1.12).

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 1.12).
- **6** Manually raise the sample tube holder gently to the sample acquisition position (see Figure 1.12) and hold the tube in that position.
- 7 Select **Boost** to transfer the sample to the flow cell.
- 8 Select Run.

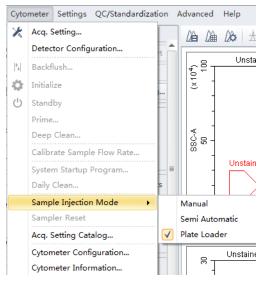
The sample runs at the designated flow rate and the Cytometer begins to acquire data.

- **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 1.12).
- **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	8	
	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 Platform instrument, refer to APPENDIX C, Sample Injection Mode Control Kit 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 12, Replacement/Adjustment Procedures.

5 Turn the Cytometer's main power switch on.

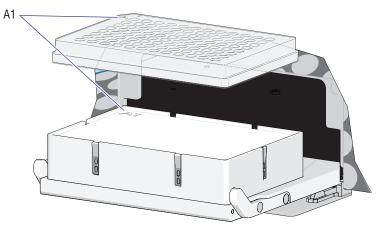
The sampler status icon located in the bottom right side of the screen changes to display *Plate Loader*.

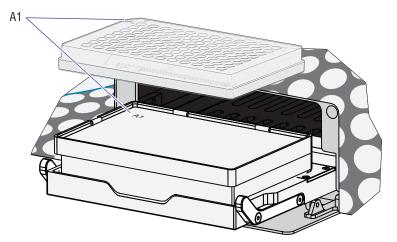


NOTE If you have a CytoFLEX LX instrument, you can turn the Cytometer's power on by selecting **Cytometer > Turn On**.

- 6 Select Initialize.
- 7 Select Eject.
- **8** Place the plate flat on the plate holder and ensure that it is secure.

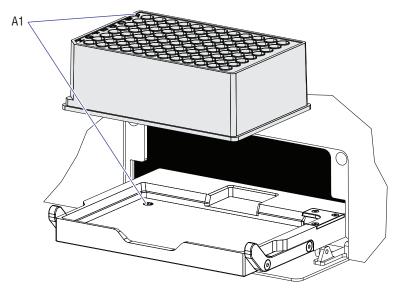
[Standard 96-Well Plate in the Plate Holder (Without Groove)]





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

[96-Well Deep Well Plate Only]



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

- **9** Select **Load** to load the plate.
- **10** Select **Run**. The plate loader automatically loads the plate holder stage and begins to acquire data.
- **11** When you are satisfied with the data, select **Record** to record the data.

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

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

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

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

The system startup program takes approximately 10 minutes if the fluidic self-check is enabled. The system startup program takes approximately 8 minutes if the fluidic self-check is not enabled.

NOTE The fluidic self-check is an optional feature only available when the sheath damper upgrade is implemented.

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 {^{0}a} \right $	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				

3 If the confirm window appears, select **Yes**.

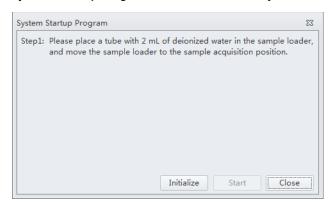
Confirm	
It's been over 24 h	ours since the last execution of "System Startup Program", execute "System Startup Program"?
	Yes No

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

System Startup Program Window in Semi-Automatic Injection Mode

System S	tartup Progran	ı			23
Step1:	Please place a	tube of 2 mL	deionized wate	er in the samp	ole loader.
			Initialize	Start	Close

System Startup Program Window in Manual Injection Mode



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

The instrument begins to prime or run the fluidic self-check. This process takes about 4 minutes if the fluidic self-check is enabled. This process takes about 1 minute if the fluidic self-check is not enabled.

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

System Startup Program	
Step2: Fluidic self-check in process	
Remainir	ng Time: 9 min 44 sec
	Initialize Stop Close

After priming or running the fluidic self-check, 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	
1	Remaining Time: 3 min 26 sec
	Initialize Stop Close

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

System Startup Program	X3
Step5: Completed.	
	Close
	(

Running the System Startup Program [With Plate Loader]

The system startup program takes approximately 10 minutes if the fluidic self-check is enabled. The system startup program takes approximately 8 minutes if the fluidic self-check is not enabled.

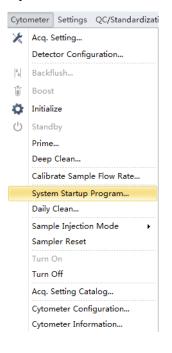
- **NOTE** The fluidic self-check is an optional feature only available when the sheath damper upgrade is implemented.
- 1 Select Initialize.

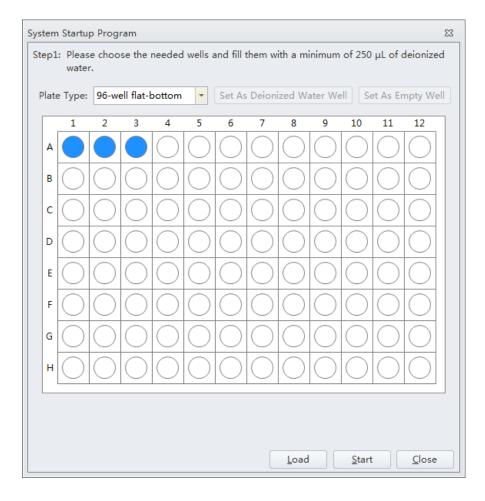
2 If the confirm window appears, select Yes.

Confirm	
?	It's been over 24 hours since the last execution of "System Startup Program", execute "System Startup Program"?
	Yes No

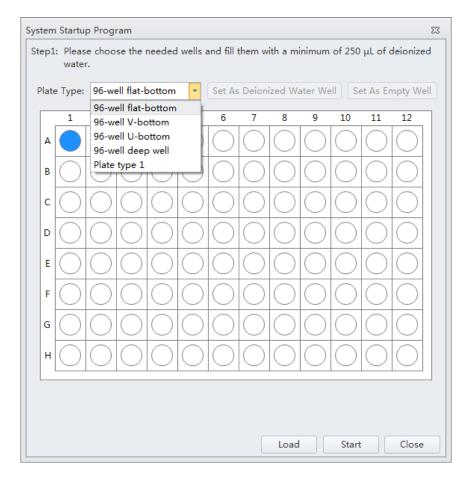
3 Select **System Startup Program** from the Cytometer menu to open the System Startup Program window.

[CytoFLEX LX Shown]



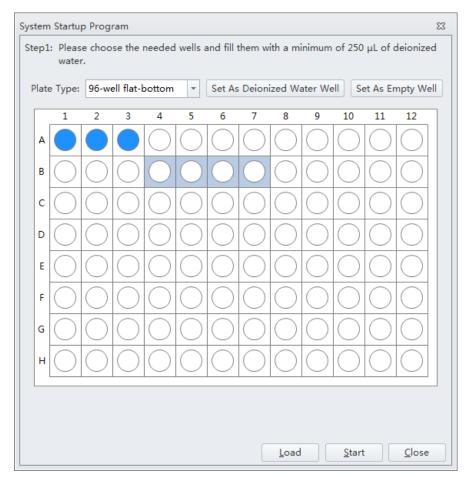


The plate loader automatically ejects the plate holder stage and the System Startup Program window appears.



4 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.



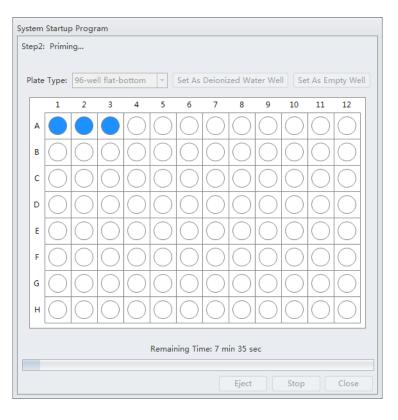
5 Follow the on screen software prompts and select the desired wells and select **Set As Deionized Water Well**.

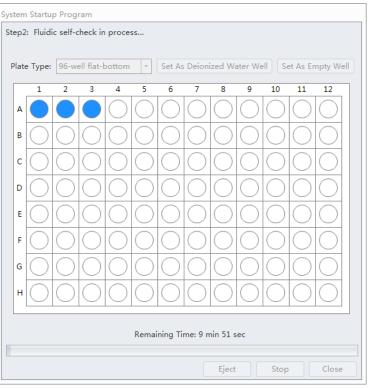
NOTE To deselect water wells, select the desired well and select Set As Empty Well.

NOTE Prepare two to six sample wells with deionized water.

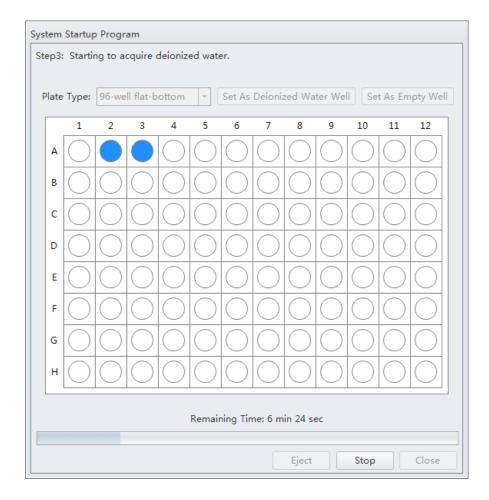
- **6** Select **Load** to load the plate.
- **7** Select **Start** to start the program. The message *Please confirm that the correct plate is placed properly and press OK* appears. Select **OK**.

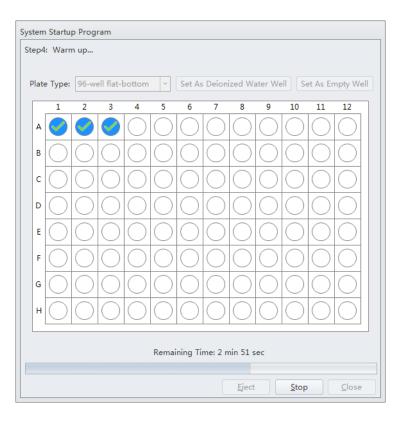
Wait for the system to initialize. The instrument begins prime or run the fluidic self-check. This process takes about 1 minute if the fluidic self-check is not enabled. This process takes about 4 minutes if the fluidic self-check is enabled.



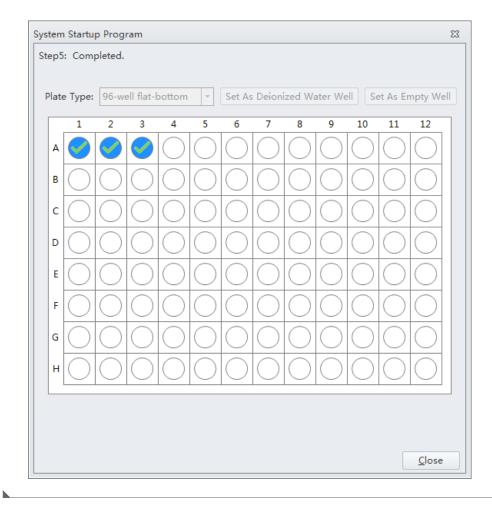


After priming or running the fluidic self-check, the system initializes again. The sample is loaded automatically. This process takes about 1 minute per well.





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



8 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.

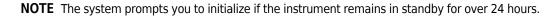
Selecting Experiments from the Start Page

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
じ Standby	% Backflush	Boost



- **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 1.12).
- **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 6, 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	Q	Restart
Ċ	Standby	Backflush	Û	Boost
•1	Next Tube	🗶 Acq. Set	ting	1

CHAPTER 5 Instrument Quality Control and Standardization

Overview

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

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

- **NOTE** Beckman Coulter recommends performing QC on a daily basis.
- **NOTE** QC target values apply exclusively to standard channels. A channel is a laser-filter combination. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, Data Acquisition and Sample Analysis to verify that the default factory detector configuration is selected before running QC.
- NOTE CytExpert QC includes an automated 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.
- **NOTE** Perform QC on the same day prior to performing the CytExpert standardization.

QC Workflow:

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

Standardization Workflow:

Obtain target acquisition - 2 settings	Run standardization sample	→	Perform standardization	→	Apply standardization settings
---	----------------------------------	---	----------------------------	---	--------------------------------------

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
- Preparing the Standardization Sample
- Generating Target Median Values
- Adding a New Standardization Item
- Performing the Standardization
- Applying the Standardized Acquisition Settings
- Standardization Target Library

Quality Control

The QC process verifies important system functions. The system:

- 1. Verifies that the unit hardware configuration matches the default configuration specified in the software. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, Data Acquisition and Sample Analysis.
- **2.** Measures the laser power of each individual laser and ensures that each laser meets the system specifications.
- **3.** Loads the QC sample and begins to acquire data.
- **4.** Verifies that the actual laser delays match those set in the software and will adjust the delay accordingly.
- **5.** Notifies you if laser delay is >2 µs from the previous setting. The software automatically changes the laser delay setting.

OR

Notifies you if laser delay is >5 µs from the previous setting. Manual laser delay adjustments are required. Refer to Setting Laser Delay in CHAPTER 12, 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.

Preparing the QC Sample

Required Materials

The following materials are required to complete the QC process:

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

CytoFLEX Daily QC Fluorospheres Preparation Process

IMPORTANT For preparation instructions for the CytoFLEX Ready to Use Daily QC Fluorospheres or the CytoFLEX Daily IR QC Fluorospheres, refer to CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process or CytoFLEX Daily IR QC Fluorospheres Preparation Process.

- **1** Take one sample tube and label it as the QC sample tube.
- **2** Add approximately 1 mL of deionized water to the sample tube.
- **3** Use the vortexer or shake vigorously to thoroughly mix the bottle of CytoFLEX Daily QC Fluorospheres.
- **4** Add 3 drops of CytoFLEX Daily QC Fluorospheres to the sample tube.
- **5** Vortex the sample tube to uniformly suspend the fluorospheres.
- **6** Place the sample tube in a dark location at 2-8 °C until ready to load the tube into the instrument for QC.
 - **NOTE** Tubes containing diluted CytoFLEX Daily QC Fluorospheres should be sealed and stored in a dark location at 2-8 °C for up to 5 days.

CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process



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

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

CytoFLEX Daily IR QC Fluorospheres Preparation Process

- **IMPORTANT** For preparation instructions for the CytoFLEX Daily QC Fluorospheres or the CytoFLEX Ready to Use Daily QC Fluorospheres, refer to CytoFLEX Daily QC Fluorospheres Preparation Process or CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process.
- **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 10 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 stored 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 or CytoFLEX Ready to Use 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

- **IMPORTANT** For preparation instructions for the CytoFLEX Ready to Use Daily QC Fluorospheres or the CytoFLEX Daily IR QC Fluorospheres, refer to CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process or CytoFLEX Daily IR QC Fluorospheres Preparation Process.
- **1** Take one 96-well plate and record the QC sample well position.
- **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 200 μ 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.

CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process



- **1** Take one 96-well plate and record the QC sample well position.
- **2** Use the vortexer to thoroughly mix the bottle of CytoFLEX Ready to Use Daily QC Fluorospheres for two or three seconds.
- **3** Add 3-4 drops of CytoFLEX Ready to Use Daily QC Fluorospheres to 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 Ready to Use Daily QC Fluorospheres should be stored sealed in a dark location at 2-8 °C for up to 5 days.

CytoFLEX Daily IR QC Fluorospheres Preparation Process

- **IMPORTANT** For preparation instructions for the CytoFLEX Daily QC Fluorospheres or the CytoFLEX Ready to Use Daily QC Fluorospheres, refer to Preparation Process CytoFLEX Daily QC Fluorospheres or CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process.
- **1** Take one 96-well plate and label one well as the QC/IR sample well.
- **2** Mix the CytoFLEX Daily IR QC Fluorospheres by inversion.

IMPORTANT Do not overfill the sample well.

- **3** Add 3-4 drops of CytoFLEX Daily IR QC Fluorospheres to the sample well.
- **4** Place the well plate in a dark location at 2-8 °C until ready to load the well plate into the instrument for QC.

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

Importing Lot-Specific Target Values

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

Lot No.	Expires	Lot No.:	5347					
5347	2015-06-26	Expires: 2 Threshold		-26				
		Channel		Mode	Value			
		FSC(Heig	FSC(Height) Manual 100000					
		Signal	-11					
		Laser	Filte		Gain	Median	Median Tolerance(
			FSC		142			
		Blue	488		59			
		Blue		/40	159			
		Blue		/42	119			
		Blue	690	/50	381	208000	0.0 5.00	
		Blue	780	/60	633			
		Red	660	/20	436	55200	0.0 5.00	5.0
		Red	712	/25	388	227000	5.00	5.(
		Red	780	/60	440	82089	2.0 5.00	5.(
		•						•

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.

Beckman Coulter Inc Beckman Coulter, Inc Wi		y Beckman Coulter , Inc. (v	2011.02)				
🔊 🔄 🗢 https://www.beckmancoulter.com/warportal/o	bage/softwareDownloadSearch				~	🔒 🗟 🐓 🗙 🔯 Live Search	
Edit View Favorites Tools Help							
Favorites 🛛 🚖 🤩 Odyssey 🔊 Suggested Sites 🔹 🔊 V	Web Silce Gallery 🔹 🎑 EBS Home 🊳 EBS Pi	rod					
Beckman Coulter Inc - Beckman Coulter, Inc.						🏠 • 🖾 - 🖃 🖶 • Page •	Safety • Tools •
				🚟 Login Con	tact Us Careers 💠 Cart (0)	te (0)	
	BECKMAN						
	COMPANY SUPPORT	Resear	ch & Discovery Di	agnostics Industrial		ο,	
	Technical Documents	& Software Dowr	iloads				
	On this page you can obtain product	t specific documentation incl	uding:	Introducing "I	My Technical Documents"		
	Instructions for Use (IFU's) Customer Notifications Compliance Documentation	Certificates of Ana Safety Data Sheet Software Downloa	s (SDS/MSDS)	Innovative electronic te Centralized location co	chnical document notifications tool. mplete with your customized preferences.		
	To obtain bibliography or literature (I and more), click here.			To learn more click on:			
				K My Technical Document			
				To access My Technical Do	cuments, please login or register.		
				10			
	Technical Documents	Ty Technical Documents	Safety Data Sheets (S	DS/MSDS) Software Downlo	ad *Indicates requir	edfields	
	Search By Product						
	Market Segment* F	Product Line*	Product Series	Product	Software Name		
	Please select one	Please select one	All Product Series	All Products	All Software		
	Lot Number L	anguage					
		English 💽	e				
	Search						
	Search By Software	Name					
	Search By Item/REF I	lumber					
	Search By Lot Number	er					
www.beckmancoulter.com/wsrportal/page/shoppingCart						Unknown Zone (Mixed)	

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** If necessary, register and log in to the Beckman Coulter website.
- **5** In the Search By Product section of the screen, select the following:
 - a. Select Research & Discovery from the Market Segment drop-down menu.
 - **b.** Select **Flow Cytometry** from the Product Line drop-down menu.
 - c. Select Instruments from the Product Platform drop-down menu.
 - **d.** Select **CytoFLEX** from the Product drop-down menu.
 - e. Select CytoFLEX QC Fluorospheres Target or CytoFLEX Ready to Use 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 Do	wnload	*Indicates required field
Search By Prod	uct				
Market Segment*	Product Line*	Product Series	Product	Softw	vare Name
Research & Discovery	Flow Cytometry	✓ Instruments	✓ CytoFLEX	Y Cyto	FLEX QC Fluorospheres T 💙
Lot Number	Language				
All	Y English	~			
Search					
Search By Soft	ware Name				
• Search By Item	REF Number				
Search By Lot N	lumber				

[CytoFLEX IR QC Fluorospheres Target]

Technical Documents	My Technical Documents	Safety Data Sheets (SDS/MSDS)	Software Download	*Indicates required field
Search By Prod	duct			
Market Segment*	Product Line*	Product Series	Product	Software Name
Research & Discovery	Flow Cytometry	Instruments	CytoFLEX	CytoFLEX Daily IR QC Fluoro
Lot Number	Language			
All	English	~		
Search				
Search By Soft	ware Name			
Search By Item	/REF Number			
Search By Lot	Number			

[CytoFLEX Ready to Use Daily QC Fluorospheres Target]

Technical Documents	My Technical Documents	Safety Data Sheets (S	SDS/MSDS) Software De	ownload	*Indicates required field:
Search By Prod	uct				
Market Segment*	Product Line*	Product Series	Product	Softwa	re Name
Research & Discovery	Y Flow Cytometry	Ministruments	CytoFLEX	M CMORLEX	Ready to Use Daily QC Fluore 😒
Lot Number	Language				
Al	English	~			
Search					
🕒 Search By Softv	vare Name				
Search By Item/	REF Number				
Search By Lot N	umber				

6 Select Search.

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

[CytoFLEX QC Fluorospheres Target]

Technical Documents	Ô	My Technical Documents	ù.	Safety Data Sheets (SDS/MS	DS) Software Down	load		*Indicates required field
Search By Proc	luct					11		
/larket Segment*		Product Line*		Product Series	Product	1	Software Name	
Research & Discovery	*	Flow Cytometry	Y	Instruments	CytoFLEX	*	CytoFLEX QC Flu	orospheres T 💌
_ot Number		Language						
All	~	English	~					
Search								
Search By Lot I								
								Vient
ftware Download Search rst Prev 1 Next Las	nt							View 25 per page
	st	Product	Lot N	o. <u>Version</u>	Item/REF NO.	1	Release Date	Language

[CytoFLEX IR QC Fluorospheres Target]

Market Segment*	Product Line*		Product Series		Product		Software Name	
Research & Discovery	Flow Cytometry	~	Instruments	~	CytoFLEX	~	CytoFLEX Daily IR	QC Fluoro
ot Number	Language							
All	English	~						
Search								
Search By Software	e Name							
Search By Item/RE	F Number							
Search By Item/RE	F Number ber							View 25 per page
Search By Item/RE Search By Lot Num	F Number ber	Lot N	<u>e. y</u>	Version	<u>Item/REF NO.</u>	B	telease Date	View 25 per page Language

[CytoFLEX Ready to Use Daily QC Fluorospheres Target]

Market Segment*	Product Line*		Product Series	Pro	duct		Software Name	
Research & Discovery	Flow Cytometry	~	Instruments	V Cyt	toFLEX	~	CytoFLEX Ready to U	e Daily QC Ruoro 🗸
Lot Number	Language							
All	 English 	~						
Search Search By Softwa	re Name							
-								
	mber							View 25 per page
Search By Lot Nu Tware Download Search Res	mber	Lot N	o. Version		Item REF NO.	5	Release Date	View 25 per page Lanceage

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

		💴 Login I Contact U	s I Careers	🃜 Cart (0)	Duote (0)
COMPANY SUPPORT	RESEARCH & DISCOVERY DIAGNOSTICS INDUSTRIAL	æ	Enter product	t, Item number c	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.tet Lot # BAH03 Download				
	Back				

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

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

	KMAN TER	Login I Contact Us I Careers) 🚝 Cart (0) 📑 Guote (0)
COMPANY SUPPO	RT RESEARCH & DISCOVERY DIAGNOSTICS INDUSTRIAL	Enter prod	uct, 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 • Downhoad the file. • Open the file and enter the values from the file as instructed in your user manuals.		
	Download Files		
	BAH03.tat Lot # BAH03 Download		
	Back		
Copyright/Tradei	Do you want to open or save BAH03.tgt (13.2 KB) from beckmancoulter.com ? Ope	n Save 🔻 Cance	II rights reserved.

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

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

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

Collecting QC Data

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

QC/Stan	dardization Setting	23
FCS F	Files	
🗹 Au	tomatically save acquired data to FCS file.	
Path:	C:\Users\Public\Documents\CytExpert QC Data	
PDF I	Files	
🗹 Au	tomatically export QC result to PDF file.	
Path:	C:\Users\Public\Documents\CytExpert QC Data	
	OK]	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	Candby	✓ [2015-01-19 10:12:06] Cytometer standby.
Connected	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 6, 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 6, Data Acquisition and Sample Analysis.

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

pert(Cyto-LX) tometer Settings Advanced Help											
ic .	a										
	P D D D .										
Task: 🧿 QC 🛛 Standardization											
				QC	Report						
🏠 Initialize 🕅 Backflush 👔 Boost											
() Standby	Bead Lot No.:	BAH	02_SE								
	Bead Expires:		-07-20				QC Date		2016-11-0	2 15:07	
Events/Sec: 0.0	Cytometer Nam						Cytomete	er SN:			
Abort(%): 0.00	Detector Config										
Events: 0	Loader Type:	Sem	i Automatic								
Time: 00:00:00	Threshold										
	Channel: FSC(Hei	ght) Mode:	Manual Val	ue: 50000							
Lot No.: *	Laser										
Current Detector Configuration:	Laser	Delay(µs)	Default De	lav(us) Diffe	rence Delay(µs)	Power(mW)	Tarr	get Powe	r(mW) F	lesult	
	NUV	72.8		72.96	-0.1		60		50-70	0	
Cyto-LX	Blue	0.0		0.00	0.0		49		40-60	õ	
	Red	36.1		36.32	-0.1	5	49		40-60	0	
eport	Yellow	-35.6	8	-35.84	0.1	6	30		20-40	0	
Detector Configuration:	Violet	-70.2	4	-70.40	0.1	5	88		70-120	0	
Cyto-LX *	Signal Value										
Date: 2016-10-03 × ~ 2016-11-02 ×	Parameter Ga	Target	%Difference		Target	%Difference		Target		0 1	
	Parameter Ga		Target Gain	Median	Median	Target Median	rCV(%)	rCV(%)	Width	Result	
Process Date Lot No. Result	FSC	252 252	0.00	206408.8	209181.4	-1.33	-		802.0	0	
2016-11-02 15:02 BAH02 🔇 -	SSC	81 81	0.00						883.2		
2016-11-02 15:05 BAH02_SE 📀	PB450	56 56	0.00				2.13				
2016-11-02 15:07 BAH02_SE	KO525	34 34	0.00			0.43	2.03				
2016-11-02 15:17 BAH02 🥥		202 202	0.00			-0.55	2.23				
		185 185	0.00			-0.15	2.32				
	Violet780	62 62	0.00				2.03				
	DAPI	69 69	0.00				0.80				
	NUV525	21 21	0.00				0.78				
		160 160	0.00				0.87				
		341 341	0.00				3.55				
	APC-A700 APC-A750	329 329 44 44	0.00			2.23	3.74				

[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.

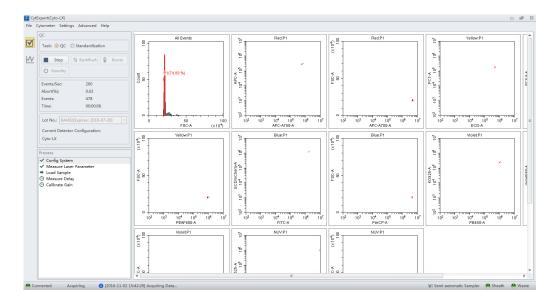
- **5** Select Initialize.
- **6** Insert the prepared QC sample tube (see CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process, or CytoFLEX Daily QC Fluorospheres Preparation Process) into the tube holder.
- 7 Select **Start** to load the sample and begin to run the QC procedure.

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

	\checkmark	Config	System
--	--------------	--------	--------

- ➡ Measure Laser Parameter
- Load Sample
- 🕒 Measure Delay
- 🕒 Calibrate Gain

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



- **8** If the sampling rate is too low, the 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 QC Fluorospheres to the sample tube and then perform the QC.
- **9** If the lot number of CytoFLEX QC Fluorospheres is new and QC fails, the following software message appears. Select **Yes**.

Confirm		
?	QC failed, do you want to save the adjusted gain as bead gain for the current batch and QC Gain?	
	Yes	

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

If the lot number of CytoFLEX QC Fluorospheres is NOT new and QC fails, refer to Step 3 of CHAPTER 5, Confirming Results, or CHAPTER 10, Troubleshooting.

If QC passes, proceed to Step 16.

10 Run Daily Clean to remove any residual fluorosphere particles if using CytoFLEX Daily QC Fluorospheres. Refer to Daily Clean in CHAPTER 11, 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	
ОК Са	incel

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.

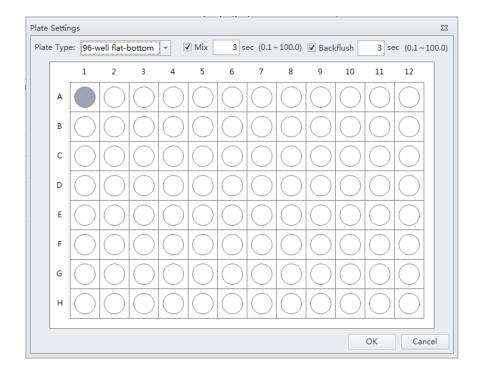
- **b.** If the icon is not green, ensure that the Cytometer USB is securely connected to the Workstation and restart the Workstation.
- **2** Verify the detector configuration. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, Data Acquisition and Sample Analysis.
 - **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 6, Data Acquisition and Sample Analysis.
- **4** Select **Start QC/Standardization** in the QC/Standardization menu to access the QC experiment.

[CytoFLEX LX Shown]

		E 🖻 🖻 🔅	ð •										
sk: 🖲 QC ု Stan	dardization												
k national (Million	ckflush 👔 Boost					QC	Report						
r minanze 14 6a	Genesii 🖉 Boost												
) Standby		Bead Lot N Bead Expin		BAH02 2019-0				0	C Date:	2	016-11-18	3 15:30	
ents/Seci	0	Cytometer	Name:					0	tometer	r SN:			
	0.00	Detector Co	onfigurat	ion: Cyto-L	x								
		Loader Typ	e:	Semi A	utomatic								
	0												
he:	00:00:00	Threshold											
		Channel: FSC	(Height)	Mode: M	anual Value	\$0000							
No.:	×	Laser											
rrent Detector Confi	ouration:	Laser	Dela	rv(us)	Default Dela	v(us) Differe	nce Delay(µs)	Power(mW)	Taro	et Power	(mW) F	lesult	
p-LX		NUV		80.80		80.80	0.00		55		50-70	0	
D-LA		Blue		0.00		0.00	0.00		51		40-60	ŏ	
ort		Red		34.72		34.72	0.00		50		40-60	ő	
		Yellow		-46.24		-46.24	0.00		30		20-40	ő	
ector Configuration:		Violet		-89.12		-89.12	0.00		90		70-120	ě	
o-LX	×			-02.12			0.00		-				
		Signal Value											
e: 2016-10-19 ×	~ 2016-11-18 *	0	Gain	Target %	Difference	Median	Target	%Difference	CV(%)	Target	Width	Result	
		Parameter			rget Gain			Target Median	CV(%)	rCV(%)	width	result	
	No. Result	FSC	149	149	0.00	256788.2	257089.0	-0.12			961.7	0	
	AH01 🥝 🔺	SSC	321	321	0.00	1632397.0	1653400.0	-1.27			1086.9	۲	
6-11-16 09:13 45:		PB450	35	35	0.00	769328.1	763724.0	0.73	1.97	5.00	1376.7	۲	
6-11-16 09:16 45:		KO525	86	86	0.00	833732.9	827384.0	0.77	2.15	5.00	1377.9	۲	
	AH01	Violet610	220	220	0.00	646996.1	648212.0	-0.19	2.34	5.00	1380.4	۲	
6-11-16 16:26 JD- 6-11-16 16:32 45:	AH01	Violet660	223	223	0.00	144380.5	142264.0	1.49	2,43	5.00	1381.6	۲	
		Violet780	270	270	0.00	505568.8	505764.0	-0.04	2.67	5.00	1407.8	۲	
	AH03-SF	DAPI	43	43	0.00	6076549.0	5884821.0	3.26	1.90	7.00	1137.6	۲	
6-11-17 13:32 BA		NUV525	114	114	0.00	4584568.0	4576057.0	0.19	1.82	7.00	921.7	۲	
6-11-17 13:32 BA		HoechstRed	150	150	0.00	1356141.0	1359745.0	-0.27	1.71	7.00	942.5	۲	
6-11-17 13:54 BA		APC	72	72	0.00	81376.7	81688.0	-0.38	2.19	5.00	1403.0	۲	
6-11-17 16:12 BA		APC-A700	109	109	0.00	2055745.0	2065561.0	-0.48	2.35	5.00	1415.8	۲	
6-11-17 16:15 BA		APC-A750	64	64	0.00	729272.6	739500.0	-1.38	2.34	5.00	1414.0	۲	
6-11-18 08:36 BA		PE	72	72	0.00	294360.6	293292.0	0.36	2.79	5.00	1325.2	۲	
	AH03-SF	ECD	95	95	0.00	310092.6	311344.0	-0.40	2.79	5.00	1323.0		
6-11-18 11:16 45:		PC5.5	162	162	0.00	379588.9	391424.0	-3.02	3.18	5.00	1324.6	۲	
6-11-18 11:18 45		PEAF680	122	122	0.00	907208.3	907040.0	0.02	3,41	5.00	1330.6	0	
	H02-SF	PC7	153	153	0.00	449356.5	453560.0	-0.93	3.02	5.00	1331.7	0	
6-11-18 15:18 BA	H02-SF Ø	FITC	107	107	0.00	1638480.0	1658561.0	-1.21	1.11	5.00	1086.1	0	
6-11-18 15:19 BA	H02-SF 🥑	ECDmChe	158	158	0.00	1113664.0	1120281.0	-0.59	1.24	5.00	1078.6		
	H02-SF	PerCP	85	85	0.00	1084768.0	1105620.0		1.65	5.00	1092.7		

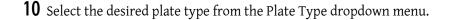
Ensure that the QC bead lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, refer to Importing Lot-Specific Target Values in CHAPTER 5, Instrument Quality Control and Standardization, then select the proper lot number.

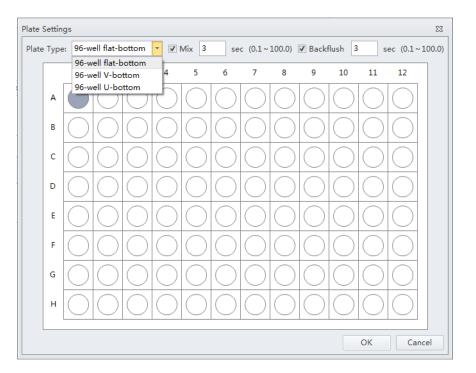
- 5 Select Initialize.
- 6 Select Eject.
- 7 Insert the prepared QC well plate (see Preparation Process CytoFLEX Daily QC Fluorospheres or CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process or CytoFLEX Daily IR QC Fluorospheres Preparation Process) into the plate holder.



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.

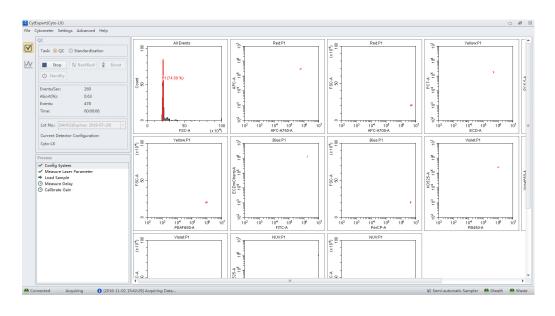
11 Select the Mix and Backflush settings in the top of the Plate Settings window.

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
- O Measure Delay
- 🕒 Calibrate Gain



During QC, the software automatically seeks the CytoFLEX 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 or prepare a new tube of CytoFLEX Ready to Use Daily QC Fluorospheres and then perform the experiment.

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

- **NOTE** If the sample flow rate is not correct, calibrate the sample flow rate before running the QC. For instructions on calibrating the sample flow rate, refer to Calibrating the Sample Flow Rate or Calibrating the Sample Flow Rate [With Plate Loader] in CHAPTER 12, Replacement/Adjustment Procedures.
- **15** If the lot number of CytoFLEX QC Fluorospheres is new and QC fails, the following software message appears. Select **Yes**.

Confirm		
?	QC failed, do you want to save the adjusted gain as bead gain for the current batch and QC Gain?	

NOTE Target gain values must be established for each new lot number of CytoFLEX QC Fluorospheres. QC could fail up to 3 times upon running each new lot number for the first time until target gain values are established. If the lot number of CytoFLEX QC Fluorospheres is NOT new and QC fails, refer to Step 3 of CHAPTER 5, Confirming Results, or CHAPTER 10, Troubleshooting.

If QC passes, proceed to Step 16.

16 Run Daily Clean to remove any residual fluorosphere particles. Refer to Daily Clean [With Plate Loader] in CHAPTER 11, 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.

Detect	tor Configurat	tior	1:			
Defau	ılt-Configurati	on				-
Date:	2016-10-23	-	~	2016-11-22	Ŧ	

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	\checkmark	
2016-11-02 15:17	BAH02	\checkmark	
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 / CytoFLEX Ready to Use 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.

Bead Lot No.:	A555		
Bead Expires:	2015-09-30	QC Date:	2015-01-20 10:23
Cytometer Name:	CytoFLEX	Cytometer SN:	AR38012
Detector Configuration	: Default-Configuration		
Loader Type:	Semi Automatic		
	Bead Expires: Cytometer Name: Detector Configuration	Bead Expires: 2015-09-30 Cytometer Name: CytoFLEX Detector Configuration: Default-Configuration	Bead Expires: 2015-09-30 QC Date: Cytometer Name: CytoFLEX Cytometer SN: Detector Configuration: Default-Configuration

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

 $\begin{array}{l} {\sf Delay:} -5.00 \mu s \leq {\sf Difference \ Delay} \leq 5.00 \mu s. \\ {\sf Gain:} \ -20.00\% \leq {\%} {\sf Difference \ Target \ Gain} \leq 20.00\%. \\ {\sf Median:} \ -5.00\% \leq {\%} {\sf Difference \ Target \ Median} \leq 5.00\%. \\ {\sf rCV:} \ {\sf rCV}(\%) \leq {\sf Target \ rCV}(\%). \\ \end{array}$

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. Laser-filter combinations that are not part of a default configuration may not have a target value defined in the QC target file and therefore will not generate a result during QC. Use the Target Library in the QC screen of CytExpert Software to view the list of laser-filter combinations that have target values assigned within a target file and therefore will return a result for QC.

QC Report

Bead Lot No.:	QC beads Lot AJ04		
Bead Expires:	2020-07-04	QC Date:	2018-07-05 11:07
Cytometer Name:	CytoFLEX	Cytometer SN:	AW42285
Detector Configuration:	Default-Configuration2		
Loader Type:	Carousel Mode		

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	0.00	0.00	0.00	46	40-60	S
Red	-40.16	-40.48	0.32	53	40-60	0
Violet	38.72	38.88	-0.16	79	70-120	0

Signal Value

Parameter	Gain	Target Gain	%Difference Target Gain	Median	Target Median	%Difference Target Median	rCV(%)	Target rCV(%)	Width	Result
FSC	85	85	0.00	207878.7	211000.0	-1.48	-	-	1133.1	0
SSC	74	74	0.00	601946.9	590000.0	2.02	-	-	1271.7	0
FITC	86	86	0.00	1630195.0	1594000.0	2.27	3.32	5.00	1251.5	0
PE	92	92	0.00	1843533.0	1885000.0	-2.20	3.38	5.00	1248.1	0
ECD	110	106	3.77	1157910.0	1163000.0	-0.44	3.34	5.00	1253.8	0
PC5.5	236	236	0.00	4499044.0	4539000.0	-0.88	3.09	5.00	1260.1	0
PC7	308	308	0.00	2218625.0	2240000.0	-0.95	3.17	5.00	1263.8	0
APC	389	389	0.00	322403.3	335000.0	-3.76	2.24	5.00	1370.9	0
APC-A700	408	408	0.00	5445790.0	5516000.0	-1.27	1.98	5.00	1374.1	0
PB450	76	76	0.00	895413.4	895000.0	0.05	1.11	5.00	1292.7	0
KO525	45	45	0.00	227806.7	230000.0	-0.95	1.08	5.00	1291.4	0
Violet610	262	262	0.00	399744.6	397000.0	0.69	1.23	5.00	1296.0	9
Violet660	290	290	0.00	104906.3	104000.0	0.87	1.46	5.00	1298.3	0
Violet780	253	253	0.00	428127.4	425000.0	0.74	1.22	5.00	1300.7	9

Specifications

 $\begin{array}{l} \mbox{Delay: -5.00 μs \le Difference Delay \le 5.00 μs.} \\ \mbox{Gain: -20.00\% \le $\%$ Difference Target Gain \le 20.00\%$.} \\ \mbox{Median: -5.00\% \le $\%$ Difference Target Median \le 5.00\%$.} \\ \mbox{rCV: rCV(\%) \le Target rCV(\%).} \end{array}$

Result

QC Passed.

Comment

No target value for CUSTOM(Red,769/45).

3 If QC fails, follow the procedure below:

NOTE Select No if the Confirm window appears.

Confirm	
?	QC failed, do you want to save the adjusted gain as bead gain for the current batch and QC Gain?
	Yes

- **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.** Verify whether the detector configuration selected for QC matches with the current detector configuration. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, Data Acquisition and Sample Analysis.
- **d.** Run Priming the Flow Cell in CHAPTER 12, Replacement/Adjustment Procedures, and retest.
- e. Run Daily Clean in CHAPTER 11, Cleaning Procedures, and retest.
- f. Run Deep Clean Procedure in CHAPTER 11, Cleaning Procedures, and retest.
- g. Repeat Steps d-e.

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

- **4** If necessary, you can select [™] (for CSV format) or [™] (for PDF format) in the top left corner of the report area to export the QC results.
- **5** Select **Close QC/Standardization** in the 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 **1** on the left side of the screen.

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

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

IJ	Charts Set	tting					23
	Detector C	onfigura	tion: De	efault-Configu	ration	-	
	Lot No.	Laser	Channe	l Alarm Bou	ndary and Scale	e Range	
		Lot	No.	Start Date	End Date		
	0	6347		2014-08-08	2014-08-08		
					ОК	Cancel	Apply

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

U Charts	Setting					23
Detector	Configura	ation: 12	23		-	•
Lot No	o, Laser	Channel	Alarm E	Boundary and	Scale Range	
	Lase	r				
			Power	Delay		
	Blue	laser				
		laser				
	Viole	et laser				
Select	All Cle	ear All				
				ОК	Cancel	Apply

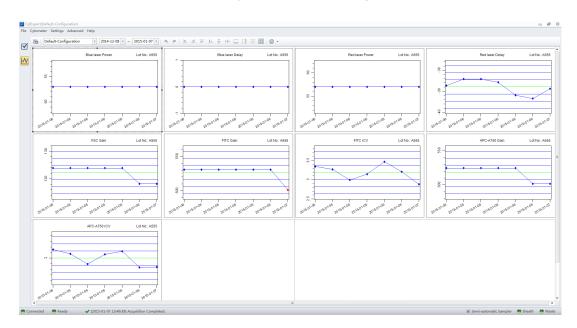
 ${\bf 5} \quad {\rm Select \ the \ Channels \ tab, \ and \ select \ each \ channel \ checkbox \ as \ needed.}$

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

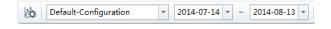
6 Select Apply.

7 Select ок.

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.

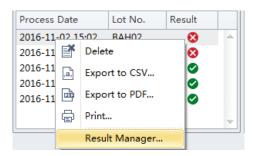


9 Select **Close QC/Standardization** in the File menu to exit the QC screen.

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.



Detector	Configuration: Default-Co	nfiguration 👻		
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	Ø
\checkmark	2016-11-18 17:52:00	Default-Configuration	123	Ø
1	2016-11-18 17:55:00	Default-Configuration	4Laser	8
	2016-11-18 17:58:00	Default-Configuration	Moni	0
	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	Ø
	2016-11-18 18:11:00	Default-Configuration	Moni	
/ Selec	t All			

Standardization

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

Preparing the Standardization Sample

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

Required Materials

The following materials are required to complete the QC process:

- CytoFLEX Daily QC Fluorospheres or CytoFLEX Ready to Use Daily QC Fluorospheres, or other material applicable for your application
- CytoFLEX Daily IR QC Fluorospheres (for systems configured with an IR laser)
- CytoFLEX Sheath Fluid
- Sample tubes (12 x 75 mm).
- Vortexer

Preparation Process

For procedures, refer to CytoFLEX Daily QC Fluorospheres Preparation Process and/or CytoFLEX Ready to Use Daily QC Fluorospheres Preparation Process and/or CytoFLEX Daily IR QC Fluorospheres Preparation Process.

Generating Target Median Values

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

NOTE Ensure the Gain and Threshold settings are optimal. Refer to Adjusting the Gain and Adjusting the Threshold in CHAPTER 6, Data Acquisition and Sample Analysis.

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

4 Change the tube name. Refer to Changing the Tube Name in CHAPTER 6, Data Acquisition and Sample Analysis.

Tube	•	
v	v v 🕼 🞞	▼ 00
	Name	Time
	unstained	2018-06-27 16:10:45
	FITC-CD3	2018-06-27 16:14:01
III (PE-CD4	2018-06-27 16:15:41
	PC5-CD3	2018-06-27 16:17:22
	PC7-CD8	2018-06-27 16:19:45
III (lymphocytes test	2018-06-27 16:26:37
III (QC beads	
4	III	•

- **5** Select **Save As** from the File menu to save the experiment.
- **6** Select **i** to delete all the remaining tubes.



Use	Show Aut	ofluoresce	nce								Area and	Height in S	Sync Ar	ea '
utofl.	Channel	-FITC%	-PE%	-ECD%	-PC5.5%	-PC7%	-APC%	-APC- A700%	-APC- A750%	-PB45 0%	-КО52 5%	-Violet 610%	-Violet 660%	-Violet 780%
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	0.0
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	0.0
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	0.0
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	0.0
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	0.0
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	0.0
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	0.0
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	0.0
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	0.0
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	0.0
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	0.0
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		0.0
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	

7 Select A. The Compensation Matrix window appears.

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

/ Use	Show Aut	ofluoresce	ence								Area and	Height in 1	Sync Ar	ea
Autofl.	Channel	-FITC%	-PerCP	-APC%	-APC- A700%	-APC- A750%	-PB45 0%	-KO52 5%	-Violet 610%	-Violet 660%	-PE%	-ECD%	-PC5.5%	-PC7%
0.00 ‡	FITC		0.00	0.00	0.00	0.00	0.00	9.00	0.00	0.00	0.00	0.00	0.00	0.0
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	0.0
0.00	APC	0.00	0 0	nfirm							0.00	0.00	0.00	0.0
0.00	APC-A700	0.00	0								0.00	0.00	0.00	0.0
0.00	APC-A750	0.00	0	Ar	e vou sure	e vou wan	t to clear	the comp	ensation r	matrix?	0.00	0.00	0.00	0.0
0.00	PB450	0.00	0		3	-					0.00	0.00	0.00	0.0
0.00	KO525	0.00	0			Yes		No			0.00	0.00	0.00	0.0
0.00	Violet610	0.00	0					-			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	0.0
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	0.0
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	0.0
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		0.0
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	

9 Load the sample tube.

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

10 Select Run.

- **11** View the plots and establish the gates. Refer to Creating Plots and Gates in CHAPTER 6, Data Acquisition and Sample Analysis.
 - **NOTE** Use the FSC channel as the trigger channel and select **Automatic** threshold.
 - **NOTE** The threshold may need to be adjusted to visualize the QC beads populations. If so, record this value for future reference.

 $12 \ {\rm Right-click} \ {\rm the} \ {\rm table} \ {\rm and} \ {\rm select} \ {\rm Statistics} \ {\rm Settings}. \ {\rm The} \ {\rm Statistics} \ {\rm Setting} \ {\rm window} \ {\rm appears}.$

Population	Median FSC-A	Median SSC-A	Export All Samples to CSV File	n PC7-A
• P1	238851.1	666696.3	Export Samples to Graphic File	1036442.2
			Export to Clipboard	
			Export All Samples to Clipboard	
			Statistics Setting	
			Сору	
			Delete	

Statistics Setting		23
Header Statistics Population	1	
Experiment Name		
🔽 Tube Name		
✓ Sample ID		
Record Time		
Volume		
Abort(%)		
Expression Edit		
Name:		
Equation:		
Comment		
	*	
Operator		
Select All Clear All		
Apply to:		
Ourrent Tube ○ All Tubes	🔲 Set As Default	OK Cancel

13 Select the Population tab and select the relevant population for the tube.

Statistics Setting	22
Header Statistics Population	
All Events	
🔽 🔵 QC beads	
 Window Snip 	
Select All Clear All	
Apply to:	
🧿 Current Tube 🔘 All Tubes	Set As Default OK Cancel

Head	der Statistic	s Populat	ion							
🔲 Pa	arent Populat	ion 🔽 Eve	nts 📝 % To	tal 🔽 % Pa	rent 🔲 Ever	nts/µL(V)				
🔲 Ev	/ents/µL(B)									
Be	eads Populati	on:			Select					
Be	eads Count:	0	Sample	e Volume: 0	.00.μ	L				
	-									
	Parameter	Mean	GeoMean	Median	rCV	rSD	CV			
	FSC-A			V						
	SSC-A			1						
Fitc-A Image: Constraint of the second sec										
mCherry-A										
PerCP-A										
APC-A										
	APC-A700									
	APC-A750									
	PE-A									
	ECD-A									
			III							
Se	lect All	lear All		0	Area 🔘 He	eight 🔘 Are	a + Height			
Pre	view									
E	vents %	Total	% Parent	Median F N	Aedian S N	Aedian Fi				
Apply	to:									
- F F 7										

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

QC beads 237489.3 660867.4 1009959.8 984541.4 10341.

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

 $15 \ {\rm Right-click} \ {\rm the \ statistics} \ {\rm table} \ {\rm and} \ {\rm select} \ {\rm Export \ tube \ to \ CSV \ file.}$

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

16 Save the experiment.

NOTE Rerun the experiment if:

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

Adding a New Standardization Item

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

Abort(%): 0.00 Events: 0 filme: 0000000 toto No: AK07(Expires: 2021-02-11) * Verter: V Standard sample FSC Parameter Gain Median Target Median %Difference Target Median Result FSC 119 202396.3 206017.6 -1.76 C SSC 322 1633009.0 1674140.0 -2.46 C FTC 143 1634233.0 1674140.0 -2.46 C FTC 143 1634233.0 1674140.0 -2.46 C FEC 273 1207103.0 1235085.0 -2.27 C	2019-09-11 09:41 Expires: 2021-02-11 Heter SN: BC01002	Initialize Net ackfluich Standbrig Standbry Construction Construction wents/Sec: 0.0 Construction Date: 2019-09-11 0.941 bort(%b): 0.00 Construction Detector Configuration: Cytometer Name: Cytometer SN: BC01002 wents: 0 0.000000 Test Kern: Standard sample Date: 2019-09-11 0.941 wents: 0 0.000000 Test Kern: Cytometer Name: Cytometer SN: BC01002 wents: 0 0.000000 Test Kern: Cytometer Name: Cytometer SN: BC01002 wents: 0 0.000000 Test Kern: Standard sample Date: Cytometer SN: BC01002 wents: 0 0.000000 Test Kern: Standard sample Date: Cytometer SN: BC01002 wents: 0 0.000000 Test Kern: Standard Stan	Initialize N Backflusk	Initialize Backfulle Backfulle <th>Initialize Backflush Backflush Standby Backflush Backflush Initialize O O Standby Backflush Backflush Initialize O O Initialize O O O Initialize O O O Initialize O O O Initialize Initialize Standard sample Date C Initialize O O O O O Initialize O O O O O O Init</th> <th>andardization</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th>	Initialize Backflush Backflush Standby Backflush Backflush Initialize O O Standby Backflush Backflush Initialize O O Initialize O O O Initialize O O O Initialize O O O Initialize Initialize Standard sample Date C Initialize O O O O O Initialize O O O O O O Init	andardization							
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Date: 2010 09 12 - 2010 00 11 -		Violet610 174 404592.5 412766.7 -1.98 © 119-09-11 09:41 AK07 Standard Violet780 258 401903.6 414027.5 -2.93 ©	Image: Construction of the standard in	Violet610 174 404592.5 412766.7 -1.98 ♥ +09-11 09:41 AK07 Standard Violet660 197 101260.7 103969.9 -2.61 ♥ •09-11 09:41 AK07 Standard Feerifications -2.93 ♥ •09-11 09:41 •0.95	Dacess Date Lot No. Task Item Rr 19-09-11 09:41 AK07 Standard Violet60 197 101260.7 103969.9 -2.61 O 91-09-11 09:41 AK07 Standard Standard Standard Standard Standard O 0	ate: 2019-08-12 - 2019-09-11 -							
	-1 08	Violetool 197 101260.7 103969.9 -2.61 C Violetool 197 101260.7 103969.9 -2.61 C Violetool 258 401903.6 414027.5 -2.93 C	19-09-11 09:41 AK07 Standard Violetob0 197 101260.7 103969.9 -2.61 ♥ Ig-09-11 09:41 AK07 Standard Violetob0 258 401903.6 414027.5 -2.93 ♥ Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Violeto60 197 1012/bit.7 103969.9 -2.61 ♥ -09-11 09:41 AK07 Standard ~ Violeto60 197 1012/bit.7 103969.9 -2.61 ♥ Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%. €5.00%. ♥ ● ●	19-09-11 09:41 AK07 Standard Nolettool 197 1012:00.7 103999:9 -2.01 ✓ Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%. Result ✓ <t< td=""><td></td><td>Violet610</td><td>174</td><td>404592.5</td><td>412766.7</td><td>-1.98</td><td>0</td></t<>		Violet610	174	404592.5	412766.7	-1.98	0	
Violet60 19/ 101260./ 103969.9 -2.61	1.50	Aldis(100 530 4013020 4140512 -5132 A	Violet sol 238 4019030 414027.3 -2.93 Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Violer/ob 238 401905.0 414027.3 -2195 ♥ Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Violet 30 2.35 401903.0 414027.3 -2.35 Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%. Result		Violet660	197	101260.7	103969.9	-2.61	0	
2019-09-11 09:41 AK07 Standard Violet780 258 401903.6 414027.5 -2.93		Enclifections	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%. Result	019-09-11 09:41 AK07 Standard A	Violet780	258	401903.6	414027.5	-2.93	0	
Specifications	-2.61	specifications			Result		Specifications						
Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	-2.61	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Result	Result			Median: -5.00% ≤ %	Difference Tar	get Median ≤ 5.00%.				
	-2.61				Standardization Passed.		Result						
Result	-2.61	Result	Standardization Paced	Standardization Passed			Standardization Pas	sed.					
K0525 34 237050.3 242110.8 -2.09 C		tet Lot No. Task Item R. Violet660 197 101260.7 103969.9 -2.61 09:41 AK07 Standard Violet780 258 401903.6 414027.5 -2.93	te Lot No. Task Item R. 09:41 AK07 Standard ↓ Violet660 197 101260.7 103969.9 -2.61 ♥ Violet780 258 401903.6 414027.5 -2.93 ♥ Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	te Lot No. Task Item R. 09:41 AK07 Standard ↓ Violet660 197 101260.7 103969.9 -2.61 ♥ Violet780 258 401903.6 414027.5 -2.93 ♥ Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	te Lot No. Task Item R. 09:41 AK07 Standard ↓ Violet660 197 101260.7 103969.9 -2.61 ♥ Violet780 258 401903.6 414027.5 -2.93 ♥ Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%. Result								
Data Lat Na Task Itan D		19-09-11 09:41 AK07 Standard ^ Violet780 258 401903.6 414027.5 -2.93 🥝	19-09-11 09:41 AK07 Standard ← Violet780 258 401903.6 414027.5 -2.93 Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	-09-11 09:41 AK07 Standard ↑ Violet780 258 401903.6 414027.5 -2.93 ♥ Specifications Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Image: 19-09-11 09:41 AK07 Standard ^ ✓ Violet780 258 401903.6 414027.5 -2.93 ✓ Specifications Median: -5.00% £ %Difference Target Median ≤ 5.00%. Result	cess Date Lot No. Task Item Re	100000000000000						
019-09-11 09:41 AK07 Standard Violet780 258 401903.6 414027.5 -2.93		Enclinetions	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%. Result	019-09-11 09:41 AK07 Standard 🔶	Violet780	258	401903.6	414027.5	-2.93	0	
Specifications	-2.61		Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%.	Median: -5.00% ≤ %Difference Target Median ≤ 5.00%. Result		Specifications						
	-2.61	Median: -5.00% ≤ %Difference Larget Median ≤ 5.00%.	Result	Result				Difference Lan	get Median ≤ 5.00%.				
	-2.61				Standardization Passed.		Result						
	-2.61		Standardization Passed.										
	-2.61		Stanuarurzativn Passev.										
	-2.61		Statituaruization Passet.										
	-2.61		Stanuarulzation Passeu.										
	-2.61												
	-2.61												
	-2.61												
	-2.61												
	-2.61												
	-2.61					· ·							
Standardization Passed.	-2.61	Standardization Passed.	•		~								

Lot No.	Expires of Ship	Lot No.:				
		Туре	Channel		Mode	Value
		Signal				
		Channel	Med	Jian	N	1edian Tolerance(%)
				Threshold Type Channel Signal	Threshold Type Channel Signal	Threshold Type Channel Mode Signal

2 Select Standardization Target Library from the Settings menu.

em:		✓ Lot No.:		▼ Expire: 2016-11-22	-
Thre	eshold				
Sta	andardization Test Se	tting	Acquisition Settin	g	
FSC	Manual:	10000 (>0)	Channel: FSC		
	1			(n	
	O Automatic		Manual	10000 (>0) 💿 Heig	ht 🔘 Area
			Automatic		
V U	se the same threshol Channel	d setting for acquisit Median	ion setting	Median Tolerance(%)	
1	FSC		0.00		5.00
	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 PB450		0.00		5.00
	PB450 KO525		0.00		5.00
	Violet610		0.00		5.00
	Violet660		0.00		5.00
	10101000		0.00		5.00

4 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.

- **5** Select either Manual or Automatic threshold from the Standardization Test Setting or Acquisition Setting section of the window.
 - **NOTE** If you select Manual threshold, enter a value greater than 0, but less than 8,388,600.
 - **NOTE** Keep the threshold setting the same as previous Step 11 in Generating Target Median Values.
 - **NOTE** Unchecking the *Use the same threshold setting for acquisition setting* checkbox allows you to specify custom threshold settings.

6 Set the channels to be standardized.

NOTE The contents of the channel, laser, and filter column come from the current detector configuration setting. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in Data Acquisition and Sample Analysis.

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

NOTE FSC is a required channel.

7 Enter the target median values saved in Step 14 into the corresponding channels.

d Sta	andardization Target	Value		٤	3 🔚 43015+ - Windows F	noto viewer				
em:	QC beads	- Lot No.: AL	01	• Expire: 2022-01-24 •	File 🔻 Print 👻	E-mail Burn 🔻	Open 🔻			
Thre	eshold									
Sta	andardization Test Se	tting	Acquisition Setting							
	 Manual: Automatic 	10000 (>0)	Channel: FSC	▼ 10000 (>0) ⊙ Height ⊙ Area						
211	se the same threshol	d setting for acquisitio	O Automatic		Tube Name: QC bead Sample ID:					
	se the sume threshold	a setting for acquisito	il setting		Population	Median FSC-A			Median PE-A	Median PC7-
	Channel	Median		Median Tolerance(%)	QC beads	237489.3	660867.4	1009959.8	984541.4	4 10341
7	FSC		237489.30	5.00	X					
V	SSC		660867.40	5.00						
1	FITC		1009960.00	5.00						
	PerCP		0.00	5.00						
	APC		0.00	5.00)					
	APC-A700		0.00	5.00						
	APC-A750		0.00	5.00						
	PB450		0.00	5.00						
	KO525		0.00	5.00						
	Violet610		0.00	5.00						
	Violet660		0.00	5.00						
1	PE		984541.40							
10	ECD		0.00							
	PC5.5		0.00							
V	PC7		1034120.00 ‡	5.00			~			
					(છ ∗ ∰		5	c x)	
	ect All			OK Cancel						

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

NOTE Verify that the target values are entered correctly.

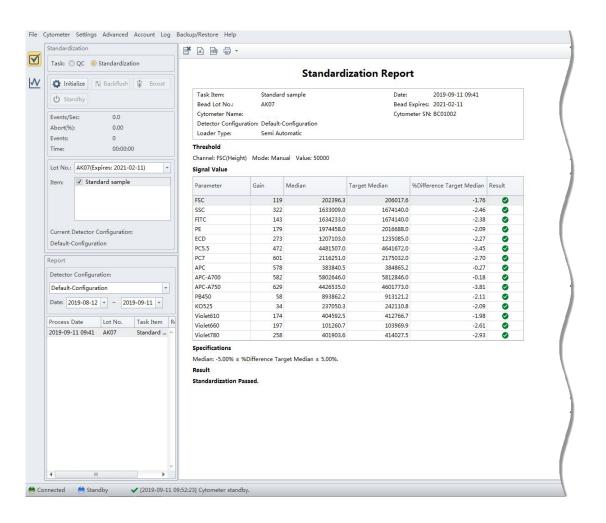
8 Select **OK** to save the target value.

The saved results display in the Standardization Target Library window. This item is ready to be run through the Standardization experiment. Refer to Performing the Standardization in CHAPTER 6, Data Acquisition and Sample Analysis.

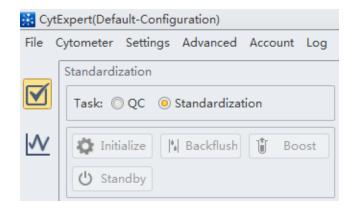
9 Select **Close** to exit the Standardization Target Library window.

Performing the Standardization

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



2 Select the **Standardization** radio button.



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

Item:	AL01(Expires: 2022-01-24)	
	Detector Configuration:	
	A REAL PROPERTY AND A REAL PROPERTY A REAL PROPERTY AND A REAL PRO	

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

4 Select the Items to be standardized.

LOT INO.:	AL01(Expires: 2022-01-24)
Item:	☑ QC beads
Current [Detector Configuration:

5 Select the proper detector configuration. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, Data Acquisition and Sample Analysis.

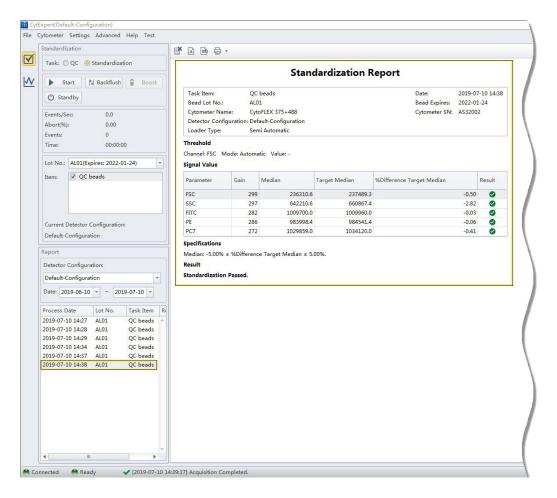
6 Select Initialize.

7 Select Start

The Process section of the screen displays the process details.

Process	
✓ Config System	
✓ Load Sample	
Calibrate QC beads Gain	

Once the process is complete, the Standardization Report displays.



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

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

Default Sett 2019-07-10 14:3 Channel Name Gain S QC beads 2019-07-10 14:3 Default-Configuration FSC 299 FITC 282 PE 286 PC7 272		Item	Date Created	Detector Configurati	Gain	Threshold	Width		
S QC beads 2019-07-10 14:3 Default-Configuration FSC 299 FITC 282 PE 286		Default Sett	2019-07-10 14:3		Channe	el Name		Gain	
S QC beads 2019-07-10 14:3 Default-Configuration SSC 297 FITC 282 PE 286		4555	2019-07-10 14:3		ESC				299
FITC 282 PE 286	S	QC beads	2019-07-10 14:3	Default-Configuration					
PE 286					1000 C				
PC7 27:					100 100 100 100 100 100 100 100 100 100				286

NOTE <u>S</u> designates test items from Standardization.

9 Run Daily Clean. Refer to Daily Clean in CHAPTER 11, Cleaning Procedures.

10 Select Close QC/Standardization.

Applying the Standardized Acquisition Settings

1 Open an experiment.

NOTE The corresponding compensation matrix should have been determined as the optimal settings. Refer to CHAPTER 7, Compensation for detailed instructions on setting compensation. 2 Select Acq. Setting from the Cytometer menu. The Acq. Setting window appears.

Acq. Setting - stain index						
Gain	Threshold	Width				
FSC		300 🗘 🕨 (1~3000)				
SSC		300 📜 🕨 (1~3000)				
FITC		273 🗘 🕨 (1~3000)				
PerCP		100 📜 🕨 (1~3000)				
APC		399 📜 🕨 (1~3000)				
APC-A	700	372 🗘 🕨 (1~3000)				
APC-A	750	387 🗘 🕨 (1~3000)				
PB450)	79 🗘 🕨 (1~3000)				
КО52	5	176 🗘 🕨 (1~3000)				
Violet	610	610 🗘 🕨 (1~3000)				
Violet	660	2158 🗘 🕨 (1~3000)				
PE		285 🗘 🕨 (1~3000)				
ECD		562 🗘 🕨 (1~3000)				
PC5.5		127 🗘 🕨 (1~3000)				
PC7		265 🗘 🕨 (1~3000)				
Set As	Set As Default					
Impo	rt <u>f</u> rom File	. Import from Catalog				
Expo	rt <u>t</u> o File	Export to Catalog	Close			

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

Default Sett 2019-07-10 14:3 4555 2019-07-10 14:3 S QC beads 2019-07-10 14:3 Default-Configuration FSC 29 SC 29 FITC 28 PE 28 PC7 27		Item	Date Created	Detector Configurati	Gain	Threshold	Width		
4555 2019-07-10 14:3 FSC 29 S QC beads 2019-07-10 14:3 Default-Configuration SSC 29 FTC 28 PE 28		Default Sett	2019-07-10 14:3		Chann	el Name		Gain	
S QC beads 2019-07-10 14:3 Default-Configuration SSC 29 FITC 28 PE 28		4555	2019-07-10 14:3						200
FITC 28 PE 28	S	QC beads	2019-07-10 14:3	Default-Configuration					
PE 28				1					
PC7 27					100000				28
					PC7				27

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

The standardized settings are applied to the sample tube.

cq. Setting - stain index		23	Acq. Setting - stair	n index
Gain Threshold	Width		Gain Thresho	ld Width
FSC	300 📜 🕨 (1~3000)		FSC	299 🗘 🕨 (1~3000)
SSC	300 📜 🕨 (1~3000)		ssc ssc	297 📜 🕨 (1~3000)
FITC	273 📜 🕨 (1~3000)		FITC	282 🛟 🕨 (1~3000)
PerCP	100 📜 🕨 (1~3000)		PerCP	100 📜 🕨 (1~3000)
APC	399 📜 🕨 (1~3000)		APC	399 📜 🕨 (1~3000)
APC-A700	372 📜 🕨 (1~3000)		APC-A700	372 📜 🕨 (1~3000)
APC-A750	387 📜 🕨 (1~3000)		APC-A750	387 2 (1~3000)
PB450	79 ÷ ► (1~3000)		PB450	79 📜 🕨 (1~3000)
K0525	176 📜 🕨 (1~3000)		КО525	176 📜 🕨 (1~3000)
Violet610	610 ÷ + (1~3000)		Violet610	610 📜 🕨 (1~3000)
Violet660	2158 📜 🕨 (1~3000)		Violet660	2158 📜 🕨 (1~3000)
PE	285 🗘 🕨 (1~3000)		PE	286 📜 🕨 (1~3000)
ECD	562 🗘 🕨 (1~3000)		ECD	562 📜 🕨 (1~3000)
PC5.5	127 🗘 🕨 (1~3000)		PC5.5	127 📜 🕨 (1~3000)
PC7	265 🗘 🕨 (1~3000)		PC7	272 📜 🕨 (1~3000)
Set As Default	Default <u>O</u> C Gain]	Set As Default	Default <u>O</u> C Gain
Import from File	Import from Catalog]	Import from Fi	le Import from Catalog
Export to File	Export to Catalog	Close	Export to File	Export to Catalog Close

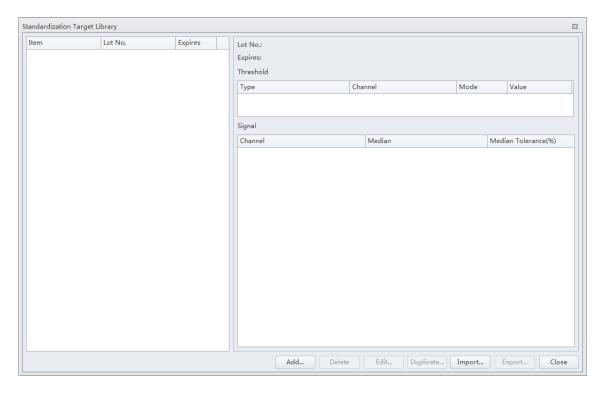
The Information window appears to notify of the corresponding channels with the changed gain as a result of the Standardization.

nformation	\$3
Acquisition Setting Import Result:	
The following gain of channels have been modified:	
FSC	
SSC	
FITC	
PE	
PC7	
	OK

5 Select OK.

Standardization Target Library

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



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

Importing a Standardization Item

- 1 Select Import... on the Standardization Target Library window.
- **2** Browse for the Item to import and select **Open .**

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

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

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

Exporting a Standardization Item

1 Browse for the Item to export.

The available items display on the left panel of the Standardization Target Library window.

2 Select **Export...** on the Standardization Target Library window.

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

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

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

Editing Standardization Item Parameters

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

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

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

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

4 Select Close to exit the Standardization Target Library window.

Duplicating Standardization Items

 Select an existing item from the Item column on the Standardization Target Library window and select Duplicate... **2** Edit Item, Lot No., Expire date and the parameters for that item and select **OK**.

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

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

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

Deleting Standardization Items

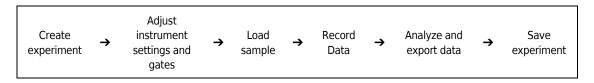
- 1 Select an item from the Item column on the Standardization Target Library window.
- 2 Select Delete. The confirm message *The target values will be permanently deleted. Are you sure?* displays. Select **Yes** to confirm.
- **3** Select Close to exit the Standardization Target Library window.

CHAPTER 6 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
- Configuring Acquisition Settings
- Load Sample and Record Data
- Analyzing and Exporting Data
- Saving the Experiment

Creating an Experiment

Creating an Experiment [Without Plate Loader]

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 4, 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 6, Data Acquisition and Sample Analysis.
- **4** Create or open an experiment using one of the following methods:
 - Create a new experiment:
 - Select New Experiment on the Start page, specify the file path, and save the experiment.
 Or
 - Select **New Experiment** in the File menu, specify the file path, and save the experiment.
 - Create a new experiment from a template:
 - Select New Experiment from Template on the Start page. Select Browse next to New Experiment and specify the file path for the new experiment, then select Browse next to Template and specify the file path to the existing template.
 Or
 - Select New Experiment from Template in the File menu, specify the file path and save the experiment.
 Or
 - Select the Template tab on the Start page and select the template from the list of
 recently used templates. Specify the file path and save the experiment.
 - Open an existing experiment:
 - If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option: Select Open Experiment on the Start page, specify the file path and save the experiment.

If you are using the CytExpert Electronic Record Management Software Option: Select **Open Experiment** on the Start page, specify the experiment file and open the experiment.

Or

 If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option: Select Open Experiment in the File menu, specify the path and save the experiment.

If you are using the CytExpert Electronic Record Management Software Option: Select **Open Experiment** in the File menu, specify the experiment file and open the experiment.

Or

 If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option: Select the Experiment tab on the Start page and select the experiment from the list of recently opened experiments. Specify the file path and save the experiment.

If you are using the CytExpert Electronic Record Management Software Option: Select the Experiment tab on the Start page and select the experiment from the list of recently opened experiments. Specify the experiment file and open the experiment.

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

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

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

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.





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 6, Data Acquisition and Sample Analysis

2 Select **.** The Plate window appears.

[CytoFLEX LX Shown]

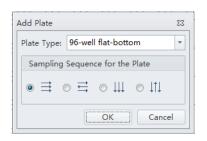
Plate									23
: † - [T	v] ļi	A	A Q		
Nam	e	Sample ID	Events	Group		Mix	Backflush	Time	

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

[CytoFLEX LX Shown]

Plat	te							23
:			· ·]]]]	U [411 A	(Å) 🕓	
	Add Plate							
	Add Plate from Template.							
	Add Plate from Layout							
	Duplicate Current Plate wi	ithout Data						
	Name Sar	mple ID	Events	Group		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:

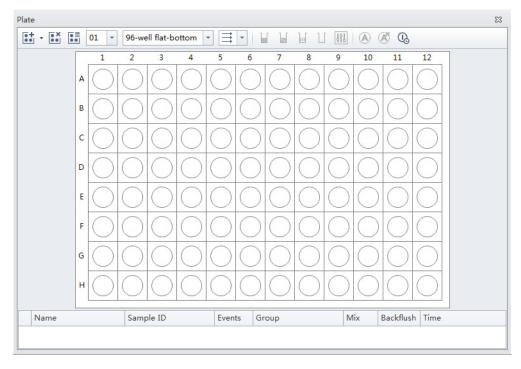
- 1. In the first row, enter the following column titles WellLabel, TubeName, SampleID, Group in sequence as shown in Figure 6.1. The table header cannot be customized.
- 2. In the WellLabel column, a maximum of 96 wells can be defined, beginning with A1-A12, B1-B12, and so on.

Figure 6.1 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.

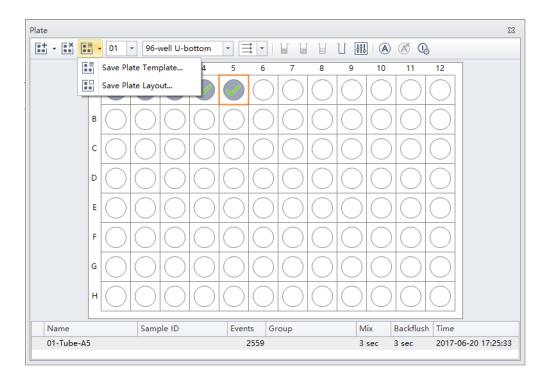
5 Select the direction of the workflow from the sampling sequence from the plate section of the window. The Plate window appears.



[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 (.) indicates auto shutdown is disabled. A yellow background (.) indicates auto shutdown 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.

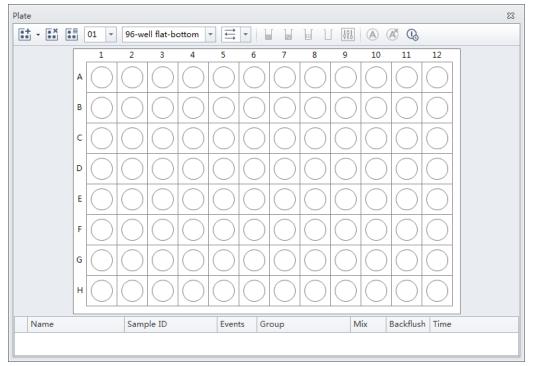


7 Select **Ο**κ.

Setting Sample Wells

Once the plate protocol is created, the plate window appears. Refer to Figure 6.2.

Figure 6.2 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 Gro	bup
refix: Tube		✓ Mix 3 sec (0.1-100) Nan	ne: 🗹 Apply to Sample
xample: 01-Tube	-A1	Backflush 3 sec (0.1-100)	pr:
Acq.Setting Cha	annel Compensation Matrix Stop	ping 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 2 (1~3000)	■	a
IR885	232 📜 🕨 (1~3000)	Automatic	
V450-PB	37 📜 🕨 (1~3000)	Logic Operator:	
V525-KrO	88 📜 🕨 (1~3000)	Secondary Threshold (Trigger Level)	
V610	232 📜 🕨 (1~3000)	Channel:	
V660	206 📜 🕨 (1~3000)	◯ Manual 1 (>0) ◯ Height ◯ Area	3
V763	261 📜 🕨 (1~3000)	Automatic	
	Default Recommende	Defau	ult Default
			Import from File Import from Catalog
			Import Acquisition Conditions from FCS File
			OK Cance

 ${\bf 7} \quad {\rm Select \ the \ desired \ acquisition \ settings \ under \ the \ Acq. \ Setting \ tab.}$

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.

aming Rules			Mix/Ba	ckflush			Group					
efix: Tub ample: 01-T	222.00		✓ Mix ✓ Back	✔ Mix 3 sec (0.1-100) ✔ Backflush 3 sec (0.1-100)				Name: V Ap Color: V				
Acq.Setting	Channel Com	npensation Matrix	Stopping Rules	Custo	m Metadata]						
Use	Channel				Label							
V	IR840-A790											
\checkmark	IR885											
\checkmark	V450-PB											
\checkmark	V525-KrO											
1	V610											
1	V660											
1	V763											
1	NUV450											
\checkmark	NUV525											
V	NUV675											
V	R660-APC											
V	R712-APCA7	700										
\checkmark	R763-APCA7	750										
\checkmark	Y585-PE											
V	Y610-ECD											
\checkmark	Y675-PC5											
Area 🗹 H	eight							Import Acquisition	Conditions from FCS File.			

9 Set compensation under the Compensation Matrix tab. Refer to CHAPTER 7, Compensation for detailed instructions on setting compensation.

ube										Group							
				V N	lix	3	sec	(0.1-100)	Nar	Name: Al					pply to Sample ID		
-Tube-A1				▼ B	✓ Backflush 3 sec (0.1-100) Color:												
Channel	Compe	ensation N	1atrix St	opping Ru	ules Cus	tom Meta	adata										
Show Auto	ofluoresce	ence										🗌 Area	and Heigl	nt in Sync	Area	-	
hannel	-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 PC5	
R840-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		
R885	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
450-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		
525-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	=	
610	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
660	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
763	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
UV450	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00		
UV525	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00		
IUV675	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00		
660-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	-	
												Imp	oort from	Library	Impo	t	
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Show Auto annel 840-A7 885 50-PB i25-KrO i10 i60 i63 JV450 JV525 JV675 i60-APC	Show Autofluoresce annel -IR840 -A790% 840-A7 880-A7 0.00 50-PB 0.00 25-KrO 0.00 10 0.00 660 0.00 7450 0.00 1V450 0.00 1V675 0.00 60-APC 0.00	Show Autofluorescence annel -IR840 -A790% -IR885 840-A7. 0.00 885 0.00 50-PB 0.00 0.00 0.00 110 0.00 0.60 0.00 60 0.00 63 0.00 1V450 0.00 1V675 0.00 60-0.00 0.00 60-0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00	Show Autofluorescence annel -IR840 -A790% -IR885 -V450 PB% 840-A7 0.00 0.00 50-PB 0.00 0.00 50-PB 0.00 0.00 110 0.00 0.00 660 0.00 0.00 633 0.00 0.00 1V450 0.00 0.00 1V525 0.00 0.00 1V675 0.00 0.00 60-APC 0.00 0.00	Channel Compensation Matrix Stopping Reserve Show Autofluorescence -V450° -V525° annel -IR840 -IR885 -V450° -V525° 840-A7 0.00 0.00 0.00 855 0.00 0.00 0.00 50-PB 0.00 0.00 0.00 25-KrO 0.00 0.00 0.00 10 0.00 0.00 0.00 63 0.00 0.00 0.00 1725 0.00 0.00 0.00 17450 0.00 0.00 0.00 60-00 0.00 0.00 0.00 17450 0.00 0.00 0.00 1755 0.00 0.00 0.00 0.00 1757 0.00 0.00 0.00 0.00 60-APC 0.00 0.00 0.00 0.00	Channel Compensation Matrix Stopping Rules Cus Show Autofluorescence	Channel Compensation Matrix Stopping Rules Custom Meta Show Autofluorescence	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence -	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence -	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence	Channel Compensation Matrix Stopping Rules Custom Metadata Show Autofluorescence Area and Height in Sym Area annel -1/8840 -1/885 -1/450 -1/525 -V610 -V660 -V76396 -NUV4 -NUV5 -NUV6 -R7712- APCAT -R763 APCAT -Y585 -V610 -V610 -V660 -V76396 -NUV5 -NUV5 -NUV6 -R7712- APCAT -R763 APCAT -Y585 -V610 -V525 -V610 -V610 -V610 -V610 -V610 -V585 -V585 -V585 -V610 -V585 -V610 -V585 -V585 -V610 -V610 -V610 -V610 -V610 -V610 -V585 -V585 -V585 -V610 -V585 -V585 -V610 -V610 -V610 -V610 -V610 -V610 -V610	

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

aming R	ules		Mix/Backflush	Group
	Tube 01-Tube-A1			(0.1-100) Name: Apply to Sample (0.1-100) Color: -
	ng Channel to Record:	Compensation Matrix Sto 10,000 Events	pping Rules Custom Metadata	
	in o Record: e to Record:	All Events * 600 sec 10 μL		
				Import Acquisition Conditions from FCS File

- **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.

t As Sample W	/ells										:
Naming Rules	5			Mix/Bac	kflush			Group			
Prefix: Tu	ibe			✓ Mix 3 sec (0.1-100)				100)	Name:		
Example: 01	-Tube-A1			✓ Backf	lush	3	sec (0.1-100)		Color:		
Acq.Setting	Channel	Compensation Matrix Stop	ping l	g Rules Custom Metadata							
Name		Valu	е								
		om Metadata				23					
	Name:	Patient 1									
	Value:	female									
			OK		Canc						
						Add	Delete				
						Add	Delete				
							Import	Acquisition	Conditions	from I	CS File
									ОК		Cancel

12 Select ок.

Modifying Well Settings

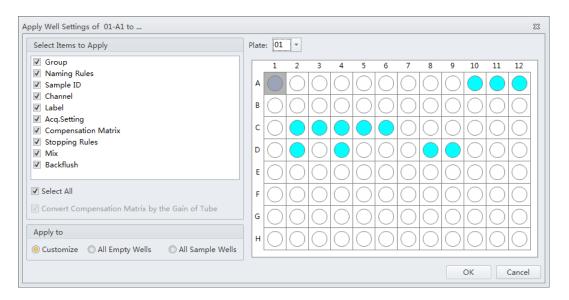
If any well settings require modification, right-click the sample well and select the setting to change.

[CytoFLEX LX Shown]

Plate														23
	•	01 -	96-wel	l flat-bo	ottom	• 🗄	•	1	¥ 1	ţţĮ	A	(A)		
		1	2	3	4	5	6	7	8	9	10	11	12]
	A				t Group		\square						_ 5	
	в	\bigcirc	\subset			Acquisiti le Wells.	ion Conc	litions \	Vells		\bigcirc		\bigcirc	
	с	\bigcirc	\subset			ng Ager ized Wa	nt Wells ter Wells	5			\bigcirc		\bigcirc	
	D	\bigcirc	\subset		s Empty cquisitic	Wells	ition				\bigcirc		\bigcirc	
	E	\bigcirc	\subset	Set N	laming I lix/Back	Rules					\bigcirc		\bigcirc	
	F	\bigcirc	\subseteq		roup / Well S	ettings t	o				\bigcirc		\bigcirc	
	G	\bigcirc	\subseteq	Copy						·····	\bigcirc		\bigcirc	
	н	\bigcirc	\subset	Paste		i - Dul			C ()		\bigcirc		\bigcirc	
Name			Sa		e Locatio	-	es and K	eset the	e Group)	E	lix	Backflus	Time	
01-Tube-	42		CE								sec	3 sec	- Inne	
					uto Acq el Auto i	uisition Acquisiti	on							

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.

Setting the Channel and Label

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

Setti	ngs	QC/Standardization	Advan			
	Set	Channel				
	Set	Label				
Set		Customized Paramete	er			
Dà	Compensation Matrix					
	Cor	mpensation Library				
	Eve	nts Display Setting				
	Lan	guage Setting				
	Ор	tions				

- 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.

pply C	hannel Setting of Tube	3 to	23
	Name	Sample ID	
1	O Tube4		
1	O Tube5		
V	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	
✓ (Tube1		
	Tube2		
☑ (Tube4		
) Tube5		
	Tube6		
) Tube7		

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

Creating Plots and Gates

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

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

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

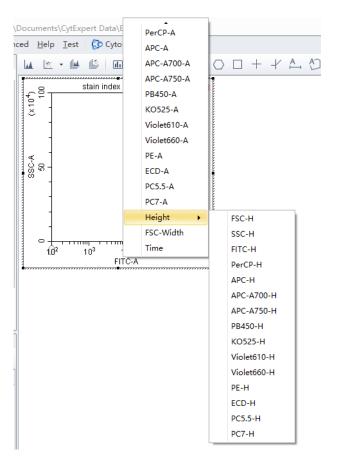
Use the *icons* to generate histograms, dot plots, density plots, pseudo color plots, and contour plot.

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

€ [₽]	Tube1: All Events 💿 🖸	°-	Tube1: All Events	8	°	Tube1: All Events	0	10 No.	ube1: All Events	2	8	Tube1: All Events	⊗ ⊠
× .		10°			2			10 III			-		
58C-A 58		PE-A			PC5.5.A			APC-A 10 ⁴			Count		
		2						2			e -		
		2 <u>-</u>			\$			2			-		
0	50 100 FSC-A (x10 ⁴)	102	10° 10° 10° FITC-A	10"	10*	10° 10° 10° PE-A	10	10" 10"	10" 10" ECD-A	10"	10 ² 10	FITC-A	10" 10"

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.

q. Setting - stain i	index	23
Gain Threshold	Width	
FSC	300 📜 🕨 (1~3000)	
SSC	300 📜 🕨 (1~3000)	
FITC	273 🗼 🕨 (1~3000)	
PerCP	100 📫 🕨 (1~3000)	
APC	399 📜 🕨 (1~3000)	
APC-A700	372 🗼 🕨 (1~3000)	
APC-A750	387 📜 🕨 (1~3000)	
PB450	79 🗼 🕨 (1~3000)	
KO525	176 🗼 🕨 (1~3000)	
Violet610	610 🗼 🕨 (1~3000)	
Violet660	2158 🗼 🕨 (1~3000)	
PE	285 📜 🕨 (1~3000)	
ECD	562 🗼 🕨 (1~3000)	
PC5.5	127 📜 🕨 (1~3000)	
PC7	265 📜 🕨 (1~3000)	

Set As Default

Export to File...

<u>D</u>efault

Import from File... Import from Catalog...

<u>Q</u>C Gain

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

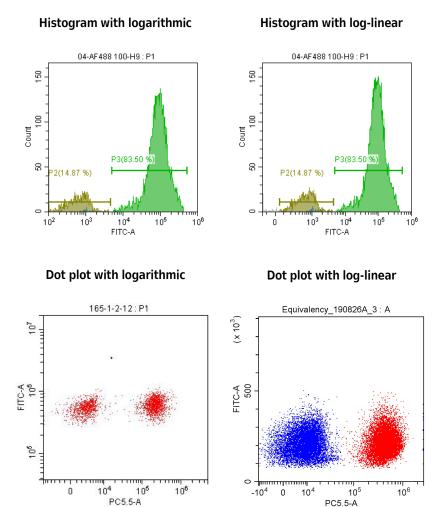
- 2) Select whether to display the axes in logarithmic or linear format for both the X-axis and Y-axis. Enter a value for log-linear coefficient if the log-linear view is desired.
- 3) Select Close.

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.

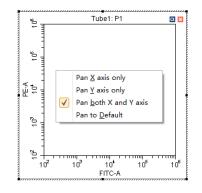
NOTE To reset the axis back to logarithmic, right-click on the axis and select **Property**. Select **X axis Default** or **Y axis Default** to reset the axis.



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

B49006AS

- 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.

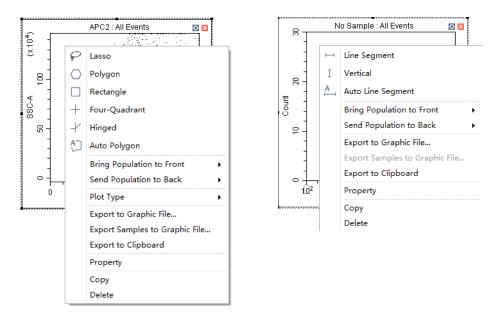


Single side pan: Modifies the axis display range dimensions when panning one axis.

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 $\vdash I \mathrel{\wp} \odot \Box + \checkmark \mathrel{\bigtriangleup} \mathrel{\circlearrowright}$ 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.

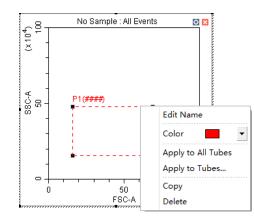


NOTE To add a vertex to a polygon gate:

- 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.

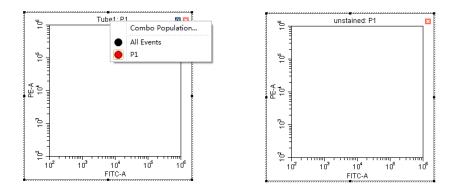
The position of the same gate in different sample tubes may vary. To change the position of a gate and apply the change to all sample tubes accordingly, you can right-click the gate and select **Apply to All Tubes**.

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.

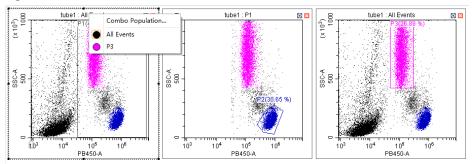
Figure 6.3 shows all gates defined in the example experiment below. Note that the only gate option in plot 1 of Figure 6.4 is P2 for the following reasons:

- 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 6.3 All Gates - Example Experiment



Figure 6.4 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			0		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.

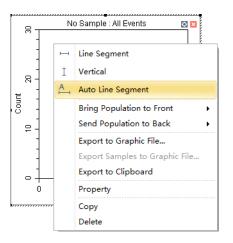
The Population Hierarchy function allows you to view how gates rank in relation to one another. To change the display color, double-click the default color and select the desired color from the drop-down color palette. To change the name of each gate, double-click the name of the desired gate. By hovering 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	% Paren
~ 🔴 Al	l Events	0	####	###
	P1	0	####	###
) P2	0	####	###
	P1 OR P2	0	####	###

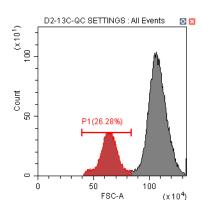
Creating and Adjusting Auto Gates

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.



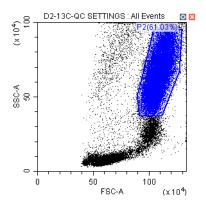
Select the population you want to gate in the histogram to automatically gate that population.



To create an auto polygon gate, select **O** from the toolbar or right-click on the 2D plot and select **Auto Polygon** from the drop down menu.

P	Lasso					
\bigcirc	Polygon					
	Rectangle					
+	Four-Quadrant					
-1	Hinged					
A	Auto Polygon					
	Bring Population to Front					
	Send Population to Back					
	Plot Type 🔸					
	Export to Graphic File					
	Export Samples to Graphic File					
	Export to Clipboard					
	Property					
	Сору					
	Delete					

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

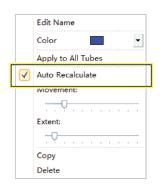
When auto recalculate is turned on, all autogates will recalculate when:

- 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 6.5 for an example of the default movement setting. Refer to Figure 6.6 for an example of the maximum movement setting.



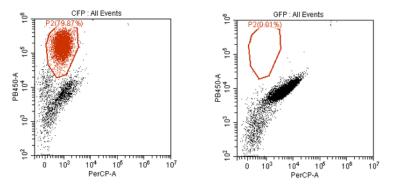
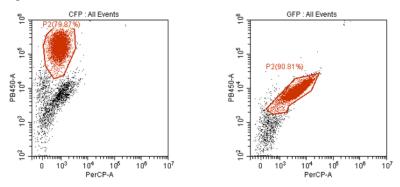


Figure 6.6 Movement - Max Setting



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 6.7 for an example of the default extent setting. Refer to Figure 6.8 for an example of the maximum extent setting.

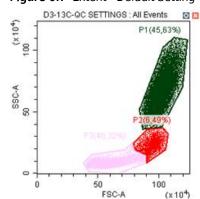
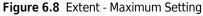
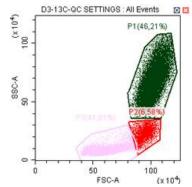


Figure 6.7 Extent - Default Setting





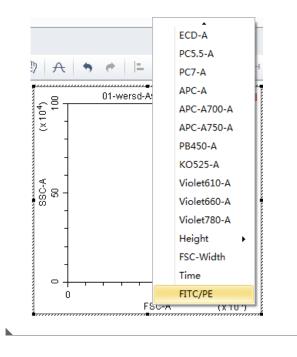
Setting Customized Parameters

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.

Set Customized Paramet	er - Blank+ST+UO26	6	\searrow		23
Name	Parameter	Oper	Parameter	Oper	Modifier
FITC/PE :	= (B525-FITC-A 💌	/ •	Y585-PE-A	·) * ·	1.00
=	= (/ -		·) * ·	1.00
Apply to:					
● Current Tube ○ All Tubes			ок	Cancel	Apply

- 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.

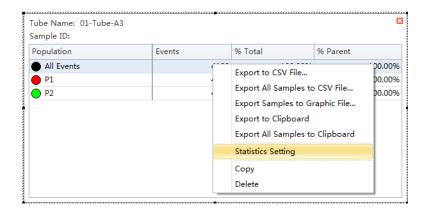


The new parameter name is displayed in the list of parameters and statistic items.

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.

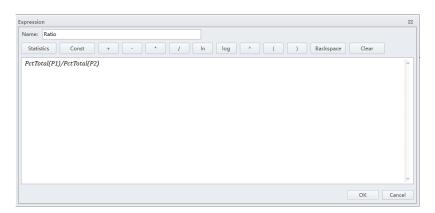


Statistics Set	ting			2
Header	Statistics	Population		
Experie	ment Name			
☑ Tube N	lame			
Sample	e ID			
Record	l Time			
Volum	e			
Abort(%)			
Expres	sion	Edit		
Name				
Equati				
Comm	ent			
			^	
			v	
Operat	tor			
Select A	ll Clear	r All		
Apply to:				
Ourrent	t Tube 🔘	All Tubes	Set As Default	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.

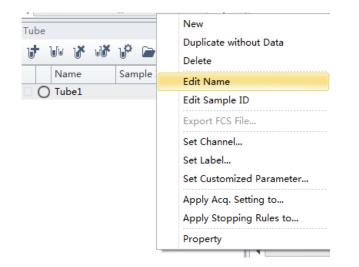
Statistics Setting	23
Header Statistics Population	
Experiment Name	
✓ Tube Name	
▼ Sample ID	
Record Time	
C Volume	
Abort(%)	
▼ Expression Edit	
Name: Ratio	
Equation: PctTotal(P1)/PctTotal(P2)	
Comment	
Operator	
Select All Clear All	
Apply to:	
© Current Tube C All Tubes Set As Default OK Can	el

P1 6799 87.13 %	00.00 87.13	 100.00		
P1 6799 87.13 %	0712	100.00	7803	
7070 0445 %	07.13	87.13	6799	
P2 7370 94.45 %	94.45	94.45	7370	
P3 6904 88.48 %	88.48	88.48	6904	

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.



Laser Settings

To access the Laser Setting window, select **Advanced** > **Laser Setting**. The Laser Setting window appears. Refer to Figure 6.9.

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

aser Setting					Σ
aser	Enable/Disable	Target Power(mW)	/	Actual Power(mV
VUV	🧿 Enable 🔘 Disable	0	Set	Read Power	-
Blue	🔘 Enable 🧿 Disable	0	Set	Read Power	-
Red	🧿 Enable 🔘 Disable	0	Set	Read Power	-
′G	🖲 Enable 🔘 Disable	0	Set	Read Power	-
/iolet	🖲 Enable 🔘 Disable	0	Set	Read Power	-
R	🖲 Enable 🔘 Disable	0	Set	Read Power	-

Figure 6.9 Laser Setting Window [CytoFLEX LX shown]

- 1. Enable/Disable: Enables or disables the laser.
- 2. Target Power (mW): Used to change the laser target power.
 - **NOTE** Laser target power can only be adjusted on the CytoFLEX LX system. The power detector has ± 1 mW tolerance. Refer to Table 6.1 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.

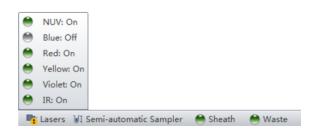
4. Read Power : Reads the current laser power before the flow cell assembly and displays the current laser power in the Actual Power (mW) column of the Laser Setting window. Refer to Table 6.1 for a list of laser ranges.

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 6.1 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 5, 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 **QC Gain** to use the instrument's QC settings.
- **NOTE** In cases where you do not specify your own default parameters, the QC 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. Setting - sta	in index	23
Gain Thresh	old Width	
FSC	1100 🗘 🕨 (1~3000)	
SSC	500 🗘 🕨 🔤 00)	
FITC	1000 🗘 🕨 📃 00)	
PerCP	1000 🗘 🕨 00)	
APC	399 🗘 🕨 🛛 00)	≡
APC-A700	372 🗘 🕨 00)	
APC-A750	387 2 00)	
PB450	79 🗘 🕨 🔤 00)	
KO525	175 2 + (1~3000)	
Violet610	610 + (1~3000)	
Violet660	2158 + (1~3000)	
DE	205 * 1 2000	-
Set As Default	Default QC Gain	
Import from I	File Import from Catalog	
Export to File.	Export to Catalog Close	•

NOTE Optimize the gain settings according to your own experimental goals. The QC Gain 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.

	ting			Σ
Gain	Threshold	Width		
Prim	ary Threshol	d (Trigge	er Level)	
Chanr	nel: FSC		•	
0 M	anual	5000	(>0)	
O Au	tomatic			
Logic (Operator:		Ŧ	
Seco	ndary Thresh	hold (Trig	gger Level)	
Chanr	nel:		-	
() () ()	anual	1	(>0)	
O Au	tomatic			
				Default
Impo	rt From File	. Imp	port From Cata	

- **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.
 - **NOTE** It is recommended to use **Manual** mode to set the threshold parameters to obtain accurate experiment data.

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.

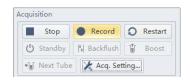
Three stop count collection conditions are available for sample recording:

- 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.

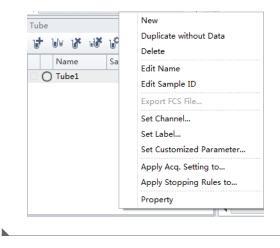
For example, if the event to record is set to record 1,000 P1 events, the software automatically stops recording when P1 events reach 1,000 events. However, the software saves all data acquired, including events outside of P1, when 1,000 P1 events is reached. You can also specify the time to store if 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	Ŧ
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 1.12).



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



Setting Plot Display Conditions

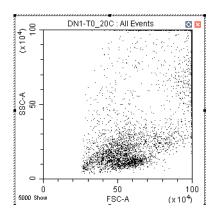
Select Events Display Setting in the Settings menu. The Events Display Setting window appears.

Events Display Setting	23
When displaying plots	:
Oisplay all events	
🔘 Display first	5000 🌲 events acquired
O Display 20	¢ percent of events acquired
ОК	Cancel Apply

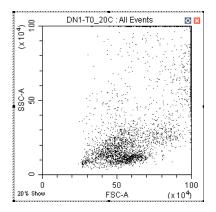
Three display options are available:

- 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*.



Load Sample and Record Data

Before Running Samples

Z	
	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 12, 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 12, 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 6, Data Acquisition and Sample Analysis.

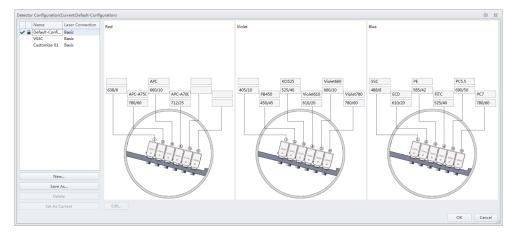
Verifying, Selecting, Editing, and Creating Detector Configuration

Risk of erroneous results. The system will read the selected Detector Configuration even if the optical filters do not match the selected Detector Configuration. You must verify the installed optical filters match the selected Detector Configuration.

- **1** Select **Detector Configuration** in the Cytometer menu to verify the correct detector configuration is selected. To change the configuration:
 - **a.** Select the desired configuration.
 - b. Select Set as Current.

A green 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.

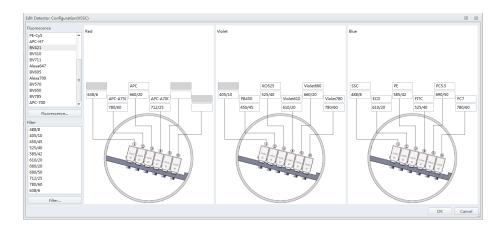
4 If a saved configuration requires changes, edit that configuration.

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.

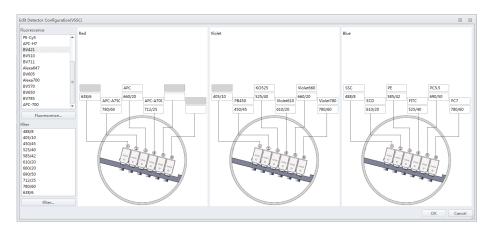


- **c.** Continue to Step 6.
- **5** If an appropriate configuration is not saved, create a new configuration.
 - a. Select Detector Configuration... in the Cytometer menu.
 - b. Select New... and name your configuration.

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.

Fluorescence	
PacBlu	-
KrO	
Violet610	
Violet660	
Violet780	
SSC488	
APC-A700	
APC-A750	
PacificBlue	
KromeOrange	=
PB450	
KO525	F.
Filter	
488/8	
405/10	
450/45	
525/40	
585/42	
610/20	
660/20	
690/50	
712/25	
780/60	
638/6	
Filter	

- 7 When finished, select **OK**.
- **8** Select the appropriate configuration.
- **9** Verify that the correct optical filters are installed in the Cytometer and match the newly created configuration.
- 10 Select Set As Current.
- 11 Select 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 deleter Compensation library QC results U charts setting Are you sure you want to delete the configuration?
	Yes

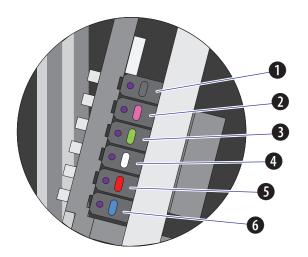
Setting Up Violet Side Scatter (VSSC) Channel

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.

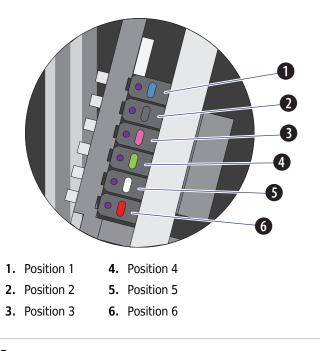
1 Open the Violet WDM lid (see Replacing the Optical Filter in CHAPTER 12, Replacement/ Adjustment Procedures) and remove the 405-nm filter, the 450-nm filter, and a third filter not required for the test, for example, the 780-nm filter.



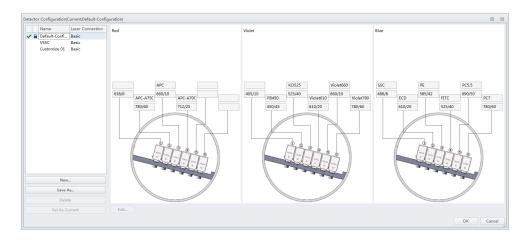
NOTE Refer to Table 6.1 to identify the WDM filter mount color codes.

- **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.



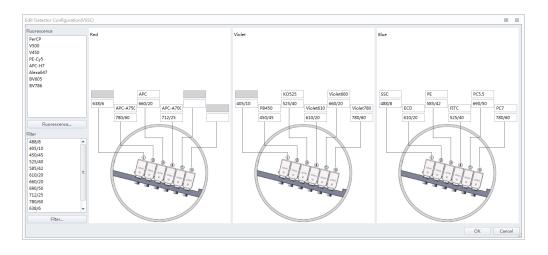
- **3** Start the CytExpert software. Refer to Logging into the Software in CHAPTER 4, Daily Startup.
- **4** Select **Detector Configuration** from the Cytometer menu. The Detector Configuration window appears.



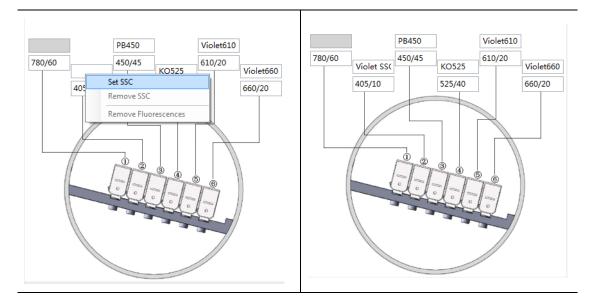
5 Select the Default Configuration and select **Save As**. The Configuration Save As window appears.

The Confi	guration Save As	23
Name:	VSSC	
	ОК Са	incel

- **6** Name the new configuration VSSC and select **OK**.
- 7 Select the VSSC configuration and select **Edit**. The Edit Detector Configuration window appears.



8 Change the filters and channel names according to the filter order in the violet WDM.



9 Right-click the VSSC channel, and select **Set SSC** to set it as the Violet SSC channel.

10 Select **OK** to save the changes and close the Edit Detector Configuration window.

11 Select Set as Current.

12 Select **OK** to save the changes and close the Detector Configuration window.

13 Create a new experiment using the VSSC configuration. Refer to Creating an Experiment.

Sampling and Collecting Data [Without Plate Loader]



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 7, Compensation.

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

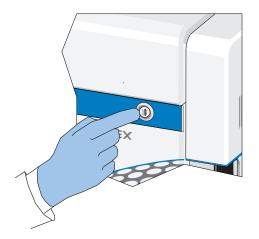
NOTE The first sample tube is already created by default.

- **2** Change the tube name if necessary. Refer to Changing the Tube Name.
- **3** Mix the sample tube intended for testing.
- **4** Ensure that the sample tube holder is in the sample loading position (see Figure 1.12). If the sample tube holder is not in the sample loading position, select **Initialize**.

<u>/ W</u>ARNING

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. Wo Create a new tube Overwrite the data	ould you like to:
	OK Cancel

- **Create new tube.** Saves the current tube and creates an additional tube.
- Overwrite the data. Overwrites the current tube data with new data.
- **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 1.12).

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 record	ed data. Would you like to:
Create a new tube	
◯ 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 contains data. Would you like to:							
© Create a new tube							
◯ Overwrite the data							
	Append data to existing file						
V Events to Record:	10000	Events					
in	All Events	-					
☑ Time to Record:	600	Sec					
Volume to Record:	10	μL					
		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 11, Cleaning Procedures to flush out the sample probe. If you are still unable to clear the sample probe, contact us.

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

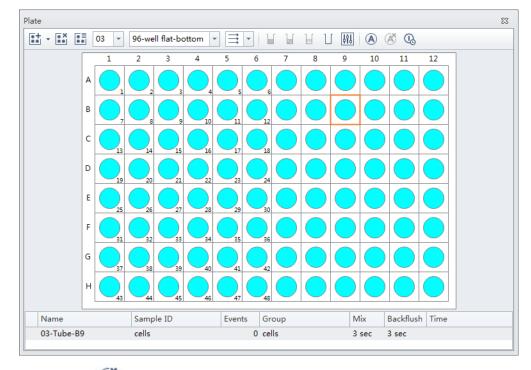
- **1** To run a single well:
 - a. Select the well in the Plate window.
 - **b.** Select **Run** to prompt the system to begin sample aspiration.

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]



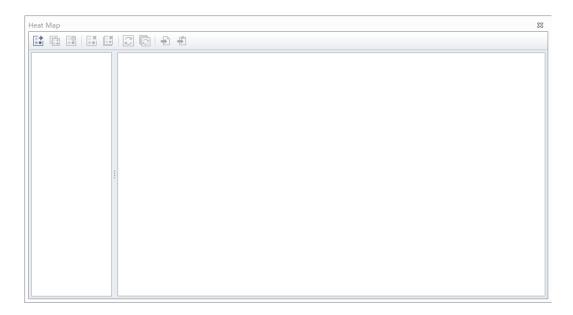
NOTE Select *intersection* to remove the auto record setting from the selected wells.

2	Select Auto Record to begin sample acquisition.
	NOTE Select to skip the current well and move to the next well for acquisition.
	NOTE Select Pause to pause acquisition. The software completes the current sample well acquisition and pauses when the current well is complete. The plate can be loaded and unloaded while the sample acquisition is paused.
	NOTE Select I Resume to continue the acquisition.

Creating a Heat Map [with Plate Loader]

IMPORTANT The heat map function is only available in plate mode.

1 Select **s** from the Tube management controls (refer to Test Tubes in CHAPTER 2, Using the CytExpert Software). The Heat Map window appears.



No.	Expression	Label		U	se Cust	om Ran	ge	Min		Max		Actua	al Ran	ge	
1					[
Dis	play Value											Add		D	elete
Color	Setting of All Parameters		We	ell											
lase (Color: 🗾 🔻 Bands:	5 (2-10)													
Per	rcentile 🛛 🔘 Fixed Range			1	2	3	4	5	6	7	8	9	10	11	12
No	Maximum Limit 🔲 No I	Minimum Limit	A												
	100 🌲 %		В												
			c	:											
-[80 ‡%		D	. —											
	60 🌲 %		E	-											
			F												
	40 🌲 %		G	i											
			н	1											
	20 🌲 %			Ĺ											

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	-

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 (C) Different settings per parameter

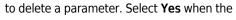
NOTE Select **Same setting for all parameters** to use the same color for all parameters. Select **Different settings per parameter** to use different colors for different parameters.

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

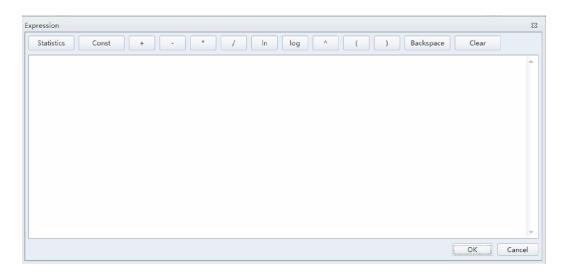
NOTE A maximum of 6 parameters can be added to a single heat map.

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



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.

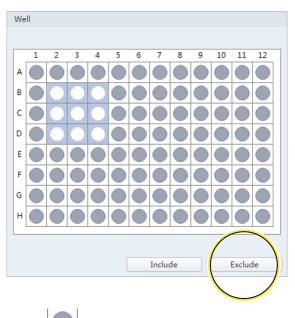


- **c.** Enter the desired expression for the selected parameter then select **OK**. The Actual Range displays in the Parameter section of the Heat Map window.
- **d.** Select the **Use Custom Ranges** checkbox if needed and enter the Min and Max ranges.

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

7 Select any wells that should be excluded from the heat map in the Wells section of the New Heat

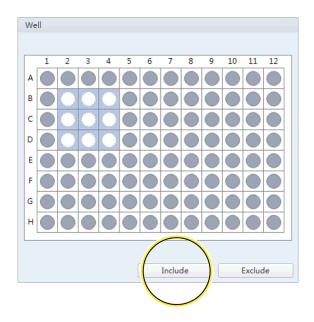
Map window then select	Exclude
------------------------	---------





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
No Maxin imit No Minimum Limit
× %
80 🗘 %
60 ‡ %
40 1 %
20 ‡ %
0 2 %

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 ‡ %
- 75 ‡ %
50 ‡ %
- 25 ‡ %
0 1 %

NOTE You can choose between 2 and 10 color bands.

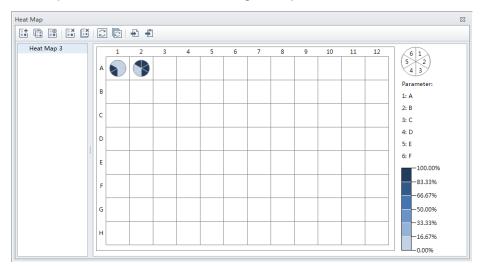
- c. Select **Percentile** to assign colors based on a percentage range.
 - **NOTE** If **Use Custom Range** is selected, the percentile is calculated according to "Min" and "Max". If **Use Custom Range** is not selected, the percentile is calculated according to "Actual Range".

Or

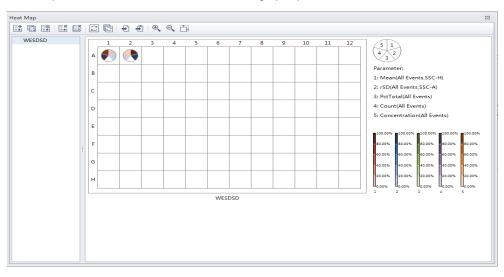
Select **Fixed Range** to assign colors based on a fixed range specified by the user. The heat map is created directly based on the result of the expression. The color of the heat map displays according to the legend range.

NOTE Fixed Range can only be used with one parameter.

- **NOTE** If **Different settings per parameter** is selected, repeat Steps a-c to assign the color setting to each parameter.
- **9** Select **OK**. The New Heat Map window closes to display the Heat Map window.

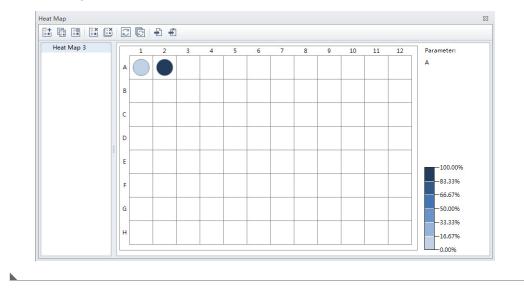


Heat Map with 6 Parameters [Same setting for all parameters]



Heat Map with 6 Parameters [Different settings per parameter]

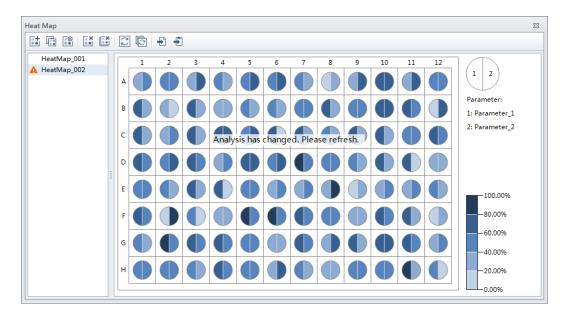
NOTE When viewing more than one parameter, the parameter location relative to the pie chart is visible in the top, right corner of the Heat Map screen.



Heat Map with 1 Parameter

Refreshing a Heat Map

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



Select 😰 from the heat map toolbar to refresh the analysis.

Modifying Existing Heat Map Settings

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

lame:	sss									🗌 Disp	lay N	lame	Plate	01	
olor Mode:	O Dif	ferent	settin	igs pe	r par	ameter									
Parameter															
No. Expres	sion	Label	Label		Use Custom Range Min					Max	Actual Range				
1 rCV(P1	rCV(P1,B525-FITC-H) ····											0.05-1.46			
🔲 Display Va	alue											Add		De	elete
Color Setting	g of All Parameter	s	Well												
Base Color:	Bands:	5 📜 (2-10)													
Percentile	C Fixed Range	e		1	2	3	4	5	6	7	8	9	10	11	12
🗌 No Maxir	num Limit 🛛 🔲 No	o Minimum Limit	A												
	100 🗘 %		В												
			с												
_	80 🗘 %		D												
			E												
	60 🗘 %														
			F												
	40 🗘 %		G												
			н												
- I	20 🗘 %														
	0 ‡ %									Inc	lude			Exclu	de

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 if 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	icel

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

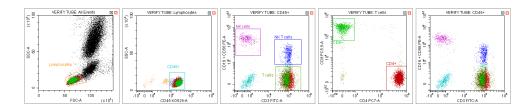
Heat maps can be exported as a graphics file (.bmp or .emf) or to a clipboard (.bmp).

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

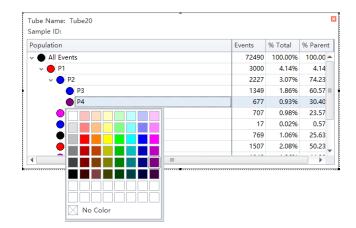
To export a heat map to a clipboard, select 🛐 from the heat map toolbar.

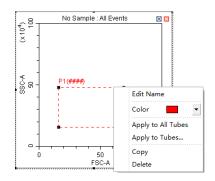
Analyzing and Exporting Data

- **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.

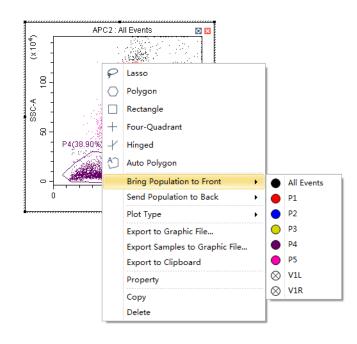


- **NOTE** Changing a gate's position does not affect the positions of other gates already established on a given sample tube. Each test tube individually records the positions of its associated gates. If you need to make a change that concerns all the tubes, you must select the gate, then right-click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

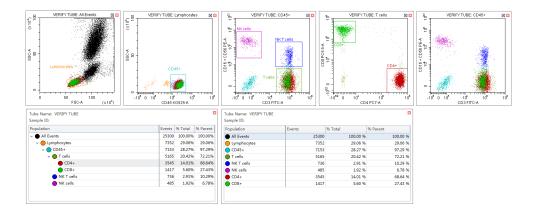
Population	Even	Export to CSV File Export All Samples to CSV File	irent
All Events			
P1		Export Samples to Graphic File	
Q1-UR		Export to Clipboard	
Q1-UL		Export All Samples to Clipboard	
Q1-LL		Statistics Setting	
Q1-LR		Сору	
		Delete	

Statistics Setting 23	Statistics Setting							
Header Statistics Population	Header Statistics Population							
□ Experiment Name	Parent Population Events, % Total % Parent Events/µL(V) Events/µL(B)							
☑ Sample ID	Beads Population:							
Record Time	Beads Count: 0 Sample Volume: 0.00 µL							
🗌 Volume								
Abort(%)	Parameter Mean GeoMean Median rCV rSD CV							
Expression Edit	FSC-A							
Name:	SSC-A							
Equation:	FITC-A							
	PE-A O O O O O							
Comment	ECD-A							
	PC5.5-A							
	PC7-A							
	APC-A							
	APC-A700							
	APC-A750							
	Select All Clear All @ Area @ Height @ Area + Height							
Operator	Preview Events % Total % Parent							
Select All Clear All								
Apply to:	Apply to:							
Current Tube Cancel Cancel 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.

Statistics Setting	1					23
Header Sta	atistics	Population				
All Eve	ents					
V 🔴 P1						
🔽 🔵 Q1-U	R					
🔲 🔴 Q1-UI	L					
🔲 🔴 Q1-LL						
V Q1-LR	R .					
🔲 🔵 Q2-UI						
🔲 🔴 Q2-UI						
🔲 🔴 Q2-LL						
🔲 🔴 Q2-LR						
🔲 🔴 Q3-UI						
🔽 🔴 Q3-UI						
Q3-LL						
🔽 🔵 Q3-LR						
Select All	Clear	All				
Apply to:						
Ourrent Tu	ube 🔘	All Tubes	🔲 Set As Defa	ault	ОК	Cancel

The final generated plots appear as below.

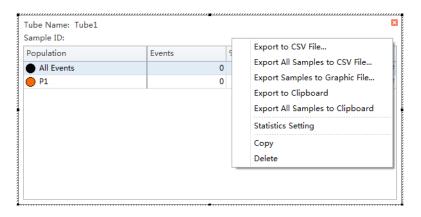


8 Right-click a plot and select **Export to Clipboard** or **Export to Graphic File** from the drop-down menu to select an image to export.

	Tube1 : Al	l Ever	nts 🛛 🔯
(x10 ²) 100 (x10 ²)			
SSC-A		P	Lasso
-		\bigcirc	Polygon
- S			Rectangle
1		+	Four-Quadrant
L	··	-1	Hinged
	50 FS	Ð	Auto Polygon
			Bring Population to Front
			Send Population to Back
			Plot Type 🔸
			Export to Graphic File
			Export Samples to Graphic File
			Export to Clipboard
			Property
			Сору
			Delete

- **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.

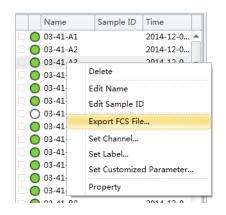
10 Export the FCS file if necessary. Refer to Exporting FCS Files.

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	-
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.

File Version: FCS 2.0 FCS 3.0 FCS 3.0 High auto-fluorescence) Channel Parameter Type FSC ① Linear Log SSC ① Linear Log FITC Linear Log PE Linear Log ECD Linear Log	
FSC Image: Constraint of the second	
SSC	
FITC O Linear O Log PE O Linear O Log	
PE O Linear O Log	
ECD Olinear Olog	
	=
PC5.5 🔘 Linear 🔘 Log	
PC7 🔘 Linear 🔘 Log	
APC 🔘 Linear 🔘 Log	
APC-A700 🔘 Linear 🔘 Log	
APC-A750 🔘 Linear 🔘 Log	_
PB450 🔘 Linear 🔘 Log	
KO525 O Linear O Log	
Violet610 🔘 Linear 🛛 🧿 Log	
🗹 Area 📝 Height	

- **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 party 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.

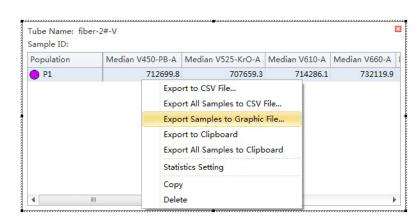
Exporting Multiple FCS Files

Step1:	Sele	ct tu	bes.			
			Name	Sample ID	Time	Т
		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-B8		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	
,		\sim	~ ~ ~ ~ ~ ~		001110.001010.00	

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 Right-click on the plot or statistics to export.

2 Select Export All Samples to Graphic File. The Export Tubes to Graphic Files window appears.

Path:	C:\L	C:\Users\lenovo\Documents								
		Name	Sample ID	Time						
	0	fiber-new-3#-N	•	2019-07-17 10:52:03	-					
	0	fiber-new-4#-N		2019-07-17 10:54:22						
	0	fiber-new-5#-N		2019-07-17 10:58:24						
	0	fiber-new-6#-N		2019-07-17 11:01:09						
	0	fiber-new-7#-N		2019-07-17 11:03:42						
	0	fiber-new-8#-N		2019-07-17 11:06:02						
	0	baseline2		2019-07-17 14:18:06						
	0	fiber-new-1#-V1		2019-07-17 14:28:18						
	0	fiber-new-5#-V1		2019-07-17 14:31:07						
1	0	TUBE 1		2019-07-17 14:40:42	=					
	0	TUBE 2		2019-07-17 14:41:09						

- **3** Select the desired tubes to export.
- **4** Select the path.
- 5 Select OK.

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

Importing and Exporting Instrument Settings

The CytExpert software supports importing and exporting instrument settings to facilitate the experiment process. Only instrument settings identical to the current configuration can be imported with current detector settings.

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

Importing Instrument Settings

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

NOTE Instrument settings can only be imported into tubes where data has not yet been recorded.

2 Select Import From File, locate the file with the required instrument settings, or select Import From Catalog to import the instrument settings.

FSC	500 2 + (1~3000)					
SSC	500 ‡ ► (1~3000)					
IR840-AF790	71 🗘 🕨 (1~3000)					
IR885	55 [^] + (1~3000)					
V450-PB	57 🗘 🕨 (1~3000)					
V525-KrO	159 📜 🕨 (1~3000)	Acq. Setting Catalog	9			
V610	394 📜 🕨 (1~3000)	Item	Date Created	Detector Configurati	Gain Threshold	Width
V660	241 + (1~3000)	Default Se	ett 2016-05-05 11:2		Channel Name	Gain
		2016-11-0	7 2016-11-07 15:1		FSC	500 -
V763	51 1 + (1~3000)				SSC	500
NUV450	69 [↑] → (1~3000)				Fitc	500
NUV525	500 2 + (1~3000)				mCherry	500
					PerCP	500
NUV675	145 🗘 🕨 (1~3000)				APC	500
R660-APC	288 2 + (1~3000)				APC-A700	500
					APC-A750	500
R712-APCA700	282 282 (1~3000)				PE	500
R763-APCA750	276 🗘 🕨 (1~3000)				ECD PC5.5	500
Y585-PE	406 + (1~3000)				PC3.5 PE-AF680	500
1363-PE	400 , (~3000)				PC7	500
Y610-mCherry	158 🗘 🕨 (1~3000)				PB450	500
Y675-PC5	507 🗘 🕨 (1~3000)				ко525	500
Y710-PC5.5	344 🗘 🕨 (1~3000)				Lm	port Delete Close
Y763-PC7	416 🗘 🕨 (1~3000)					
B525-FITC	180 🗘 🕨 (1~3000)					
B610-ECD	130 🗘 🕨 (1~3000)					
B690-PerCP	1103 . + (1~3000)					
Set As Default	Default Recommended					
Import from File	Import from Catalog					

[CytoFLEX LX Shown]

3 Select Close.

Exporting Instrument Settings

- 1 Select the desired sample tube to export. Then select \swarrow 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 Importing and Exporting Compensation in CHAPTER 7, 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
ß	Batch Export to PDF File

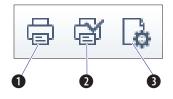
• **Print Preview.** Used to access the Preview screen.

	- B 2
● 段 B (
$ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	
Page 1 of 3	1005 🕤 ——— 🔿

- 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 Print				Σ
	Name	Sample ID	Time	
	Tube1		2018-05-09 13:09:20	
	Tube2		2018-05-09 13:12:54	
	Tube3		2018-05-09 13:13:33	
	Tube4		2018-05-09 13:17:16	
Select All				
🔲 Merge Pri	inting			
in merge Ph	inung			
			OK Car	icel

- **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
	Tube1		2018-05-09 13:09:20
	Tube2		2018-05-09 13:12:54
	🔵 Tube3		2018-05-09 13:13:33
	🔵 Tube4		2018-05-09 13:17:16
Select	All		

- **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 9, Daily Shutdown.

CHAPTER 7

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 7.1 and Figure 7.2 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.

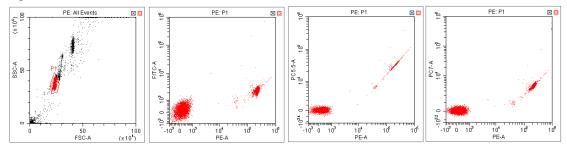
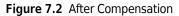
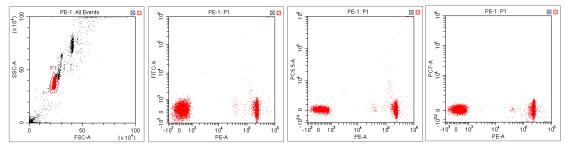


Figure 7.1 Before Compensation





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 6, 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.

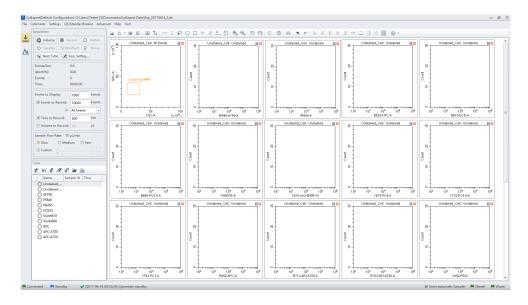
If a negative population is not present in each single color tube, then an unstained control tube is recommended.

- **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.

Area	🔘 Height				
Use	Tube	Label	Lot No.	Sample T	уре
1	Unstained_Cell			Cell	🔘 Bead
\checkmark	Unstained_Bead			Cell	🔘 Bead
\checkmark	FITC			Ocell	🔘 Bead
\checkmark	PerCP			Ocell	🔘 Bead
\checkmark	APC			Ocell	🔘 Bead
\checkmark	APC-A700			Ocell	🔘 Bead
\checkmark	APC-A750			Cell	🔘 Bead
\checkmark	PB450			Cell	🔘 Bead
\checkmark	KO525			Cell	🔘 Bead
\checkmark	Violet610			Cell	Bead
\checkmark	Violet660			Cell	🔘 Bead
\checkmark	PE			Cell	🔘 Bead
\checkmark	ECD			Cell	🔘 Bead
\checkmark	PC5.5			Cell	🔘 Bead
\checkmark	PC7			Cell	🔘 Bead

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 present in the panel
- 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

The design of CytoFLEX system makes Gain independent compensation possible. CytExpert software automatically recalculates the compensation matrix according to gain. Manual adjustment may be applied but not necessary because it may introduce inaccurate results.

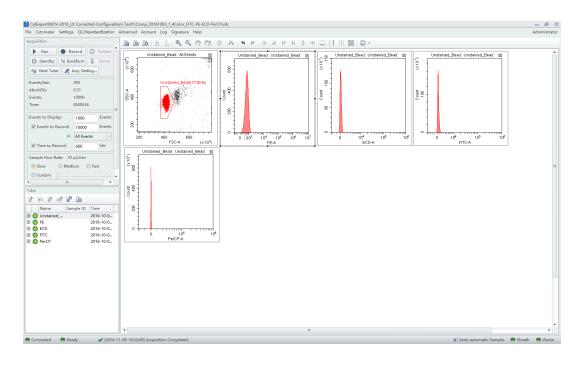
Defining the Negative Population Using Unstained Samples

1 Confirm that the instrument has been initialized. Refer to Initializing the Instrument in CHAPTER 4, Daily Startup.

A CAUTION

Risk of erroneous results. Calculations based on excessively small volumes of sampled data can be inaccurate. Ensure that more than 1,000 positive events and more than 1,000 negative events are sampled. If the ratio of positive cells is comparatively low, increase the number of acquisition events to a suitable amount.

2 Import the gain setting and apply the setting to all tubes. Refer to Adjusting the Gain in CHAPTER 6, 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 6, 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 1.12).

- **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 number of positive cells is comparatively low, increase the number of acquisition events to a suitable amount.

- **4** Move the gate in the FSC/SSC plot so that it encloses the desired population. Move the positive gate in the plot so that it encloses the positive population. If necessary, move the positive gate so that it encloses the positive population.
 - **NOTE** Figure 7.3 shows an example of selecting the positive population when the negative population is defined by the unstained sample.

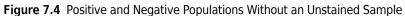


Figure 7.3 Positive Population Selected from the Single-Stained Sample

1. Positive population

NOTE Figure 7.4 shows an example of selecting both the 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.

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	mpensation Setup										
<i>l</i> ≙	Cor	Compensation Calculation										
<i>L</i> ∕≙	Compensation Matrix											
	Eve	Events Display Setting										
	Lan	guage Setting										
	Ор	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	

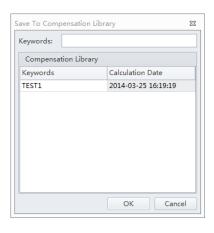
NOTE The primary fluorescence channels are listed in columns; the secondary fluorescence channels are listed in rows.

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.
- **NOTE** The Compensation range is from -500 to 500 depending on the spillover percentage calculated. The CytExpert software displays the value in either black, or orange, or red as a visual clue.
 - Black denotes the value falls within 0 99.99, which is acceptable.
 - Orange denotes the value falls within 100 399.99, or -99.99 to -0.01, which requires special attention.
 - Red denotes the value is within 400 500 or -500 to -100, which is unacceptable.
- **3** Select **Save As** to export the compensation matrix as a .comp file and specify where to save it.

NOTE The compensation matrix can also be imported for use in other experiments.

- **4** Select **Save To Compensation Library** to save the single color compensation values in the compensation library.
- 5 Specify the key words and select **OK**.



NOTE The settings stored in the compensation library are specific to the detector configuration. The compensation library can only be applied when the detector configurations are the same.

At any time, saved compensation experiments can be reopened and the compensation values recalculated.

6 Select Close.

Creating a Compensation Experiment [With Plate Loader]

Before creating a compensation experiment, you must verify the instrument's detector configuration settings (see Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, 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.

mpens	ation on:				Plate Type: 96-well flat-bottom 🔹 Sampling Sequence:
Area	O Height				Set As Sample Well Set the Sample Well As Empty
Use	Tube	Label	Lot No.	Sample Type Loca	on Set Tube Unstained_Cell to Well
1	Unstained_Cell			⊙ Cell 🔘 Bead	1 2 3 4 5 6 7 8 9 10 11 12
\checkmark	Unstained_Bead			🔘 Cell 🔘 Bead	
\checkmark	FITC			Cell Bead	
1	PerCP			💿 Cell 🔘 Bead	
1	APC			Cell O Bead	
1	APC-A700			💿 Cell 🔘 Bead	
1	APC-A750			Cell OBead	
1	PB450			Cell Bead	
1	KO525			💿 Cell 🔘 Bead	
1	Violet610			💿 Cell 🔘 Bead	
\checkmark	Violet660			💿 Cell 🔘 Bead	F <u>0000000000000000000000000000000000</u>
1	PE			Cell OBead	
1	ECD			Cell Bead	
\checkmark	PC5.5			💿 Cell 🔘 Bead	
1	PC7			💿 Cell 🔘 Bead	
					☑ Mix 3 sec ☑ Backflush 3 sec

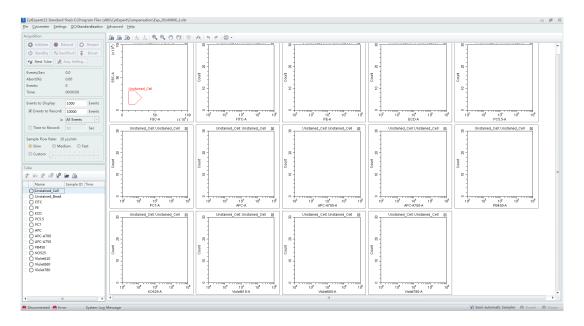
5 Select the Mix and Backflush settings in the bottom, right of the Compensation Setup window.

- **6** Assign the well locations.
 - **a.** Select the fluorochrome.
 - **b.** Select the desired sample well location for the fluorochrome.
 - c. Select Set As Sample Well.

NOTE The well location populates in the location column.

- **d.** Repeat Steps a-c for each fluorochrome.
- 7 Select OK.

After confirmation, the software automatically generates the following compensation experiment.



NOTE Select Area to calculate compensation based on the Area measured. Alternatively, select Height to calculate compensation based on the Height measured.

NOTE If the plate settings require modification, select

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 Sampling and Collecting Data [With Plate Loader] in CHAPTER 6, 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 7, 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.

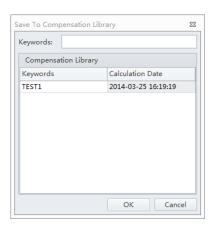
The Compensation Matrix window appears, displaying the calculated compensation values.

Channel	-FITC%	-PE96	-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	

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 6, 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.

Tube	J.	v° /	<u>/</u> @	
Name		Samp	le ID	Time
O Uns ⁺⁻ⁱ	Imp	ort FC	S File	
O NU	Exp	ort FCS	File	
	Арр	ly Stop	ping F	Rules to
0 R66	Prop	perty		
C R712-AF				
V675-PC				
C Y710-PC	5.5			
○ Y763-PC				
B525-FI B610-EC				

(in front of a test tube indicates that the corresponding data have been imported.

Tube	•		
U.	Vr V vV V	۵	
	Name	Sample ID	Time
	Unstained_Cell		
	FITC		
-) pe		
) PC5.5		
) PC7		
) ко525		

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.

NOTE The CytoFLEX platform uses the positive-negative method to calculate compensation.

$$B(k+1) = \frac{S(k) - B(k)}{1024} + B(k)$$

Where S(k) denotes the real-time background noise being measured.

Where B(k) denotes the background noise being filtered.

where B(k+1) denotes the noise background base being filtered at the next point.

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 ²⁰/₂ 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 apply the gain independent compensation capability, i.e. importing the compensation values with or without recalculating the compensation matrix 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 🕅
Import compensation matrix and convert based on the current gain.
Import compensation matrix only.
 Import compensation matrix and gain.
OK Cancel

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.

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

[CytoFLEX LX Shown]

Use	Show Aut	tofluoresce	ence																		Ar	rea
utofl.	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%	-¥763- 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.
0.00	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.
0.00	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	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.
0.00	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.
0.00	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.
0.00	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.
0.00	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.
0.00	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.
0.00	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.
0.00	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.
0.00	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.
0.00	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.
0.00	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	

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

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.

Key	words List		Select fluorencense			
v	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	
		- - - -	APC		CD3 20140805-Comp4 2014-08-05 14:09	
>			APC-A700			
>			APC-A750			
>	20140805-Comp4		PB450		20140805-Comp2 2014-08-05 1	
			KO525		20140805-Comp2 2014-08-05 1	
			Violet610			
			Violet660			
				Violet780		

 $\mathbf{3}$ 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 Autofluorescence														ea								
utofl.	Channel	-AF79 0%	-PF840	-PB45 0%	-КО52 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
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
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
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
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
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
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
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
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
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
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	

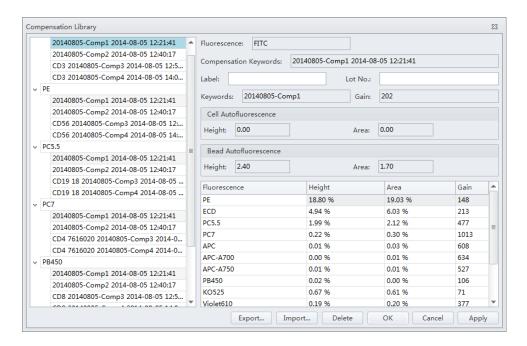
4 Select Save.

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.

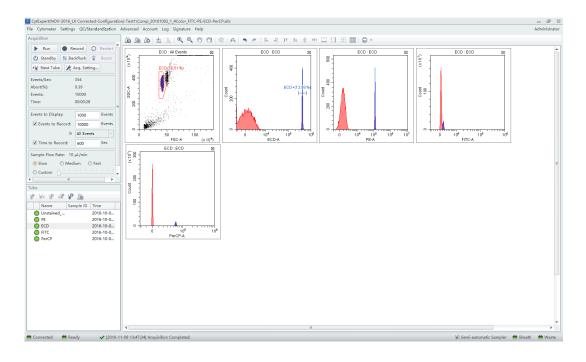
Adding Channels for Compensation

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.

CHAPTER 8 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 6, 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.

🗱 CytExpert(Default-Configuration)-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 <u>f</u> rom Template			
	New Compensation			
	Open Experiment Ctrl+O			
	Open Com <u>p</u> ensation			
	<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	+		
	Recent Temp <u>l</u> ate			
	Recent Compensation	•		
	<u>C</u> lose Experiment			
	E <u>x</u> it			

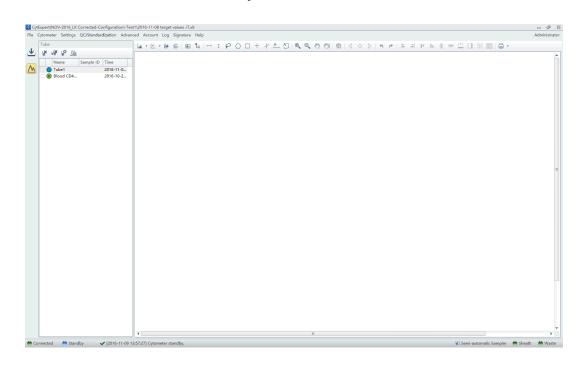
Imported data files appear in the Tube screen.

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 \bigwedge on the left to enter the Analysis screen.

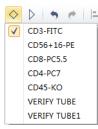


- **2** Copy plots obtained during data acquisition.
 - **a.** If you need original plots used during data acquisition, select 坐 to access the Acquisition screen.
 - **b.** Select the appropriate plots.
 - **c.** Right-click the selected plots and select **Copy** from the drop-down menu or press Ctrl+C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

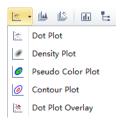
The CytExpert software supports histogram and dot plot data overlay functionality, allowing you to combine data from differing sources onto the same histogram or dot plot.

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

 Image: Image

Or

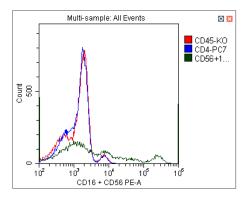
Select **Dot Plot Overlay** under the dot plot icon drop-down list to create a new dot plot overlay.



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	> • <i>e</i> =
	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 6, 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 12, Replacement/Adjustment Procedures) prior to collecting data for volumetric analysis:

- Select the *cells/µL(V)* checkbox to calculate concentration directly.
 - **NOTE** The direct calculation of concentration can be affected by several conditions such as, the sample's viscosity and sample mixing. Uncalibrated sample volume uptake rates may lead to erroneous results.
- If using reference beads to calculate the concentration, select the *cells/µL(B)* checkbox and select the gated **Beads Population**. Enter the total number of reference beads as the **Beads Count**, as well as the sample volume in total. The software automatically calculates the original sample concentration based on the input values. (You can also enter the reference bead concentration directly in the beads count field and set the sample volume as 1.)

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

- The sample concentration is 2×10^4 - 10^7 units/mL.
- Samples are thoroughly mixed before loading and that they exhibit no apparent subsidence throughout the testing process.
- The detection rate is maintained at less than 10,000 events/second throughout the sampling process. When the detection rate does not exceed the stated event rate, running at medium to high acquisition speeds are considered more accurate.
- A constant sampling rate is maintained when recording data.
- You acquire at least 10 µL of sampling volume.

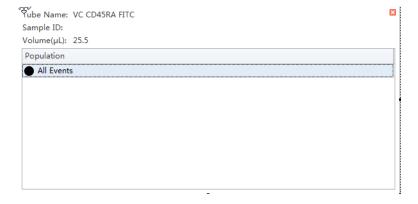
In the Statistics Setting screen, select **Volume** and the concentration item to see the corresponding information in the statistics table.

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

[CytoFLEX LX Shown]

Statistics Setting 83	Statistics Setting	
Header Statistics Population	Header Statistics Population	
□ Experiment Name	Parent Population I Events I % Total I % Parent Events/µL(V)	
✓ Sample ID	Beads Population: Select	
Record Time	Beads Count: 0 Sample Volume: 0.00 µL	
Volume		1
Abort(%)	Parameter Mean GeoMean Median rCV rSD	(
Etypession Edit	FSC-A	[
Name:	SSC-A	[
Equation:	FITC-A	[
	PE-A D D D D	[
Comment	ECD-A	
	PC5.5-A	l
	PC7-A	
	APC-A	
	APC-A700	1
· · · · · · · · · · · · · · · · · · ·	Select All Olear All O Area O Height O) Area + H
Operator	Preview	
	Events % Total % Parent	
Select All Clear All		
Apply to:	Apply to:	
Current Tube All Tubes Set As Default OK Cancel	Current Tube All Tubes Set As Default OK	C

[CytoFLEX LX Shown]



Adjusting Compensation Settings

Data compensation can be carried out at any time. You can select the desired tube in the tube list

on the left side of the screen and select in the compensation controls, or select **Compensation Setup** in the Compensation menu. Refer to Adjusting Compensation in CHAPTER 7, Compensation, for detailed instructions on adjusting compensation settings.

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Exporting Results

Refer to CHAPTER 6, Data Acquisition and Sample Analysis.

Data Review Exporting Results

CHAPTER 9 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 11, Cleaning Procedures.
- **2** If necessary, empty all waste liquid from the waste container. Refer to Emptying the 4 L Waste Container in CHAPTER 12, 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 11, 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 in CHAPTER 6, 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 11, Cleaning Procedures.

CHAPTER 10 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
- 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 Platform flow cytometer can be configured with 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 10.1 and Figure 10.2 for the Laser Warning Label on the Cytometer optical bench.
- See Figure 10.3 and Figure 10.4 for the Laser Warning Label on the optical bench (located Inside the Cytometer).

See Figure 10.6 and Figure 10.7 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 10.1.

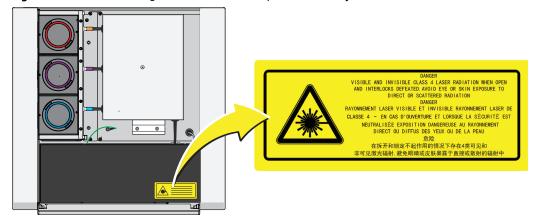


Figure 10.1 Laser Warning Label on the Laser Optical Bench [CytoFLEX]

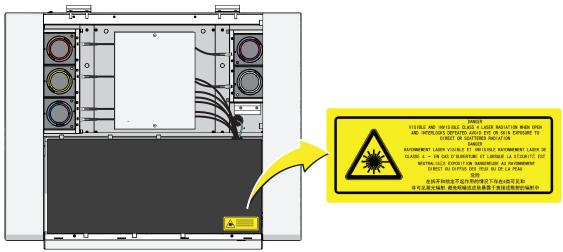


Figure 10.2 Laser Warning Label on the Laser Optical Bench [CytoFLEX LX]

Figure 10.3 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX and CytoFLEX S]

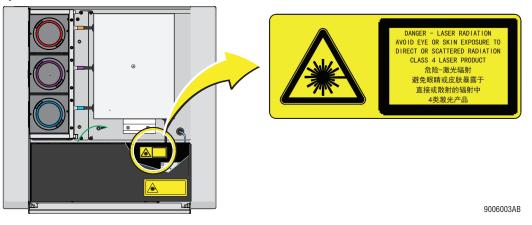
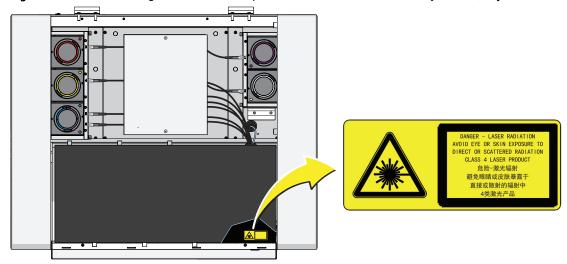


Figure 10.4 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX LX]



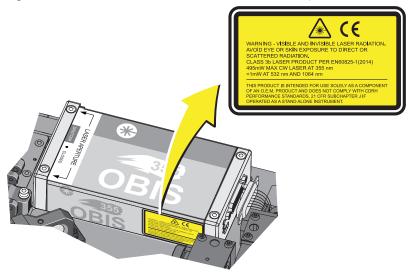
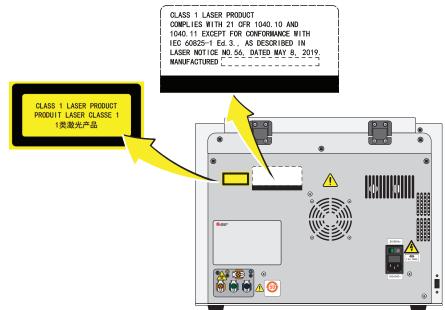


Figure 10.5 Laser Warning Label on the 355-nm Laser [CytoFLEX LX]

Figure 10.6 Laser Warning Labels on the Cytometer Back Cover [CytoFLEX and CytoFLEX S]



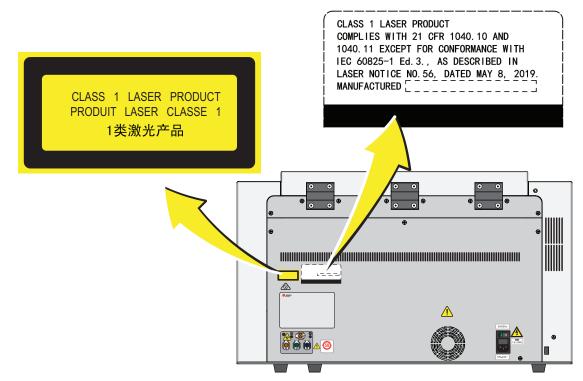


Figure 10.7 Laser Warning Labels on the Cytometer Back Cover [CytoFLEX LX]

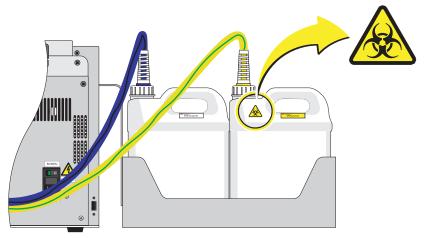
Hazard Labels and Locations

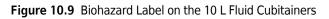
Carefully read the hazard warning labels on the instrument. The hazard labels are located on the instrument as indicated.

NOTE If a label is missing or unclear, contact us.

Biohazard Label and Location

Figure 10.8 Biohazard Label on the 4 L Fluid Containers





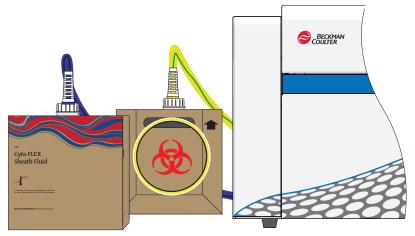
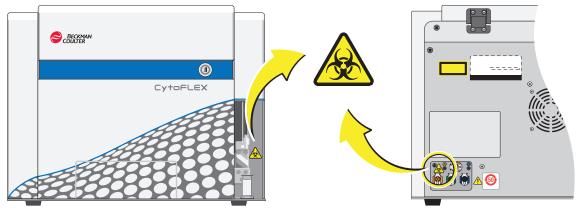
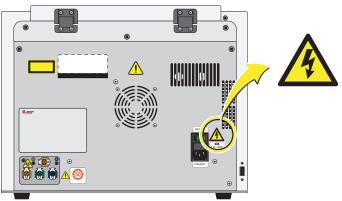


Figure 10.10 Biohazard Label Located in the Sample Station and on the Back of the Cytometer [CytoFLEX Shown]



Electrical Shock Hazard Label and Location

Figure 10.11 Electrical Shock Hazard Label by the Power Switch [CytoFLEX or CytoFLEX S]



Caution Labels and Location

Figure 10.12 Caution Labels [CytoFLEX or CytoFLEX S]

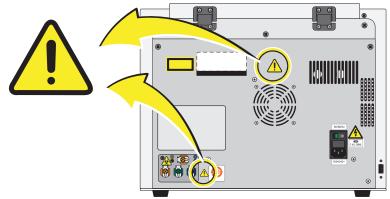


Plate Loader Hazard Labels and Location

Figure 10.13 Plate Loader Hazard Labels

Disposal Precaution



🕂 WARNING

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

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

Troubleshooting Table

Table 10.1 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]	 Ensure the power switch is in the on position on the back of the Cytometer. [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. 	 Select Turn On in the Cytometer menu. [CytoFLEX LX]
		3. Ensure that the power cable is securely connected to the back of the Cytometer.
		4. Replace the fuse. Refer to Replacing the Fuse in CHAPTER 12, Replacement/ Adjustment Procedures.
		5. If the problem persists, contact us.
The Workstation cannot be turned on.	 The power cable is not securely connected. The Workstation was restarted too fast. 	1. Ensure that the power cable is securely connected to the back of the Cytometer.
		2. Unplug the power cable. Wait 10 seconds, then plug the power cable back in. Then, restart the computer.
		3. If the problem persists, contact us.

Problem	Probable Cause	Corrective Action
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 1.22. Restart the software. Restart the Workstation. Refer to Initializing the Instrument in CHAPTER 4, 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
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. 	 If the problem persists, contact us. 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 1.22. Restart the Workstation. Refer to Initializing the Instrument in CHAPTER 4, Daily Startup. Restart the software. WARNING Risk of biohazardous contamination if you have skin contact with the waste container, its contents, and its associated tubing. The waste 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 12, Replacement/Adjustment Procedures. If the problem persists, contact us.

Table 10.1	Troubleshooting	(Continued)
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Problem	Probable Cause	Corrective Action	
The fluid status information displays red for <i>Sheath</i> and/or <i>Waste</i> even though the sheath fluid container is full and the waste container is empty.	 Instrument data connection error. The sensor connection is not working properly. The sensor does not work properly. The sheath fluid/waste harnesses have been secured on the wrong container. 	 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 1.22. Restart the software. 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. Replace the sheath fluid harness and/or the waste harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 12, Replacement/ Adjustment Procedures. 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 4, Daily Startup. If the problem persists, contact us. 	
The sample flow rate is unstable.	 The sample probe is clogged. The sample contains aggregates or clumps. There are air bubbles in the flow cell. The sample peristaltic pump tubing is aged. The sample peristaltic pump tubing is not properly connected. 	 Run Prime. Refer to Priming the Flow Cell in CHAPTER 12, Replacement/ Adjustment Procedures. Run Daily Clean. Refer to Daily Clean in CHAPTER 11, Cleaning Procedures. Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 11, Cleaning Procedures. Filter the sample using an appropriately sized mesh aperture filter. Ensure that the sample tubing is properly connected. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 12, Replacement/Adjustment Procedures. Replace the sample probe and sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 12, Replacement/Adjustment Procedures. If the problem persists, contact us. 	

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 6, 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 12, 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 12, Replacement/Adjustment Procedures. If the problem persists, contact us.
Laser power is low.	Communication error	 Reinitialize. Refer to Initializing the Instrument in CHAPTER 4, 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 12, 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 12, 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 12, Replacement/Adjustment Procedures. If the problem persists, contact us.

Table 10.1 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 12, Replacement/ Adjustment Procedures. Run the Deep Clean procedure. Refer to Deep Clean Procedure in CHAPTER 11, 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 12, 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 12, 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 12, Replacement/Adjustment Procedures. If the problem persists, contact us.

Table 10.1	Troubleshooting	(Continued)
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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 6, Data Acquisition and Sample Analysis. Increase the gain setting. Refer to Adjusting the Gain in CHAPTER 6, 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 securely connected. Run Prime. Refer to Priming the Flow Cell in CHAPTER 12, Replacement/ Adjustment Procedures. Reinitialize. Refer to Initializing the Instrument in CHAPTER 4, 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 11, 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 12, 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 12, Replacement/Adjustment Procedures. If the problem persists, contact us

Table 10.1 Troubleshooting (Continued)

Problem	Probable Cause	Corrective Action
Data populations are not where they are expected.	 The detector configuration setting is incorrect. The optical filter is not placed correctly. QC was not completed. Gain and threshold is not set correctly. 	 Ensure that the detector configuration is set correctly. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, 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 6, Data Acquisition and Sample Analysis. Ensure that the optical filter is installed correctly. Refer to Replacing the Optical Filter in CHAPTER 12, Replacement/ Adjustment Procedures. Follow the QC procedure. Refer to CHAPTER 5, Instrument Quality Control and Standardization. Review the gain and threshold settings. Refer to Adjusting the Gain and Adjusting the Threshold in CHAPTER 6, Data Acquisition and Sample Analysis. Review the display ranges. Refer to Creating Plots and Gates in CHAPTER 6, 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 ^(W) to modify compensation in the desired plot.

Table 10.1	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 6, Data Acquisition and Sample Analysis. Increase the gain setting. Refer to Adjusting the Gain in CHAPTER 6, Data Acquisition and Sample Analysis

Table 10.1 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 6, Data Acquisition and Sample Analysis. Threshold. Refer to Adjusting the Threshold in CHAPTER 6, Data Acquisition and Sample Analysis. Compensation settings. Refer to CHAPTER 7, Compensation)
The sample probe is too low.	The sample probe is not securely connected.	 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 12, 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 12, Replacement/Adjustment Procedures. Ensure the sample pump cover is securely fastened. If the problem persists, contact us.

Table 10.1	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 12, 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 1.22. If the problem persists, contact us.
Software installation fails.	Multiple issues.	Contact us.

Table 10.1 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 contains aggregates or clumps. The sample probe is clogged. The sample line is clogged. 	 Add 1 drop of CytoFLEX Daily QC Fluorospheres or CytoFLEX Daily IR QC Fluorospheres to the QC solution. Then, rerun QC. Prepare a new sample of QC Fluorospheres. Then, rerun QC. NOTE Mix the fluorospheres dropper bottle thoroughly before dispensing it into a tube.
		 Reload the target value file. Refer to Importing Lot-Specific Target Values in CHAPTER 5, Instrument Quality Control and Standardization. Then, rerun QC. Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 11, Cleaning Procedures. Then, rerun QC.
		5. Run Prime. Refer to Priming the Flow Cell in CHAPTER 12, Replacement/ Adjustment Procedures. Then, rerun QC.
		6. Run Daily Clean. Refer to Daily Clean in CHAPTER 11, 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 12, Replacement/Adjustment Procedures. Then, rerun QC. If problem persists, contact us.

Table 10.1	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 waste container are on the same level as the Cytometer. Rerun QC. Refer to CHAPTER 5, Instrument Quality Control and Standardization. Run Prime. Refer to Priming the Flow Cell in CHAPTER 12, 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 11, Cleaning Procedures. Then, rerun QC. Run Deep Clean. Refer to Deep Clean Procedure in CHAPTER 11, Cleaning Procedures. Then, rerun QC. If the problem persists, contact us.

Table 10.1 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 12, Replacement/Adjustment Procedures. Calibrate the sample probe sampling position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 12, 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 12, 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 12, 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.

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 direc	tory		⇒⊙
Backup Directory:	F:\Backup CytFLEX	Next > Cl	

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:

War	ning	
	!	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.

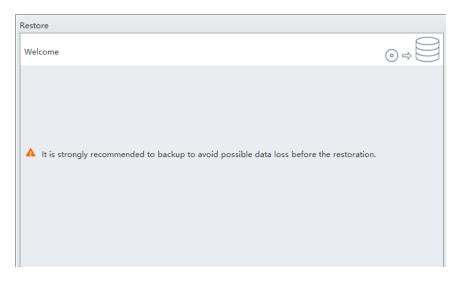
Backup	
Prechecking	
Prechecking the backup data, please wait a while.	

The backup starts automatically after the prechecking.

Backup is in prog	ress		
Backing up			
Total Size:	240.66 KB		
Size Remaining:	868 bytes		
Time Remaining:	About 0 Second(s)		
		< Back Next >	Cancel
ackup			
	4		
	3		
	4		
	4		
3ackup succeeded			
Backup succeeded	e CytExpert data succeeded.		
Backup succeeded		-111241	
Backup succeeded	e CytExpert data succeeded.	.111241	
Backup succeeded	e CytExpert data succeeded.	-111241	
	e CytExpert data succeeded.	-111241	
Backup succeeded	e CytExpert data succeeded.	.111241	Finish

5 Select Finish.

Restore



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

NOTE Ensure the data is backed up before the restoration.

- 2 Select Next.
- **3** Select _____ and browse to the desired directory to restore.

elect the restore p	oint		
lelect the restore p	Sinc		
Backup Directory:	D:\spring backup		
Restore Points			
Backup Time		CytExpert Version	Size
2017-06-02 11:22:2	2	2.1.0.66	240.66 KB
2017-06-02 13:08:1	6	2.1.0.66	240.66 KB

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

Warn	ng
4	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

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 prog	gress				•	
Restoring the Cyt	Expert data					
	240.66 KB					
Size Remaining:						
Time Remaining:	Calculating					
				Next >	C	ancel

Restore	
Restore succeeded	
The CytExpert data has been restored successfully.	
	Finish

6 Select **Finish**. The following system prompt appears.

The CytExpert will be restarted to complete the re-	store.
OK	

7 Select ок.

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 2017-07-11 00:00:00 🔻	before: Next > Close

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



3 Select the desired date and time.

Log Clean-up	
Set clean-up condition	
Clean up experiment operation log and system operation log 2017-07-11 00:00:00 ▼	before: 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 No

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 c	ompleted successfully.

6 Select Finish.

Chapter 11 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

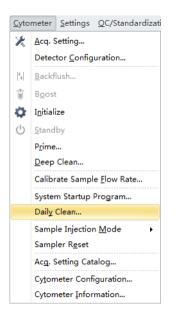
Daily Clean should be performed during instrument startup and instrument shutdown to clean the sample line.

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.

Perform a Daily Clean when the system reminder prompts you to do so. Otherwise, the reminder will prompt you repeatedly.

Confirm	
2	To keep sample line clear, a Daily Clean is required within 24 hours from running sample. Select "Yes" to run the Daily Clean now. Select "No" to defer the Daily Clean and CytExpert will remind you.

- **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 4, 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: 🛛 🚦 🗘 minutes (1~100)	
	Run
	Kun

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 Clean	23
Step2: Please load a tube with deionized wa Duration:	
	Run
Daily Clean	
Step2: Please load a tube with deionized wa	ater.
Duration: 5 🗍 minutes (1~10)0)
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]

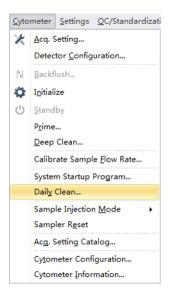
Daily Clean should be performed during instrument startup and instrument shutdown to clean the sample line.

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.

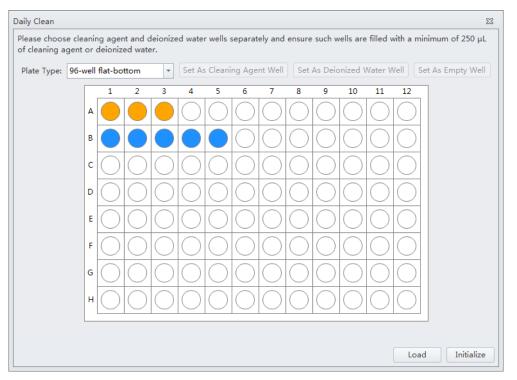
Perform a Daily Clean when the system reminder prompts you to do so. Otherwise, the reminder will prompt you repeatedly.

Co	onfirm	
	?	To keep sample line clear, a Daily Clean is required within 24 hours from running sample. Select "Yes" to run the Daily Clean now. Select "No" to defer the Daily Clean and CytExpert will remind you.
L		

- **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 4, 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



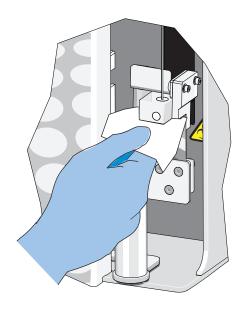
Carry out semi-automatic cleaning for the sample injection device once a week.

1 Ensure that the system has been shut down properly. Refer to Shutting Down the Instrument in CHAPTER 9, 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 12, 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 12, Replacement/Adjustment Procedures.

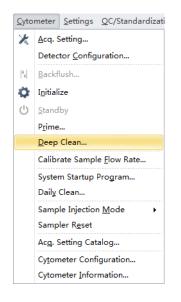
Deep Clean Procedure

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 12, 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 12, 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.

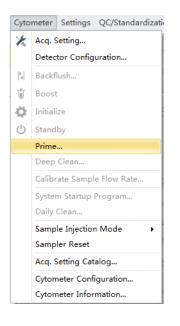


5 The status bar prompts that a deep clean is under way. Wait for the Deep Clean process to finish. The following software message appears:

Information	22	
Deep	p 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 4, 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 12, 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 in CHAPTER 12, 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.

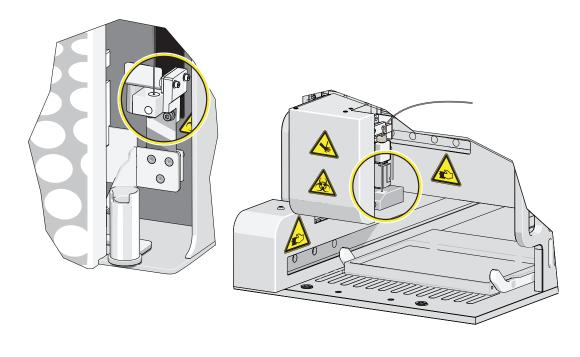
- **8** Dispose of the sodium hypochlorite solution in accordance with your local regulations and acceptable laboratory procedures.
- **9** Use deionized water to rinse the waste container and the waste harness. Ensure that there is no sodium hypochlorite residue.

10 Place the waste container back into the Fluid Container holder.

Nonscheduled Cleaning

Surface Cleaning and Disinfection

- **1** Wipe all external surfaces 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 12, 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 12, 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 in CHAPTER 12, Replacement/Adjustment Procedures).
- **5** Clean and disinfect all surfaces. Refer to Surface Cleaning and Disinfection.

🕂 WARNING

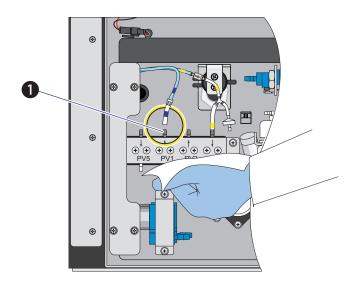
Risk of biohazardous contamination if you have skin contact with the waste container, its contents, and its associated tubing. The waste container and its associated tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the contents of 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 12, 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 12, Replacement/Adjustment Procedures).
- **10** Power down and disconnect all the cables and sheath fluid and waste harnesses.

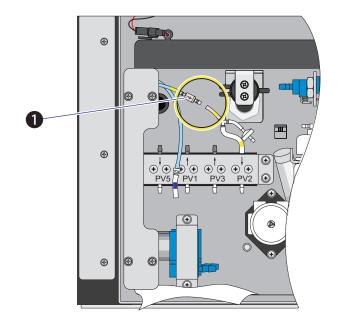
Risk of instrument damage. The Cytometer can suffer irreparable damage if it is exposed to subfreezing temperatures while it still contains liquid. Always drain the flow cell after cleaning the Cytometer if the Cytometer will be stored or transported in subfreezing temperatures.

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 choke to vent the flow cell, allowing it to drain.

- 1. Choke
- $13 \ {\rm Verify\ that\ liquid\ has\ stopped\ dripping\ from\ the\ blue-labeled\ tubing.}$

 $\ensuremath{\textbf{NOTE}}$ 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 choke.
- **17** Reinstall the right-side cover (see Right-Side Cover Removal and Reinstallation in CHAPTER 12, Replacement/Adjustment Procedures).
- **18** Ensure that the optical filters are seated properly.
- **19** Ensure that the top cover is tightly closed.

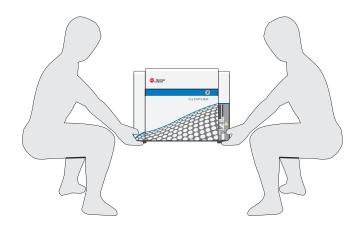
20 If the instrument is to be transported or stored, put the instrument and the Plate Loader module (if applicable) into the Beckman Coulter supplied packing and comply with the requirements in Instrument Transportation and Storage in APPENDIX A, Instrument Installation, regarding correct placement during 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 12 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
 - Replacing the 10 L Sheath Fluid Cubitainer
 - Emptying the 4 L Waste Container
 - 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

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.

- <image>
- **4** Remove the two screws securing the front cover and pull the front cover forward.

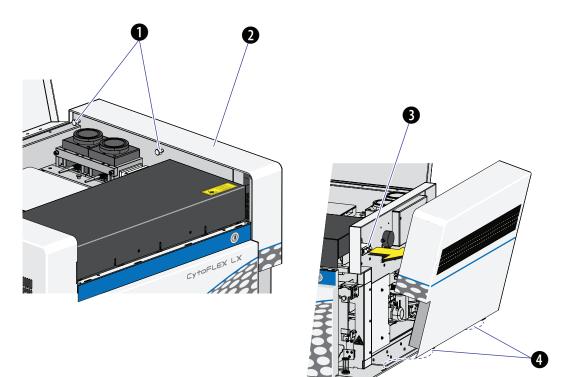
- 1. Securing screws
- 2. Front cover
- **5** Lift the front cover up and out of the slots in the frame.

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

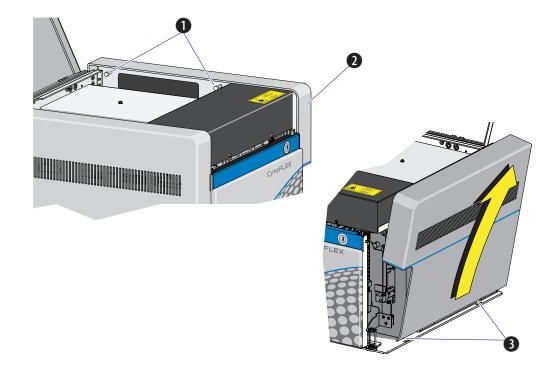
IMPORTANT The CytoFLEX LX flow cytometer only requires you to unfasten the thumbscrews the first time. There is a retaining clip that is used to secure the right-side cover without the need for the thumbscrews, if desired. Once the thumbscrews are unfastened, the right-side cover can simply be pulled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

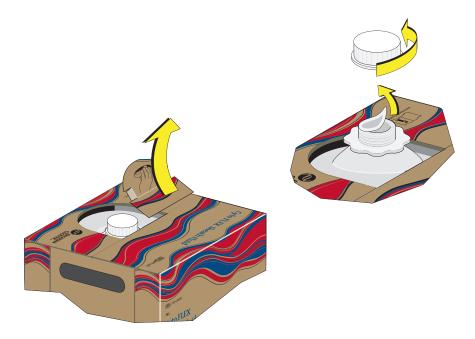
- 1 Confirm that the instrument is turned off or is in the standby state.
- **2** If necessary, remove any cardboard cutouts from the new CytoFLEX Sheath Fluid cubitainer. If you do not need a new cubitainer, skip to Step 7.
- **3** Locate the spigot inside the cardboard cutout.
- **4** Remove the cap 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.

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.

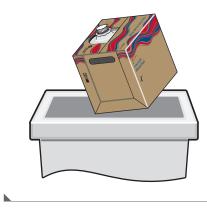


4 Inspect the pickup tube assembly and replace it if necessary.

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



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



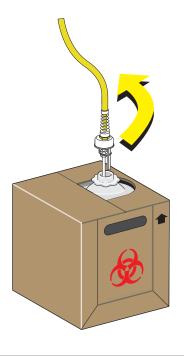
1 Confirm that the instrument is turned off or is in the standby state.

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



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.

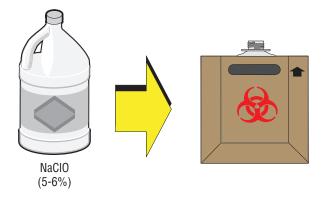


- **4** Empty the waste cubitainer according to your laboratory's procedures.
 - **NOTE** Take proper precautions to avoid spills if you are emptying the waste cubitainer into a sink, drain, or larger container. When moving the waste cubitainer to dispose of its contents, be sure the cap is secure to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

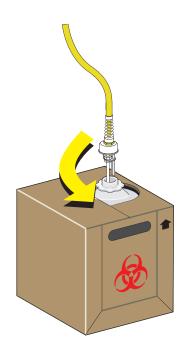
🕂 WARNING

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 Amaintenance Message from the status bar to access the

Maintenance window. The expired item appears with a warning triangle | \triangle to the left of the item listed.

Or

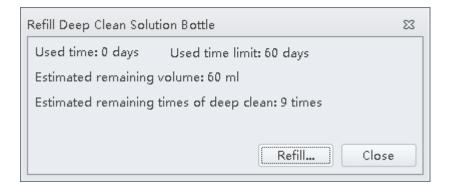
Select Maintenance in the Advanced menu. The Maintenance window appears. The expired

item is displayed with a warning triangle	A	• to the left of the item listed.

laintenance		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.

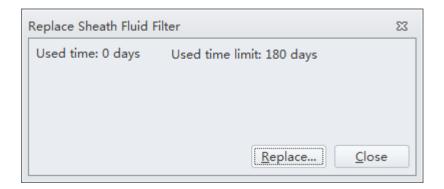
3 Select **Detail**. The Refill Deep Clean Solution Bottle window appears.



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		
Used time: 0 days	Jsed time limit: 180 days	
	<u>R</u> eplace <u>C</u> lose	

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

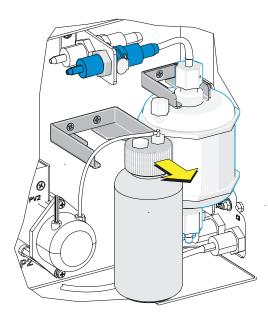
Replace Peristaltic Pump Tubing		
Maintenance Date:]	
Use time limit:	180	days
	<u>o</u> k	<u>C</u> ancel

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.

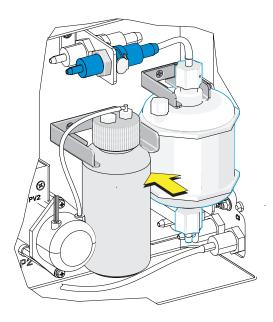
Risk of chemical injury from Contrad® 70 reagent. To avoid contact with the Contrad® 70 reagent, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

- **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.



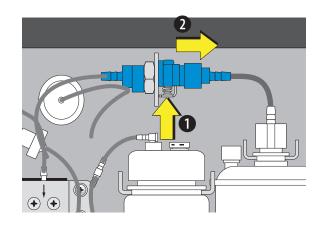
- 7 Reinstall the right-side cover (see Right-Side Cover Removal and Reinstallation), and fasten the thumbscrews.
- **8** Reset the maintenance reminder tracker. Refer to Managing the Maintenance Reminder.

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.

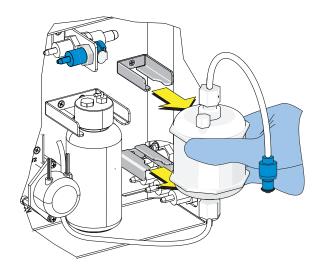
3 Press the spring piece on the quick connector and the Cytometer on the upper side of the filter, and disconnect the quick connector.



4 Repeat Step 3 for the quick connector behind the quick connector removed in the previous step.

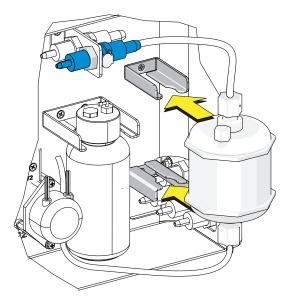
NOTE The spring is on the opposite side.

5 Remove the sheath fluid filter from the bracket.

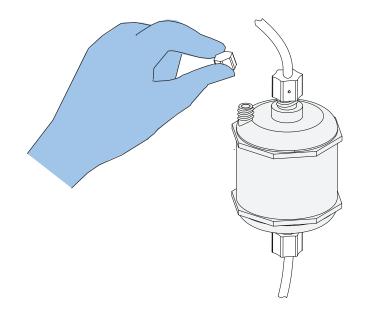


6 Connect the new, unused filter using the quick connector springs.

7 Attach the filter to the filter bracket.



8 Remove the vent cap and set it aside.

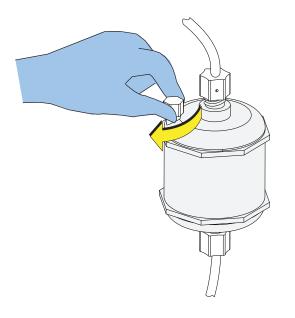


- **9** Turn on the Cytometer and open the software.
- $10 \hspace{0.1 cm} \text{Select Prime in the Cytometer menu.}$

<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 4, 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.

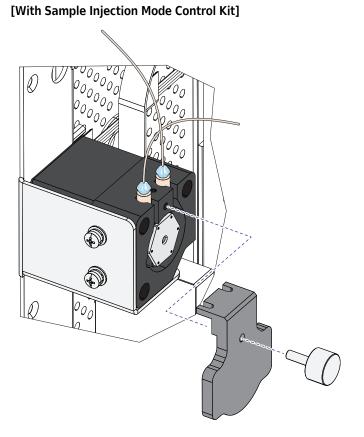
IMPORTANT If you need to replace the single tube sample probe assembly and you have the Sample Injection Mode Control Kit installed on your CytoFLEX instrument, pull the probe out of the CytoFLEX Sample Injection Mode Control Kit and push the new probe into place.

Beckman Coulter recommends replacing the tubing of the sample peristaltic pump every six months, or sooner. High sample volume, degradation of the stability of the sample flow, and increase of the CV may indicate the need to replace the tubing more frequently.

NOTE Ensure you replace the sample probe with a sample probe that has the same color jewel.

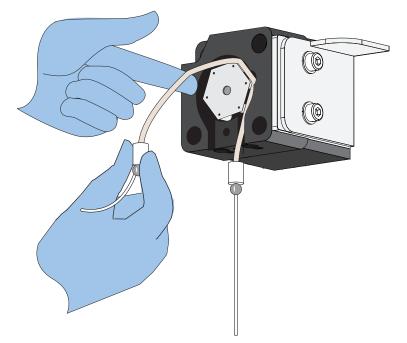
- **1** Place the instrument in standby state.
- **2** Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation.
- **3** Remove the front cover. Refer to Front Cover Removal and Reinstallation.

- [Without Sample Injection Mode Control Kit]
- **4** Remove the sample pump cover thumbscrew and the sample pump cover.

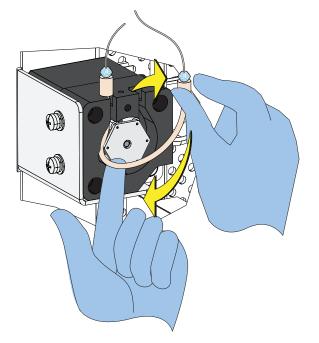


 ${\bf 5} \quad {\rm Take \ out \ the \ sample \ peristaltic \ pump \ tubing.}$

[Without Sample Injection Mode Control Kit]



[With Sample Injection Mode Control Kit]

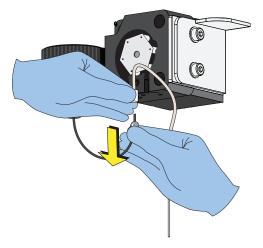


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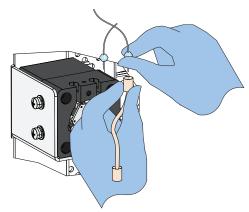
Risk of optical misalignment. Removing the PEEK tubing from the bottom of the flow cell could cause misalignment of the optical components. Do not remove the PEEK tubing from the bottom of the flow cell.

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

[Without Sample Injection Mode Control Kit]



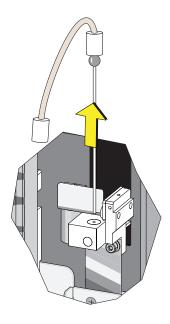
[Without Sample Injection Mode Control Kit]: Proceed to Step 7.



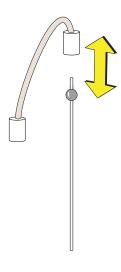
[With Sample Injection Mode Control Kit]

[With Sample Injection Mode Control Kit]: Skip to Step 9.

7 [Without Sample Injection Mode Control Kit]: Lift the sample probe out of the wash station.

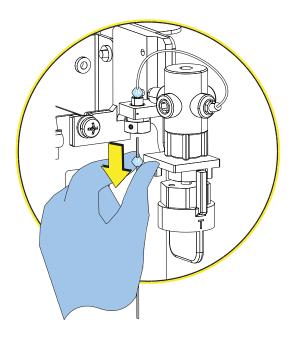


8 [Without Sample Injection Mode Control Kit]: Remove the sample peristaltic pump tubing from the sample probe.



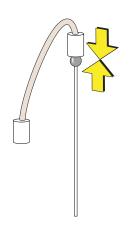
Skip to Step 10.

9 [With Sample Injection Mode Control Kit]: Remove the sample probe from the metal block if you need replace the sample probe.

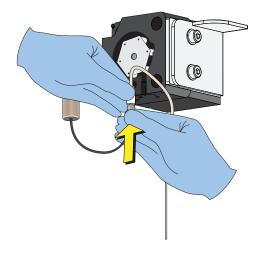


Proceed to Step 10.

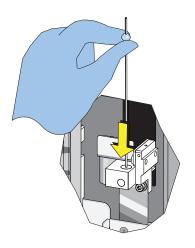
- 10 Dispose of the old sample probe and/or sample peristaltic pump tubing in accordance with your local regulations and acceptable laboratory procedures.
 [Without Sample Injection Mode Control Kit]: Proceed to Step 11.
 [With Sample Injection Mode Control Kit]: Skip to Step 13.
- **11** [Without Sample Injection Mode Control Kit]: Connect the sample peristaltic pump tubing to the sample probe.



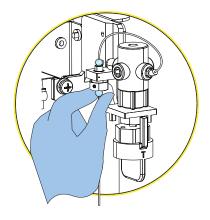
12 [Without Sample Injection Mode Control Kit]: Connect the sample PEEK tubing to the sample peristaltic pump tubing.



13 [With and Without Sample Injection Mode Control Kit]: Slide the sample probe into the wash station.

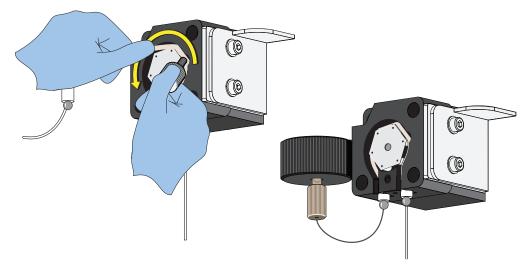


[Without Sample Injection Mode Control Kit]: Skip to Step 15. [With Sample Injection Mode Control Kit]: Proceed to Step 14. **14** [With Sample Injection Mode Control Kit]: Connect the sample probe to the metal block.



Proceed to Step 15.

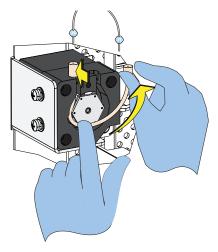
- **15** Install the sample peristaltic pump tubing, taking care not to use any sharp tools, ensuring that the tube is fully inserted into the groove.
 - **NOTE** Use the sample pump cover thumbscrew to insert the sample peristaltic pump tubing into the groove while rotating the sample pump.



[Without Sample Injection Mode Control Kit]

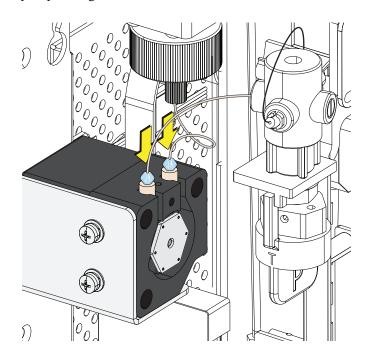
[Without Sample Injection Mode Control Kit]: Skip to Step 17.





[With Sample Injection Mode Control Kit]: Proceed to Step 16.

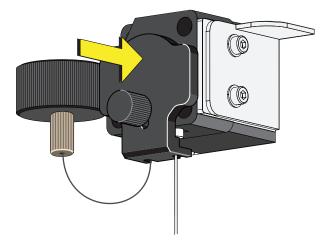
16 [With Sample Injection Mode Control Kit]: Connect the PEEK tubing to the sample peristaltic pump tubing.



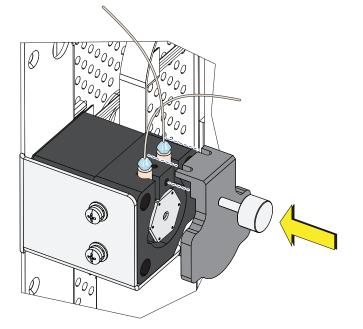
Proceed to Step 17.

17 Install the sample pump cover.

[Without Sample Injection Mode Control Kit]



[With Sample Injection Mode Control Kit]



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

[With and Without Sample Injection Mode Control Kit]: Proceed to Step 18.

18 Reinstall the right-side cover (see Right-Side Cover Removal and Reinstallation), and lock with the screw.

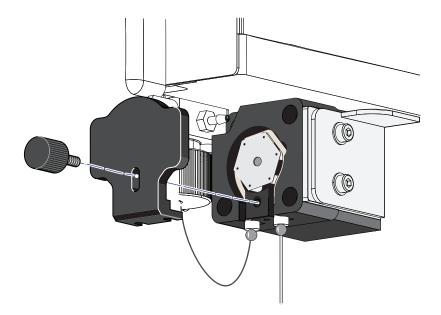
Replacing the Sample Probe Assembly [With Plate Loader]



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

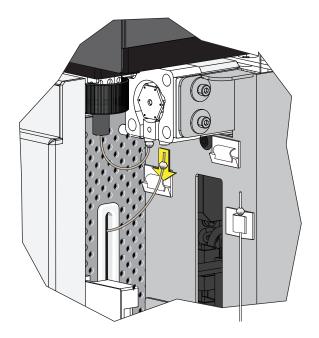
Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample 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 12, Replacement/Adjustment Procedures.
- **3** Remove the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 12, Replacement/Adjustment Procedures.
- **4** Remove the sample pump cover thumbscrew and the sample pump cover.



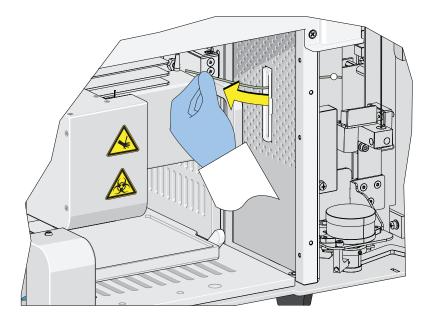
Risk of optical misalignment. Removing the PEEK tubing from the bottom of the flow cell could cause misalignment of the optical components. Do not remove the PEEK tubing from the bottom of the flow cell.

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

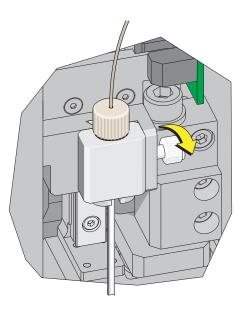


The plate loader PEEK tubing can be deformed, which could affect sample flow. When routing the plate loader PEEK tubing to and from the Sample Station, be careful not to pinch, crimp, stretch, or break the tubing.

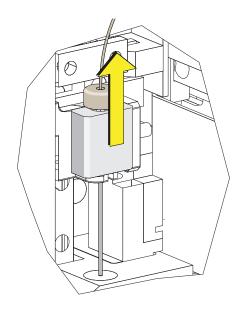
6 Pull the plate loader PEEK tubing through the slot so the tubing sits inside the instrument.



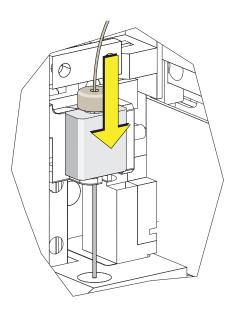
7 Loosen the white plastic thumbscrew behind the plate loader sample probe.



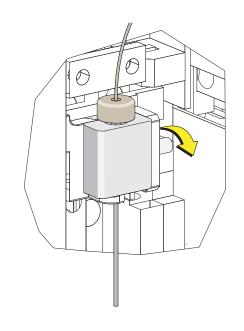
8 Lift the plate loader sample probe assembly straight up to remove it from the probe holder.



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.



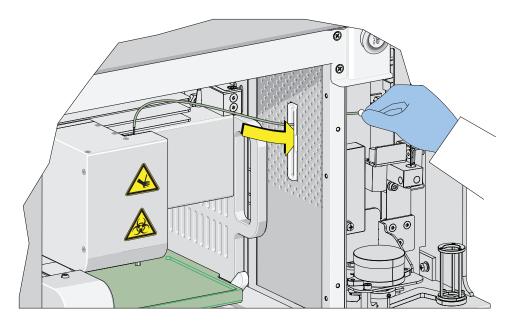
10 Tighten the white plastic thumbscrew.

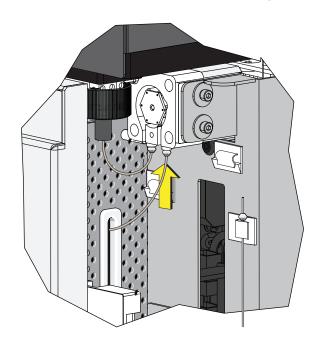




The plate loader PEEK tubing can be deformed which could affect sample flow. When routing the plate loader PEEK tubing to and from the Sample Station, be careful not to pinch, crimp, stretch, or break the tubing.

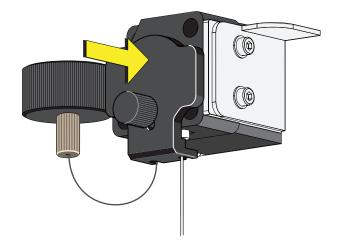
11 Slide the new plate loader PEEK tubing through the slot into the single tube sample station area.





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 12, Replacement/Adjustment Procedures), and lock with the screw.

15 Close the top cover.

16 Turn the Cytometer's main power switch on.

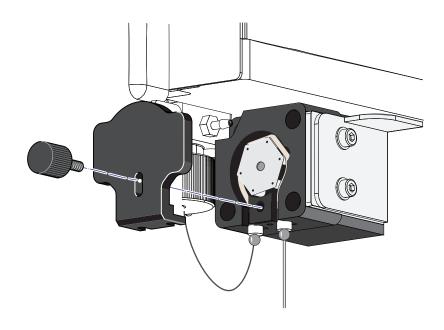
Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [CytoFLEX With Plate Loader]



🕂 WARNING

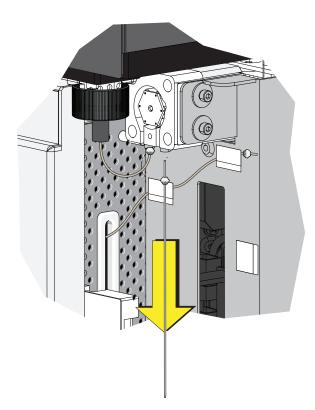
Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately.

- **NOTE** If you have the Sample Injection Mode Control Kit installed on your CytoFLEX flow cytometer, 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.
- Switch to the Plate Loader sample injection mode. Refer to Selecting the Plate Loader Sample Injection Mode [With Plate Loader] in CHAPTER 4, Daily Startup.
- **2** Lift the top cover.
- **3** Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation in CHAPTER 12, Replacement/Adjustment Procedures.
- **4** Remove the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 12, Replacement/Adjustment Procedures.

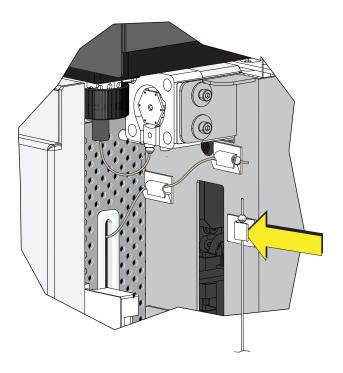


5 Remove the sample pump cover thumbscrew and the sample pump cover.

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



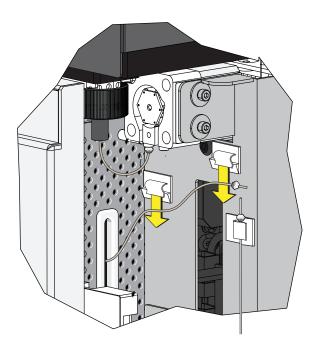
7 Place the sample probe in the white clip located on the right side of the sample station.



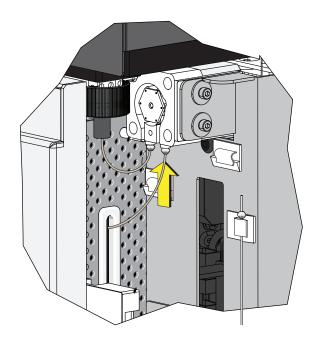
<u>A</u> CAUTION

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.

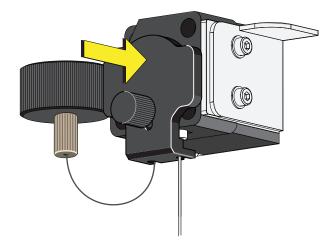
8 Remove the plate loader PEEK tubing from the two white clips located at the top of the sample station.



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 12, 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

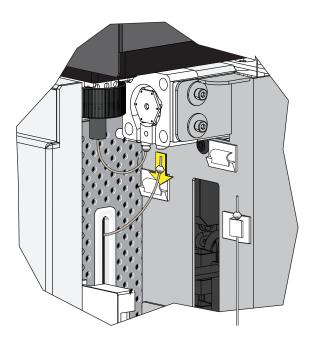
Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately.

NOTE If you have the Sample Injection Mode Control Kit installed on your CytoFLEX flow cytometer, 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 4, Daily Startup.
- **2** Lift the top cover.
- **3** Remove the right-side cover. Refer to Right-Side Cover Removal and Reinstallation in CHAPTER 12, Replacement/Adjustment Procedures.
- **4** Remove the front cover. Refer to Front Cover Removal and Reinstallation in CHAPTER 12, Replacement/Adjustment Procedures.

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

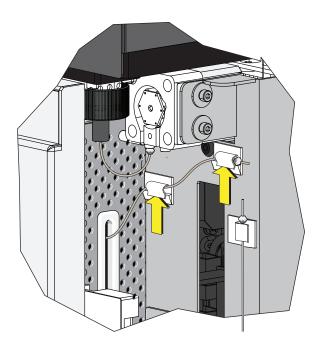
6 Remove the plate loader PEEK tubing from the sample peristaltic pump tubing.



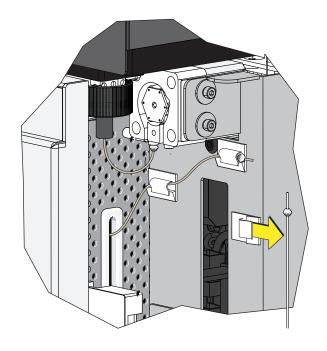
<u>A</u> CAUTION

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

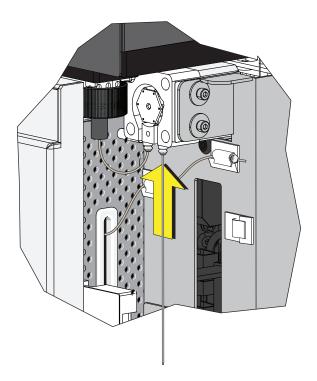
7 Place the plate loader PEEK tubing in the two white clips located at the top of the sample station.



8 Remove the sample probe from the white clip located on the right side of the sample station.



9 Connect the sample probe to the sample peristaltic pump tubing.



10 Run Daily Clean in CHAPTER 11, Cleaning Procedures.

Inspecting the Liquid Flow Path for Leaks

WARNING

The liquid flow tubing can be 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 4, 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.

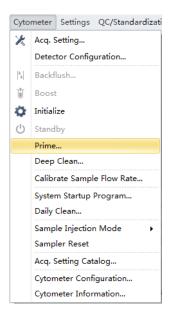
Priming the Flow Cell

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.

[2016-11-22 16:31:03] Completed Prime.

3 Run Daily Clean to clean the sample line. Refer to Daily Clean in CHAPTER 11, Cleaning Procedures

Replacing the Plate Holder [With Plate Loader]

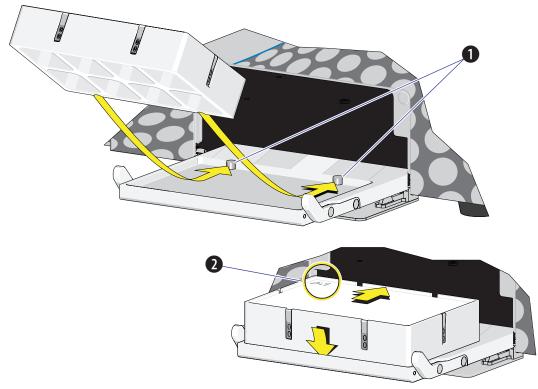
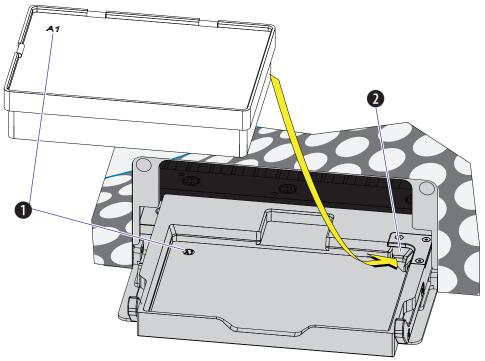


Figure 12.1 Plate Holder Stage (Standard Plate Loader)

- 1. Pins
- 2. Position A1

Figure 12.2 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 12.1 and Figure 12.2) to prevent instrument damage.

Slide the notches on the bottom of the plate holder (refer to Figure 1.17) over the pins (refer to Figure 12.1, and Figure 12.2).

Plate Loader Module Removal and Reinstallation [With Plate Loader]



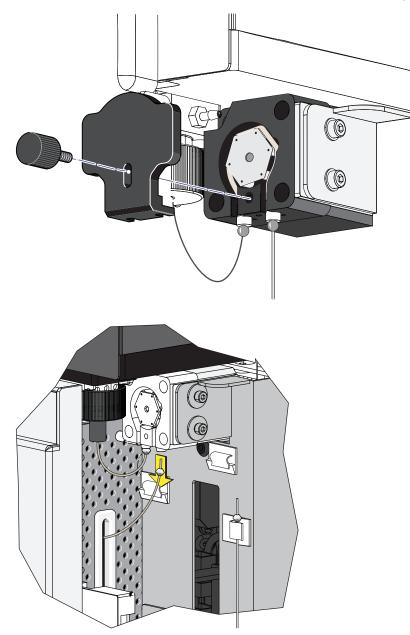
🕂 WARNING

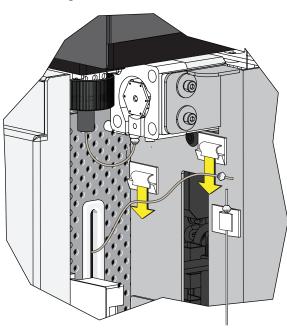
Risk of contamination from biohazardous material. Always wear PPE when performing this procedure as you can contact components with blood residue.

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.



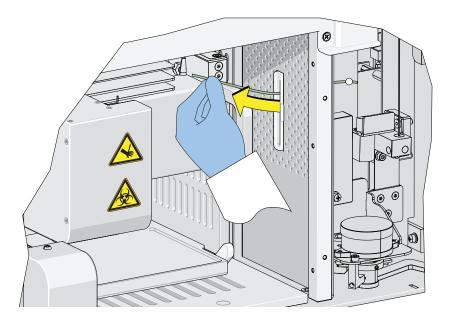


• If the plate loader PEEK tubing is in the plate loader PEEK tubing clips, remove the tubing from the clips.

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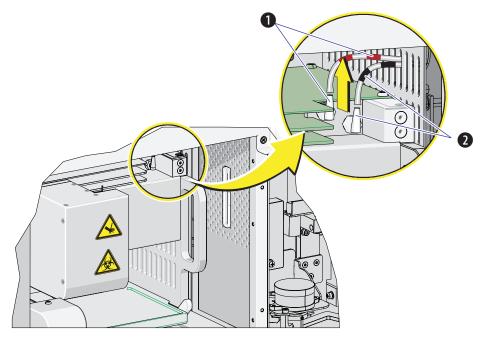
The PEEK tubing can be deformed which could affect sample flow. When routing the Plate Loader PEEK tubing to and from the Sampling Station, be careful not to pinch, crimp, stretch, or break the tubing.

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 12.3.

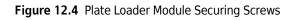
Figure 12.3 Removing the Tubings from the Fluidics Module to the Plate Loader

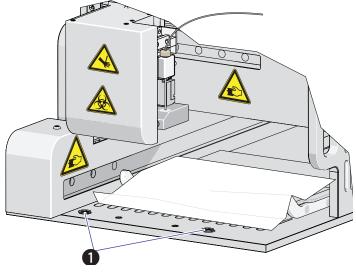


1. Red

2. Black

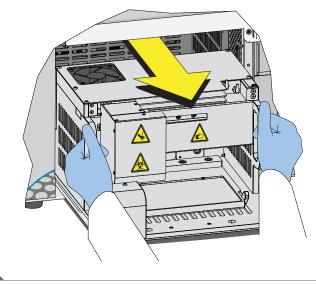
7 Remove the two countersunk M4 x 10 Phillips-head screws in the Plate Loader module. Refer to Figure 12.4.





- 1. Securing screws
- **8** Slide the Plate Loader module out of the Cytometer. Refer to Figure 12.5.



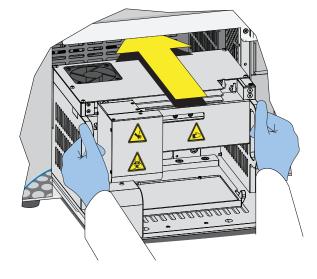


Installation

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 into the Cytometer. Refer to Figure 12.6.

Figure 12.6 Installing the Plate Loader into the Cytometer



4 Install the two countersunk M4 x 10 Phillips-head screws in the Plate Loader module. Refer to Figure 12.7.

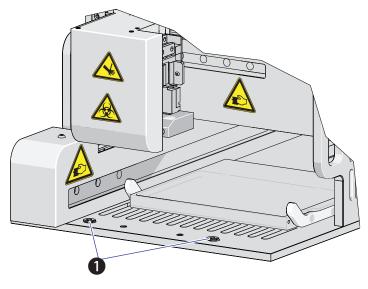
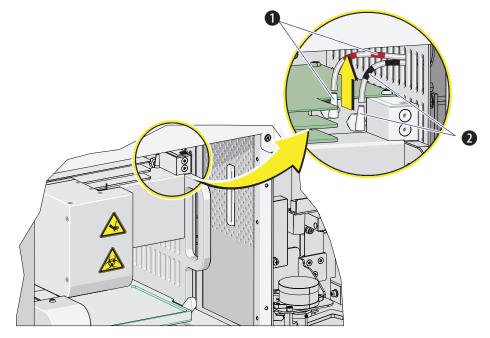


Figure 12.7 Plate Loader Module Securing Screws

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 12.8.

Figure 12.8 Connecting the Tubings from the Fluidics Module to the Plate Loader

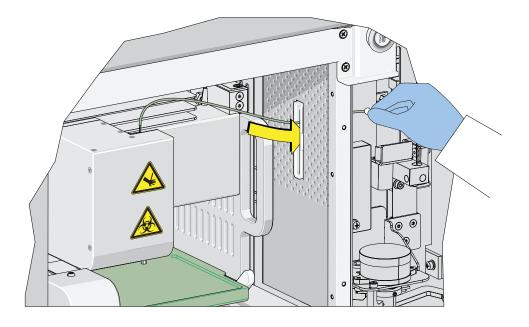


1. Red

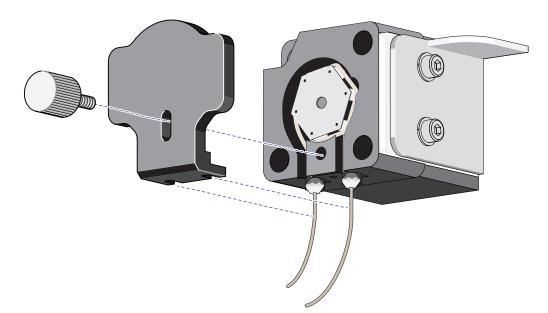
2. Black

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.



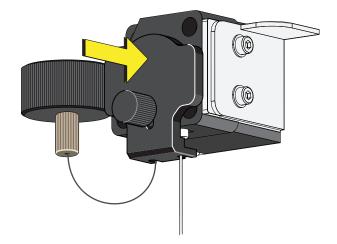
7 Remove the sample pump cover thumbscrew and the sample pump cover.



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 12, 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 5, 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.

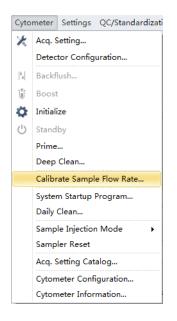
З Select ок.

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):
Next 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 1.12).

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 Cancel
Previous Run Cancel

Calibrate Sample Flow Rate(Fast)
Step2: Start acquiring sample.
Acquisition Duration(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

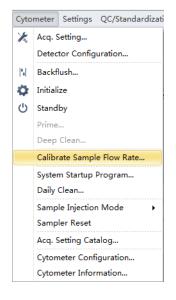
If deviation occurs, the setting is automatically corrected.

The weight before acquisition	n: 3.5000 g.
Acquisition duration: 3 minut	e(s).
The weight after acquisition:	3.3500 g.
The calibration result is out o	of error limit!
Click on "OK" button to set a	as calibrated flow rate.
According to this value ca	librate other flow rate
According to this value ca	sibiate other now rate

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.



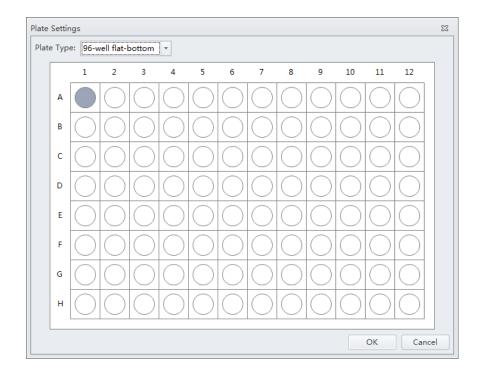
3 Select the sampling speed that needs to be calibrated and select **Calibrate**. The Calibrate Sample Flow Rate window appears.

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.

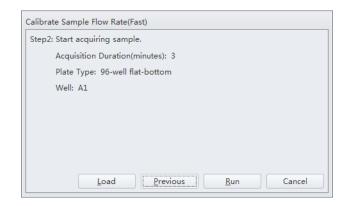
NOTE Do not exceed three minutes when using the fast sampling rate.

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
Next 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 Rat	te(Fast)				
Step3: Please enter the weight 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 OK

Setting Laser Delay

Laser delay is preset for QC. Only change the laser delay if the software prompts you that there is a difference in the actual delay and the default delay.

Error	23	
	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	

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

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

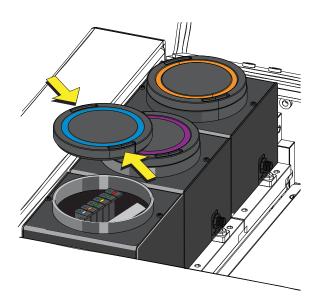
- **2** Set the current to the actual delay for the specified detector stated in the error message received.
- **3** Select **Set as Default**.

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.

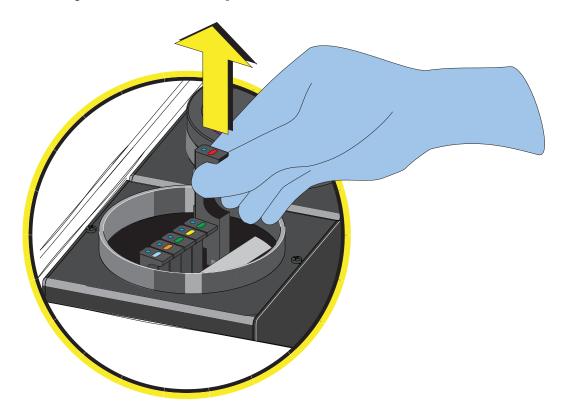


AUTION

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 6, Data Acquisition and Sample Analysis.

Set this new configuration as the current configuration.

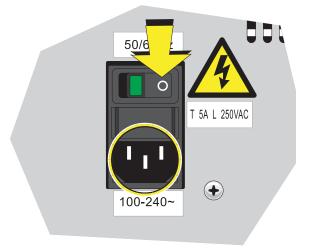
Replacing the Fuse

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

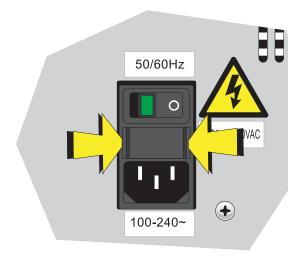


Risk of personal injury. A shock hazard exists if the power cable is connected. Turn off the Cytometer and disconnect the primary power cable before performing these procedures.

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.



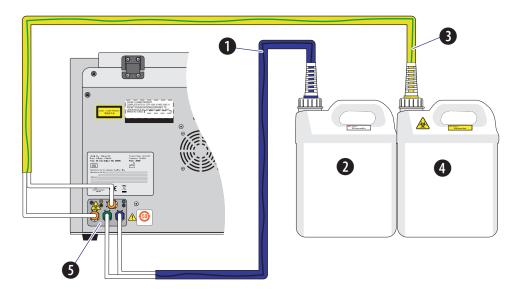
4 Insert the fuse holder.

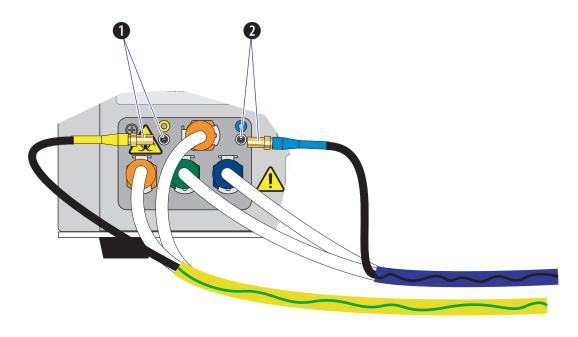
5 Reconnect the power cable.

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.

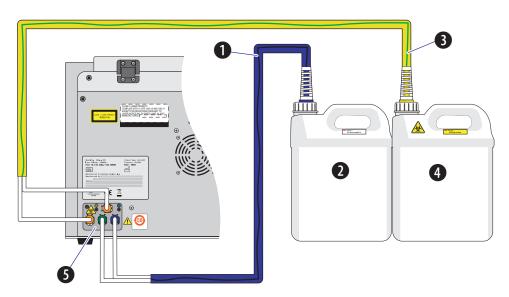
- 1 Confirm that the instrument is turned off or is in the standby state.
- **2** Remove sheath and/or waste pickup tubing from the appropriate container.
- 3 Disconnect the blue harness (1) from the sheath fluid container (2) and/or the yellow harness (3) from the waste container (4) from the fluid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

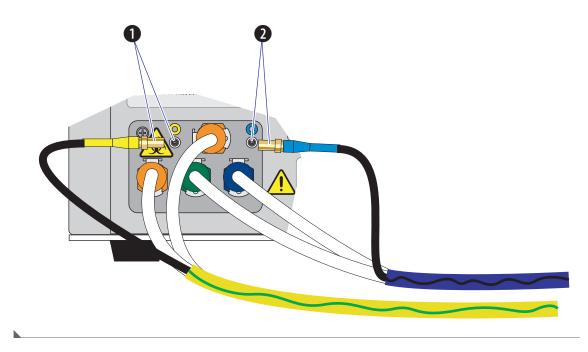




Disconnect the waste (1) and/or sheath (2) level sensors.

- **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 4, Daily Startup.

2 Select **Cytometer Configuration** in the Cytometer menu. The Cytometer Configuration window appears.

Cytometer Configuration		
Backflush Duration		
Manual/Semi Automatic: 3 sec (1 ~ 600)		
Plate Loader: 3 sec (1 ~ 600)		
Semi Automatic		
Sample Mixing		
Duration: 1.0 sec (0.1 ~ 5.0)		
Default OK Cance	9	

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. For **[CytoFLEX and/or CytoFLEX S]** with Sample Injection Mode Control Kit, perform an additional 6 second backflush or alternatively running daily clean after running QC.

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 12, Replacement/Adjustment Procedures.

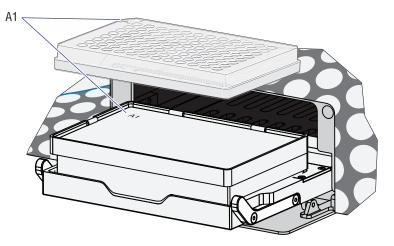


- 23 Plate Type Library Active Name Plate Mode Last Calibration Time Default \checkmark 96-well flat-bottom Standard 0 - \checkmark 96-well V-bottom Standard 0 - \checkmark 96-well U-bottom 0 Standard _ \bigcirc \checkmark 96-well deep well Deep Well -Add... Edit... Duplicate... Delete ОК Cancel
- **3** Select **Plate Type Library** in the Advanced menu. The plate loader automatically ejects the plate holder stage and the Plate Type Library window appears.

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

[Standard 96-Well Plate]: Proceed to Step 4. [96-Well Deep Well Plate]: Skip to Step 5.

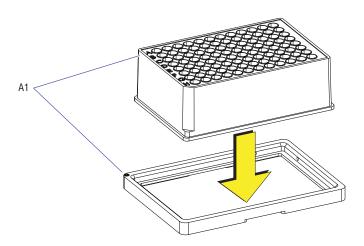
4 [Standard 96-Well Plate]: Select the plate type and place the plate on the plate holder. Skip to Step 11.



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

NOTE Ensure plate well A1 aligns with position A1.

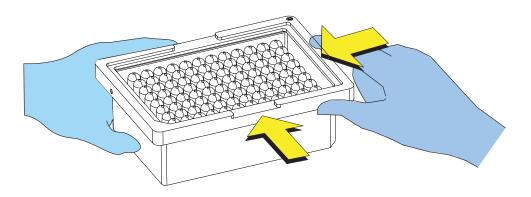
- **5 [96-Well Deep Well Plate]**: Select the 96-Well Deep Well plate type.
- **6 [96-Well Deep Well Plate]**: Place a 96-well deep well plate into the calibration frame.



NOTE Ensure plate well A1 aligns with position A1.

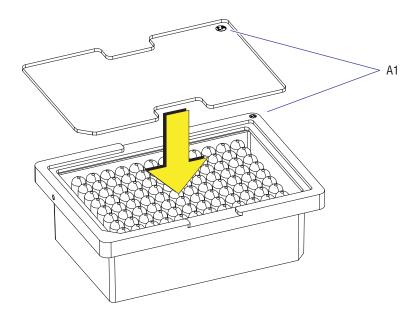
NOTE The calibration frame and the transparent plate are used to assist the calibration in X-axis and Y-axis. They are delivered together with the Plate Loader DW.

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.



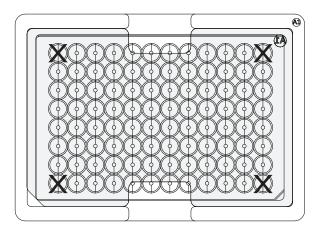
NOTE Ensure the deep well plate is correctly placed into the calibration frame.

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

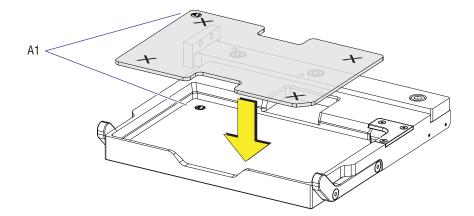


NOTE Ensure the A1 of the transparent plate aligns with position well A1.

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.

 $11 \ \text{Select } \textbf{Edit} \text{ to access the calibrate icon. The Edit Plate Type window appears.}$

[Standard 96-Well Plate]

Edit Plate Type		22
Name:	96-well V-bottom	I
Plate Mode:	Standard	v
Mix:	5 sec	
Remarks:		
		Y
Last Calibration Time:	-	
Calibrate		OK Cancel

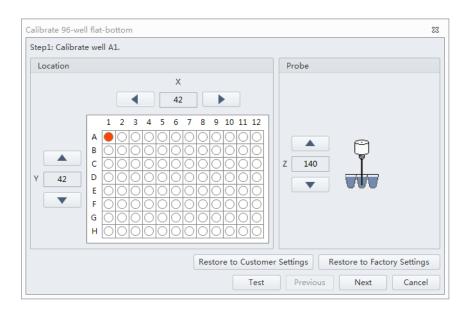
[96-Well Deep Well Plate]

Edit Plate Type		23
Name:	96-well deep well	
Plate Mode:	Deep Well	
Mix:	10 sec	
Remarks:		
		•
		r
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.

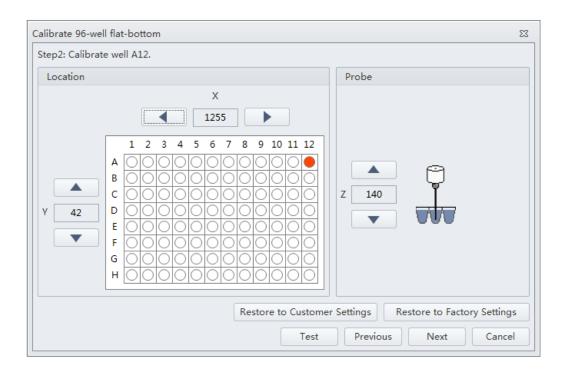
16 Select **Test** to verify the sample probe position.

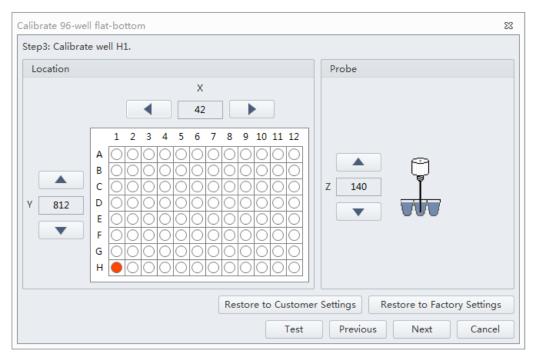
Listen for the click to ensure the probe has made contact with the bottom of the well. Readjust the sample probe position by moving the probe 3 steps up. This is the correct position for the Z-axis of the probe.

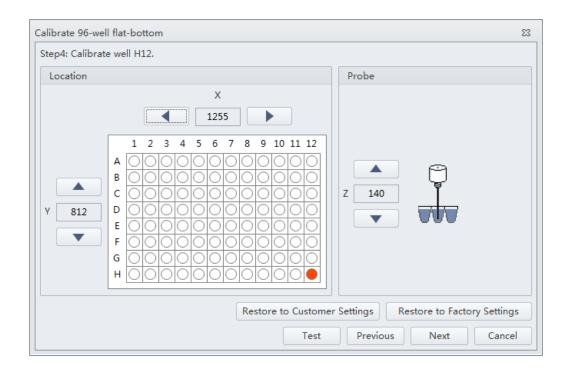
NOTE [Transparent Plate]: The sample probe cannot come in contact with the surface of the transparent plate in the Z-axis. This is normal. Do not adjust the sample probe positions in the X-axis. The probe position in the Z-axis will be calibrated using a deep well plate.

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.

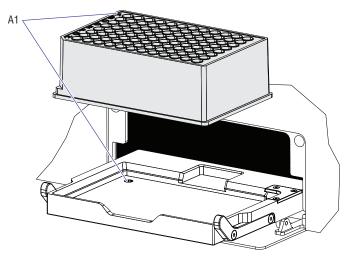
alibrate Plate 1	23
tep 5: Calibration completed.	
Click on the "Finish" button to save the data and exit, or click on the "Cancel" button to discard the data.	
Previous Finish Can	cel

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]

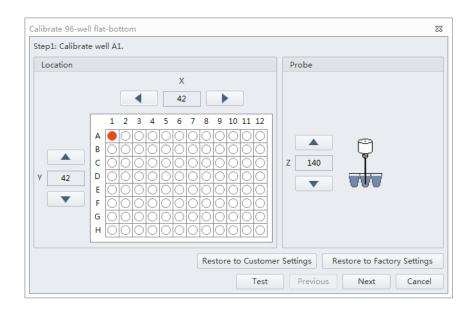


NOTE Ensure plate well A1 aligns with position A1.

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	
Plate Mode:	Deep Well	
✓ Mix:	10 sec	
Remarks:		
		-
Last Calibration Time:		
Calibrate	ОК	Cancel

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.



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.

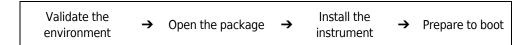
Listen for the click to ensure the probe has made contact with the bottom of the well. Readjust the sample probe position by moving the probe 3 steps upper. This is the correct position for the Z-axis of the probe.

- 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 12, 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 Platform]
- Upgrading the CytExpert Software
- Reinstalling the CytExpert Software

Instrument Transportation and Storage

Refer to Preparing the Instrument for Transport or Storage in CHAPTER 11, 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 83 kg, gross weight 103 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

A CAUTION

Risk of instrument damage. Place the instrument on a level surface. Failing to do so places the system is in danger of toppling and can result in damage. Take all necessary precautions throughout the process of storing or transporting the instrument.

- The tabletop must be smooth and level.
- Minimum tabletop load bearing capacity [CytoFLEX]: 50 kg
- Minimum tabletop load bearing capacity [CytoFLEX LX]: 100 kg.
- The tabletop must not vibrate or shake.
- Minimum tabletop dimensions [CytoFLEX]: 120 cm x 80 cm; minimum vertical space above tabletop: 80 cm
- Minimum tabletop dimensions [CytoFLEX LX]: 200 cm x 80 cm; minimum vertical space above tabletop: 100 cm
- Position the instrument in such a manner that it will facilitate disconnection of the power cable at the instrument end.

Ventilation and Cleaning

- **IMPORTANT** If necessary, use ventilation equipment, but airflow must not be allowed to blow directly on the system, as it can affect the reliability of the data.
- Ensure that the working environment is well ventilated for proper heat dissipation.
- Maintain a clearance of at least 20 cm from the back of the instrument for heat dissipation.
- Keep the environment as dust free as possible.
- Avoid direct exposure to sunlight.
- Avoid placing near heat sources or exposing to drafts.
- Avoid corrosives or flammable gases.

Power Source

A 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.

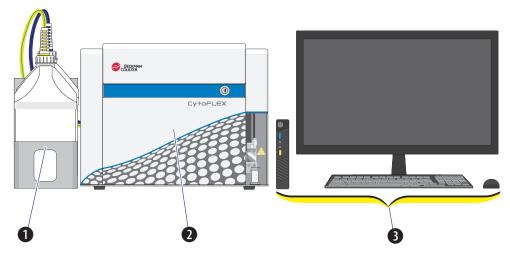
1 Remove the Cytometer, the Fluid Containers, the accompanying holder, the computer, the monitor, and the keyboard and mouse from each respective box placing them flat on the instrument worktable.

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.

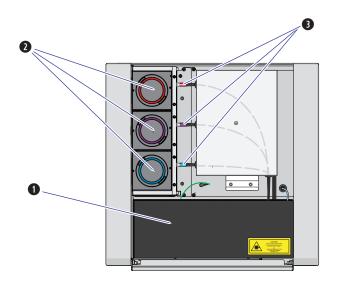
2 Ensure a minimum clearance of 20 cm on both sides and to the back of the Cytometer to maintain enough room to access the on/off controls for the Cytometer devices.

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

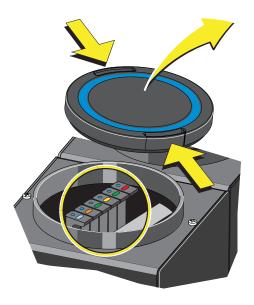


[CytoFLEX Without Plate Loader Shown]

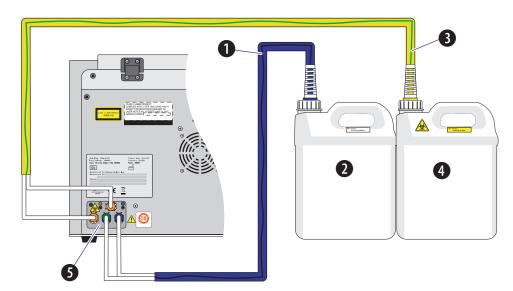
- 1. Fluid Containers. Place on the left side of the Cytometer.
- 2. Cytometer. Place between the Fluid Containers and the Workstation.
- **3.** Workstation. Place on the right side of the Cytometer.
- 4 Open the top cover of the Cytometer. Check inside to verify that the optical bench cover (1) is tightly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

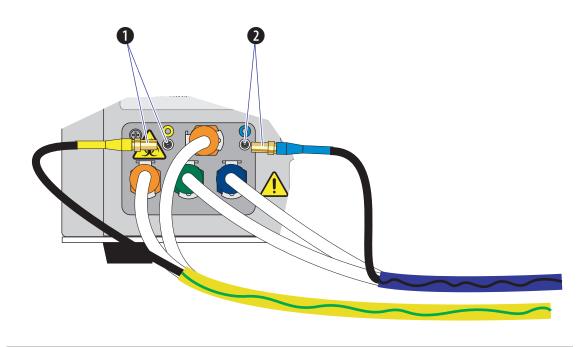


- **6** Insert sheath and waste pickup tubing into the appropriate container.
- 7 Connect the blue harness (1) from the sheath fluid container (2) and the yellow harness (3) from the waste container (4) to the fluid connector panel (5) on the back right corner of the Cytometer according to the color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

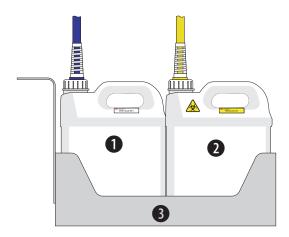


А

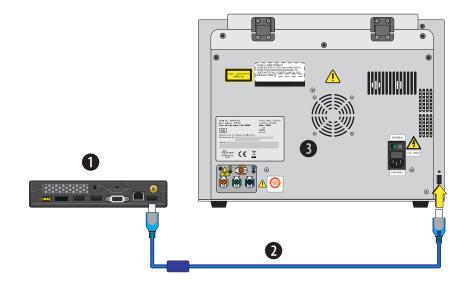
8 Connect the waste (1) and sheath (2) level sensors.



9 Place the sheath fluid container (1) and the waste container (2) in the Fluid Container holder (3).



10 Set up the supplied computer (1) and connect the USB cable (2) from the back of the Cytometer (3) to a USB port on the back of the computer.



11 Plug the Cytometer power cable into the back of the Cytometer.

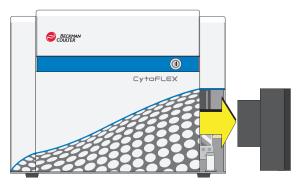


12 Connect the computer keyboard, mouse, and monitor.

A

13 Remove the foam support from the instrument.

[CytoFLEX Without Plate Loader Shown]



- **14** Add Deep Clean solution to the Deep Clean solution bottle. Refer to Step 1 and Steps 3 7 of the Adding the Deep Clean Solution in CHAPTER 12, Replacement/Adjustment Procedures.
- **15** Clean the sheath fluid container. Refer to Cleaning the 4 L Sheath Fluid Container in CHAPTER 11, Cleaning Procedures.
- **16** Fill the Sheath fluid container. Refer to Filling the 4 L Sheath Fluid Container in CHAPTER 12, Replacement/Adjustment Procedures.

🕂 WARNING

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

- **17** Add 400 mL of 5 to 6% bleach to the waste container.
- **18** Install the CytExpert software. Refer to Installing the Software [CytoFLEX Platform].
- **19** Turn on the instrument. Refer to Turning On the Instrument in CHAPTER 4, Daily Startup.
- **20** Open the CytExpert software. Refer to Logging into the Software in CHAPTER 4, Daily Startup.
- **21** Run the System Startup Program. Refer to Running the System Startup Program [with the Single Tube Loader] in CHAPTER 4, Daily Startup.

- **22** Prime the instrument three times. Refer to Priming the Flow Cell in CHAPTER 12, Replacement/ Adjustment Procedures.
- **23** Prepare a QC sample. Refer to Preparing the QC Sample in CHAPTER 5, Instrument Quality Control and Standardization.
- **24** Import the lot-specific target values files. Refer to Importing Lot-Specific Target Values in CHAPTER 5, 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 5, Instrument Quality Control and Standardization.
- **26** If the lot number is new and QC fails, the following software message appears. Select **Yes**.

QC failed, do you want to save the adjusted gain as bead gain for the current batch and QC Gain? Yes No

- **NOTE** Target gain values must be established for each new lot number of CytoFLEX QC Fluorospheres. QC could fail up to 3 times upon running each new lot number for the first time until target gain values are established.
- **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 Installer
CytExper	t Installation Opt	ions	
Please select the	CytExpert installation option:		
O De	fault		
	er Management		
) Ele	ectronic Record Management		
		uctions for Use manual for details rega e manual at: <u>www.beckmancoulter.com</u>	
		<	Back 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 Platform]

The installation process workflow is as follows:

Install the CytExpert software	→	Install instrument configuration files	→	Start the software
--------------------------------	---	--	---	--------------------

The CytExpert software can be installed on any computer that meets the minimum specifications (see Instrument Specifications in CHAPTER 1, System Overview) for analysis-only use.

А

Required Materials

The following materials are required to install the CytExpert software:

- CytoFLEX platform flow cytometer.
- Workstation.
- CytExpert software installation USB.
- Authorized Beckman Coulter CytoFLEX USB configuration key.

Installing the CytExpert Software

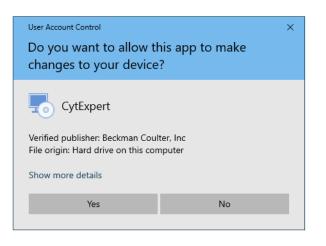
IMPORTANT Follow this procedure when installing the CytExpert software for the first time.

- 1 Ensure the workstation and the monitor are powered on.
- **2** Insert the software USB into the computer.

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



3 Select **CytExpert_Setup_X.X.exe**. The User Account Control window appears.



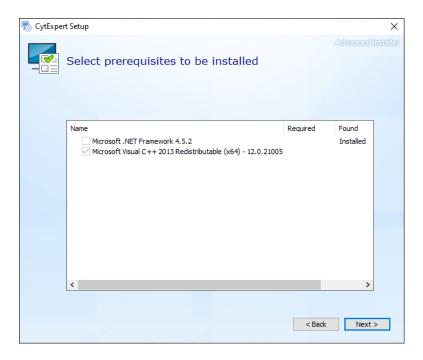
4 Select **Yes**. The CytExpert Setup Welcome window appears.



5 Select Next.

Α

6 Select both support program checkboxes in the CytExpert Setup Prerequisites window.



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

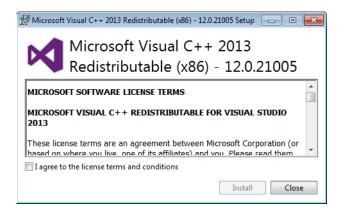
rosoft .NET Framework 4.5.2		- • •
Framework 4.5.2 Setup Please accept the license terms to	continue.	
MICROSOFT SOFTWARE SU	JPPLEMENTAL LICEN	ISE TERMS
.NET FRAMEWORK AND AS MICROSOFT WINDOWS OP		GE PACKS FOR
Microsoft Corporation (or ba affiliates) licenses this supple Microsoft Windows operating may use this supplement. Yo	ement to you. Íf you a g system software (th	are licensed to use ne "software"), you
I have read and accept the lice	nse terms.	
Download size estimate:	0 MB	
Download time estimates:	Dial-Up: 0 minu Broadband: 0 r	

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

9 Select **Install**. The Installation is Complete window appears when installation has finished.



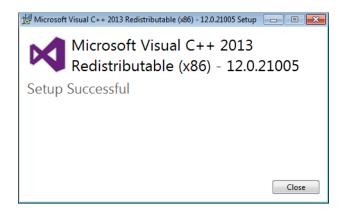
 $10\,$ Select Finish. The Microsoft Visual C++ 2013 Redistributable Setup window appears.



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

Α

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



13 Select **Close**. The Welcome to the CytExpert Setup Wizard window appears.



 $14 \hspace{0.1in} \text{Select Next. The Beckman Coulter License Terms window appears.}$

So CytExpe	ert Setup		
	Advanced Installer		
\neq	Read the Beckman Coulter License Terms		
	To continue you must read and accept the terms of this agreement. If you do not want to accept the Beckman Coulter License Terms, dose this window to cancel the installation.		
	BECKMAN COULTER, INC.		
	SOFTWARE END-USER LICENSE AGREEMENT		
	IMPORTANT: READ THIS END-USER LICENSE AGREEMENT:		
	THIS END-USER LICENSE AGREEMENT ("Agreement") GOVERNS THE USE OF		
	CERTAIN COMPUTER PROGRAMS ASSOCIATED WITH THE CORRESPONDING		
	PRODUCT ("Product") OF BECKMAN COULTER, INC. OR ITS AFFILIATES ("BCT").		
	YOUR USE OF SUCH COMPUTER PROGRAMS, YOUR PURCHASE OF THE PRODUCT. AND/OR YOUR USE OF THE PRODUCT CONSTITUTES ASSENT AND		
	ACCEPTANCE OF THIS AGREEMENT BY YOU (collectively "You" or "Your") AND		
	SIGNIFIES YOUR AGREEMENT TO BE BOUND BY THE TERMS AND CONDITIONS		
	OF THIS AGREEMENT. IF YOU DO NOT AGREE WITH ALL OF THE TERMS AND \checkmark		
	I accept the terms of this agreement.		
	< Back Next >		
	COUR INCAS		

15 Read the Beckman Coulter Customer End User License Agreement.

16 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.

A

17 Select **Next**. The Choose a file location window appears.

CytExpe	ert Setup		- • •
	Choose a file locatio	n	
	To install in this folder, click "Next".	To install to a different folder, enter it	below or click "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	1	< Back Next >

18 Select Next. The Installation Options window appears.

🗟 CytExpert Setup	×
Adv	anced Installer
CytExpert Installation Options	
Please select the CytExpert installation option:	
Operault	
🔘 User Management	
Electronic Record Management	
Please refer to the CytoFLEX Series Instructions for Use manual for details regarding each in option. Download the Instructions for Use manual at: <u>www.beckmancoulter.com/ifu</u>	stallation
< Back	Next >

19 Select the desired installation option. Refer to CytExpert Software Installation Options.

20 Select **Next**. The Begin installation of CytExpert window appears.



 $\label{eq:21} \textbf{Select Install} \text{ to begin installing the software. The Installation Progress window appears.}$

CytExper	t Setup	E
5	Installation Progress	Advincadi Indellar
	Copying new files	
	Installing CytExpert	

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

А

22 The following software prompt appears. Select **Οκ**.



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

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

🗟 CytExpert Setup		
	CytExpert has been successfully installed.	Advanced/installer
	Gose	

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

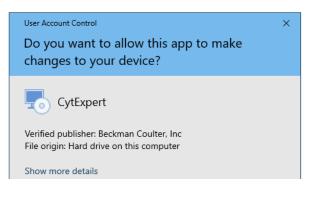
Risk of erroneous results or instrument damage. Only install the configuration file that matches your instrument. Installing an incorrect configuration file could cause erroneous results or instrument damage.

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 Platform].

1 Select and run **CytExpert_X.X_Config_Setup_XXXX.exe**. The User Account Control window appears.

NOTE XXXX refers to the serial number of the instrument.



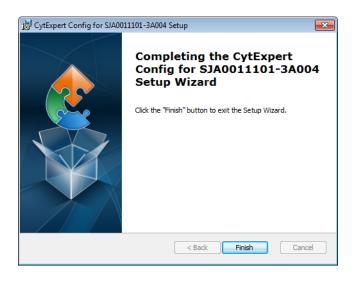
2 Select **Yes**. The CytExpert Config Welcome window appears.



3 Select **Next**. The CytExpert Config Ready to Install window appears.



- **4** Verify that the serial name displayed at the top of the window is correct.
- **5** Select **Install**. When the installation has finished, the Completing the CytExpert Config Setup Wizard window appears.



6 Select Finish.

7 If you get the following message, select **OK**. When you launch CytExpert, the system will automatically upgrade your configuration file.



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 4, 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 previous version.

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.

А

Refer to CytExpert Software Installation Options for the differences between each software option available.

A CAUTION

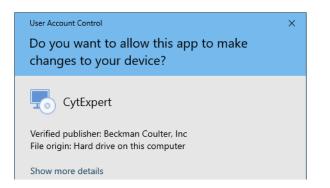
Risk of data loss. Reinstalling the CytExpert Software could overwrite your database. Ensure you backup your database prior to software reinstallation.

1 Insert the software USB into the computer.

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

🖡 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.

CytExpert Setup				
Z	Advanced linstallar Read the Beckman Coulter License Terms			
	To continue you must read and accept the terms of this agreement. If you do not want to accept the Beckman Coulter License Terms, dose this window to cancel the installation.			
	BECKMAN COULTER, INC.			
	SOFTWARE END-USER LICENSE AGREEMENT			
	IMPORTANT: READ THIS END-USER LICENSE AGREEMENT:			
	THIS END-USER LICENSE AGREEMENT (" <i>Agreement</i> ") GOVERNS THE USE OF CERTAIN COMPUTER PROGRAMS ASSOCIATED WITH THE CORRESPONDING PRODUCT (" <i>Product</i> ") OF BECKMAN COULTER, INC. OR ITS AFFILIATES (" <i>BCT</i> "). YOUR USE OF SUCH COMPUTER PROGRAMS, YOUR PURCHASE OF THE PRODUCT, AND/OR YOUR USE OF THE PRODUCT CONSTITUTES ASSENT AND ACCEPTANCE OF THIS AGREEMENT BY YOU (collectively " <i>You</i> " or " <i>Your</i> ") AND SIGNIFIES YOUR AGREEMENT TO BE BOUND BY THE TERMS AND CONDITIONS OF THIS AGREEMENT. IF YOU DO NOT AGREE WITH ALL OF THE TERMS AND			
	I accept the terms of this agreement.			

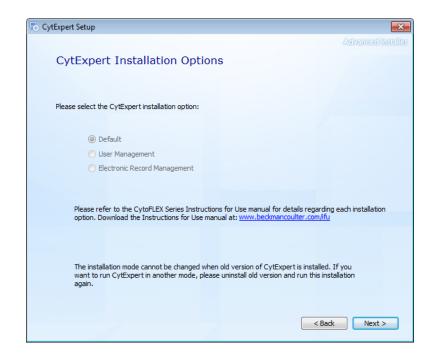
5 Read the Beckman Coulter Customer End User License Agreement.

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 10, 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	merð Ínsteller
Click Remove to remove CytExpert from your computer. If you want to review or change any 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.

А

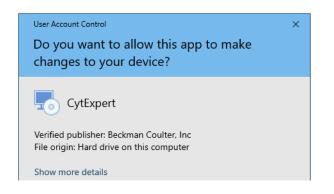
8 Select **Remove**. When software removal is complete, the software displays the message *CytExpert has been successfully configured*.



9 Select Close.

10 Navigate back to the software USB folder.

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



12 Select **Yes**. The Welcome to CytExpert Setup Wizard window appears.



 $\textbf{13} \hspace{0.1 cm} \text{Select Next. The Beckman Coulter License Terms window appears.}$

Solution CytExpe	rt Setup	x
Z	Read the Beckman Coulter License Terms	itillar
	To continue you must read and accept the terms of this agreement. If you do not want to accept the Bedoman Coulter License Terms, dose this window to cancel the installation.	
	BECKMAN COULTER, INC.	
	SOFTWARE END-USER LICENSE AGREEMENT	
	IMPORTANT: READ THIS END-USER LICENSE AGREEMENT:	
	THIS END-USER LICENSE AGREEMENT (" <i>Agreement</i> ") GOVERNS THE USE OF CERTAIN COMPUTER PROGRAMS ASSOCIATED WITH THE CORRESPONDING PRODUCT (" <i>Product</i> ") OF BECKMAN COULTER, INC. OR ITS AFFILIATES (" <i>BCT</i> "). YOUR USE OF SUCH COMPUTER PROGRAMS, YOUR PURCHASE OF THE PRODUCT, AND/OR YOUR USE OF THE PRODUCT CONSTITUTES ASSENT AND ACCEPTANCE OF THIS AGREEMENT BY YOU (collectively " <i>Fou</i> " or " <i>Four</i> ") AND SIGNIFIES YOUR AGREEMENT TO BE BOUND BY THE TERMS AND CONDITIONS OF THIS AGREEMENT. IF YOU DO NOT AGREE WITH ALL OF THE TERMS AND	
	I accept the terms of this agreement.	

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.

ScytExpe	t Setup		- • -
	Choose a file location		Advenced (natellar
	To install in this folder, click "Next". To in	istall 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	
		< <u>B</u> ar	k Next >

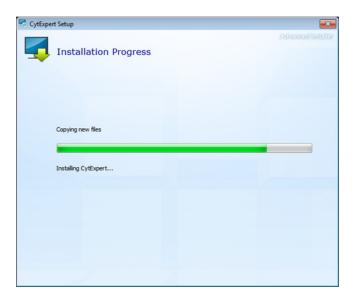
17 Select **Next**. The Installation Options window appears.



- **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 **Οκ**.



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.

SytExpert Setup		
		Advanced Installer
	*	
	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 available in the CytExpert Default software option or the CytExpert User Management software option. Refer to Figure 2.3 and Figure 2.4 for comprehensive software menu trees and details on which menu item applies to each software option.

Experiment Management

CAUTION

Risk of file corruption. Do not add, delete, or modify data from the Windows Explorer directory. Manage all data changes using Experiment Explorer to ensure file indexing remains intact.

Closed File System

The closed file system provides audit trail capability for CytExpert experiment files. The closed file system provides a secure layer between the actual Windows Explorer files and the CytExpert users to retain file integrity.

Three file types are managed by the closed file system:

- Experiment files
- Compensation experiment files
- Experiment template files

The Experiment Explorer dialog can be accessed by selecting **File > Experiment Explorer**. The Experiment Explorer dialog functions similarly to Windows Explorer.

periment Explorer							۰	23
Workspace							Enter text to search	٦
Open New Folder [Delete Rename Impo	ort Experiment	Export Experin	nent				
🗸 📄 Workspace (335.99 GB (Name	Date modified 🔺	Modified by	Туре	Date created	Author	Signature Status	1
> 퉬 Experiment1	Experiment1			Folder				
퉬 Compensation	Compensation			Folder				
퉬 Templates	鷆 Templates			Folder				
	🙀 Exp_20180509_3.xit	2018-05-09 14:4	Admin	Experi	2018-05-09 14:4	Admin	Unsigned	
	Exp_20180508_1(1).xit	2018-05-14 14:2	Admin	Experi	2018-05-14 14:2	Admin	Unsigned	
	Exp_20180508_1(2).xit	2018-05-14 14:4	Admin	Experi	2018-05-14 14:4	Admin	Unsigned	
	Exp_20180509_1(1).xit	2018-05-14 15:2	Admin	Experi	2018-05-14 15:2	Admin	Unsigned	
	Exp_20180509_2(1).xit	2018-05-14 15:2	Admin	Experi	2018-05-14 15:2	Admin	Unsigned	
	Exp_20180509_3(1).xit	2018-05-14 15:2	Admin	Experi	2018-05-14 15:2	Admin	Unsigned	
	🔢 Exp_20180514_1.xit	2018-05-14 16:0	Admin	Experi	2018-05-14 16:0	Admin	Unsigned	
	Comp_20180515_1.xitc	2018-05-15 09:2	Admin	Compe	2018-05-15 09:1	Admin	Unsigned	
	template1.xitm	2018-05-15 09:2	Admin	Template	2018-05-15 09:2	Admin		
	4							

Experiments, compensation experiments, and experiment templates created in other modes 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 set up at least one experiment directory for experiment files.

NOTE Only one experiment directory can be created per drive.

Setting Up the Experiment Directory

1 Select Settings > Set Experiment Directory. The Set Experiment Directory window appears.

Set Experime	nt Directory	-		23
Name	Path	Free Spa	ce	
	Add Rename Dele	te	Close	,

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

Add New	Experiment Directory	23
Name:		
Path:		
	OK Can	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	Experiment Director	y	_ 0 %
	Name	Path	Free Space
E	Kelly	C:\CytoFLEX-S_AS24001_Data	411.68 GB free
E	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.

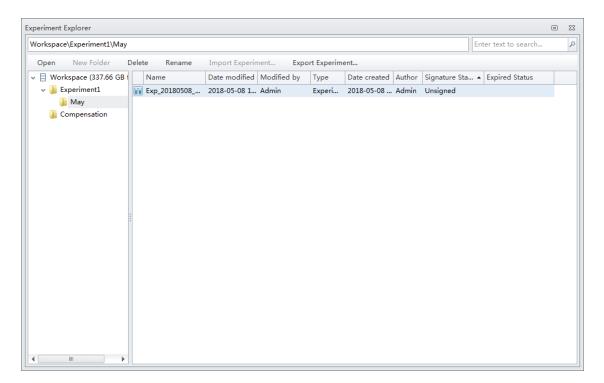
iment Explorer							۰	
rkspace\Experiment1						Enter text to	search	
pen New Folder Delete Rename Im	port Experiment	Export Experir	nent					
Workspace (336.24 GB Name	Date modified	Modified by	Туре	Date created	Author	Signature Status	Expired Sta	atu
New Folder			Folder					
Rename								
Import Experiment								

Select **Rename** from the right-click drop down menu or from the Experiment Explorer toolbar to rename a subfolder.

Select **Delete** from the right-click drop down menu or from the Experiment Explorer toolbar to delete a subfolder.

Experiment Related Operations

The Experiment Explorer dialog appears in place of the Windows File Explorer in the following operations: New/Open Experiment, New/Open Compensation Experiment, Save As, Save Experiment As Template, Recent Template, and New Experiment from Template.



Importing an Experiment/Template

Use the following procedure to import experiment (.xit), compensation (.xitc), or experiment from template (.xitm) files into the system.

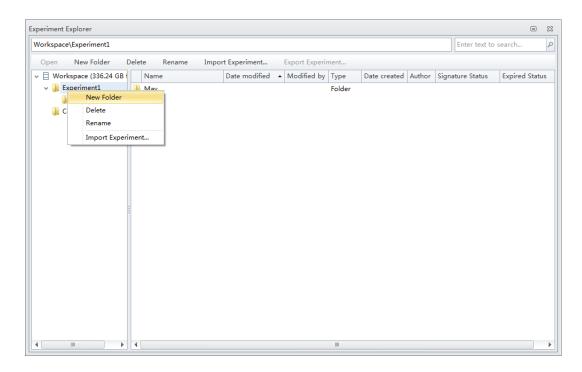
1 Select File > Experiment Explorer. The Experiment Explorer window appears.

xperiment Explorer										۰	23
Workspace\Experiment1\May									Enter text to search		P
Open New Folder D	Delete	Rename	Import Experin	nent Expo	rt Experime	ent					
👻 📄 Workspace (336.37 GB (Nan	ne	Date modified	Modified by	Туре	Date created	Author	Signature Sta.	. • Expired Status		
🗸 퉬 Experiment1	Exp.	_20180508	2018-05-08 1	Admin	Experi	2018-05-08	Admin	Signed	Unexpired		
퉲 May	Exp.	_20180509	2018-05-09 1	Admin	Experi	2018-05-09	Admin	Unsigned			
Compensation	Exp.	_20180509	2018-05-09 1	Admin	Experi	2018-05-09	Admin	Validated	Unexpired		
	Exp.	_20180509	2018-05-09 1	Admin	Experi	2018-05-09	Admin	Validated	Unexpired		
:	:										
	:										
4											

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

Open New Folder Delet Rename Import Experiment. Export Experiment. Export Experiment. Date created Author Signature Sta Expired Status Workspace (336.33 G8 Name Date modified Modified by Type Date created Author Signature Sta Expired Status May Experiment1 Exp.20180508.1xit 2018-05-08 17:0 Admin Experi 2018-05-09 Admin Unexpired May New Folder (1).xit 2018-05-09 14:4 Admin Experi 2018-05-09 Admin Unexpired Unexpired New Folder (1).xit 2018-05-09 13:1 Admin Experi 2018-05-09 Admin Validated Unexpired Unexpired Experiment Lxit 2018-05-09 14:0 Admin Experi 2018-05-09 Admin Validated Unexpired Unexpired Lxit 2018-05-09 14:0 Admin Experi 2018-05-09 Admin Validated Unexpired Unexpired Lxit 2018-05-09 14:0 Admin Experi 2018-05-09<	orkspace\Experime	ent1\May								Enter text to	search
May Experiment1 Experise 2018-05-08 1.xit 2018-05-08 1.xit 2018-05-08 1.xit 2018-05-08 May Unexpired May May New Folder (1).xit 2018-05-09 14.4 Admin Experi 2018-05-09 Admin Unexpired Delete Lxit 2018-05-09 13:1 Admin Experi 2018-05-09 Admin Vialidated Unexpired Lete Lxit 2018-05-09 14:0 Admin Experi 2018-05-09 Admin Vialidated Unexpired	Open New Fol	lder De	elete Rename	Imp	oort Experiment	Export Experi	ment				
May New Folder 1.1).xit 2018-05-09 14:4 Admin Experi 2018-05-09 Admin Unsigned Delete Lxit 2018-05-09 13:1 Admin Experi 2018-05-09 Admin Validated Unexpired Rename Lxit 2018-05-09 14:0 Admin Experi 2018-05-09 Admin Validated Unexpired	Uvrkspace (33	6.33 GB 1	Name		Date modified	Modified by	Туре	Date created	Author	Signature Sta 🔺	Expired Status
New Folder (1).xit 2018-05-14 Experi 2018-05-14 Momin Unsigned Delete .xit 2018-05-09 13:1 Admin Experi 2018-05-09 Admin Validated Unexpired Rename .xit 2018-05-09 14:0 Admin Experi 2018-05-09 Admin Validated Unexpired	🗸 鷆 Experiment:	1	Exp_20180508	1.xit	2018-05-08 17:0	Admin	Experi	2018-05-08	Admin	Signed	Unexpired
Lxit 2018-05-09 13:1 Admin Experi 2018-05-09 Admin Validated Unexpired Rename Lxit 2018-05-09 14:0 Admin Experi 2018-05-09 Admin Validated Unexpired		New F	older								
r.xit 2018-05-09 14:0 Admin Experi 2018-05-09 Admin Validated Unexpired											
				2.xit	2018-05-09 14:0	Admin	Experi	2018-05-09	Admin	Validated	Unexpired

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



A progress bar appears when importing files.

CytExpert	
Importing Exp_20180509_1.xit	0% Finished

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

orkspace\Experiment1\May							Enter text to	search
Open New Folder [Delet	e Rename Impo	ort Experiment	Export Experir	ment			
Workspace (336.24 GB		Name			Туре	Date created Author	Signature Status	Expired Status
Experiment1		Exp 20180508 1.xit	2018-05-08 17:0		Experi	2018-05-08 Admin	Signed	Unexpired
May		Exp_20180509_1.xit	2018-05-09 13:1	Admin	Experi	2018-05-09 Admin	Validated	Unexpired
Compensation		Exp_20180509_2.xit	2018-05-09 14:0	Admin	Experi	2018-05-09 Admin	Validated	Unexpired
		Exp_20180509_3.xit	2018-05-09 14:4	Admin	Experi	2018-05-09 Admin	Unsigned	
		Exp_20180508_1(1).xit	2018-05-14 14:2	Admin	Experi	2018-05-14 Admin	Unsigned	
		Exp_20180508_1(2).xit	2018-05-14 14:4	Admin	Experi	2018-05-14 Admin	Unsigned	
		Exp_20180509_1(1).xit	2018-05-14 15:2	Admin	Experi	2018-05-14 Admin	Unsigned	
		Exp_20180509_2(1).xit	2018-05-14 15:2	Admin	Experi	2018-05-14 Admin	Unsigned	
		Exp_20180509_3(1).xit	2018-05-14 15:2	Admin	Experi	2018-05-14 Admin	Unsigned	
	4							

NOTE Users can import .xit, .xitc, and .xitm files individually or multiple files at a time.

Exporting an Experiment/Template

Vorksp	ace\Experiment1\May									Enter text to search	
Open	New Folder [Dele	te Rename	Import Experir	nent Evo	rt Experime	ant				_
	orkspace (336.37 GB		Name	Date modified		-		ا مر الم	Cianata a Cha	• Expired Status	_
_	Experiment1					Туре			·		
× 🚛	May	-	Exp_20180508				2018-05-08 Ad		-	Unexpired	
	Compensation		Exp_20180509			1 C C C C C C C C C C C C C C C C C C C			<u> </u>		
	Compensation	-	Exp_20180509							Unexpired	
			Exp_20180509	2018-05-09 1	Admin	Experi	2018-05-09 Ad	lmin	Validated	Unexpired	

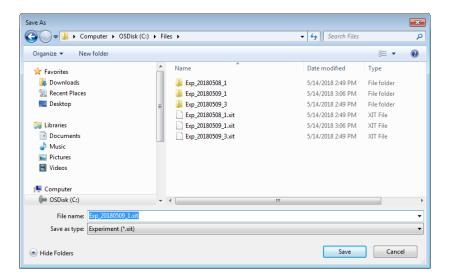
1 Select File > Experiment Explorer. The Experiment Explorer window appears.

2 To export a single experiment:

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

Workspace\Experiment1\May					
Open New Folder D	elete Rename	Import Experin	nent Expo	rt Experim	ent
🗸 📄 Workspace (336.37 GB f	Name	Date modified	Modified by	Туре	Date create
🗸 퉬 Experiment1	Exp_20180508	0010 05 00 1	A 1 - 1	Experi	2018-05-08
🌗 May	Exp_20180509	Open		Experi	2018-05-09
Compensation	Exp_20180509	Delete		Experi	2018-05-09
	Exp_20180509	Rename		Experi	2018-05-09
		Set Retention	Period		
		Export Experir	nent		

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



A progress bar appears when exporting files.

CytExpert	
Exporting Exp_20180509_1.xit	33% Finished

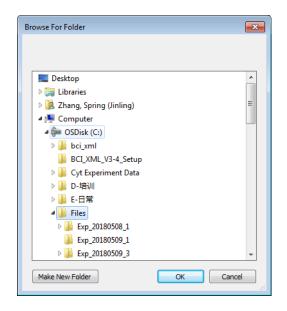
Once the export is complete, the exported file displays in the target folder. OR

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.

orkspace\Experiment1\May							Enter text	to search
Open New Folder D	elet	e Rename Imp	oort Experiment	Export Exper	iment			
Workspace (336.31 GB		Name	Date modified	Modified by	Туре	Date created Autho	r Signature Status	Expired Status
🗸 퉬 Experiment1		Exp_20180508_1(1).xit	2018-05-14 14:2	Admin	Experi	2018-05-14 Admi	Unsigned	
🌗 May		Exp_20180508_1(2).xit	2018-05-14 14:4	Admin	Experi	2018-05-14 Admi	Unsigned	
Compensation		Exp_20180508_1.xit	2018-05-08 17:0	Admin	Experi	2018-05-08 Admi	Signed	Unexpired
		Exp_20180509_1.xit	2018-05-09 13:1	Admin	Experi	2018-05-09 Admi	Validated	Unexpired
			2019 05 00 14-0	Admin	Experi	2018-05-09 Admi	Validated	Unexpired
		Exp_20180509_	pen	nin	Experi	2018-05-09 Admi	Unsigned	
			ename et Retention Period					
			port Experiment					
1								
	4							

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



A progress bar appears when exporting files.

	33% Finis
Exporting Exp_20180509_1.xit	33% FINIS

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.

В

9

Figure B.1 Experiment Operation Log Window

×p	periment Operation Log				7
	Query Criteria				
- 1	Experiment: Workspace\Expe	eriments\Exp_20161122_1.xi	it		Current Experiment Select
- 1	Experiment ID: BACBF084B1984	55CA734FEDA3FD4A39E			
Ι,	User: (ALL)	0.	•		
	Time Range: () All time				
		2016-10-22 00:00:00	~ 2016-11-22 23:59:	59 -	
	Costonized	2010 10 11 00:00:00			
1	Query				Print & Expo
1	Operation	Username	User Full Name	Timestamp	Record
1	1 Add plate	Admin	Administrator	2016-11-22 15:32:49	Add a new plate 01.
	2 Add Tube	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 Tube	Admin	Administrator	2016-11-22 15:35:53	Add a new tube 01-Tube-A10.
	7 Add Tube	Admin	Administrator	2016-11-22 15:35:53	Add a new tube 01-Tube-A11.
	8 Add Tube	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.
I	10 Modify Well Type	Admin	Administrator	2016-11-22 15:36:02	Modify plate 01 well C3 type from empty well to sample well.
		Admin	Administrator	2016-11-22 15:36:02	Modify plate 01 well C4 type from empty well to sample
	11 Modify Well Type	Admin	Administration		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.
- 5. Query : Runs the query on the specified query criteria.

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

Figure B.2 Select Experiment Profile Window

ect 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 Fl
BCA319BDA9E4735AD90FA052EE8	Exp_20161024_1.xit	Kelly	Admin	Administrator	2016-10-24 10:3	Admin	Administrator	2016-10-27 14:23:37	No
5318583279441898E787ECCD774	Exp_20161025_1.xit	Kelly	TechSupport	TechSupport	2016-10-25 12:3	TechSupport	TechSupport	2016-10-25 12:36:05	No
42C64D8F9048AF983AA1C3EF62	2010-10-17_KF_fite-ecd-pc7 stdzn setup.xit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:38	No
LDDB2C8DF5C447B960699E4DF3B	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.sit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:38	No
589F05D2BF4ED9BE00787D9816A	Exp_20161004_1_Test Flow Plate.xit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:41	No
D10415C8464688926514F2E4676	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
458627FBD6746988C89C1F58EB3F	FITC-PE-ECD-PC7KO_Exp_20160928_1.ait	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:42	No
33340035E34E0BA16240337289D	QC Bead_Exp_20160928_1.wit	Kelly	Admin	Administrator	2016-10-27 08:1	Admin	Administrator	2016-10-27 08:13:42	No
S8C708D0372423AA8D5684608E4	KF_FITC_PE_ECD_PC7_KO_Exp_20160927_2.slt		Admin	Administrator	2016-10-27 08:1	TechSupport	TechSupport	2016-10-27 15:13:11	Vec
32D80023D9E4538B17B47955F005	laser delay_Exp_20160927_1.sit		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	temp1ait	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
CF82D4C3A7A45358668320CDDD3	Comp_20161027_2.xitc	Kelly	Admin	Administrator	2016-10-27 14:2	Admin	Administrator	2016-10-27 14:27:17	No
D64C01D042148E98A451A20AD83	Danaitm		Admin	Administrator	2016-10-27 14:3	TechSupport	TechSupport	2016-10-27 15:13:12	Yes
SS2E814A9864D90ADOF9DESD1AB	Exp_20161027_1.xk	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
0683D78C654446580F6EE97C1EC5	Exp_20161027_2.nit	Kelly\Mouse 10Color\Experiment	Kelly	Kelly	2016-10-27 15/2	Kelly	Kelly	2016-10-27 15:25:27	No
EE499EC40D4C649EE273CFA7285	Comp_20161027_1.xitc	Kelly/Mouse 10Color/Compens	Kelly	Kelly	2016-10-27 15:2	Kelly	Kelly	2016-10-27 15:27:06	No
0531C63FB994973901626465A70B	Comp_20161027_1.xitc	TechSupport\Rat 6color	Kelly	Kelly	2016-10-27 15:2	Kelly	Kelly	2016-10-27 15:29:18	No
312EAE81D4F42A3B3FCAC83D5A9	Comp_20161027_2.xitc	TechSupport\Rat 6color	Kelly	Kelly	2016-10-27 15:3	Kelly	Kelly	2016-10-27 15:30:08	No
904336A439343ABB490C347D18A	Exp_20161027_1.xit	TechSupport\Rat 6color	TechSupport	TechSupport	2016-10-27 15:3	Kelly	Kelly	2016-10-27 15:50:02	No

- 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.

6

В

100%			a •							
			Operation Lo			Operation	Username	User Full Name	Timestamp	Record
		Report Gene	erated Date: 2016-10-2	8 14:25:52	1:	Open Experiment	TechSupport	TechSupport	2016-10-27 15:39:55	Open experiment Exp_20161027_1, experiment path:TechSupport\Rat 6color\Exp_20161027_1.sit
	TechSupport	Rat Gcolor\Exp_2016102 343ABB490C347D18A907			1:	Add Gate	TechSupport	TechSupport	2016-10-27 15:40:01	Add e now Line segment gate P1 at worksheet Collection mode.
User: Time Range	All Users All Time			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.nt
Timezone:		astern Time (US & Canad	a)	Ð	14	Close Experiment	TechSupport	TechSupport	2016-10-27 15:40:14	Close experiment Exp_20161027_1, experiment path:TechSupport\Rat 6color\Exp_20161027_1.xit
Query Re	eration Usern	me User Full Name	Timestamp	Record	19	o 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	TechSup		2016-10-27 15:37:53	Create new experiment Exp_20161027_1, experiment path: TechSupport\Rat	10	i Add Gate	Kelly	Kelly	2016-10-27 15:49:57	Add a new Line segment gate P2 at worksheet Collection mode.
2 Add	gram	port TechSupport	2016-10-27 15:38:08	dcolor(Exp_20161027_1.xit. Add a new Histogram x : SSC-A, y : Count at worksheet Collection mode.	1	7 Save Experiment	Kelly	Kelly	2016-10-27 15:50:01	Save experiment Exp_20161027_1, experiment path:TechSupport\Rat 6color\Exp_20161027_1.ait
3 Add	gram TechSup	port TechSupport	2016-10-27 15:38:08	Add a new Histogram x : FITC-A, y : Count at worksheet Collectionmode.	11	Close Experiment	Kelly	Kelly	2016-10-27 15:51:20	Close experiment Exp_20161027_1, experiment path:TechSupport\Rat 6color\Exp_20161027_1.xit
4 Add Histo	TechSup	port TechSupport	2016-10-27 15:38:09	Add a new Histogram x : PerCP-A, y : Count at worksheet Collection mode.						
s Add Histo	gram TechSup	port TechSupport	2016-10-27 15:38:09	Add a new Histogram x : FSC-A, y : Count at worksheet Collection mode.						
6 Add Histo	gram TechSup	port TechSupport	2016-10-27 15:38:09	Add a new Histogram x : APC-A, y : Count at worksheet Collection mode.						
7 Edit1 Nam		port TechSupport	2016-10-27 15:38:38	Change Tubel name from Tubel to Tech Support Tubel.						
8 Click Butte Acqu Tube	on to ire	port TechSupport	2016-10-27 15:39:06	Click Record button, Tech Support Tubel record 731 events.						
			1							

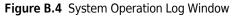
Figure B.3 Print and Export Preview Window

- 1. **Print:** Prints the report.
- 2. Export to PDF or CSV: Exports the report as a PDF or CSV file.
- 3. Report View: Displays the detailed report view.

System Operation Log

The System Operation Log generates system log records for settings, configuration, maintenance, and QC.

Select Log > System Operation Log to open the System Operation Log window. Refer to Figure B.4.



stem	Operation Log				۵	Σ
Quer	ry Criteria					
User:	(ALL) (3 -				
Time	Range: 2016-09-28 00:00:00	· ~ 2016-10-28	3 23:59:59 👻			
Qu	ery				Print & Export	+
					· · · · · · · · · · · · · · · · · · ·	_
#	Operation	Username	User Full Name	Timestamp	Record	
	1 Modify Sample Injection M	Admin	Administrator	2016-10-24 09:55:39	Modify sample injection mode from plate loader mode to semi automation mode.	ſ
	2 Modify Laser Settings	TechSupport	TechSupport	2016-10-24 11:19:15	Modified laser settings.	
	3 Modify Laser Settings	TechSupport	TechSupport	2016-10-24 11:23:22	Modified laser settings.	
	4 Modify Sample Injection M	Admin	Administrator	2016-10-24 12:18:18	Modify sample injection mode from semi automation mode to munual mode.	-
	5 Import QC Target File	Admin	Administrator	2016-10-24 15:28:55	Import QC target file D:\BAH02.tgt, lot No.: BAH02.	
	6 Run QC	Admin	Administrator	2016-10-24 15:31:36	Run QC, lot No.: BAH02.	
	7 Run QC	Admin	Administrator	2016-10-24 15:32:29	Run QC, lot No.: BAH02.	
	8 Run QC	Admin	Administrator	2016-10-24 15:33:35	Run QC, lot No.: BAH02.	
	9 Modify Sample Injection M	Admin	Administrator	2016-10-24 15:39:42	Modify sample injection mode from munual mode to semi automation mode.	
1	0 Run QC	Admin	Administrator	2016-10-24 15:40:20	Run QC, lot No.: BAH02.	
1	1 Run QC	Admin	Administrator	2016-10-24 15:44:10	Run QC, lot No.: BAH02.	
1	2 Run QC	Admin	Administrator	2016-10-24 16:10:50	Run QC, lot No.: BAH02.	
1	3 Delete QC Result	Admin	Administrator	2016-10-24 16:12:44	Delete QC result, lot No.: BAH02, date: 2016-10-24.	
1	4 Run QC	Admin	Administrator	2016-10-25 08:28:52	Run QC, lot No.: BAH02.	
1	5 Run QC	Admin	Administrator	2016-10-27 08:57:23	Run QC, lot No.: BAH02.	
1	.6 Run QC	Admin	Administrator	2016-10-27 09:00:44	Run QC, lot No.: BAH02.	
1	7 Run QC	Admin	Administrator	2016-10-27 09:10:54	Run QC, lot No.: BAH02.	
1	.8 Run QC	Admin	Administrator	2016-10-27 09:18:44	Run QC, lot No.: BAH02.	
1	9 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 Operation Log				۵
Query Criteria				
User: (ALL) Time Range: 2016-10-09 00:00	 .00 ▼ 2016-11-0 	09 23:59:59 *		
Query				Print & Expor
# Operation	Username	User Full Name	Timestamp	Record
1 Login	Admin	Administrator	2016-11-04 16:18:49	Login successfully.
2 Change password	Admin	Administrator	2016-11-04 16:19:05	Change password.
3 Logout	Admin	Administrator	2016-11-04 16:33:00	Logout.
4 Login			2016-11-04 16:41:09	Login failed (Username: Admin).
5 Login	Admin	Administrator	2016-11-04 16:41:13	Login successfully.
6 Logout	Admin	Administrator	2016-11-04 16:43:42	Logout.
7 Login	Admin	Administrator	2016-11-04 16:44:36	Login successfully.
8 Login	Admin	Administrator	2016-11-04 16:48:40	Login successfully.
9 Logout	Admin	Administrator	2016-11-04 17:07:29	Logout.
10 Login	Admin	Administrator	2016-11-07 09:35:10	Login successfully.
11 Logout	Admin	Administrator	2016-11-08 17:08:07	Logout.
12 Login			2016-11-08 17:10:11	Login failed (Username: Admin).
13 Login	Admin	Administrator	2016-11-08 17:10:14	Login successfully.
14 Logout	Admin	Administrator	2016-11-08 18:01:52	Logout.
15 Login			2016-11-09 07:28:53	Login failed (Username: Admin).
16 Login	Admin	Administrator	2016-11-09 07:29:05	Login successfully.
17 Create user	Admin	Administrator	2016-11-09 07:37:02	Create new user: [Username=Sarah, Full Name=Sarah L, Role=Operator, Enabled=Yes].
18 Modify user informatio	n Admin	Administrator	2016-11-09 07:39:32	Modify user usability (Username: Sarah) from [Yes] to [No].
19 Modify user informatio	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

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 Experiments

1 Select **File > Save** to save the experiment.

2 Select Signature > Sign. The Sign window appears.

Sign	83
Username:	Admin
Password:	
Comment:	
	V
	OK Cancel

3 Enter your user name and password.

Sign			23
Username:	Admin		
Password:	•••••		
Signature:			-
Comment:			
			-
			•
Enforce a	as final signature		
		ОК	Cancel

4 Select the appropriate signature.

Sign			23
Username:	Admin		
Password:	•••••		
Signature:	Approval		-
Comment:	Analysis Review		
	Approval		
			v
Enforce a	as final signature		
		ОК	Cancel

NOTE Only the highest signature level can skip to the final approval by selecting **Enforce as the final signature**.

5 If necessary, enter comments related to the experiment.

6 Select OK.

The signature status displays in the top-left corner of the software screen.

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

 I
 This experiment is signed. Editing and saving the experiment will retract the electronic signature.

If the experiment has been signed by all required signatures or the **Enforce as final signature** is selected, the status displays: *The experiment has been validated*.

7 Select Signature > Signature Records to view the electronic signature details.

Admin (Administrator)Analysis2018-05-09 13:20:48 (UTC+08:00)checkedAdmin (Administrator)Review2018-05-09 13:21:58 (UTC+08:00)reviewedAdmin (Administrator)Approval2018-05-09 13:48:38 (UTC+08:00)1000000000000000000000000000000000000	Signer	Signature Name	Date	Comment	
	Admin (Administrator)	Analysis	2018-05-09 13:20:48 (UTC+08:00)	checked	
Admin (Administrator) Approval 2018-05-09 13:48:38 (UTC+08:00)	Admin (Administrator)	Review	2018-05-09 13:21:58 (UTC+08:00)	reviewed	
	Admin (Administrator)	Approval	2018-05-09 13:48:38 (UTC+08:00)		

Rejecting Experiment

1 Select Signature > Reject.

Reject		23
Username:	Admin	
Password:		
Comment:		
	OK	el

NOTE You cannot reject an experiment if it has been validated.

2 If necessary, enter comments related to the experiment.

3 Select **OK**. The following message appears: Are you sure you want to reject the signature?

4 Select **Yes** to confirm the rejection of your signature or **No** to cancel.

Setting the Signature Retention Period

The signature retention period becomes effective immediately once an experiment has been signed. The experiment cannot be deleted or edited within the retention period.

Select **Settings** > **Set Retention Period** to set the signature retention period for an experiment.

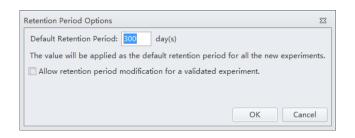
Set Retention Period	23
Retention Period: 300 day(s)	
The retention period starts from the first signature of the experiment.	
OK Cance	

NOTE The default signature retention period is 300 days. The allowable range is 1-9999 days.

Select **Settings > Set Retention Period** to navigate to the Experiment Explorer window to set the signature retention period for multiple experiments.

							1	-		-
orkspace\Experiment1\May								Enter text to search		1
Open New Folder 🛛	Delet	te Rename	Import Experin	nent Expo	ort Experime	nt				
Uvrkspace (337.97 GB		Name	Date modified	Modified by	Туре	Date created Author	Signature Sta	 Expired Status 		
🗸 퉬 Experiment1		Exp_20180508	2018-05-08 1	Admin	Experi	2018-05-08 Admin	Signed	Unexpired	_	
퉬 May		Exp_20180509	2018-05-09 1	Admin	Experi	2018-05-09 Admin	Unsigned			
퉬 Compensation		Exp_20180509	2018-05-09 1	Admin	Experi	2018-05-09 Admin	Validated	Unexpired		
		Exp_20180509	Open	I	Experi	2018-05-09 Admin	Validated	Unexpired		
			Delete							
			Rename							
			Set Retention	n Period						
			Export Exper	riment						

Select **Settings > Retention Period Options** to default signature retention period for experiments.



NOTE Select **Allow retention period modification for a validated experiment** to allow modifying the signature retention period for a validated experiment.

Signature Setting

The Signature Setting allows you to set the signature hierarchy, the signature name, and the maximum signature count for each signature level.

1 Select **Signature > Signature Setting**. The Signature Setting window appears.

Signature Setting			22
Signatures	Signatures		ture Details
2	Signature Name Analysis Review Approval	Allow s	Analysis um Signature Count: 1 ignature for roles: iistrator
			Add Edit Delete Close

NOTE You can add a maximum of 3 signature levels.

2 Select the signature name to be edited from the Signatures section of the Signature Setting window. Then, select **Edit**. The Edit Signature window appears.

Edit Signature	8
Name: Analysis	
Maximum Signature Count: 1	
Allow signature for roles:	
Administrator	
Role 1	
The role without permission of "Sign/R in above list.	eject" is not displayed ×
	OK Cancel

- **3** Enter the signature name.
- **4** Select the desired Maximum Signature Count.
 - **NOTE** The signature count means the allowable number of signatures for the signature name. You can choose between 1 and 10. The default value is 1.

5 Select a role for the signature from the *Allow signature for roles* section of the Edit Signature window.

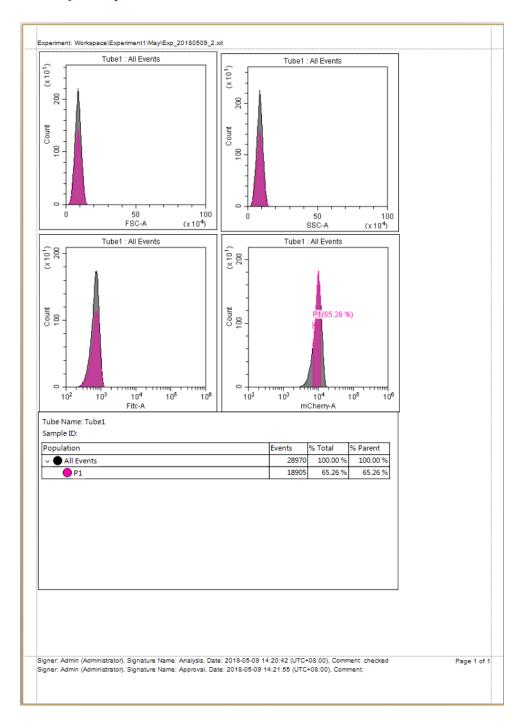
Edit Sign	ature	23				
Name:	Analysis1					
Maximu	m Signature Count: 3					
Allow si	gnature for roles:					
	✓ Administrator					
Role	e 1					
	The role without permission of *Sign/Reject* is not displayed × in above list.					
	OK Cance					

- **NOTE** Each role can sign or reject only if the sign/reject permission is assigned and the role signature is selected. The available roles displayed in the window depend on the permission setting assigned in Role Manager. To assign sign/reject permissions to a role, refer to Modifying User Roles in the Role Window in CHAPTER 2, Using the CytExpert Software.
- **6** Select **Ο***κ*.
- 7 Select Close.

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 Printing Graphics in CHAPTER 6, Data Acquisition and Sample Analysis.



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 4, Daily Startup.

Locking the Account

Refer to Locking the Account in CHAPTER 4, 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]

Perfor	mance [CytoFLEX or CytoFLEX S 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 or CytoFLEX S With Plate Loader DW]			
Carryover	Plate Loader format<1.0%		
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		
	backflush: <54 min.	ond acquisition with 5 second mixing and 4 second 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.

F	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 ≤1.0%			
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			
	backflush: <60 min.	nd acquisition with 6 second mixing and 4 second ond acquisition with 6 second mixing and 4 second		

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

Sample Injection Mode Control Kit Components

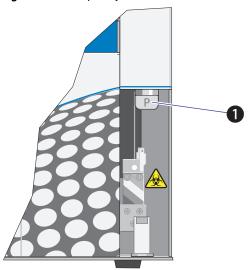


Figure C.1 Sample Injection Mode Control Knob

1. Switch module knob

NOTE The **P** indicates the instrument is set to Plate Loader sample injection mode. The **T** indicates the instrument is set to the Semi-Automatic or manual sample injection mode.

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



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.

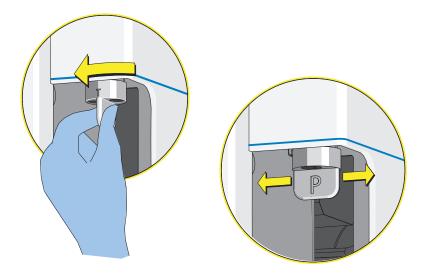
Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

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 12, 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 4, Daily Startup.

2 Turn the switch module knob to the left until the flat side of the knob is parallel to the front panel and the **P** is facing you. Ensure that the knob is turned as far as it will go.



The system is now ready for use in the Plate Loader sample injection mode.

NOTE If you do not see any events upon running an acquisition:

- 1. Select **stop** to stop the acquisition.
- 2. Ensure that the switch module knob is turned as far as it will go.
- **3.** Rerun the acquisition.
- 4. If the problem persists, contact us.

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



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.

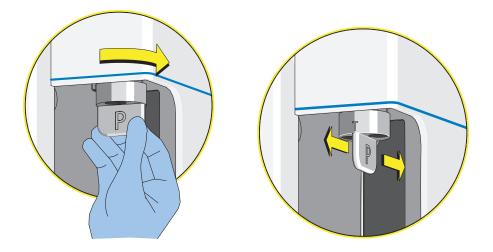
Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

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 12, 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 4, Daily Startup.

2 Turn the switch module knob to the right 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 12, Replacement/Adjustment Procedures.

Table D.1 Deep Well Plate [with Plate Loader DW	Table D.1	Deep Well Plate	[with Plate	Loader DW
---	-----------	-----------------	-------------	-----------

Name	Volume	Material	Manufacturer	PN
96-well deep well plate	1 mL	Polystyrene	Beckman Coulter	267001
96-well deep well plate	1 mL	Polypropylene	Beckman Coulter	267006
96-well deep well plate	2 mL	Polypropylene	Beckman Coulter	140504

NOTE Ensure that the following specifications are met when you select the 96-well deep well plates:

- The inner well diameter is \geq 5.8 mm.
- The plate height is \leq 45.5 mm.

Deep Well Plate Specimen Collection Plate Specifications

APPENDIX E Custom Optical Filters

Overview

IMPORTANT A custom optical filter could influence the performance of other channels due to the band pass and the reflection efficiency. Beckman Coulter recommends the operator to validate the performance of the custom optical filter within their configuration prior to accepting experimental results.

The optical filter is critical for optimal experiment results. When selecting an optical filter glass piece, the filter should comply with the following specifications.

Length	14.5 mm ± 0.1 mm
Width	6.1 mm ± 0.1 mm
Thickness	2.0 mm ± 0.1 mm
Incident angle	14 ° ± 2 °
Material	Fused silica, N-BK7, or equivalent
Surface quality	60/40 on both surfaces
Parallelism	\leq 20 arc seconds
Optical density	Make sure the optical density of the optical filter glass is big enough to block the wavelengths of the installed lasers to decrease the background noise.

Table E.1 CytoFLEX Platform Optical Filter Specifications

NOTE When installing a custom filter, you need to create a new detector configuration. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, Data Acquisition and Sample Analysis. For QC, refer to CHAPTER 5, Instrument Quality Control and Standardization.

Installing a Custom Optical Filter

IMPORTANT Keep the optical filter mounting fixture for future use.

The optical filter mounting fixture is used to align the custom optical filter glass piece with the optical filter holder. The optical filter mounting fixture and the optical filter holder are optional accessories that can be purchased separately.

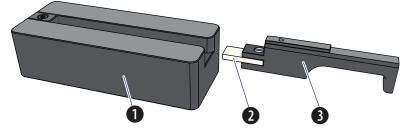


Figure E.1 Optical Filter Mounting Fixture and Optical Filter

- **1.** Optical filter mounting fixture
- 2. Custom optical filter glass piece
- 3. Optical filter holder

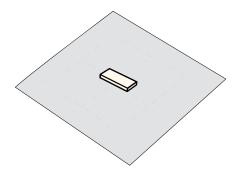
Before you start to install the custom optical filter glass piece, ensure the following items are available:

- Custom optical filter glass piece
- Optical filter mounting fixture
- Optical filter holder
- 3.0-mm flat-head screwdriver
- Lens papers
- Absolute ethanol

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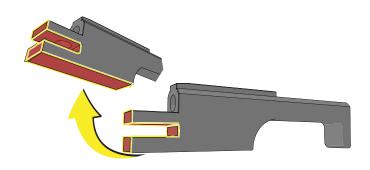
Risk of damage to the optical filter. When cleaning or replacing a filter, handle with care to avoid scratching the glass surface and to prevent the filter from falling. Use optical lens paper and absolute ethanol to clean the optical filters.

1 Place the new custom optical filter glass piece on a piece of clean lens paper.



2 Moisten a piece of lens paper with several drops of absolute ethanol.

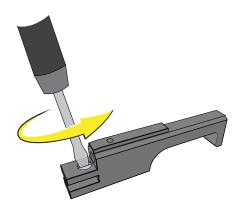
3 Using the moistened lens paper gently clean any spots on the surface of the optical filter glass piece and the socket of the optical filter holder.



4 Allow the custom optical filter glass piece to dry and check the optical filter glass piece for streaks.

NOTE If streaks remain, repeat Steps 2-3 until the custom optical filter glass piece is clean. If the optical filter glass piece is scratched, replace the optical filter glass piece as needed.

5 Using the 3.0-mm flat-head screwdriver, loosen the screw on the optical filter holder.

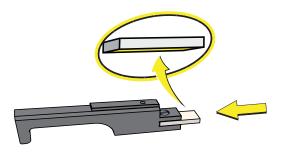


Risk of damage to the optical filter glass piece. Push the optical filter glass piece straight into the optical filter holder socket carefully. Inserting the optical filter glass piece at an angle could chip the edge of the optical filter glass piece.

<u>A</u>CAUTION

Risk of damage to the optical filter glass piece. Do not touch the filter glass piece. Touching the optical filter glass can obscure and/or scratch the optical filter. Always handle the optical filter glass piece with lens paper.

6 Push the clean optical filter glass piece straight into the optical filter holder socket with the band pass coating surface facing down.

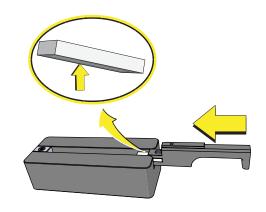


NOTE If you are unsure about which surface is the band pass coating surface, consult the supplier of your custom filter glass piece.

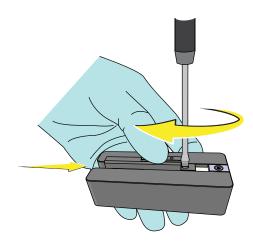
7 Using the 3.0-mm flat-head screwdriver, fasten the screw on the filter holder. Do not fully tighten the screw until adjustments are completed below.

Risk of damage to the optical filter. Push the filter all the way into the mounting fixture carefully. Inserting the filter at an angle could chip the edge of the filter glass piece.

8 Slide the optical filter into the slot of the optical filter mounting fixture until it touches the end of the fixture.



9 Hold the optical filter and tighten the screw using the 3-mm flat-head screwdriver.



- **10** If necessary, label the optical filter holder with the corresponding band pass information.
- **11** Install the optical filter into the WDM. Refer to Replacing the Optical Filter in CHAPTER 12, Replacement/Adjustment Procedures.

- **12** Edit the detector configuration to ensure the correct display of axis labels. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, Data Acquisition and Sample Analysis.
- **13** Run QC to check whether the custom filter has impacted the performance of the default channels. Refer to CHAPTER 5, Instrument Quality Control and Standardization.

NOTE QC will not provide a result for laser-filter combinations that do not have a target value.

14 If necessary, you need to adjust the filter screw slightly and repeat QC until optimal performance is achieved. Refer to Step 9 and Steps 11-13.

APPENDIX F WDM Beam Splitter

Overview

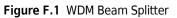
IMPORTANT This chapter applies to CytoFLEX LX instruments with the WDM beam splitter installed. The WDM beam splitter is installed by your Beckman Coulter Service Representative. The instrument configuration must have the IR laser and WDM activated to use the WDM beam splitter.

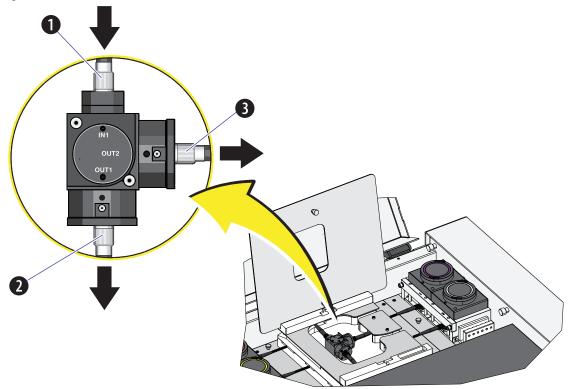
The WDM beam splitter splits the light from the UV, Near UV or Violet laser into the original WDM and the Infrared WDM. This results in the following new channels:

- V(S)712 for the Violet laser line
- N(S)740 and N(S)819 for the Near UV laser line
- U(S)740 and U(S)819 for UV laser lines.

Refer to Table 1.7.

NOTE The labels on the beam splitter ports indicate the direction that the fluorescence is transmitted. Refer to Figure F.1.





- 1. Fiber inlet: Connects to either violet, UV or NUV laser.
- 2. Beam outlet 1: Transfers the light with wavelength longer than 695 nm to the IR WDM.
- 3. Beam outlet 2: Transfers the light with wavelength shorter than 695 nm back to the original WDM.

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WDM		Fluorescent Channel (Standard)	Fluorescent Channe (WDM Beam Splitter)	
		405/30 BP	405/30 BP	
355 nm	355 nm	525/40 BP	525/40 BP	
		675/30 BP	675/30 BP	
	808 nm	840/20 BP	740/35 BP	
		885/40 BP	819/44 BP	
		450/45 BP	450/45 BP	
	375 nm	525/40 BP	525/40 BP	
		675/30 BP	675/30 BP	
375 nm		840/20 BP	740/35 BP	
	808 nm	885/40 BP	819/44 BP	
		405/10 BP ^a	885/40 BP ^b	
		450/45 BP	405/10 BP (VSSC)	
		525/40 BP	450/45 BP	
405 nm	405 nm	610/20 BP	525/40 BP	
		660/10 BP	610/20 BP	
		763/43 BP	660/10 BP	
		840/20 BP	712/25 BP	
	808 nm	885/40 BP	763/43 BP	

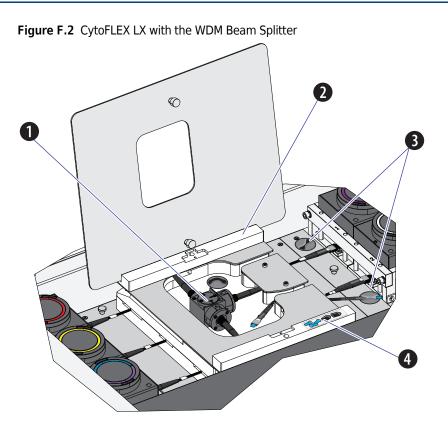
Table F.1 Configuration Comparison

a. This channel has no detector.

b. This channel has no detector.

NOTE The 712/25 BP filter, the 740/35 BP filter, and the 819/44 BP filter are supplied in the WDM beam splitter package.

Modifying Detector Configuration [With WDM Beam Splitter]



- 1. WDM beam splitter
- 2. Cover holder
- 3. Clips for idle fibers
- 4. Holder for idle fiber caps

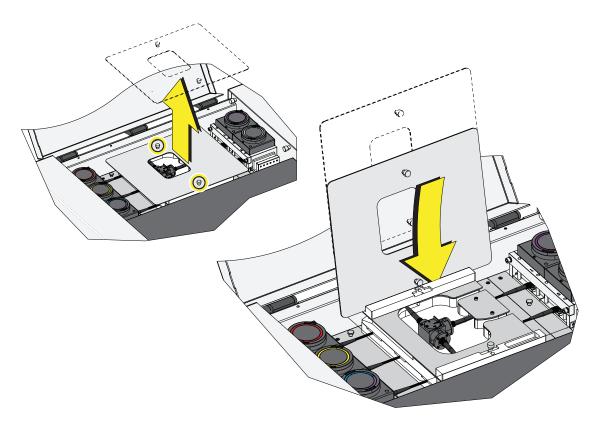
Table F.2 Optical Fibers with Indicator

Optical Fibers	Indicator	Name
	1	Long violet fiber
	2	Long UV/NUV fiber
	3	Red fiber
6	4	Yellow fiber
	5	Blue fiber
	6	Long IR fiber
	Violet color ring	Short violet fiber
	White color ring	Short UV/NUV fiber
	Red color ring	Short IR fiber

NOTE The short IR fiber, short violet fiber, and the short UV/NUV fibers are delivered with the WDM beam splitter package.

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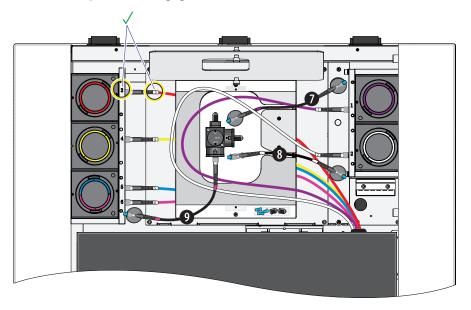
- **1** Open the top cover of the instrument.
- **2** Loosen the two thumbscrews securing the beam splitter cover and put the cover in the cover holder.



Risk of data integrity damage.

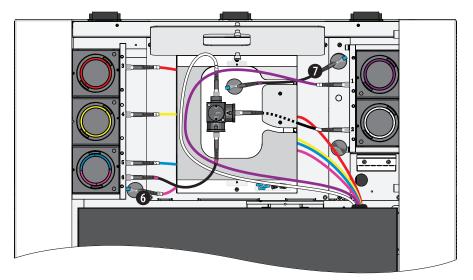
- During use, verify that the optical fibers are securely connected to the WDM. A loose connection can alter the optical path and affect fluorescence.
- Do not kink the optical fibers.
- **3** Connect the correct optical fibers to the corresponding WDMs as needed. Ensure the indicator number or the color ring of the optical fiber matches the corresponding WDM. Refer to Table F.2.

[WDM Beam Splitter-Not engaged]



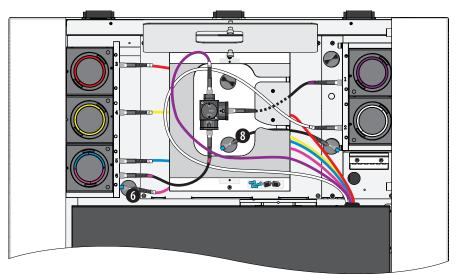
NOTE The short IR fiber (9), short violet fiber (7), and the short UV/NUV fiber (8), are idle.

[UV/NUV Splitter]



NOTE The long IR fiber (6), and the short violet fiber (7) are idle.

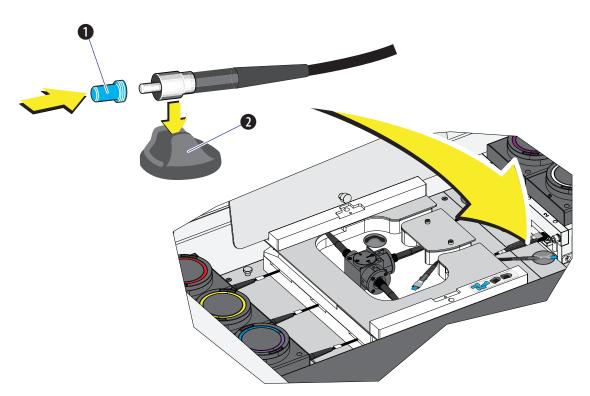
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[Violet Splitter]

NOTE The long IR fiber (6), and the short UV/NUV fiber (8) are idle.

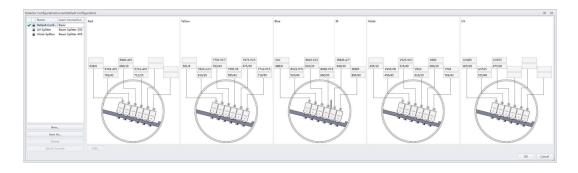
4 Put the blue fiber cap (1) on the head of idle fibers and fix the idle fibers in place with the fiber clips (2).



5 Change the optical filters in the WDM accordingly. Refer to Table F.1, Configuration Comparison for the positions of the corresponding optical filters. Refer to Replacing the Optical Filter in CHAPTER 12, Replacement/Adjustment Procedures.

NOTE The optical system does not require realignment once the filters are changed.

6 Select the proper detector configuration. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 6, Data Acquisition and Sample Analysis.



- 7 Verify the installed optical filters match the selected detector configuration.
- **8** Run QC. Refer to CHAPTER 5, Instrument Quality Control and Standardization.

Good Practices for Cyber Security



The following procedures are the only validated and approved recommendations for cyber privacy and security. Contact your IT professional for assistance.

- Changes below require Administrator Level Windows access.
- Before connecting to an external device such as a hard drive, USB, or DVD/CD, verify it does not have a virus or malware.

NOTE Beckman Coulter recommends using different anti-virus software to ensure that all viruses or malware can be identified.

- Disable all the unused USB ports on the instrument computer.
- Close all unnecessary applications while operating the CytExpert software.

<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 computer with software authorized by Beckman Coulter.
- You install software that is not an original copyrighted version. Only use software that is an original copyrighted version to prevent virus contamination.

This chapter contains information on:

- Drive Encryption
- Protection from Malware Software
- Operating System Updates
- System Hardening



Your Windows 10 system is equipped with BitLocker. Use BitLocker disk encryption software to prevent unauthorized access to your hard drive, refer to BitLocker Keys. For instructions on BitLocker Encryption or BitLocker Decryption, see Enabling BitLocker or BitLocker Decryption.

IMPORTANT BitLocker is the only approved encryption method.

BitLocker Keys

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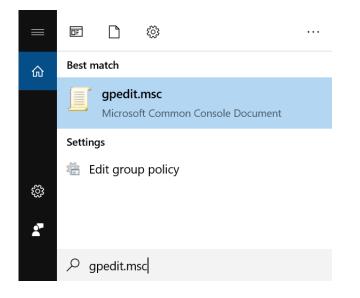
Risk of data loss. Store your BitLocker key in a safe place. For instructions on saving your BitLocker key, refer to Backing Up Your Recovery Key. If you lose your BitLocker Key, you will lose access to your entire hard drive including all of your data. Beckman Coulter is not responsible for security keys and will not be able to recover your data off your hard drive. Work with your IT department in creating and storing the keys in a safe place.

Changing Encryption Strength Level for BitLocker

BitLocker defaults to XTS-AES 128-bit encryption method. To alter the encryption, follow the steps below before enabling BitLocker. Changes to the encryption level will only impact the encryption of new volumes. Work with your IT department to change the Encryption Strength Level.

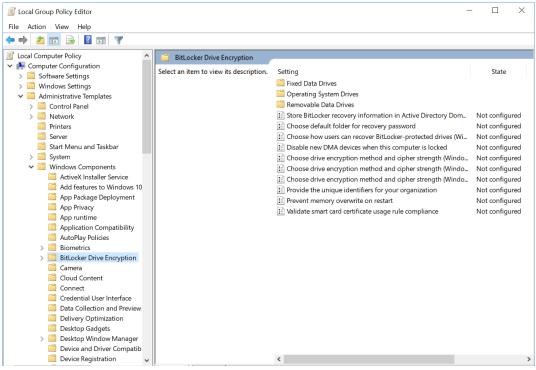
Changing the encryption strength for BitLocker

1 Type **gpedit.msc** from the Windows search bar to locate the gpedit Control Panel.



- 🗾 Local Group Policy Editor \times File Action View Help (+ +) 🖬 🗟 🗟 🖬 🧾 Local Computer Policy Iocal Computer Policy Computer Configural
 Software Settings Select an item to view its description. Name 🞥 Computer Configuration > 📋 Windows Setting: 🛃 User Configuration > 📋 Administrative Te 🗸 💰 User Configuration > 📋 Software Settings > 📋 Windows Setting: > 📋 Administrative Te > \Extended \Standard
- **2** Click **gpedit.msc**. The Local Group Policy Editor window appears.

3 Navigate to Computer Configuration > Administrative Templates > Windows Components > BitLocker Drive Encryption.



4 Double-click "Choose drive encryption method and cipher strength (Windows 10.....)". Refer to Figure G.1.

Local Group Policy Editor				- 🗆	\times
File Action View Help					
🗢 🏟 🛃 📰 🔒 🛛 🖬 🐨					
Icoal Computer Policy ∧ ✓ R. Computer Configuration > ≥ Software Settings > > Windows Settings	BitLocker Drive Encryption Choose drive encryption method and cipher strength (Windows 10 [Version 1511] and later)	Setting	State	Comment	
 ✓ Administrative Templates > ☐ Control Panel > ☐ Network ☐ Printers ☐ Server ☐ Start Menu and Taskbar 	Edit <u>policy setting</u> Requirements: At least Windows Server 2016, Windows 10	Operating System Drives Encourable Data Drives Encourable Data Drives Store BitLocker recovery information in Active Directory Domain Services (Windows Server 2 Choose default folder for recovery password Drobse how users can recover BitLocker-protected drives (Windows Server 2008 and Windo Disable new DMA devices when this computer is locked	Not configured Not configured Not configured Not configured	No No No	
System Windows Components ActiveX Installer Service Add features to Windows 10 App Package Deployment App Privage App Privage App Unitive App Unitive AutoPlay Policies Simetrics	Description: This policy setting allows you to configure the algorithm and cipher strength used by BitLocker Divite Encryption. This policy setting is applied when you turn on BitLocker. Changing the encryption method has no effect if the divise already encrypted, or if the civity already encrypted, or if encryption is in progress.	Choose drive encryption method and cipher strength (Windows & Windows Stever 2012, Win- Choose drive encryption method and cipher strength (Windows Vista; Windows Stever 2018, Win- Choose drive encryption method and cipher strength (Windows Vista; Windows Stever 2008, Choose drive encryption method and cipher strength (Windows Vista; Windows Stever 2008, Provide the unique identifies for your organization Prevent memory overwrite on restat Validate smart card certificate usage rule compliance	Not configured Not configured Not configured Not configured Not configured Not configured	No No No No No	
BitLocker Drive Encryption Camera Cloud Content Connect Connect Connect Data Collection and Preview Builds Data Collection and Preview Builds	If you enable this policy setting you will be able to configure an encryption algorithm and key cipher strength for fixed data drives, operating system drives, and removable data drives individually. For fixed and operating system drives, we recommend that you use the XTS- AES algorithm. For removable	,			
C Desktop Gadgets	Extended Standard	ć			-

Figure G.1 Choosing Drive Encryption Method and Cipher Strength

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5 Select **Enabled** and use the drop-down arrows to select the encryption method for the operating system drive, fixed data drive, and for the removable data drives, see Figure G.2.

Choose drive encryption method ar	id cipher strengt	h (Windows 10 [Version 1511] and later)	\times
Choose drive encryption method an Previous Setting Next Setting	nd cipher streng	th (Windows 10 [Version 1511] and later)	
Not Configured Comment: Enabled Disabled			< >
Supported on:	At least Windo	ws Server 2016, Windows 10	< >
Options:		Help:	
Select the encryption method for opera drives: XTS-AES 256-bit Select the encryption method for fixed XTS-AES 256-bit Select the encryption method for removed drives: XTS-AES 256-bit	data drives:	This policy setting allows you to configure the algorithm and cipher strength used by BitLocker Drive Encryption. This policy setting is applied when you turn on BitLocker. Changing the encryption method has no effect if the drive is already encrypted, or if encryption is in progress. If you enable this policy setting you will be able to configure an encryption algorithm and key cipher strength for fixed data drives, operating system drives, and removable data drives individually. For fixed and operating system drives, we recommend that you use the XTS-AES algorithm. For removable drives, you should use AES-CBC 128-bit or AES-CBC 256-bit if the drive will be used in other devices that are not running Windows 10 (Version 1511). If you disable or do not configure this policy setting, BitLocker wil use AES with the same bit strength (128-bit or 256-bit) as the "Choose drive encryption method and cipher strength (Windows	
		OK Cancel Apply	

Figure G.2 Choosing the Encryption Level

NOTE The default is set to XTS-AES 128-bit.

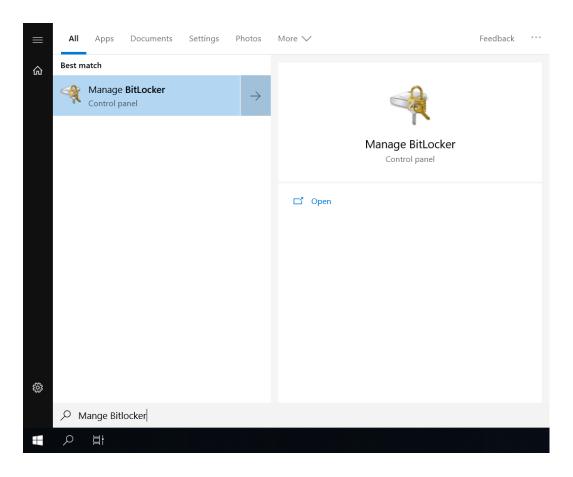
6 Select Apply.

.

- 7 Select **OK** and close all windows.
- **8** Restart the computer and proceed to Enabling BitLocker.

Enabling BitLocker

1 Type Manage BitLocker from the Windows search bar to locate the Manage BitLocker Control Panel.



G

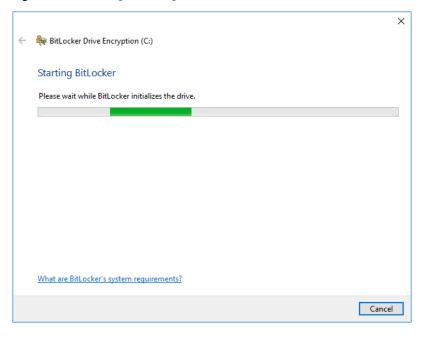
- 🍕 BitLocker Drive Encryption \times _ $\leftarrow ~
 ightarrow ~ \star$ \P > Control Panel > System and Security > BitLocker Drive Encryption Q ・ じSearch Control Panel ? Control Panel Home BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protecting your drives with BitLocker. Operating system drive Windows (C:) BitLocker off Furn on BitLocker 5 Fixed data drives Removable data drives - BitLocker To Go Insert a removable USB flash drive to use BitLocker To Go. See also **TPM** Administration 💎 Disk Management Privacy statement
- 2 Double-click Manage BitLocker to open the BitLocker Drive Encryption window.

3 Select **Turn on BitLocker** from the Control Panel screen.

← → ✓ ↑ 👎 > Cont	ol Panel > System and Security > BitLocker Drive Encryption	~ Ū	Search Control Panel	Q
Control Panel Home	BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protecting your drives with BitLocker.			(
	Operating system drive			
	Windows (C:) BitLocker off		\odot	
	Fixed data drives		\bigcirc	
	DATA1 (D:) BitLocker off Removable data drives - BitLocker To Go Insert a removable USB flash drive to use BitLocker To Go.		U	
See also TPM Administration Disk Management				

4 BitLocker verifies that your PC meets the requirements. Refer to Figure G.3.

Figure G.3 Checking PC Configurations



5 Select **Print the recovery key** to back up the recovery key. For instructions, refer to Backing Up Your Recovery Key.

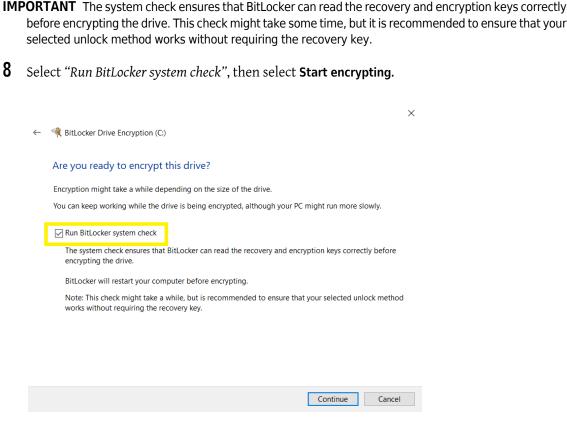
		\times					
\leftarrow	RitLocker Drive Encryption (C:)						
	How do you want to back up your recovery key?						
	Some settings are managed by your system administrator.						
	A recovery key can be used to access your files and folders if you're having problems unlocking your PC. It's a good idea to have more than one and keep each in a safe place other than your PC.						
	ightarrow Save to your Microsoft account						
	ightarrow Save to a file						
	\rightarrow Print the recovery key						
	How can I find my recovery key later?						
	Next Cance	1					

6 Select "Encrypt used disk space only (faster and best for new PCs and drives)", then select **Next**.

	×
🔶 litLocker Drive Encryption (C:)	
Choose how much of your drive to encrypt	
choose now much of your unve to encrypt	
If you're setting up BitLocker on a new drive or a new PC, you only need to encrypt the par that's currently being used. BitLocker encrypts new data automatically as you add it.	rt of the drive
If you're enabling BitLocker on a PC or drive that's already in use, consider encrypting the Encrypting the entire drive ensures that all data is protected–even data that you deleted bu contain retrievable info.	
Encrypt used disk space only (faster and best for new PCs and drives)	
○ Encrypt entire drive (slower but best for PCs and drives already in use)	
Next	Cancel

7 Select "New encryption mode (best for fixed drives on this device)", then select Next.

		\times
~	🄫 BitLocker Drive Encryption (C:)	
	Choose which encryption mode to use	
	Windows 10 (Version 1511) introduces a new disk encryption mode (XTS-AES). This mode provides additional integrity support, but it is not compatible with older versions of Windows.	
	If this is a removable drive that you're going to use on older version of Windows, you should choose Compatible mode.	
	If this is a fixed drive or if this drive will only be used on devices running at least Windows 10 (Version 1511) or later, you should choose the new encryption mode	
	• New encryption mode (best for fixed drives on this device)	
	\bigcirc Compatible mode (best for drives that can be moved from this device)	
	Next Cance	1



NOTE BitLocker restarts your computer before encrypting.

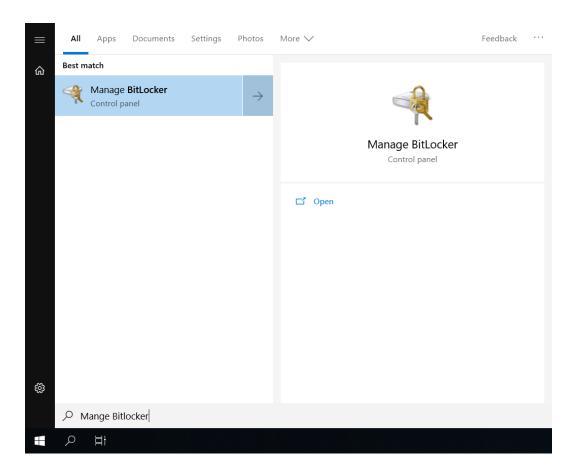
9 Select **Restart now** to start the BitLocker Encryption.



10 Enter your password in the Password screen.

NOTE After logging in you will see a Window that says **Encrypting in Progress**.

G



$11 \ \text{Open the Manage BitLocker Control Panel from the Windows search bar.}$

12 Under Operating System Drive, you will see the BitLocker is Encrypting the drive. See Figure G.4.

BitLocker Drive Encryption			– 🗆 ×
← → 🕐 📙 → Control Panel → System and Security → BitLocker Drive Encryption			Search Control Panel 🔎
Control Panel Home	BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protec	ting your drives with BitLocker.	0
	() For your security, some settings are managed by your system ad	ninistrator.	
	Operating system drive		
	Windows (C:) BitLocker Encrypting		\bigcirc
	Sack up your reco Turn off BitLocker		
	Fixed data drives		
	Removable data drives - BitLocker To Go Insert a removable USB flash drive to use BitLocker To Go.		
See also TPM Administration Disk Management Privacy statement			

Figure G.4 BitLocker Encrypting the Drive

13 Once the BitLocker has encrypted the drive, the BitLocker will be on. See Figure G.5.

Figure G.5 BitLocker Successfully On

🕆 👎 > Contro	I Panel > System and Security > BitLocker Drive Encryption	~ Ŭ	Search Control Panel	
Control Panel Home	BitLocker Drive Encryption			
	Help protect your files and folders from unauthorized access by protecting your drives with BitLocker.			
	For your security, some settings are managed by your system administrator.			
	Operating system drive			
	Windows (C:) BitLocker on		\bigcirc	
	Suspend protection Back up your recovery key Turn off BitLocker			
	Fixed data drives			
	DATA1 (D:) BitLocker off		\odot	
	Removable data drives - BitLocker To Go			
	Insert a removable USB flash drive to use BitLocker To Go.			
See also				
TPM Administration				
Disk Management Privacy statement				

Backing Up Your Recovery Key

IMPORTANT The Back Up Your Recovery Key to your Microsoft Account is not supported at this time.

1 Type Manage BitLocker from the Windows search bar to locate the Manage BitLocker Control Panel, see below.

≡	All	Apps	Documents	Settings	Photos	More 🗸	Feedback	
ሴ	Best m	atch						
	Ą	Manage Control p	e BitLocker banel		\rightarrow			
						Manage BitLocker Control panel	r	
						다 Open		
ŝĝ								
	ρM	lange Bit	locker					
	Q	Ξi						

2 Double-click **Manage BitLocker** to open the BitLocker Drive Encryption window.

4	BitLocker Drive Encryption			-	□ ×
÷	\leftarrow \rightarrow \checkmark \Uparrow Control Panel \Rightarrow System and Security \Rightarrow BitLocker Drive Encryption \checkmark				Q
	Control Panel Home	BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protecting your drives with BitLocker For your security, some settings are managed by your system administrator.	r.		0
		Operating system drive			
		Windows (C:) BitLocker on		\bigcirc	
		Suspend protection Sack up your recovery key Turn off BitLocker			
		Fixed data drives			
		DATA1 (D:) BitLocker off		\odot	
		Removable data drives - BitLocker To Go Insert a removable USB flash drive to use BitLocker To Go.			
-	See also TPM Administration Disk Management Privacy statement				

3 Select *"Backup your recovery key"*. The following window appears.

		\times
🍕 Bi	tLocker Drive Encryption (C:)	
	How do you want to back up your recovery key?	
	A recovery key can be used to access your files and folders if you're having problems unlocking your PC. It's a good idea to have more than one and keep each in a safe place other than your PC.	
	ightarrow Save to your cloud domain account	
	\rightarrow Save to a USB flash drive	
	\rightarrow Save to a file	
	\rightarrow Print the recovery key	
	How can I find my recovery key later?	
	Cancel	

IMPORTANT The Recovery Key can only be saved to a USB or external drive. You cannot save to a location on your PC.

- **4** Select a destination to save the Recovery Key.
 - **Save to a file**: Select "*Save to a file*". The following screen appears.

Save Print Output As						×
$\leftarrow \ \ \rightarrow \ \ \lor \ \ \uparrow$ This	PC > Documents			∨ Ü S	earch Documents	Q
Organize New folder						≣ • ()
A Quick access	Name	Date modified	Туре	Size		
Desktop	Key.pdf	6/15/2020 11:45 PM	PDF File	176 KB		
Downloads *		✓ Ø Search Documents Ø Image: Size Ø Size				
🖹 Documents 🖈						
📰 Pictures 🛛 🖈						
📕 Backing Up Your						
🧼 DATA1 (D:)						
🧢 This PC						
3D Objects						
Desktop						
Documents						
🖶 Downloads						
Music						
E Pictures						
File name: Key.pdf						~
Save as type: PDF Do	cument (*.pdf)					nts
				-		
∧ Hide Folders					Save	Cancel

• **Print the recovery key**: Select "*Print the recovery key*". The following screen appears.

🖶 Print	×
General	
Select Printer	🚍 Send To OneNote 2013
Microsoft Print to PDF	🛱 Send To OneNote 2016 🐨 Snagit 12
<	>
Status: Ready Location: Comment:	Preferences Find Printer
Page Range All Selection Current Page	Number of copies:
O Pages:	Collate

1. Select "Microsoft Print to PDF", and then select **Print**.

Save Print Output A	s)
· -> · 🛧 🗎 :	> This PC > Docume	nts			~ Ō	Search Document	s	P
)rganize 🔻 New	folder							?
🖈 Quick access	Name	^	Date modified	Туре	Size			
Desktop	*		No items mate	h your search.				
Downloads	*							
😫 Documents	*							
Pictures	*							
BitLocker								
👌 Music								
Videos								
💻 This PC								
💣 Network								
-								
File name								
Save as type: P	DF Document (*.pdf)							
Hide Folders						Save	Cancel	

- **2.** Select the location that you want the file saved. In the File name, type **BitLocker Key** and in the Save as type, ensure that it is listed as.pdf.
- **5** Select **Save** then select **Finish**.

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BitLocker Decryption

IMPORTANT Turning off the BitLocker feature decrypts all the data on the drive. Decrypting your operating system means that you have removed BitLocker from your system. The BitLocker key will change each time BitLocker is re-enabled. Only the latest BitLocker key will work. Previous BitLocker keys will be invalid and will not unlock the system. Please note this is a time-consuming process. However, you can also suspend BitLocker instead of decrypting the drive, see Suspending BitLocker Drive Encryption.

Turning off BitLocker

1 Type Manage BitLocker from the Windows search bar to locate the Manage BitLocker Control Panel.

≡	All	Apps	Documents	Settings	Photos	More 🗸	Feedback	
ሴ	Best m	atch						
	Ą	est match Image BitLocker Control panel		\rightarrow				
						Manage BitLocker Control panel		
						C Open		
ŝ								
**	РM	lango Pit	lockor					
			IUCKEI					
	Q							

2 Double-click **Manage BitLocker** to open the BitLocker Drive Encryption.

RitLocker Drive Encryption			-	×
$\leftarrow \rightarrow \vee \uparrow$ $\ref{aneroid}$ > Control Pane	I > System and Security > BitLocker Drive Encryption	νŪ	Search Control Panel	P
Control Panel Home	BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protecting your drives with BitLocker. for your security, some settings are managed by your system administrator.			7
	Operating system drive			
	Windows (C:) BitLocker on Suspend protection Back up your recovery key Turn off BitLocker		\odot	
	Fixed data drives			
	DATA1 (D:) BitLocker off		\odot	
	Removable data drives - BitLocker To Go Insert a removable USB flash drive to use BitLocker To Go.			
See also				
 TPM Administration Disk Management Privacy statement 				

3 Select **Turn off BitLocker**.

4	BitLocker Drive Encryption			-		×
~	→ 🐳 ↑ 🔫 > Control Panel >	System and Security > BitLocker Drive Encryption	νŪ	Search Control Panel		P
		litLocker Drive Encryption lelp protect your files and folders from unauthorized access by protecting your drives with BitLocker.				?
		For your security, some settings are managed by your system administrator.				
	C	Dperating system drive				
		Windows (C:) BitLocker on		6)	
		Suspend protection Back up your recovery key Turn off BitLocker				
	F	ixed data drives				
		DATA1 (D:) BitLocker off		\odot)	
	Ŗ	Removable data drives - BitLocker To Go				
	See also	Insert a removable USB flash drive to use BitLocker To Go.				
•	TPM Administration					
•	Disk Management					
	Privacy statement					

?

The following message appears.

BitLocker Drive Encryption	×
Turn off BitLocker	
Your drive will be decrypted. This might take a long tim you can keep using your PC during the decryption pro	
Turn off BitLocker Ca	incel

Insert a removable USB flash drive to use BitLocker To Go.

IMPORTANT Drive decryption might take a long time, but you can keep using your PC during the decryption process.

4 Select **Turn Off BitLocker**. The BitLocker Drive Decryption screen displays.

BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protecting your drives with BitLocke	r.
Operating system drive	
Windows (C:) BitLocker Decrypting	\bigcirc
\$	
Fixed data drives	
Removable data drives - BitLocker To Go	

← → × ↑ 🏘 > Contro	I Panel > System and Security > BitLocker Drive Encryption	v ⊙	Search Control Panel	
Control Panel Home	BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protecting your drives with BitLocker.			
	Operating system drive			
	Windows (C:) BitLocker off		\odot	
	Turn on BitLocker			
	Fixed data drives			
	Removable data drives - BitLocker To Go Insert a removable USB flash drive to use BitLocker To Go.			
See also				
TPM Administration				
Disk Management				
Privacy statement				

After the decryption has completed, the following screen displays.

Suspending BitLocker Drive Encryption

IMPORTANT Suspending BitLocker Drive Encryption is a temporary method for removing BitLocker protection without decrypting the Windows drive. Use Suspend BitLocker if you need to update the BIOS firmware or Windows boot loader/startup files that requires access to the drive in a pre-boot environment. This will help prevent BitLocker from locking the drive and can avoid a lengthy decryption process.

Suspending BitLocker

1 Type **Manage BitLocker** from the Windows search bar to locate the Manage BitLocker Control Panel.

≡	All	Apps	Documents	Settings	Photos	More 🗸	Feedback	
ሴ	Best m	atch						
	Ą	Manage Control p	e BitLocker anel		\rightarrow			
						Manage BitLocker Control panel		
						⊏r Open		
ŝ								
ω.	νN	lange Bit	locker					
	Q	Ξi						

2 Double-click **Manage BitLocker** to open the BitLocker Drive Encryption window.

4	BitLocker Drive Encryption		-		×
\leftarrow	→ 🗸 ↑ 代 > Control Panel	> System and Security > BitLocker Drive Encryption V	Search Control Panel		Q
Control Panel Home	Control Panel Home	BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protecting your drives with BitLocker.			0
		For your security, some settings are managed by your system administrator.			م
		Operating system drive			
		Windows (C:) BitLocker on	\bigcirc)	
		Suspend protection Suspend protection Sack up your recovery key Turn off BitLocker			
		Fixed data drives			
		DATA1 (D:) BitLocker off	\odot)	
		Removable data drives - BitLocker To Go Insert a removable USB flash drive to use BitLocker To Go.			
	See also				
	TPM Administration				
•	Disk Management Privacy statement				

3 Select Suspend Protection.

🗅 🗸 🧄 💊 Contre	ol Panel > System and Security > BitLocker Drive Encryption	✓ ひ Search Control Panel	م
Control Panel Home	BitLocker Drive Encryption Help protect your files and folders from unauthorized access by protecting your drives with BitLocker.		
	For your security, some settings are managed by your system administrator.		
	Operating system drive		
	Windows (C:) BitLocker on	\odot	
	Fixed data drives		
	Removable data drives - BitLocker To Go Insert a removable USB flash drive to use BitLocker To Go.		
See also			
TPM Administration Disk Management			
Privacy statement			

A confirmation message "Do you want to suspend BitLocker protection?" displays.

BitLocker Drive Encryption	\times
Do you want to suspend BitLocker protection	1?
If you do, your data won't be protected. You might need to temporarily suspend BitLocker if you're updating your PC's firmware, hardware, or operating system. If you forget to resume BitLocker protection, it'll resume automatically the next time you restart your PC.	5
Yes No	

IMPORTANT If you choose to suspend BitLocker, your data will not be protected.

4 If you want to proceed, select **Yes** and the following popup information will display.

A	Protection is suspended
<u>/!</u>	Protection of C; by BitLocker Drive Encryption is suspended. Click to
	manage BitLocker settings.
	BitLocker Drive Encryption Notification Utility

If you do not want to proceed, select **No**.

NOTE There are certain situations where you may want to suspend BitLocker such as updating your PC's firmware, hardware, or operating system. If you forget to resume BitLocker after these updates are completed, BitLocker will resume automatically the next time you restart your PC.

Restarting BitLocker Protection

1 Select **Resume Protection**.

Recovering Your BitLocker Key

There might be situations where the BitLocker Key is requested from your computer, such as:

- Your drive is placed onto another computer
- Computer requests your recovery key, it may be due to one of the following but is not limited to:
 - Hardware changes
 - Firmware changes
 - Other Windows updates

BitLocker Recovery

1 BitLocker Recovery window will be displayed upon powering on your system.

Entor tk	no recovery lieu for this drive
Enter tr	ne recovery key for this drive
1	
	information on how to retrieve this key, go to dows.microsoft.com/recoverykeyfaq from another PC or mobile device.
Use the n	umber keys or function keys F1-F10 (use F10 for 0).
Recovery	key ID: 86B0A9C0-0C54-44CA-81AC-D0482036E519
Press E	nter to continue
Press E	sc for more recovery options

2 Enter your key from your saved text file or printed copy, then select **Enter**.

Enter the recovery key for this drive
For more information on how to retrieve this key, go to http://windows.microsoft.com/recoverykeyfaq from another PC or mobile device.
Use the number keys or function keys F1-F10 (use F10 for 0).
Recovery key ID: 86B0A9C0-0C54-44CA-81AC-D0482036E519
Press Enter to continue
Press Esc for more recovery options



CAUTION

The instrument workstation is vulnerable to malware, viruses, and unauthorized access if the instrument PC is connected directly to the laboratory network without installation of all approved OS and malware patches.

IMPORTANT Only the version of the McAfee Application Control (Version 8.3) has been validated to work with the CytoFLEX platform instrument.

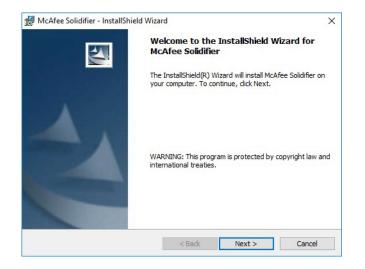
For more information on Configuration of McAfee Application Control, refer to McAfee Application Control Guide.

Installing McAfee Application Control

IMPORTANT The McAfee Application Control Software (version 8.3) which has been proved compatible with the CytExpert software, should be purchased separately from McAfee. Download the McAfee Application Control Software, access McAfee website at www.mcafee.com.

The following procedure can assist you to install the McAfee Application Control software. For more instructions, access the McAfee for help.

- **2** Right-click **Setup-win-8-10-2012-amd64-xxx.exe**, and select **Run as administrator**. The McAfee Installer opens.



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3 Select **Next**. The License Agreement displays.



Read the license agreement and select I accept the terms in the license agreement, then select Next.

4 Enter the username and the serial number.

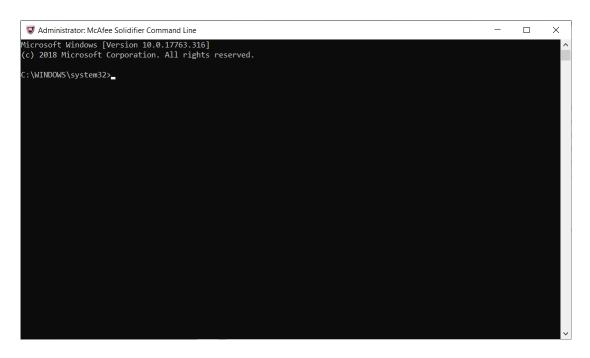
	Customer Information	
•	Please enter your information.	
<u>U</u> ser Na	ime:	
<u>O</u> rganiz	ation:	
Insta	all without license kay May one add the license kay after installation)	
	all without license key (You can add the license key after installation)	
	all without license key (You can add the license key after installation) lumber:	
<u>S</u> erial N	lumber:	
<u>S</u> erial N		
<u>S</u> erial N	lumber:	
<u>S</u> erial N	lumber:	

If you select **Install without license key**, follow the procedures below to add the license key after the installation.

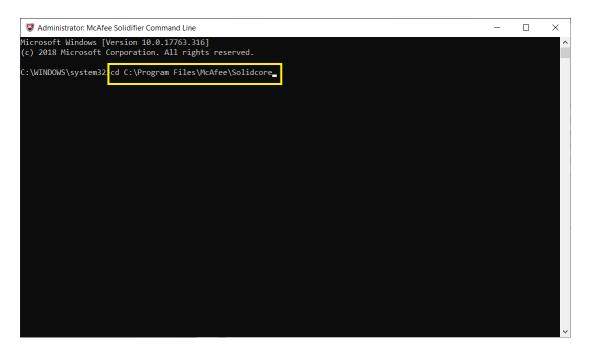
a. Right-click MCATEC on your desktop, and select Run as administrator.

1	Open
McAf	Open file location
Solidi Comm	🖓 Run as administrator
Line	Pin to Start
	Scan with Windows Defender
	Pin to taskbar
	Restore previous versions
	Send to >
	Cut
	Сору
	Create shortcut
	Delete
	Rename
	Properties

The following window displays.



b. Enter cd C:\Program Files\McAfee\Solidcore.



 $\textbf{c.} \quad \text{Enter the license key in the console.}$

🐺 Administrator: McAfee Solidifier Command Line	-	×
Microsoft Windows [Version 10.0.17763.316] (c) 2018 Microsoft Corporation. All rights reserved.		^
C:\WINDOWS\system32>cd C:\Program Files\McAfee\Solidcore		
C:\Program Files\McAfee\Solidcore>sadmin license add "Application Control" license added.		
C:\Program Files\McAfee\Solidcore>_		

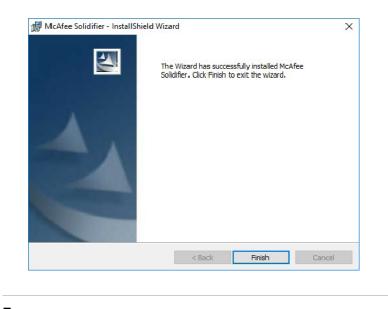
The license key is added.

Administrator: McAfee Solidifier Command Line Microsoft Windows [Version 10.0.17763.316]		^
(c) 2018 Microsoft Corporation. All rights reserved.		
C:\WINDOWS\system32>cd C:\Program Files\McAfee\Solidcore		
C:\Program Files\McAfee\Solidcore>sadmin license list (Application Control,)		
C:\Program Files\McAfee\Solidcore>		

5 Select Next.

🖟 McAfee	Solidifier - InstallShield Wizard	(
W	Destination Folder Click Next to install to this folder, or click Change to install to a different folder.	
Ø	Install McAfee Solidifier to: C:\Program Files\McAfee\Solidcore\ Change	
	Create Desktop Shortcut	
	Run for post installation customization: Browse	
InstallShield -	< Back Next > Cancel]
HcAfee	Solidifier - InstallShield Wizard – – × Installing McAfee Solidifier The program features you selected are being installed.	
1	Please wait while the InstallShield Wizard installs McAfee Solidifier. This may take several minutes. Status:	
InstallShield –	< Back Next > Cancel	-

6 Select the destination folder and select **Next**. The system starts to install McAfee Solidifier.



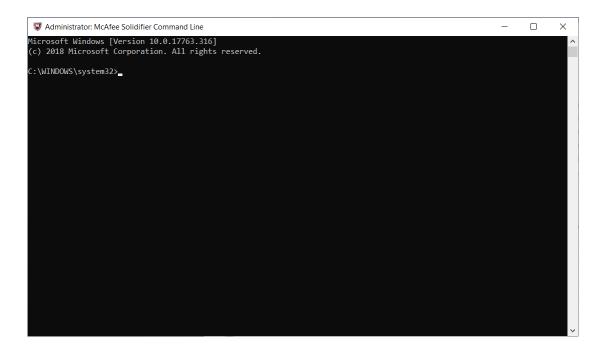
- 7 Select Finish.
- **Enabling McAfee Application Control**

The enabled McAfee Application Control Software prevents your system from executing unauthorized applications on your instrument workstation.

IMPORTANT Ensure all the necessary applications have been installed prior to enabling the McAfee®Application Control.



1 Right-click solution on your desktop, and select **Run as administrator**. The following window displays.



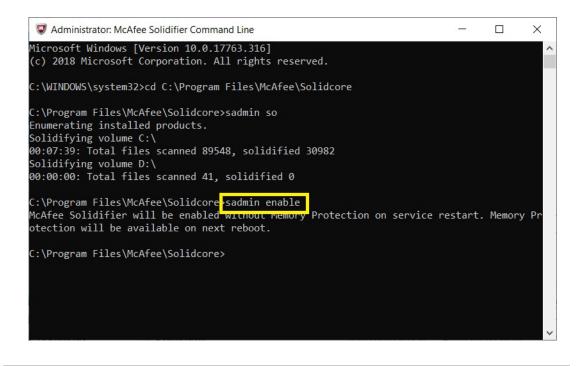
2 Enter cd C:\Program Files\McAfee\Solidcore.

✓ Administrator: McAfee Solidifier Command Line - □ Microsoft Windows [Version 10.0.17763.316] (c) 2018 Microsoft Corporation. All rights reserved. C: \WINDOWS\system32 cd C:\Program Files\McAfee\Solidcore_			
(c) 2018 Microsoft Corporation. All rights reserved.	S Administrator: McAfee Solidifier Command Line	-	Х
C:\WINDOWS\system32:cd C:\Program Files\McAfee\Solidcore_			^
	C:\WINDOWS\system32:cd C:\Program Files\McAfee\Solidcore_		
			~

3 Enter **sadmin so** in the console to scan the system and create a white list. The solidification process takes about 30 minutes depending on your system size and CPU performance.

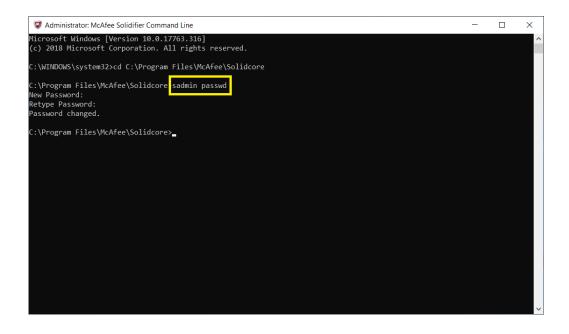
S Administrator: McAfee Solidifier Command Line - sadmin so		\times
Microsoft Windows [Version 10.0.17763.316] (c) 2018 Microsoft Corporation. All rights reserved.		^
C:\WINDOWS\system32>cd C:\Program Files\McAfee\Solidcore		
C:\Program Files\McAfee\Solidcore Enumerating installed products. Solidifying volume C:\ 00:01:52: Total files scanned 15792, solidified 3651		
• • • • • • • • • • • • • • • • • • •		
		~

4 Enter **sadmin enable** to enable the McAfee Application Control.



IMPORTANT Beckman Coulter recommends setting a password to manage the authority of enabling/disabling the McAfee Application Control.

5 Enter **sadmin passwd** to set a password to lock the McAfee Application Control authority.



6 Restart the system.

Exporting the Certificate

Follow procedures below to export the certificate of CytExpert software into your PC.

1 Right-click on the application **CytExpert for xxxx Setup**, and select **Properties**. The following window displays.

CytExpert		Setup.exe	Properties)
Security General	Deta Compa		Previous Versi Digital Signat	
	CytExpert	ubiity	1 Setup.exe	ures
Type of file:	Application (.exe)			
Description:	This installer datab	ase contains th	ne logic and data re	
Location:				
Size:	144 MB (151,881,	320 bytes)		
Size on disk:	144 MB (151,883,	776 bytes)		
Created:	202(9:03:11		
Modified:	2020	9:03:24		
Accessed:	202(, 9	9:03:24		
Attributes:	Read-only	Hidden	Advanced	
		ОК	Cancel	Apply

2 Select the **Digital Signatures** tab. Then select the signature and select **Details** to view the signature information.

ytExpert Demo	Properties		
Security	Deta	ils	Previous Versions
General	Compa	tibility	Digital Signatures
Signature list			
Name of sign	Digest algorit	Timestamp	
Beckman Co	sha256	Monday, Ju	ine 15,
			Details

💿 CytExp	ert Demo P	roperties					\times
	Security General		Details mpatibility	P	Previous Ver Digital Sign		
Signa	Digital Sig	nature De	tails			?	×
Be	General	Advanced					
	Name: E-mail	This digital	nature Informa signature is OK. Beckman Coulte Not available Monday, June	er, Inc.	6:02:06 PM View Cer	tificate	
	Counter	rsignatures					
		-	E-mail addre Not available	Timesta Monday,	mp , June 15,		
					Deta	iils	

The Digital Signature Details window displays.

3 Select View Certificate.

🐖 Certificate	×				
General Details Certification Path					
Certificate Information This certificate is intended for the following purpose(s): • Ensures software came from software publisher • Protects software from alteration after publication					
* Refer to the certification authority's statement for details.					
Issued to: Beckman Coulter, Inc.					
Issued by: Symantec Class 3 SHA256 Code Signing CA					
Valid from 7/22/2019 to 7/30/2021					
Install Certificate Issuer Statemen	nt				
0	<				

Certificate	>
General Details Certification	n Path
<u>S</u> how <all></all>	~
Field Serial number Signature algorithm Signature hash algorithm Signature hash algorithm Signature hash algorithm Susuer Valid from Valid to	Value V3 193ef093a3d59325c55ea sha256RSA sha256 Symantec Class 3 SHA25 Monday, July 22, 2019 5: Friday, July 30, 2021 4:5
Subject	Beckman Coulter, Inc., Be 🗸
	Edit Properties Copy to File
	ОК

4 Select the Details tab and then select Copy to File.

The Certificate Export Wizard window appears.

← 🛛 🐓 Certificate Export Wizard

Welcome to the Certificate Export Wizard

This wizard helps you copy certificates, certificate trust lists and certificate revocation lists from a certificate store to your disk.

A certificate, which is issued by a certification authority, is a confirmation of your identity and contains information used to protect data or to establish secure network connections. A certificate store is the system area where certificates are kept.

To continue, click Next.

Next Cancel

 \times

5 Select Next to export the certificate.
--

Certificate Export Wizard	×
Export File Format Certificates can be exported in a variety of file formats.	
Select the format you want to use:	
O DER encoded binary X.509 (.CER)	
Base-64 encoded X.509 (.CER)	
Cryptographic Message Syntax Standard - PKCS #7 Certificates (.P7B)	
Include all certificates in the certification path if possible	
O Personal Information Exchange - PKCS #12 (.PFX)	
Include all certificates in the certification path if possible	
Delete the private key if the export is successful	
Export all extended properties	
Enable certificate privacy	
Microsoft Serialized Certificate Store (.SST)	
Next	Cancel

6	Select Base-64-encoded X.509 and then select Next.
---	--

←	Certificate Export Wizard	×
	File to Export Specify the name of the file you want to export	
	File name: Browse	

Next Cancel	Next

- 🍮 Save As \times \rightarrow \checkmark \uparrow \blacksquare > This PC > Desktop > マ ひ Search Desktop ← Q •=== • ? Organize

 New folder 📕 Export Certificati ^ Name Date modified Size Туре Installing McAfe SOLIDCOR830-303_WIN 6/15/2020 11:26 PM File folder This PC 3D Objects 📃 Desktop Documents Downloads 👌 Music Pictures Videos 🔩 Windows (C:) 🧼 DATA1 (D:) Multiple USB Drive (E:) 🖆 USB Drive (E:) File name: BEC \sim Save as type: DER Encoded Binary X.509 (*.cer) \sim Save Cancel ∧ Hide Folders
- $\textbf{7} \quad \text{Select a destination to save the certificate and then select \textbf{Save}.}$

8 Select Next. ★
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File to Export
Becify the name of the file you want to export
File name:
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Next	Cancel

9 Select **Finish** when the success message displays.

Importing BEC Certificate

Follow the procedures below to add the certificate of CytExpert software into the McAfee Certificate list. All the certified applications will be regarded as trusted and authorized by the McAfee Application Control.

IMPORTANT McAfee Application Control recognize only X.509 certificates.

1 Copy the certificate *BEC.cer* from the CytExpert software disk into your instrument PC.



Right-click Run as administrator. The following window 2 displays.



3 Enter cd C:\Program Files\McAfee\Solidcore.



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4 Enter sadmin cert add C:\BEC.cer to add the certificate.



Switching McAfee Application Control to Update Mode

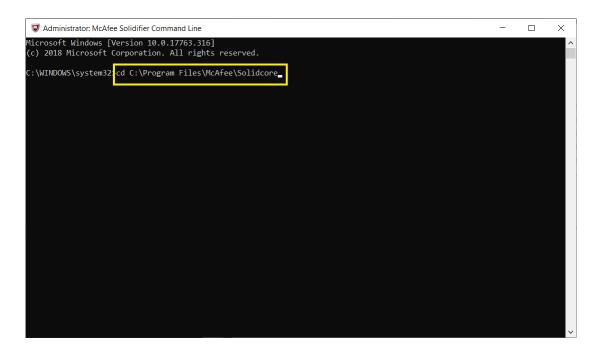
The update mode allows the McAfee Application Control to scan the instrument workstation and add the newly-installed application into the white list automatically. Switch the McAfee Application Control to the update mode when you execute scheduled or emergency changes on your instrument workstation.

1

Right-click solution on your desktop, and select **Run as administrator**. The following window displays.

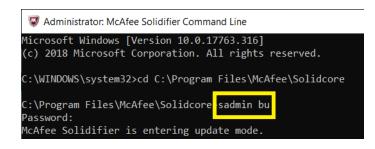


2 Enter cd C:\Program Files\McAfee\Solidcore.

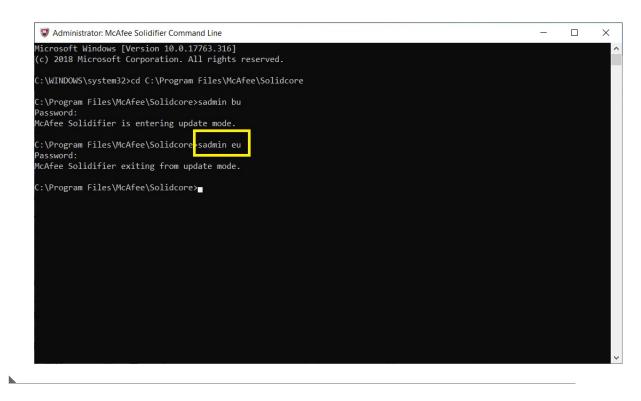


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3 Enter **sadmin bu** to switch the McAfee Application Control to Update Mode.



- **4** Execute scheduled or emergency changes, patch installations, or software updates for your instrument workstation.
- **5** Enter **sadmin eu** to exit the Update Mode.



Disabling McAfee Application Control

IMPORTANT Disabling McAfee Application Control is a temporary method in special cases, for example, Windows or firmware changes. This will help stop McAfee Application Control from blocking unauthorized applications. Beckman Coulter recommends enabling the McAfee Application Control in most cases.

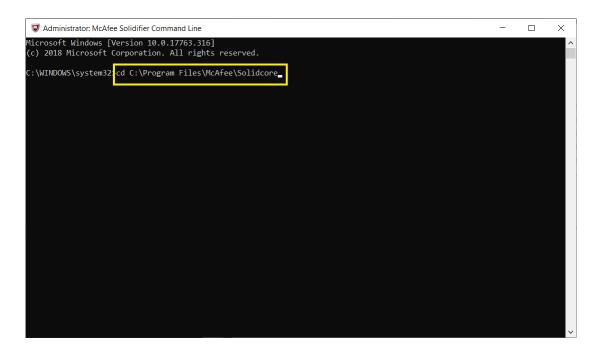
All the applications installed in this mode will not be included in the McAfee white list.

1

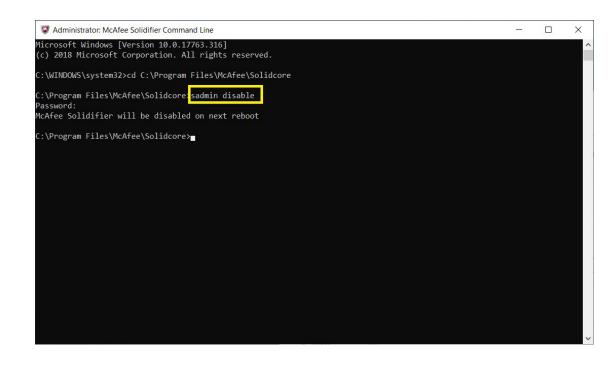
Right-click solution on your desktop, and select **Run as administrator**. The following window displays.



2 Enter cd C:\Program Files\McAfee\Solidcore.



3 Enter sadmin disable in the console.



- 4 Select Enter.
- **5** Restart the System.

6 Enter **sadmin status** to view the status of McAfee Application Control.

Administrate	or: McAfee Solidifier Co	mmand Line							-	\times
			rved.							^
C:\WINDOWS\sy	/stem32>cd C:\Prog	ram Files\McAfee\	Solidcore							
	iles\McAfee\Solidc	ore>sadmin disabl	e							
<pre> Administrator: McAfee Solidifier Command Line Microsoft Windows [Version 10.0.17763.316] (c) 2018 Microsoft Corporation. All rights reserved. C:\WINDOWS\system32>cd C:\Program Files\McAfee\Solidcore C:\Program Files\McAfee\Solidcore>sadmin disable Password: McAfee Solidifier will be disabled on next reboot C:\Program Files\McAfee\Solidcore? sadmin status McAfee Solidifier on reboot:</pre>										
NTFS	Unsolidified	Attached	D:\							
C:\Program Fi	iles\McAfee\Solidc	ore>								
<pre>(c) 2018 Microsoft Corporation. All rights reserved. C:\WINDOWS\system32>cd C:\Program Files\McAfee\Solidcore C:\Program Files\McAfee\Solidcore>sadmin disable Password: McAfee Solidifier will be disabled on next reboot C:\Program Files\McAfee\Solidcore Sadmin status McAfee Solidifier: McAfee Solidifier: McAfee Solidifier on reboot: Disabled ePO Managed: PO Managed: Secovered [fstype] [status] [driver status] [volume] * NTFS Solidified Attached D:\</pre>										
										~



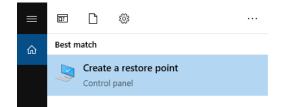
🕂 WARNING

Risk of software incompatibility. Only operating system updates posted to the Beckman Coulter website should be installed on the instrument controller PC. Do not install patches from any other source.

Install the update on one instrument and verify functionality before installing on other instruments.

Enabling System Protection

1 In the Start Menu, type **Restore** to locate the **Create a Restore Point**.



2 Double-click **Create a Restore Point** to open the System Properties window.

System Properties	×						
Computer Name Hardware Advanced Syste	m Protection Remote						
Use system protection to undo unwanted system changes.							
System Restore							
You can undo system changes by reverting your computer to a previous restore point.	System Restore						
You can undo system changes by reverting your computer to a previous restore point. System Restore Protection Settings							
Available Drives	Protection						
Windows (C:) (System)	On						
System Restore							
nave system protection turned on.							
ОК	Cancel Apply						

3 Select Configure.

System Properties	×							
Computer Name Hardware Advanced System Protection Rem	note							
Use system protection to undo unwanted system changes.								
System Restore								
You can undo system changes by reverting your computer to a previous restore point.	ore							
Protection Settings	_							
Use system protection to undo unwanted system changes. System Restore You can undo system changes by reverting your computer to a previous restore point. System Restore								
and delete restore points.								
OK Cancel	<u>A</u> pply							

The following window appears.

System Protection for Windows (C:)	×
Restore Settings	
By enabling system protection, you can undo undesired changes by reverting your computer to a previous point in time.	
Turn on system protection	
O Disable system protection	
Disk Space Usage	
You can adjust the maximum disk space used for system protection. As space fills up, older restore points will be deleted to make room for new ones.	
Current Usage: 1.01 GB	
Max Usage:	
5% (11.77 GB)	
Delete all restore points for this drive. Delete	
OK Cancel Apply	

4 Select **Turn on system protection**, then select **Apply**.

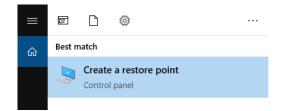
5 Close the System Properties window by selecting **Οκ**.

Creating a Restore Point

A restore point is used to remove all software and update installations that occurred after it was created. If installing an update on your PC causes any loss of functionality, the restore point can be used to place the system back into the operable state present before the update installation. In all cases, a system restore with any restore point would not cause loss of any data (i.e. LMD files) on the system.

Risk of unexpected software modification. To ensure a system recovery point is available that is indicative of the most recent software configuration, the user should create a system restore point immediately prior to running the operating system update installer. Otherwise, a user would need to utilize the most recent automatically created restore point which might be up to 72 hours old and not include all recent software modifications.

1 In the Start Menu, type **Restore** to locate the **Create a Restore Point**.



2 Double-click **Create a Restore Point** to open the System Properties window.

System Properties	×							
Computer Name Hardware Advanced System Protein	ction Remote							
Use system protection to undo unwanted system changes.								
System Restore ————								
You can undo system changes by reverting your computer to a previous restore point.	System Restore							
Protection Settings								
Available Drives Protect	tion							
Windows (C:) (System) On								
Configure restore settings, manage disk space, and delete restore points.	Configure							
Create a restore point right now for the drives that have system protection turned on.	Create							
ОК	Cancel Apply							

- **3** Type a name for the restore point and select **Create**.
- **4** Wait for the restore point creation to complete and then select **Close**.
- **5** Close the System Properties window by selecting **OK**.

Downloading Operating System Updates

In order to download and install operating system updates, you will need to access the Beckman Coulter website at www.beckmancoulter.com.

IMPORTANT Account registration is required for new users.

Go to www.beckmancoulter.com.

- 2 Using the menu bar in the in the upper right hand corner of the screen, hover over **Login**, then scroll down to select **Software Downloads**.
- **3** To search by product:
 - 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.

4 Select Search.

- **IMPORTANT** Print out the Release Notes for each update as they will not be available when the next update is released. The Component Name section of the release notes identifies the Microsoft updates that are included in the download. This information may be requested by your IT team and will be needed to recover from a failed update.
- 5 Each operating system update is cumulative and only the most recent validated set of updates will be available for download. Select the first file in the results to view the release notes and

then select Download

If more than one update is listed in the search results, select **Back**, and repeat this step for each update.

If the operating system updates cannot be not downloaded directly to the instrument controller system, transfer the downloaded files via an encrypted external media or secured network drive to the instrument controller PC.

Notification Options

To receive notification when a new operating system update is validated and available for download, you must sign up for an account on www.beckmancoulter.com and follow the procedure for setting up subscriptions.

Sign up for subscriptions as outlined below:

- Select the Document Language consistent with your IFU language.
- Select Research & Discovery from Market Segment.
- Select Flow Cytometry from Product Line.
- Select Instruments from Product Series.
- Select CytoFLEX from Product.

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If you do not sign up for product notifications, you will have to check periodically for newly released operating system updates, see Downloading Operating System Updates.

NOTE Beckman Coulter will only validate and post updates for Windows 10 LTSC 2019.

Installing the Operating System Updates

CAUTION

Risk of software incompatibility. Beckman Coulter can only support operating system updates on unmodified systems. Application of domain policies or other configuration changes may impact compatibility.

IMPORTANT If more than one update is available for your product, install the updates in the order indicated by the sequence number as outlined in the file naming convention below.

RSU-<Operating System> <Product Name>-<Year><Month>_<Sequence #>_<Sequence Revision#> Example: RSU-W10-CytoExpert-2021Sep_1_0

- 1 Ensure you have a recent copy of your BitLocker key, refer to Backing Up Your Recovery Key.
- **2** Login to Windows as a user with Administrator access.
- **3** Close all program and save open files.
- **4** Power down the instrument.

5 Open the first operating system update file (For example: RSU-W10LTSC-CytoFLEX-2020Jun_1_0) on the Instrument Controller PC by double-clicking on the file. If the Microsoft Windows Defender Smart Screen prompt appears, select **More Info** and **Run Anyway** to launch the installer. This process may take several minutes. See Figure G.6.

CytoFLEX S	RT Operating System Update Install	- 🗆 🗙
Extract	: windows10,0-kb4539571-x64 24d9621cc8:	1434610770c25f5ba3
Show detail	s	

Figure G.6 Security Update Setup Installing

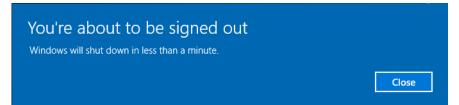
NOTE The security update installer will automatically detect and install only the necessary updates.

6 Once the operating system update setup has completed, select **OK** to restart the system and complete the update process. See Figure G.7 and Figure G.8.

Figure G.7 Security Update Setup Successfully Installed

	:
Press OK to restart the system and complete the update process.	
ОК	

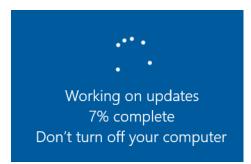




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7 Wait while the system completes the installation of the security updates. This process may take several minutes and will return you to the Windows login screen. See Figure G.9.

Figure G.9 Working on Completing Security Updates



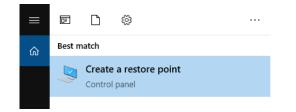
- **8** Install any additional downloaded operating system updates (sequence numbers above 1) by repeating steps 4 to 6.
- **9** Verify functionality of instrument software before installing operating system updates on any other system. If functionality is impaired, see Recovering from Failed Operating System Updates.

Recovering from Failed Operating System Updates

These steps below should only be followed if the instrument software or operating system functionality is impaired after an operating system update was applied.

- **1** Power down the instrument and close all programs. Do not turn your workstation off.
- 2 In the start menu, type view update and select View your Update History.
- **3** Select Uninstall Updates.
- **4** Highlight the update listed under Microsoft Windows that matches the cumulative security update KB number in the release notes. Refer to Component Name section of the Release Notes, see Step 5, Downloading Operating System Updates.
- **5** Select **Uninstall** and select **Yes** when prompted to verify the uninstall. This process may take several minutes to complete.

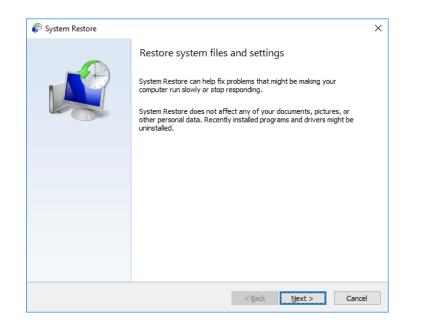
- **6** When prompted to restart, select **Restart Now**.
- **7** Once the PC has been rebooted, login and verify Microsoft update has been removed by repeating Steps 2 and 3. Verify the KB selected in Step 4 is no longer listed.
- **8** Power on the instrument and verify restored software functionality.
 - If software functionality has been restored, continue using your instrument without the update installed. Contact us for troubleshooting the update incompatibility. Do not proceed to Step 9.
 - If software functionality has not been restored, continue to Step 9.
- **9** In Start Menu, type **Restore** and select **Create a Restore Point**.



 $10 \ {\rm Double-click} \ {\rm Create} \ {\rm a} \ {\rm Restore} \ {\rm Point} \ {\rm to} \ {\rm open} \ {\rm the} \ {\rm System} \ {\rm Properties} \ {\rm window}.$

System Properties	\times							
Computer Name Hardware Advanced System Protection Remote								
Use system protection to undo unwanted system changes.								
System Restore								
You can undo system changes by reverting your computer to a previous restore point. System Restore								
Protection Settings —	_							
Available Drives Protection								
🛀 Windows (C:) (System) On								
Configure restore settings, manage disk space, Configure								
Create a restore point right now for the drives that Create Create								
OK Cancel Apply	/							

11 Select **System Restore**. The following window appears.



12 Select **Next**. The following window appears.

🌮 System Restore		×
Restore your computer to t	he state it was in before the selected	event
Current time zone: GMT+8:00		
Date and Time	Description	Туре
5/6/2020 4:11:35 PM	Automatic Restore Point	System
Show <u>m</u> ore restore points	[Scan for <u>affected</u> programs
	< <u>B</u> ack	<u>N</u> ext > Cancel

🕂 WARNING 🛛

Risk of software changes. All software installations and operating configuration system changes that occurred after the selected restore point time stamp will be removed during the restore process.

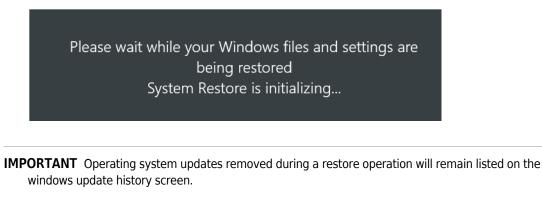
 $13 \hspace{0.1 cm} \text{Select the most recent Restore Point and select Next. The following window appears.}$

🌮 System Restore		×
	Confirm your restore point	
	Your computer will be restored to the state it was in before the event in the Description field below.	
	Time: 5/6/2020 4:11:35 PM (GMT +8:00)	
	Description: System: Automatic Restore Point	
	Drives: Windows (C:) (System)	
	Ecan for affected programs	
	If you have changed your Windows password recently, we recommend that you create a password reset disk.	
	System Restore needs to restart your computer to apply these changes. Before you proceed, save any open files and close all programs.	
	< Back Finish Cance	ł

14 Select **Finish**. The confirm message appears.

٢	×
	Once started, System Restore cannot be interrupted. Do you want to continue?
	System Restore cannot be undone until after it has completed. If System Restore is being run in safe mode or from the System Recovery Options menu, it cannot be undone.
	Yes No

15 Select **Yes**. Wait while the system restores. This process takes about several minutes to return to the Windows login screen.



- **16** Log in to the system again and test the functionality of software.
- **17** Contact us for assistance in troubleshooting the update incompatibility or for assistance restoring functionality.

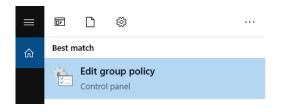


Enabling Automatic Log-Off

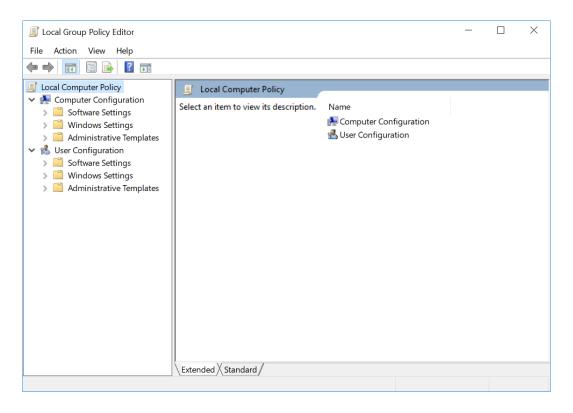
IMPORTANT Automatic log-Off can protect your instrument PC from unauthorized operation when you leave the PC temporarily. Follow the steps below to enable the Screen Saver protection.

Enabling Screen Saver Protection

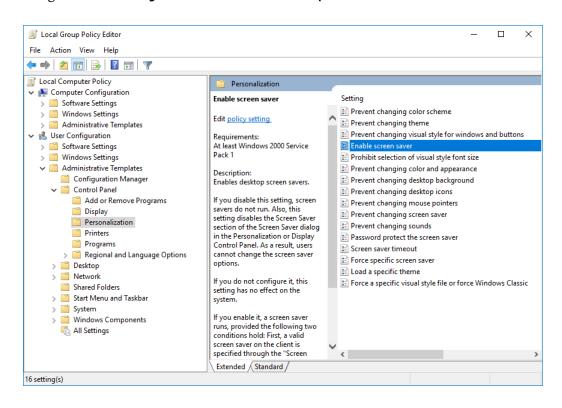
1 Type **Edit group policy** from the Windows search bar to locate the Edit group policy editor.



2 Select **Edit group policy**. The Local Group Policy Editor window displays.



(J



3 Navigate to User Configuration > Administrative Templates > Control Panel > Personalization.

4 Select **Enable screen saver**. The following window appears.

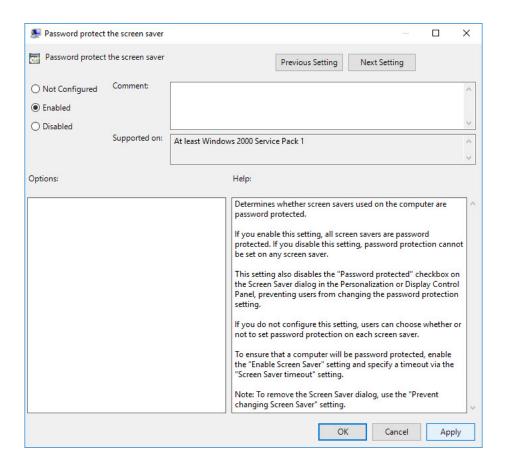
Enable screen sav	er			_		×
Enable screen sav	/er		Previous Setting	Next Setting		
 Not Configured Enabled 	Comment:					^
O Disabled	Supported on:	At least Windows 2000	Service Pack 1			~ ~
Options:		Help:				
		If you settin dialog users If you syster If you condi specif throu screer or Co	es desktop screen savers. disable this setting, scree g disables the Screen Save j in the Personalization or cannot change the screer do not configure it, this s m. enable it, a screen saver tions hold: First, a valid so gh Control Panel on the o n saver timeout is set to a ntrol Panel. see the "Prevent changing	er section of the Scree Display Control Pane a saver options. Setting has no effect of runs, provided the fol reen saver on the clir Saver executable nam client computer. Seco nonzero value throug	en Saver el. As a resu on the lowing two ent is e" setting o nd, the gh the setti	o or
			0	K Cancel	Арр	ly

- **5** Select **Enabled** and **Apply**.
- **6** Select **OK** to close the Enable screen saver window.

Local Group Policy Editor		- 🗆 ×
File Action View Help		
Þ 🔿 🙍 🔜 🔒 🛛 🖬 🛛 🔻		
Local Computer Policy Image: Market Configuration	Personalization	
Software Settings	Password protect the screen save	r Setting
 > ⁽¹⁾ Windows Settings > ⁽¹⁾ Administrative Templates > ⁽²⁾ User Configuration > ⁽²⁾ Software Settings > ⁽¹⁾ Windows Settings 	Edit <u>policy setting</u> Requirements: At least Windows 2000 Service Pack 1	Prevent changing color scheme Prevent changing theme Prevent changing visual style for windows and buttons Enable screen saver Prohibit selection of visual style font size
 Administrative Templates Configuration Manager Control Panel Add or Remove Programs Display Personalization Printers Programs Regional and Language Options Desktop 	Description: Determines whether screen savers used on the computer are password protected. If you enable this setting, all screen savers are password protected. If you disable this setting, password protection cannot be set on any screen saver. This setting also disables	Prevent changing color and appearance Prevent changing desktop background Prevent changing desktop icons Prevent changing mouse pointers Prevent changing sounds Prevent changing sounds Prevent changing sounds Second Se
 Network Shared Folders Start Menu and Taskbar System System Windows Components All Settings 	the "Password protected" checkbox on the Screen Saver dialog in the Personalization or Display Control Panel, preventing users from changing the password protection setting.	Force a specific visual style file or force Windows Classic
	setting, users can choose whether	<
5 setting(s)		

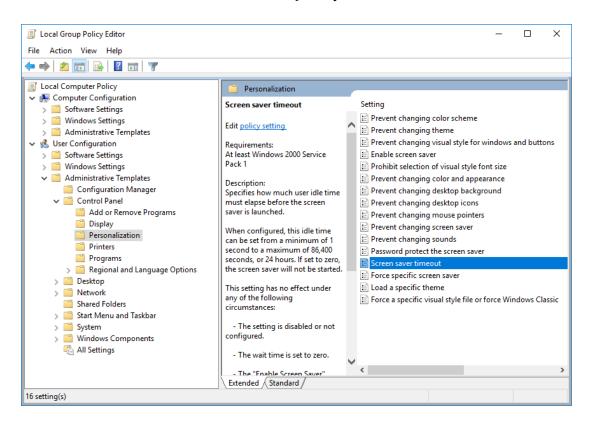
7 Select **Password protect the screen saver** from the Local Group Policy Editor window.

8 Select **Enabled** and **Apply**.



9 Select **OK** to close the window.

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10 Select **Screen saver timeout** from the Local Group Policy Editor window.

The Screen saver timeout window appears.

Screen saver time	out				_		×
Screen saver time	out			Previous Setting	Next Setting		
Not Configured	Comment:						~
O Enabled							
 Disabled 	Constant						~
	Supported on:	At least Window	ws 2000 Servic	e Pack 1			^
							~
Options:			Help:				
Number of seconds to saver Seconds: 0	o wait to enable th	e screen	saver is lau When conf second to a the screen a This setting circumstan - The set - The wa - The "En - Neither Screen Save	igured, this idle time o a maximum of 86,400 s saver will not be starte g has no effect under a	an be set from a neconds, or 24 hou ed. any of the followin configured. ting is disabled. cutable name" set computer's Person	ninimum of rs. If set to z g ting nor the alization or	1 ero,
				ОК	Cancel	Ap	ply

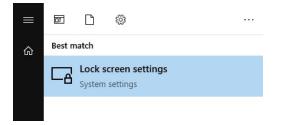
11 Select **Enabled** and **Apply**.

👰 Screen saver time	out		— 🗆 X
Screen saver time	out		Previous Setting Next Setting
 Not Configured Enabled Disabled 	Comment: Supported on:	At least Windo	ws 2000 Service Pack 1
Options:			Help:
Number of seconds to saver Seconds: 900	o wait to enable th	ie screen	Specifies how much user idle time must elapse before the screen saver is launched. When configured, this idle time can be set from a minimum of 1 second to a maximum of 86,400 seconds, or 24 hours. If set to zero, the screen saver will not be started. This setting has no effect under any of the following circumstances: - The setting is disabled or not configured. - The wait time is set to zero. - The "Enable Screen Saver" setting is disabled. - Neither the "Screen Saver executable name" setting nor the Screen Saver dialog of the client computer's Personalization or Display Control Panel specifies a valid existing screen saver
L			OK Cancel Apply

12 Select \mathbf{ok} to close the window.

Setting Lock Screen

1 Type Lock screen settings from the Windows search bar to locate Screen saver settings.



The Settings window appears.

Settings		-	×
命 Home	Lock screen		
Find a setting $\ensuremath{\mathcal{P}}$ Personalization	5:12		
🖾 Background	Tuesday, June 16		
⑦ Colors	Background		
Ca Lock screen	Picture ~		
🖉 Themes	Choose your picture		
A Fonts			
₽ Start			
🖵 Taskbar	Browse		
	Choose an app to show detailed status +		
	Choose apps to show quick status		
	+ + + + + +		
	Show lock screen background picture on the sign-in screen		
	On On		
	Screen timeout settings		
	Screen saver settings		

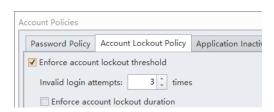
Screen Saver Settings	×
Screen Saver	
Screen saver	
Blank V Settings Preview	
Wait: 15 🚔 minutes 🗹 On resume, display logon screen	
Power management Conserve energy or maximize performance by adjusting display brightness and other power settings. <u>Change power settings</u>	
OK Cancel A	pply

2 Scroll down to the bottom and select **Screen saver settings**. The Screen Saver Settings window appears.

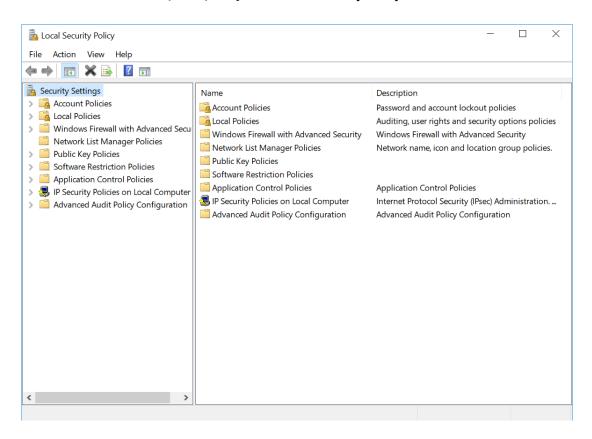
- **3** Select Apply.
- **4** Select **OK** to close the window.

Setting Account Lockout Policy

1 Type Local Security Policy from the Windows search bar to locate the Local Security Policy.



2 Double-click **Local Security Policy** to open the Local Security Policy window.



(J

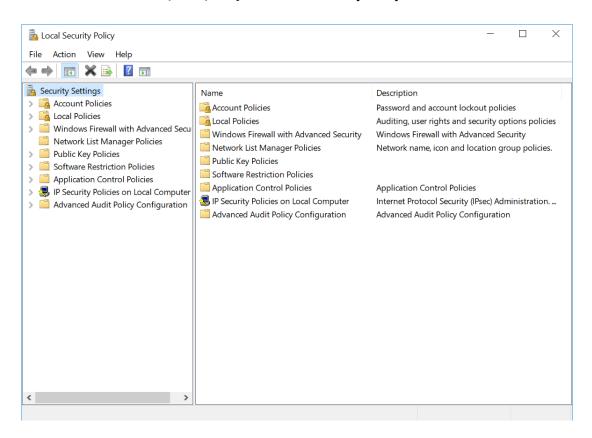
- here a contract and the security Policy _ Х File Action View Help 🗢 🄿 🙍 📆 🔂 👘 🚡 Security Settings Policy Security Setting Account Policies Account lockout duration 15 minutes 🐴 Password Policy 📖 Account lockout threshold 10 invalid logon attempts 済 Account Lockout Policy 📓 Reset account lockout counter after 15 minutes > 📑 Local Policies 🛛 🧮 Windows Defender Firewall with Adva Network List Manager Policies Public Key Policies Software Restriction Policies Application Control Policies IP Security Policies on Local Computer Advanced Audit Policy Configuration
- **3** Navigate to **Security Setting > Account Policies > Account Lockout Policy**. Configure the Security Settings as below.

Setting Password Policy

1 Type Local Security Policy from the Windows search bar to locate the Local Security Policy.



2 Double-click **Local Security Policy** to open the Local Security Policy window.



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- here a security Policy \times File Action View Help 🗢 🄿 🙎 🖬 🗟 🖬 hecurity Settings Policy Security Setting Account Policies Enforce password history 10 passwords remembered Assword Policy 🗓 Maximum password age 90 days 📑 Account Lockout Policy Minimum password age 0 days > A Local Policies Minimum password length 10 characters 🣔 Windows Defender Firewall with Advai Reserved must meet complexity requirements Enabled Network List Manager Policies Disabled 🔯 Store passwords using reversible encryption Public Key Policies > Software Restriction Policies Application Control Policies > IP Security Policies on Local Computer > > Advanced Audit Policy Configuration
- **3** Navigate to Security Setting > Account Policies > Password Policy.

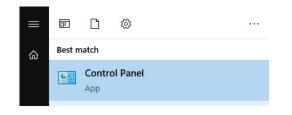
- **4** Customize the password policy according to the following.
 - Enforce password history: 10 passwords remembered
 - Maximum password age: 90 days
 - Minimum password age: optional
 - Minimum password length: 10 characters
 - Password must meet complexity requirements: enabled

The password must contain all four of the following character types:

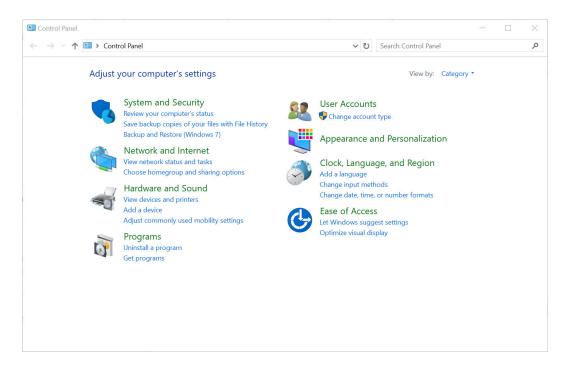
- letter in upper case (A -Z)
- letter in lower case (a -z)
- numbers (0-9)
- special character (i.e.,!, @, #, \$,%, ^, &)
- Store passwords using reversible encryption: optional

Disabling Remote Access

1 Type **Control Panel** from the Windows search bar to locate the **Control Panel** app.



2 Select **Control Panel** to open the Control Panel window.



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- System Control Panel _ × v ひ System ← → × ↑ 🖭 > Control Panel × ~ System Edit the system environment variables Show which operating system your computer is running View advanced system settings View system resource usage in Task Manager Allow remote access to your computer Check processor speed Create a restore point Rename this computer See the name of this computer Show how much RAM is on this computer Sound Adjust system volume Change system sounds Security and Maintenance Review your computer's status and resolve issues View reliability history Troubleshooting Perform recommended maintenance tasks automatically Programs and Features () Show which programs are installed on your computer User Accounts Give other users access to this computer Tackbar and Navigation
- **3** Type **Security** from the search bar to locate **Allow remote access to your computer**.

4 Select **Allow remote access to your computer**. The System Properties window appears.

omputer Name Hardware Advanced S	System Protection	Remote
Remote Assistance		
	1 . U.S	
Allow Remote Assistance connection	ons to this comput	ler
What happens when I enable Remote /	Assistance?	
		Advanced
Remote Desktop		
Remote Desktop		
Choose an option, and then specify who	o can connect.	
Don't allow remote connections to the second s	his computer	
Allow remote connections to this co	moutor	
	mputer	
Allow connections only from com Desktop with Network Level Auti		
		,
		Select Users
Help me choose		001001 00010

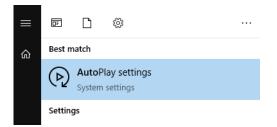
5 Select **Don't allow remote connections to this computer** from the Remote tab.

				-	
Computer Name	Hardware	Advanced	System Prote	ction Remote	
Remote Assis	lance				
Allow Rem	ote Assistar	nce connecti	ons to this comp	outer	
What happen:	s when I ena	ble Remote	Assistance?		
				Advan	iced
- Remote Deskt	lon				
Remote Deski	op				
Choose an op	tion, and the	en specify wh	o can connect.		
Don't allow	remote con	nections to t	his computer		
	ote connectio	ons to this co	mputer		
			nputers running in (recommende		top
Help me choo	ISE			Select	Jsers

6 Select **OK** to close the window.

Disabling Auto Play

1 Type **Auto Play settings** from the Windows search bar to locate the **Auto Play settings**.



 Home Find a setting Use AutoPlay for all media and devices On 		
On		
Devices Choose AutoPlay defaults		
Bluetooth & other devices Removable drive		
B Printers & scanners Choose a default ∨		
Mouse Memory card		
Choose a default		
Touchpad		
Typing Honor V9 spring		
Open device to view files (File Explorer) V		
d ³ Pen & Windows Ink		I
AutoPlay Related settings		
Default app settings		
Have a question?		

 ${\color{black} 2} \quad \text{Select Auto Play settings. The Settings window appears.}$

3 Select **off** to turn off Auto Play.

ettings		- 0
分 Home	AutoPlay	
Find a setting	Use AutoPlay for all media and devices	Related settings
Devices	Off Off	Default app settings
	Choose AutoPlay defaults	
Bluetooth & other devices	Removable drive	
Printers & scanners	Take no action \sim	
) Mouse	Memory card	
Typing	Take no action \lor	
ନ୍ତି AutoPlay		
USB		

4 Close the window.

Disabling Unnecessary Services

It is recommended to disable all the unnecessary services while operating the CytExpert software. Refer to Table G.1.

Bluetooth Support Service	Microsoft FTP Service (If	Windows Media Player Network
	available)	Sharing Service
Geo-location Service	Microsoft iSCSI Initiator Service	Windows Mobile Hotspot Service
Infrared monitor service	Remote Procedure Call (RPC)	WinHTTP Web Proxy Auto-
	Locator	Discovery Service
Internet Connection Charing	Douting and Dougate Access	When Live Come Cours
Internet Connection Sharing	Routing and Remote Access	Xbox Live Game Save
Link-Layer Topology Discovery	SSDP Discovery	Xbox Live Networking Service
Mapper		5
LxssManager (If available)	UPnP Device Host	

Table G.1 List of Unnecessary Services

1 Type **Computer Management** from the Windows search bar to locate the Computer Management App.

		ß	\$ <u>2</u>	
ŵ	Best m	atch		ł
		Comp App	uter Management	

2 Select Computer Management.

Computer Management	×
File Action View Help	
Computer Management (Local Name	Actions
 System Tools Task Scheduler Shared Folders Shared Folders Some Local Users and Groups Device Manager Toisk Management Services and Applications 	Computer Management (L More Actions

3 Navigate to Services and Applications > Services.

🛃 Computer Management						– 🗆 X
File Action View Help						
🗢 🄿 🙍 📷 🙆 🔒						
🜆 Computer Management (Local	O. Services					Actions
 V System Tools C Task Scheduler 	Select an item to view its description.	Name	Description	Status	Startu ^	Services 🖌
> 🛃 Event Viewer		ActiveX Installer (AxInstSV)	Provides Us		Manu	More Actions
> 👸 Shared Folders		Adobe Acrobat Update Serv		Running	Autor	
> 🔊 Local Users and Groups		Adobe Genuine Monitor Ser	Adobe Gen	Running	Autor	
> 🔊 Performance		Adobe Genuine Software In	Adobe Gen	Running	Autor	
📇 Device Manager		AdobeUpdateService		Running	Autor	
✓ 😫 Storage		AllJoyn Router Service	Routes AllJo		Manu	
📅 Disk Management		App Readiness	Gets apps re		Manu	
Services and Applications		Application Identity	Determines		Manu	
Services		Application Information	Facilitates t	Running	Manu	
WMI Control		Application Layer Gateway	Provides su		Manu	
		Application Management	Processes in		Manu	
		AppX Deployment Service (Provides inf		Manu	
		🍓 Auto Time Zone Updater	Automatica		Disabl	
		🧟 Background Intelligent Tran	Transfers fil		Manu	
		Background Tasks Infrastru	Windows in	Running	Autor	
		Sase Filtering Engine	The Base Fil	Running	Autor	
		BitLocker Drive Encryption	BDESVC hos	Running	Manu	
		Block Level Backup Engine	The WBENG		Manu	
		Bluetooth Handsfree Service	Enables wir		Manu	
		Bluetooth Support Service	The Bluetoo	Running	Manu	
		BranchCache	This service		Manu	
		Certificate Propagation	Copies user		Manu	
		Client License Service (ClipS	Provides inf		Manu	
		CNG Key Isolation	The CNG ke	Running	Manu	
		COM+ Event System	Supports Sy	-	Autor	
		<			>	
< >	Extended Standard /					
	(

4 Select the service you want to disable.

🗢 🔿 🙍 📊 🖄	🛛 📷 🕨 💷 💷								
🌆 Computer Management (Local	O Services							Actions	
 System Tools Task Scheduler 	Internet Connection Sharing (ICS)	Name	Description	Status	Startup Type	Log On As	^	Services	
Scheduler Idsk Scheduler IdskScheduler Idsk Scheduler Idsk Scheduler Idsk Scheduler	<u>Start</u> the service	Intel Bluetooth Service	Intel(R) Wir Intel(R) Con	Running	Automatic Manual	Local Syste Local Syste		More Actions	
		Intel(R) Content Protection				Local Syste		Internet Connection Sharin	
	Description: Provides network address translation, addressing, name resolution and/or	Intel(R) HD Graphics Contro		Running	Automatic	Local Syste		More Actions	
Device Manager		Internet Connection Sharin	Provides ne	Kunning	Manual (Trig	Local Syste			
Storage		IP Helper	Provides tu	Running	Automatic	Local Syste			
📅 Disk Management	intrusion prevention services for a home or small office network.	A IP Translation Configuratio	Configures		Manual (Trig	Local System			
Services and Applications	nome of small office network.	A IPsec Policy Agent	Internet Pro	Running	Manual (Trig	Network S			
Services		Juniper Network Connect S		Running	Automatic	Local Syste			
i WMI Control		KtmRm for Distributed Tran		,	Manual (Trig	Network S			
		Language Experience Service	Provides inf		Manual	Local Syste			
		Lenovo Hotkey Client Loader		Running	Automatic	Local Syste			
		kenovo Microphone Mute		Running	Automatic	Local Syste			
		🍓 Lenovo Platform Service		Running	Automatic	Local Syste			
		🍓 Lenovo PM Service		Running	Automatic	Local Syste			
		🖏 Link-Layer Topology Discov	Creates a N		Manual	Local Service			
		kocal Profile Assistant Service	This service		Manual (Trig	Local Service			
		🍓 Local Session Manager	Core Windo	Running	Automatic	Local Syste			
		🆏 MessagingService_76a30	Service sup		Manual (Trig	Local Syste			
		🍓 Microsoft (R) Diagnostics H	Diagnostics		Manual	Local Syste			
		🍓 Microsoft Account Sign-in	Enables use	Running	Manual (Trig	Local Syste			
		🍓 Microsoft App-V Client	Manages A	Running	Automatic	Local Syste			
		Microsoft iSCSI Initiator Ser	Manages In		Manual	Local Syste			
		Microsoft Office Click-to-R	Manages re	Running	Automatic	Local Syste			
		Microsoft Passport	Provides pr		Manual (Trig	Local Syste			
		Microsoft Passport Container	Manages Io		Manual (Trig	Local Service			
		Microsoft Policy Platform L			Manual	Local Syste			
		Alternation Part Policy Platform P			Manual	Local Syste	~		

The following window appears.

Internet Connec	ction Sharing (ICS) Properties (Local Computer)	×
General Log O	On Recovery Dependencies	
Service name:	SharedAccess	
Display name:	Internet Connection Sharing (ICS)	
Description:	name resolution and/or intrusion prevention services for a home or small office network.	
Path to execut C:\WINDOWS	able: 5\System32\svchost.exe -k netsvcs -p	
Startup typ <u>e</u> :	Manual ~	
Service status:	Stopped	
<u>S</u> tart	Stop <u>P</u> ause <u>R</u> esume	
You can specif from here.	fy the start parameters that apply when you start the service	
Start para <u>m</u> ete	rs:	
	OK Cancel Apply	

5 Select **Disabled** from the Startup type drop-down.

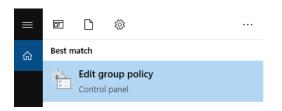
Internet C	onnectio	on Sharing (ICS) Properties (Local Computer)	Х
General	Log On	Recovery	Dependencies	
Service	name:	SharedAcc	ess	
Display	name:	Internet Co	nnection Sharing (ICS)	
Description: name resolution and/or intrusion prevention services for a home or small office network.				
	executabl DOWS\S		host.exe -k netsvcs -p	
Startup	typ <u>e</u> :	Manual	~	
		Automatic Automatic Manual	(Delayed Start)	
Service	status:	Disabled Stopped		
<u>S</u>	tart	Stop	Pause Resume	
You car from her		he start parar	neters that apply when you start the service	
Start pa	ra <u>m</u> eters:]
			OK Const	
			OK Cancel <u>Apply</u>	

6 Select Apply.

7 Select **OK** to close the window.

Disabling Unauthorized Applications

1 Type **Edit group policy** from the Windows search bar to locate the **Edit group policy editor** control panel.



2 Select **Edit group policy editor**. The Local Group Policy Editor window displays.

Local Group Policy Editor			_	×
File Action View Help Image: Constraint of the second seco				
 Local Computer Policy Computer Configuration Software Settings Windows Settings Administrative Templates Software Settings Software Settings Windows Settings Administrative Templates 	Local Computer Policy Select an item to view its description. Extended X Standard	Name		

3 Navigate to Computer Configuration > Administrative Templates > Windows Components > Store.

J Local Group Policy Editor	- 0	×
File Action View Help		
🔶 🤿 🔀 🔜 🔒 🛛 🖬 🛛 🍸		
Microsoft Edge Microsoft Secondary Authe Microsoft User Experience V Microsoft User Experience V Microsoft User Experience V Microsoft User Experience V Online Assistance Portable Operating System Presentation Settings Search Search Search Search Search Search Search Search Search Search Sound Recorder Sound Recorder Speech Store	to view its description. Setting State Turn off Automatic Download of updates on Win8 machines Not configured Turn off Automatic Download and Install of updates Not configured Turn off the offer to update to the latest version of Windows Not configured Disable all apps from Windows Store Turn off the Store application Not configured Only display the private store within the Windows Store app Not configured	
Sync your settings	<	>
< > Extended (St		
6 setting(s)		

4 Select Disable all apps from Windows Store.

e Action View Help				_
MDM * Microsoft account Microsoft Edge Microsoft Secondary Auther Microsoft Secondary Auther Microsoft User Experience V NetMeeting OneDrive Online Assistance Portable Operating System Presentation Settings Remote Desktop Services RSS Feeds Search Security Center Shutdown Options Smart Card Software Protection Platforn Sonual Recorder Speech	Store Disable all apps from Windows Store Edit policy setting. Requirements: At least Windows Server 2016, Windows 10 Description: Disable turns off the launch of all apps from the Windows Store that came pre-installed or were downloaded. Apps will not be updated. Your Store will also be disabled. Enable turns all of it back on. This setting applies only to Enterprise and Education editions of Windows.	Setting Turn off Automatic Download of updates on Win8 machines Turn off Automatic Download and Install of updates Turn off the offer to update to the latest version of Windows Disable all apps from Windows Store Turn off the Store application Only display the private store within the Windows Store app	State Not configured Not configured Not configured Not configured	
Store Sync your settings Tablet PC		<		

The following window appears.

Disable all apps fr	rom Windows Stor	re						×
Disable all apps fi	rom Windows Stor	e		Previous Setting	Next Set	ting		
Not Configured	Comment:							~
○ Enabled								
○ Disabled	C							~
	Supported on:	At least Window	ws Server 201	5, Windows 10				^
Options:			Help:					~
			that came updated. Y	ns off the launch of ore-installed or were our Store will also b is setting applies o Windows.	e downloaded. e disabled. Ena	Apps will ble turns	l not be all of it	
				(ОК С	ancel	App	ly

- **5** Select **Enabled** and **Apply**.
- **6** Select **OK** to close the window.

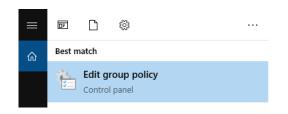
7 Disable the following applications. Refer to Steps 4-6.

- Turn off Automatic Download and Install of updates.
- Turn off the offer to update to the latest version of Windows.
- Turn off the Store application.

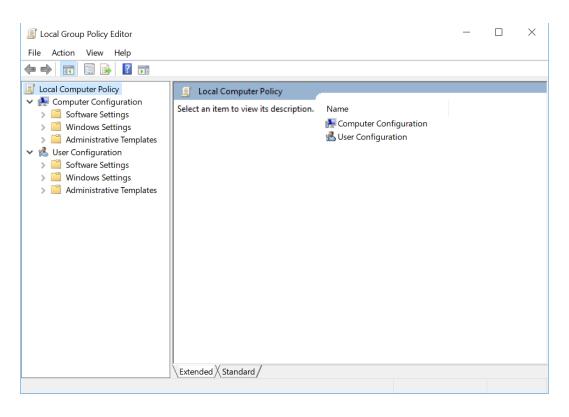
Enabling SMB Digitally Sign Communications

The Server Message Block (SMB) protocol provides the basis for file and print sharing and many other networking operations, such as remote Windows administration. To prevent man-in-the-middle attacks that modify SMB packets in transit, follow the instructions below to enable the SMB Digitally Sign Communications.

1 Type **Edit group policy** from the Windows search bar to locate the **Edit group policy editor** control panel.



2 Select **Edit group policy editor**. The Local Group Policy Editor window displays.



G

Local Group Policy Editor	- 0	Х
File Action View Help		
🗢 🔿 🖄 🔜 🛛 🖬		
 Computer Configuration Software Settings Windows Settings Name Resolution Policy Scripts (Startup/Shutdown) Deployed Printers Security Settings Account Policies Local Policies Local Policies Local Policies Security Options Windows Defender Firewall with Advanced S Network List Manager Policies Public Key Policies Software Restriction Policies Software Restriction Policies Application Control Policies Advanced Audit Policy Configuration Policy-based QoS Advanistrative Templates Software Configuration 	Policy Accounts: Administrator account status Accounts: Block Microsoft accounts Accounts: Guest account status Accounts: Limit local account use of blank passwords to co Accounts: Rename administrator account Accounts: Rename guest account Accounts: Rename guest account Audit: Audit the access of global system objects Audit: Audit the use of Backup and Restore privilege Audit: Shut down system immediately if unable to log secur DCOM: Machine Access Restrictions in Security Descriptor D DCOM: Machine Launch Restrictions in Security Descriptor Maccess Allowed to format and eject removable media Devices: Allowed to format and eject removable media Devices: Restrict CD-ROM access to locally logged-on user Devices: Restrict floppy access to locally logged-on user only Domain controller: Allow server operators to schedule tasks Domain controller: Allow server operators to schedule tasks Domain controller: Allow server channel binding token requi	Not Ena Not Disi Not Not Not
< >>	<	>

3 Navigate to Computer Configuration > Windows Settings > Security Settings > Security Options.

4 Select Microsoft network server: Digitally sign communications (always).

J Local Group Policy Editor		- 0	\times
ile Action View Help			
• 🔿 🙍 📷 🗙 🖼 🗟 🖬			
 Computer Configuration Computer Configuration Computer Settings Windows Settings Windows Settings Deployed Printers Security Settings Security Settings Account Policies Local Policies Local Policies User Rights Assignment Security Options Windows Defender Firewall with Advanced S Public Key Policies Software Restriction Policies Software Restriction Policies Software Restriction Policies Software Restriction Policies Modivare Advanced Policies Software Restriction Policies Software Restriction Policies Software Advanced Audit Policy Configuration Policy-based QoS Policy-based QoS Monicy-based QoS 	Policy Policy Interactive logon: Number of previous logons to cache (in case domain controller Interactive logon: Prompt user to change password before expiration Interactive logon: Require Windows Hello for Business or smart card Interactive logon: Smart card removal behavior Interactive logon: Interactive logon logon low: Smart card low anonymous SID/Name translation Interval access: Do not allow anonymous enumeration of SAM accounts and sha Interval access: Do not allow anonymous enumeration of SAM accounts and sha Interval access: Do not allow anonymous second second second cardentials for network a Interval acce	5 days Disabled Disabled No Action Disabled Enabled Disabled 15 minutes Not Defined Disabled Enabled Not Defined Disabled Enabled Disabled Disabled	
Viser Configuration	Network access: Let Everyone permissions apply to anonymous users Network access: Named Pipes that can be accessed anonymously Network access: Remetly accessible remistry names	System\CurrentControlS	

The following window appears.

Microsoft network server: Digitally sign communications ($?$	<
Local Security Setting Explain	
Microsoft network server: Digitally sign communications (always)	
Modifying this setting may affect compatibility with clients, services, and applications. For more information, see <u>Microsoft network server: Digitally sign</u> <u>communications (always)</u> . (Q823659)	
OK Cancel Apply	

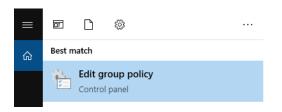
5 Select **Enabled** and **Apply**. The Confirm window appears.

Confin	m Setting Change				
	You are about to change this setting to a value that may affect compatibility with clients, services, and applications.				
	For more information, see <u>Microsoft network server: Digitally sign</u> communications (always). (Q823659)				
	Do you want to continue with the change?				
	Yes No				

 $\textbf{6} \quad \text{Select } \textbf{Yes.} \text{ Then select } \textbf{OK} \text{ to close the window.}$

Enabling Installation Restriction

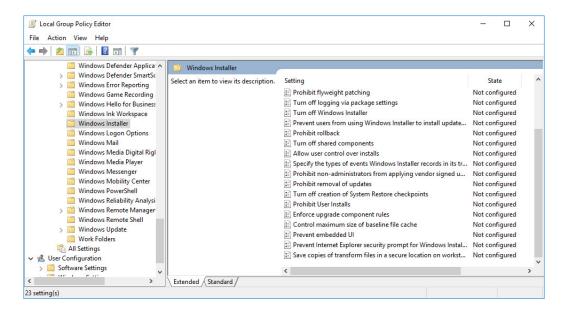
1 Type **Edit group policy** from the Windows search bar to locate the **Edit group policy editor** control panel.



2 Select **Edit group policy editor**. The Local Group Policy Editor window displays.

File Action View Help Image: Computer Policy Image: Computer Configuration Image: Compu	Jocal Group Policy Editor			_	×
 Computer Configuration Software Settings Windows Settings Software Settings Mindows Settings Administrative Templates 					
	 Computer Configuration Software Settings Windows Settings Administrative Templates User Configuration Software Settings Windows Settings 	Select an item to view its description.	E Computer Configuration		

3 Navigate to Computer Configuration > Administrative Templates > Windows Components > Windows Installer.



4 Select **Allow user control over installs**.

Cocal Group Policy Editor				- 0	×
ile Action View Help					
• 🔿 🖄 📷 🔒 📓 📷 🛛 🍸					
Windows Clotendar Windows Clotendar Windows Color System Windows Customer Experience Improvement Program Windows Defender Antivirus Windows Defender Antivirus Windows Encording and Breadcasting Windows Game Recording and Breadcasting Windows Into Workspace Windows IntelWorkspace Windows Mail Windows Mail Windows Mail Digital Rights Management Windows Media Di	Vindows Installer Allow user control over installs Edit policy setting Requirement: At least Windows 2000 Description: This policy setting permits users to change installation options that typically are available only to system administrators. If you enable this policy setting, some of the security features of Windows Installer are bypassed. If you enable to a dent configure If you disable or do not configure If you disable or do not configure	Setting Prohibit flyweight patching Turun off logging via package settings Turun off logging via package settings Turun off windows Installer Prevent users from using Windows Installer to install update Prohibit rollback Mlow user control over installs Allow user control over installs Prohibit thermoval of events Windows Installer records in its tr Prohibit removal of updates Turun off reation of System Restore checkpoints Prohibit User installs Enforce upgrade component rules Control maximum size of baseline file cache Prevent embedded UI	State Not configured Not configured	Comment No No No No No No No No No No No No No	t '
>	this policy setting, the security	Prevent Internet Explorer security prompt for Windows Instal Save copies of transform files in a secure location on workst	Not configured Not configured	No	
> windows opdate	Extended / Standard /	<			>

The following window appears.

Allow user contro	ol over installs	— 🗆 X
Allow user contro	ol over installs	Previous Setting <u>N</u> ext Setting
○ Not <u>C</u> onfigured	Comment:	^
○ <u>E</u> nabled		
Disabled		v
	Supported on:	At least Windows 2000
Options:		Help:
		This policy setting permits users to change installation options that typically are available only to system administrators. If you enable this policy setting, some of the security features of Windows Installer are bypassed. It permits installations to complete that otherwise would be halted due to a security violation. If you disable or do not configure this policy setting, the security features of Windows Installer prevent users from changing installation options typically reserved for system administrators, such as specifying the directory to which files are installed. If Windows Installer detects that an installation package has permitted the user to change a protected option, it stops the installation and displays a message. These security features operate only when the installation program is running in a

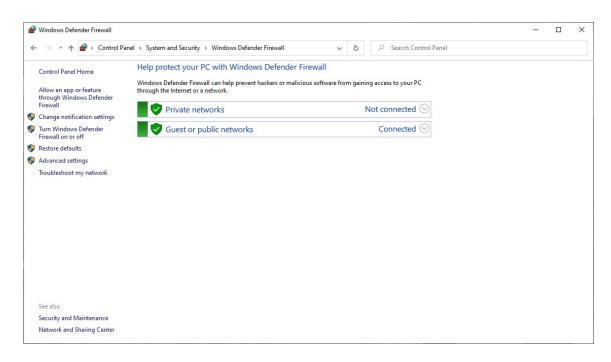
- **5** Select **Disabled** and **Apply**.
- **6** Select **OK** to close the window.
- 7 Disable install with elevated privileges. Refer to Steps 4-6.

Enabling Firewall Defender

1 Type **Windows Defender Firewall** from the Windows search bar to locate the Windows Defender Firewall app.



2 Select Turn Windows Defender Firewall on or off.



3 Select **Turn on Windows Defender Firewall** for both Private network and Public network settings.

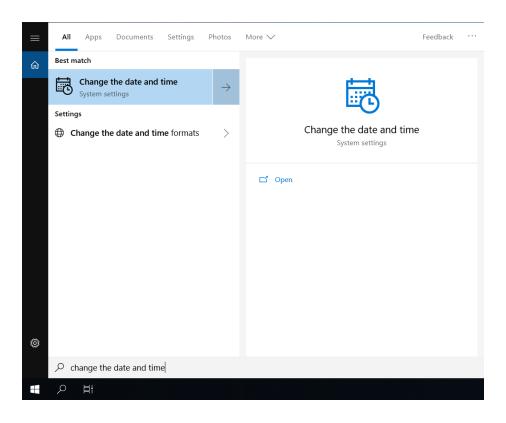
	_		
> Windows Defender Firewall > Customize Settings v 👌 🔎 Search Control Panel			
Customize settings for each type of network			
You can modify the firewall settings for each type of network that you use.			
Private network settings			
Turn on Windows Defender Firewall Block all incoming connections, including those in the list of allowed apps Kin Notify me when Windows Defender Firewall blocks a new app			
O Turn off Windows Defender Firewall (not recommended)			
Public network settings			
 Block all incoming connections, including those in the list of allowed apps Notify me when Windows Defender Firewall blocks a new app 			
O Turn off Windows Defender Firewall (not recommended)			
OK Cancel			
	Customize settings for each type of network You can modify the firewall settings for each type of network that you use. Private network settings Image: Setting the setting of the set of	Customize settings for each type of network You can modify the firewall settings for each type of network that you use. Private network settings Image: Setting the firewall of the setting the set of allowed apps in the list of allowed apps in the setting the set of	 Windows Defender Firewall > Customize Settings v o P Search Control Panel Customize settings for each type of network You can modify the firewall settings for each type of network that you use. Private network settings I Block all incoming connections, including those in the list of allowed apps Notify me when Windows Defender Firewall I Slock all incoming connections, including those in the list of allowed apps I Turn off Windows Defender Firewall I Block all incoming connections, including those in the list of allowed apps Notify me when Windows Defender Firewall

4 Select OK.

Enabling Network Time Protocol

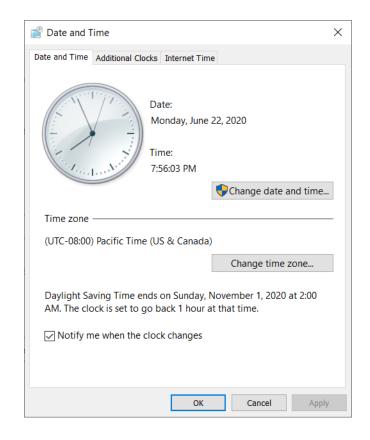
To ensure the accuracy of date and time information included in logs, follow the instructions below to enable the Network Time Protocol.

1 Type **Change the date and time** from the Windows search bar.



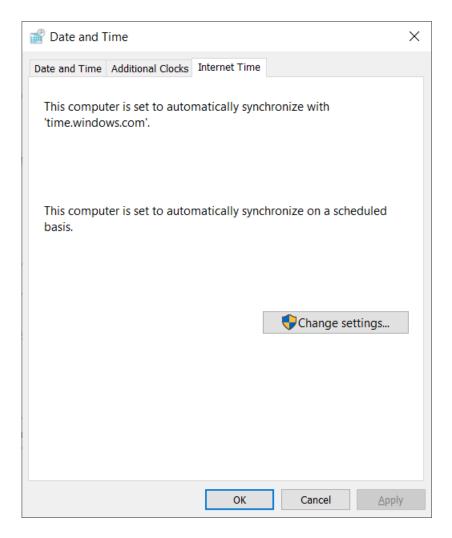
2 Double-click **Change the date and time**. The Date and Time Settings window appears.

Settings		- 🗆 ×
û Home	Date & time	
Find a setting ρ	Date and time	Relatec settings Date, time, & regional formatting
Time & Language	1:06 PM, Wednesday, November 4, 2020	Add clocks for different time zones
🗟 Date & time	Set time automatically On	
Region	Set time zone automatically	
₄ [≇] Language	Change date and time	
G Speech	Change	
	Time zone (UTC+08:00) Beijing, Chongqing, Hong Kong, Urumci V	
	Adjust for daylight saving time automatically	
	Off Show additional calendars in the taskbar	
	Don't show additional calendars	



 $\textbf{3} \quad \text{Select Set time automatically to access the Date and Time window.}$

4 Select **Change settings** from the Internet Time tab.



The Internet Time Settings window displays.

imit Internet Time Settings						
Configure Internet time settings:						
Synchronize with an Internet time server						
Server: time.windows.com VDpdate now						
This computer is set to automatically synchronize on a scheduled basis.						
OK Cancel						

5 Select **Synchronize with an internet time server**, and select **OK**. The PC is set to automatically synchronize with the internet time server.

APPENDIX H Table of Hazardous Substances

Table of Hazardous Substances

The Hazardous Substances Names and Concentration are shown in Table H.1, Table H.2, and Table H.3.

电子电气产品号码 EEP Part Number:	产品名称 Product Name: CytoFLEX 产品型号 Product Model Number:					
		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 H.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.

电子电气产品号码 EEP Part Number:	产品名称 Product Name: CytoFLEX S 产品型号 Product Model Number:					
	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 H.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 S]

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.

Т

电子电气产品号码 EEP Part Number:	产品名称 Product Name: CytoFLEX LX 产品型号 Product Model Number:					
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 H.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]

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 CV — coefficient of variation DNA — deoxyribonucleic acid 				
± — plus or minus					
< — less than					
> — greater than	DW — deep well				
\leq — less than or equal to	ECD — Energy Coupled Dye				
μ — micron	 EFUP — Environmentally friendly Use Period EMF — enhanced metafile format EMR — electromagnetic radiation FAPD — Fiber Array Photo Detector FCS — flow cytometry standard 				
μL — microliters μm — micrometer					
A — ampere					
AC — alternating current					
APC — Allophycocyanin					
APC-A700 — Allophycocyanin-Alexa	FITC — Fluorescein isothiocyanate				
Fluor™ 700 tandem dye	FSC — forward scatter				
APC-A7500 — Allophycocyanin-Alexa Fluor™ 750	GB — gigabyte				
APC-Cy7 — Allophycocyanin-Cyanin 7	GHz — gigahertz				
API — Application Programming Interface	Gr Wt — gross weight H — humidity				
Acq. — Acquisition					
BCI — Beckman Coulter Incorporated	Hz — hertz				
BMP — bitmap	IEC — International Electrotechnical Commission				
	IR — infrared				

RAM — random access memory **kg** — kilograms **rCV** — robust coefficient of variation **KO** — Krome Orange **LED** — light emitting diode **RH** — relative humidity **RoHS** — Restriction of Hazardous Substances L — liter 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

PEEK — polyether ether ketone

PC5 — Phycoerythrin-Cy[™]5 tandem dye

PC7 — Phycoerythrin-Cy[™]7 tandem dye

PC5.5 — Phycoerythrin-Cy[™]5.5 tandem dye

PerCP — Peridinin-Chlorophyll

PI — Propidium Iodide

PE — Phycoerythrin

PN — part number

nm — nanometer

Nt Wt — net weight

PB — Pacific Blue[™] dye

QC — quality control

Index

Symbols

define, Abbreviations-1 n define, Abbreviations-1 °C define, Abbreviations-1 °F define, Abbreviations-1 μ define, Abbreviations-1 μL define, Abbreviations-1 μm define, Abbreviations-1 ± define, Abbreviations-1 < define, Abbreviations-1 define, Abbreviations-1

Numerics

10 L sheath fluid cubitainer replacing, 12-7 10 L waste cubitainer (CytoFLEX LX) emptying, 12-10 4 L sheath fluid container to clean, 11-10 4 L sheath fluid container (cleaning) routine maintenance, 11-10 4 L sheath fluid container (CytoFLEX) filling, 12-6 4 L waste container to clean, 11-11 4 L waste container (cleaning) routine maintenance, 11-11 4 L waste container (CytoFLEX) emptying, 12-9

A

А define, Abbreviations-1 about this manual, xxxix AC define, Abbreviations-1 account locking, 4-9 unlock, 2-27 account menu, 2-21 account policies, 2-31 acoustic noise level, 1-38 Acq. define, Abbreviations-1 acquisition screen, 2-3 collection, 2-5 navigation, 2-4 plot area, 2-8 status bar, 2-9 test tubes, 2-7 acquisition settings to configure, 6-38 add vertex to auto polygon gate, 6-30 adding Deep Clean solution, 12-17 standardization item, 5-36 adding channels for compensation, 7-22 adjust compensation manually, 7-16 adjusting autogate movement, 6-31 compensation, 7-16 compensation settings, 8-8 gain, 6-41 threshold, 6-43 adjusting auto gates, 6-28 adjustment nonscheduled, 12-61 routine, 12-2 advanced menu, 2-20 alarm

Fluidics module, 1-19 mute, 1-19 alarm does not sound when the waste container is full or the sheath fluid container is low and the software status display is red, 10-10 alerts caution define, xvii important define, xvii note define, xvii warning define, xvii analysis screen, 8-1 analysis screen, 2-9 to open, 8-3 analyzing data, 6-70 APC define, Abbreviations-1 APC-A7500 define, Abbreviations-1 APC-Cy7 define, Abbreviations-1 API define, Abbreviations-1 applying standardization in QC, 5-40, 5-43 auto gates adjusting, 6-28 creating, 6-28 auto polygon gate add vertex, 6-30 auto recalculate turn off, 6-30 turn on, 6-30 auto sampling system Deep Clean solution peristaltic pump, 1-19 auto shutdown, 6-8, 9-2, 11-6 auto startup, 9-2 autogate adjusting movement, 6-31 extent, 6-32 movement, 6-31 automated software feature, 3-6

В

backflush settings to change, 12-76 backup, 10-22

manual injection mode, 4-10 band-pass filter, 1-7, 1-9, 1-11, 1-12, 1-14, Abbreviations-1 band-pass filters, 1-6 bar-code labels used on sample carousel, 3-1 BCI define, Abbreviations-1 beam shaping laser, 3-3 Beckman Coulter Customer Support Center, contacting, ii BMP define, Abbreviations-1 bold face font conventions, xli bottle Deep Clean solution, 1-19 BP define, Abbreviations-1

С

calculating compensation values, 7-8, 7-13 concentration, 8-7 sample volume, 8-7 calculation of the automatic compensation experiment is incorrect, 10-16 calculations compensation, 7-1 calibrating plate position [with plate loader], 12-78 sample flow rate, 12-61 sample flow rate with plate loader, 12-65 call center, contact information, ii carousel, sample bar-code labels, 3-1 description, 3-1 See also MCL caution define, xvii CDRH define, Abbreviations-1 cell illumination description, 3-4 CFSE define, Abbreviations-1 change password, 2-27

changing mixer and backflush settings, 12-76 tube name, 6-38 changing sample probe from plate loader to single tube sample station, 12-43 from single tube sample station to plate loader, 12-38 channel to set, 6-16 channels adding for compensation, 7-22 fluorescence, 6-49, 6-50 to set. 6-16 characteristics performance, 1-41 check waste and reagent levels (10 L fluid cubitainers), 4-4 waste and reagent levels (4 L fluid containers), 4-2 cleaning 4 L sheath fluid container, 11-10 4 L waste container, 11-11 nonscheduled, 11-13 routine, 11-1 sample probe, 11-8 sample station, 11-7 surfaces, 11-13 cleaning and ventilation installation environment validation, A-2 cleaning solution to prepare, 9-1 cleaning the 4 L sheath fluid container routine maintenance, 11-10 cleaning the 4 L waste container routine maintenance, 11-11 cleaning the sample station routine maintenance, 11-7 closed file system electronic record management, B-2 cm define, Abbreviations-1 collecting data, 6-55 QC data, 5-12 QC data with plate loader, 5-15 collection acquisition screen, 2-5 collection conditions

to set, 6-44 compensation adding channels, 7-22 calculations, 7-1 to adjust, 7-16 to manually adjust, 7-16 compensation controls compensation experiment screen, 2-11 compensation experiment to create, 7-2 compensation experiment screen, 2-11 compensation controls, 2-11 compensation experiment with plate loader creating, 7-10 compensation library to manage, 7-21 compensation matrix to create from previously acquired data, 7-14 to generate, 7-4, 7-13 compensation sample prepare, 7-4, 7-13 compensation settings adjust, 8-8 exporting, 7-19 importing and exporting, 6-80 compensation settings from compensation library to import, 7-18 compensation settings from compensation matrix files to import, 7-16 compensation values to calculate, 7-8, 7-13 components Cytometer, 1-2 Fluid Containers/Cubitainers, 1-2 fluidics system, 1-17 instrument, 1-1 main, 1-2 optical, 1-4 plate holder, 1-27 plate loader, 1-23 sample injection mode control kit, C-2 Workstation, 1-2 concentration calculation, 8-7 sample volume, 8-7 concentration calculation is incorrect, 10-17 concluding

experiment, 6-83 configuration system, 1-31 configuring acquisition settings, 6-38 confirming results, 5-20 connection indicator light in the lower left corner of the software screen is red and displays Disconnected and Error, 10-10 contact information, Beckman Coulter Customer Support Center, ii conventions bold face font, xli the term press, xli the term select, xli conventions used, 1-xli copying experiments, 8-1 previously acquired experiment, 8-1 create a compensation experiment start page operations, 2-2 create a new experiment from a template start page operations, 2-2 create a new screen start page operations, 2-2 creating compensation experiment, 7-2 compensation experiment with plate loader, 7-10 compensation matrix from previously acquired data, 7-14 detector configuration, 6-48 dot plot overlays, 8-5 heat map (plate loader), 6-60 histogram overlays, 8-5 Levey-Jennings charts, 5-24 new experiment, 6-1 new experiment with plate loader, 6-1, 6-3 plots and gates, 6-19 user roles, 2-29 users, 2-25 creating auto gates, 6-28 CSV define. Abbreviations-1 custom filter, E-1 custom statistics setting, 6-34 customer-replaceable parts

customized parameters setting, 6-33 CV define, Abbreviations-1 cyber security, G-53 backing up your recovery key, G-13 BitLocker decryption, G-17 BitLocker encryption, G-6 BitLocker keys, G-2 changing encryption strength, G-2 disabling McAfee application control, G-49 drive encryption, G-2 enabling McAfee application control, G-31 exporting certificate, G-35 good practices in cyber security, G-1 Importing certificate, G-45 installing McAfee Application Control, G-26 malware detection software, G-25 operating system patching, G-57, G-61 recovering your BitLocker key, G-23 suspending BitLocker drive encryption, G-21 switching McAfee Application control to update mode, G-47 CytExpert installation options, A-12 CytExpert API test client setup, 2-48 CytExpert certificate, G-35 CytExpert software reinstall, A-29 upgrade, A-24 Cytobank, 2-14

instrument, 1-36 dimensions, 1-36

instrument, 1-37 dimensions, 1-37 Cytometer, 1-2, 1-3, A-6 Cytometer cannot be turned on, 10-9 cytometer menu, 2-17

D

daily shutdown routine maintenance, 11-1 daily shutdown with plate loader routine maintenance, 11-5 daily start up

ordering, H-1

routine maintenance, 11-1 daily start up with plate loader routine maintenance, 11-5 data analyzing, 6-70 collecting, 6-55 exporting, 6-70 sampling, 6-55 to import, 8-1 data populations are normal on one laser, but too low on another laser, 10-14 data populations are not where they are expected, 10-15 data sheets, material safety how to order, 1-35 data storage description, 3-6 Deep Clean procedure, 11-8 routine maintenance, 11-8 Deep Clean solution to add, 12-17 to prepare, 12-17 Deep Clean solution bottle fluidics module, 1-19 Deep Clean solution peristaltic pump auto sampling system, 1-19 default detector configuration, 6-49 default password, 2-24, A-24 default username username default, A-24 define %, Abbreviations-1 caution, xvii important, xvii note, xvii quality control (QC), 5-1 symbols, -xix warning, xvii defining the negative population, 7-5 delete experiment directory (electronic record management), B-4 heat map parameter, 6-62 deleting heat map (plate loader), 6-69 standardization items, 5-48 user roles, 2-30

users, 2-26 detector (WDM) optical components, 1-4 detector configuration to create, 6-48 to edit, 6-48 to select, 6-48 to verify, 6-48 detector configuration default, 6-49 detector unit wavelength division multiplexor (WDM), 1-5 disabling lasers, 6-40 disinfection surfaces, 11-13 disposal precaution, 10-8 waste, A-4 DNA define, Abbreviations-1 document overview, xxxix dot plot overlays to create, 8-5 downloading security updates, G-57 drive decryption enabling BitLocker, G-17 drive encryption, G-2 BitLocker keys, G-2 BitLocker recovery, G-23 changing encryption strength level for BitLocker, G-2 enabling BitLocker, G-6 dual-parameter plots, 2-34 duplicating standardization item parameters, 5-47 DW define, Abbreviations-1

Ε

ECD define, Abbreviations-1 editing detector configuration, 6-48 standardization item parameters, 5-47 EFUP define, Abbreviations-1 electronic devices fuse, 1-32, 1-34 load button, 1-32, 1-34 power switch, 1-32, 1-34 electronic record management closed file system, B-2 electronic signature, B-17 experiment directory management, B-3 experiment management, B-2 experiment operation log, B-12 experiment related operations, B-5 exporting an experiment/template, B-9 folder hierarchy management, B-4 importing an experiment/template, B-6 log, B-12 software menu, B-1 system operation log, B-15 user management, B-26 user management operation log, B-16 electronic signature electronic record management, B-17 retract, B-20 EMF define, Abbreviations-1 emptying 10 L waste cubitainer (CytoFLEX LX), 12-10 4 L waste container (CytoFLEX), 12-9 EMR define, Abbreviations-1 enabling lasers, 6-40 environment validation installation, A-2 environment validation (installation) power source, A-3 temperature and humidity, A-4 ventilation and cleaning, A-2 worktable, A-2 exchange detector configuration WDM beam splitter, F-4 exit the software start page operations, 2-2 experiment concluding, 6-83 sign, B-17 experiment (new) creating, 6-1 experiment directory (electronic record management) delete, B-4 rename, B-4 setup, B-3 experiment directory management electronic record management, B-3

experiment management electronic record management, B-2 experiment operation log electronic record management, B-12 experiment related operations electronic record management, B-5 experiment saving, 6-83 experiment selection start page, 4-27 experiment settings, 2-45 experiment signature print, B-25 experiments to copy, 8-1 exporting compensation settings, 6-80, 7-19 data, 6-70 FCS files, 6-75 heat map (plate loader), 6-69 instrument settings, 6-78, 6-79 plots of multiple tubes as picture files, 6-77 results, 8-9 standardization item, 5-47 statistics table of multiple tubes as a picture file, 6-77 user logs, 2-33 exporting and experiment/template electronic record management, B-9 extent autogate, 6-32

F

FAPD define, Abbreviations-1 FCS define, Abbreviations-1 FCS files exporting, 6-75 files menu, 2-15 filling 4 L sheath fluid container (CytoFLEX), 12-6 filling sheath fluid container, 4-2 filter, E-1 optical, 1-5 sheath fluid, 1-19 FITC define, Abbreviations-1 FL See fluorescent light (FL)

flow cell hydrodynamic focusing, 3-2 illustration, 3-3 to prime, 12-48 flow cell waste out fluidic connection, 1-20, 4-3, 4-5 Fluid Container holder, 1-17 Fluid Containers, 1-2, 1-3, A-6 Fluid Containers/Cubitainers component, 1-2 fluidics system, 1-18 fluidics system component, 1-17 fluid sensor holder cutout, 1-17 fluid status information displays red for Sheath and/or Waste even though the sheath fluid container is full and the waste container is empty, 10-11 fluidic connections flow cell waste out, 1-20, 4-3, 4-5 sheath fluid in, 1-20, 4-3, 4-5 sheath fluid level sensor connector, 1-20, 4-3, 4-5 sheath return, 1-20, 4-3, 4-5 waste level sensor connector, 1-20, 4-3, 4-5 waste out, 1-20, 4-3, 4-5 Fluidics module alarm, 1-19 fluidics module Deep Clean solution bottle, 1-19 fluidics system, 1-19 fluidics system component, 1-17 sheath fluid filters, 1-19 fluidics system components, 1-17 Fluid Containers/Cubitainers, 1-18 fluidics module, 1-19 fluidics system components Fluid Containers/Cubitainers, 1-17 fluidics module, 1-17 fluorescence channels, 6-49, 6-50 fluorescent light (FL) cell illumination, 3-4 collection, 3-5 when to use, example, 3-4 focusing, hydrodynamic description, 3-2 folder hierarchy management electronic record management, B-4 forward scatter (FS) cell illumination, 3-4

four-quadrant gates for dual-parameter plots, 2-35 front cover removal, 12-2 FS *See* forward scatter FSC define, Abbreviations-1 functions software, 2-1 fuse electronic devices, 1-32, 1-34 to replace, 12-72

G

gain to adjust, 6-41gate settings, 2-47 gates to create, 6-19 gates for dual-parameter plots four-quadrant gates, 2-35 lasso gates, 2-35 polygon gates, 2-35 rectangle gates, 2-35 gates for single-parameter plots line-segment gates, 2-35 vertical gates, 2-35 GB define, Abbreviations-1 generating compensation matrix, 7-4, 7-13 Ghz define, Abbreviations-1 Gr. Wt. define, Abbreviations-1 graphic and gating styles, 2-34 graphics printing, 6-80

Η

H define, Abbreviations-1 hazard laser, 10-1 hazards laser beam, 10-2 radiation, 10-3 hazards/precautions, 10-1 heat map (plate loader) creating, 6-60 deleting, 6-69 exporting, 6-69 modifying, 6-68 refreshing, 6-67 heat map parameter deleting, 6-62 help menu, 2-22 help, Beckman Coulter Customer Support Center, ii histogram types of display, 3-7 histogram overlays to create, 8-5 how to use your manual, xxxix humidity and temperature installation environment validation, A-4 hydrodynamic focusing description, 3-2 Hz define, Abbreviations-1

I

IEC define, Abbreviations-1 important define, xvii importing compensation settings, 6-80 compensation settings from compensation library, 7-18 compensation settings from compensation matrix files, 7-16 data. 8-1 instrument settings, 6-78 lot-specific target values, 5-7 previously acquired data, 8-1 standardization item, 5-46 importing an experiment/template electronic record management, B-6 information ordering, 1-35 initializing the instrument, 4-28 inspecting materials unpacking, A-4 inspection

liquid flow tubing, 12-47 pre-boot, 4-1 inspection when unpacking, A-4 installation environment validation, A-2 instrument, A-1 software, A-12 installation category, 1-37 installation options CytExpert, A-12 installing custom filter, E-1 instrument configuration file, A-22 Plate Loader module, 12-51 software, A-13 installing security updates, G-61 installing the instrument and connecting the equipment unpacking inspection, A-5, A-11 instrument components, 1-1 initializing, 4-28 inspecting materials, A-4 installation, A-1 maintenance, 12-1 parameters, 1-38 shut off, 9-1 transportation and storage, A-1 unpacking, A-4 instrument characteristics, 1-36 instrument components Cytometer, 1-2 Fluid Containers/Cubitainers, 1-2 Workstation, 1-2 instrument configuration unpacking inspection, A-4 instrument configuration file to install, A-22 instrument dimensions, 1-36, 1-37 instrument operations cannot be performed in the Acquisition screen, 10-18 instrument settings exporting, 6-79 importing, 6-78 importing and exporting, 6-78 instrument specifications, 1-36 instrument transport or storage to prepare, 11-14 IR

define, Abbreviations-1 items adding for standardization, 5-36 deleting for standardization, 5-48 duplicating for standardization, 5-47 editing for standardization, 5-47 exporting for standardization, 5-47 importing for standardization, 5-46

Κ

kg define, Abbreviations-2 KO define, Abbreviations-2

L

L define, Abbreviations-2 labels to set, 6-16 language settings, 2-48 laser beam shaping, 3-3 hazards, 10-1 settings, 6-38 target power settings (CytoFLEX LX only), 6-40 laser beam hazards, 10-2 laser delay to set, 12-69 laser delay values are out of range, 10-13 laser lines, 1-38 laser power is low, 10-12 laser target power settings (CytoFLEX LX only), 6-40 laser wavelength, 1-38 lasers disable, 6-40 enable, 6-40 lasso gates for dual-parameter plots, 2-35 launching the software, 2-1 LED define, Abbreviations-2 lenses beam shaping, 3-3 cross-cylindrical, 3-3

Levey-Jennings charts to create, 5-24 light collection, separation and measurement description, 3-4 line segment gates for single-parameter plots, 2-35 liquid flow tubing inspection, 12-47 liquid flow tubing inspection routine maintenance, 12-47 LI define, Abbreviations-2 load button electronic devices, 1-32, 1-34 locking account, 4-9 log electronic record management, B-12 user management operation, 2-33 log in software, 4-6 log menu, 2-22 logs exporting, 2-33 viewing, 2-33 lot-specific target values to import, 5-7 LWH define, Abbreviations-2

Μ

m define, Abbreviations-2 main components, 1-2 maintenance instrument, 12-1 See also routine maintenance; nonscheduled maintenance maintenance reminder to manage, 12-14 malware detection software, G-25 managing compensation library, 7-21 maintenance reminder, 12-14 manual about, xxxix adjust compensation, 7-16 manual injection mode running small sample volumes, 4-10

manuals updating, iii material safety data sheets (MSDS/SDS) how to order, 1-35 MB define, Abbreviations-2 McAfee application certificate, G-45 McAfee application control disabling, G-49 enabling, G-31 update mode, G-47 MCL (Multi-Tube Carousel Loader) loading the sample, 3-1 menu account, 2-21 acquisition and analysis screen, 2-15 advanced, 2-20 cytometer, 2-17 files, 2-15 help, 2-22 log, 2-22 QC/Standardization, 2-20 settings, 2-19 signature, 2-22 software, 2-14 menu tree software, 2-14 MFI define, Abbreviations-2 MHz define, Abbreviations-2 min define, Abbreviations-2 mixer is not functioning, 10-18 mixer settings to change, 12-76 mL define, Abbreviations-2 mm define, Abbreviations-2 modifying heat map (plate loader), 6-68 user roles, 2-30 users, 2-26 well settings with plate loader, 6-14 modifying well settings, 6-14 applying existing well settings to additional wells, 6-15

moving well location [with plate

loader], 6-16 movement adjusting for autogates, 6-31 autogate, 6-31 moving well location [with plate loader], 6-16 MSDS (material safety data sheets) how to order, 1-35 multiple tube plots export as picture files, 6-77 multiple tube statistics table

export as picture files, 6-77 mute alarm, 1-19 mute alerter icon, 1-19 mW define, Abbreviations-2

Ν

NA define, Abbreviations-2 NaClO define, Abbreviations-2 NaN3 define, Abbreviations-2 navigation acquisition screen, 2-4 QC screen, 2-13 negative population to define using unstained samples, 7-5 new experiment creating, 6-1 new experiment with plate loader creating, 6-1, 6-3 nm define, Abbreviations-2 no changes occurred after manually adjusting compensation settings, 10-15 no data acquisition, 10-14 nonscheduled cleaning, 11-13 nonscheduled maintenance adding the Deep Clean solution, 12-17 calibrating the sample flow rate, 12-61 calibrating the sample flow rate with plate loader, 12-65 cleaning the sample probe, 11-8 preparing the instrument for storage or transport, 11-14

replacing the fuse, 12-72 replacing the optical filter, 12-70 nonscheduled replacement/adjustment, 12-61 note define, xvii Nt. Wt. define, Abbreviations-2

0

open a compensation experiment start page operations, 2-2 open an experiment start page operations, 2-2 open software, 4-6 opening analysis screen, 8-3 operating system patching, G-53 optical components, 1-4 optical bench optical component, 1-4 optical components detector (WDM), 1-4 optical bench, 1-4 optical fiber, 1-4 optical fiber, 1-16 optical filter, 1-5 to replace, 12-70 ordering customer-replaceable parts, H-1 ordering information, 1-35 overlay dot plots, 2-34 overlay histograms, 2-34 overview document, xxxix

Ρ

page setup settings, 2-47 parameter (heat map) delete, 6-62 parameters description, 3-7 duplicating standardization items, 5-47 editing standardization items, 5-47 instrument, 1-38 TIME, 3-7 password change, 2-27

default, 2-24, A-24 reset, 2-27 PB define, Abbreviations-2 PC5 define, Abbreviations-2 PC5.5 define, Abbreviations-2 PC7 define, Abbreviations-2 PE define, Abbreviations-2 PEEK define, Abbreviations-2 PerCP define, Abbreviations-2 performance characteristics, 1-41 sample injection mode control knob, C-1 performing daily quality control, 5-1 PI define, Abbreviations-2 picture files to export, 6-77 plate holder components, 1-27 plate holder with plate loader replacing, 12-49 plate loader components, 1-23 Plate Loader module installing, 12-51 removing, 12-51 plate loader PEEK tubing replacing, 12-32 plate loader PEEK tubing (replacing) routine maintenance, 12-32 plate position [with plate loader] calibrating, 12-78 plate type, 2-36 plate type library, 2-36 plot area acquisition screen, 2-8 plot display conditions to set, 6-46 plot settings, 2-46 plots dual-parameter, 2-34 overlay dot plots, 2-34 overlay histograms, 2-34

single-parameter, 2-34 to create, 6-19 to set, 8-3 types of display, 3-7 PN define, Abbreviations-2 policies account, 2-31 pollution degree, 1-38 polygon gates for dual-parameter plots, 2-35 population amplitude is decreasing and CV values are increasing, 10-13 populations are drifting, 10-12 position sample tube holder, 1-22 power source installation environment validation, A-3 power source inspection pre-boot, 4-5 power switch electronic devices, 1-32, 1-34 pre-boot inspection, 4-1 check waste and reagent levels (10 L fluid cubitainers), 4-4 check waste and reagent levels (4 L fluid containers), 4-2 power source inspection, 4-5 workstation connections inspection, 4-5 precaution disposal, 10-8 precautions safety, -xviii precautions/hazards, 10-1 preparation process QC sample (CytoFLEX Daily IR QC Fluorospheres), 5-4 QC sample (CytoFLEX Daily IR QC Fluorospheres) with plate loader, 5-6 QC sample (CytoFLEX Daily QC Fluorospheres), 5-3, 5-29 QC sample (CytoFLEX Daily QC Fluorospheres) with plate loader, 5-5 QC sample (CytoFLEX Ready to Use Daily QC Fluorospheres), 5-4, 5-6 preparing compensation sample, 7-4, 7-13 Deep Clean solution, 12-17

instrument for storage or transport, 11-14

QC sample, 5-3 QC sample with plate loader, 5-5 Standardization sample, 5-28 the cleaning solution, 9-1 preparing QC sample required materials, 5-3, 5-29 preparing QC sample with plate loader required materials, 5-5 press conventions, xli previously acquired data to import, 8-1 previously acquired experiment to copy, 8-1 priming flow cell, 12-48 print experiment signature, B-25 printing graphics, 6-80 procedure Deep Clean, 11-8 product description, 1-1

Q

0C applying standardization, 5-40, 5-43 define, Abbreviations-2 result manager, 5-27 standardization, 5-29 QC aborted due to low event rate, 10-19 QC data collecting, 5-12 QC data with plate loader collecting, 5-15 QC experiment screen, 2-12, 2-13 QC failed, 10-20 OC menu tree software, 2-15 QC report screen, 2-12 QC sample preparation, 5-3 QC sample (CytoFLEX Daily IR QC Fluorospheres) preparation process, 5-4 QC sample (CytoFLEX Daily IR QC Fluorospheres) with plate loader preparation process, 5-6 QC sample (CytoFLEX Daily QC Fluorospheres)

preparation process, 5-3, 5-29 QC sample (CytoFLEX Daily QC Fluorospheres) with plate loader preparation process, 5-5 QC sample (CytoFLEX Ready to Use Daily QC Fluorospheres) preparation process, 5-4, 5-6 QC sample with plate loader preparation, 5-5 QC screen navigation, 2-13 QC software menu tree, 2-15 QC/Standardization menu, 2-20 Quality Control, 5-2 quality control, 5-2 perform daily, 5-1 quality control (QC) define, 5-1

R

radiation hazards, 10-3 RAM define, Abbreviations-2 rCV define, Abbreviations-2 reagent levels (10 L fluid cubitainers) check, 4-4 reagent levels (4 L fluid containers) check, 4-2 recovery key, G-13 rectangle gates for dual-parameter plots, 2-35 refreshing heat map (plate loader), 6-67 reinstall CytExpert software, A-29 reinstallation procedures right-side cover, 12-5 removal procedures front cover, 12-2 right-side cover, 12-4 removing Plate Loader module, 12-51 rename experiment directory (electronic record management), B-4 replacement nonscheduled, 12-61

routine, 12-2 replacing 10 L sheath fluid cubitainer, 12-7 fuse, 12-72 optical filter, 12-70 plate holder with plate loader, 12-49 plate loader PEEK tubing, 12-32 sample peristaltic pump tubing for sample loading, 12-22 sheath fluid filter, 12-18 sheath fluid harness and/or waste harness, 12-74 replacing sheath fluid cubitainer, 4-4 replacing the peristaltic pump for sample loading routine maintenance, 12-22 replacing the sample probe for plate loader routine maintenance, 12-32 replacing the sheath fluid filter routine maintenance, 12-18 required materials QC sample preparation, 5-3, 5-29 QC sample preparation with plate loader, 5-5 software installation, A-13 reset password, 2-27 restore, 10-22 result manager QC, 5-27 results confirming, 5-20 to export, 8-9 retention period setting, B-21 retract electronic signature, B-20 RH define, Abbreviations-2 right-side cover reinstallation, 12-5 removal, 12-4 RoHS define, Abbreviations-2 role management, 2-27 roles creating, 2-29 deleting, 2-30 modifying, 2-30 routine cleaning, 11-1 routine maintenance

cleaning the 4 L sheath fluid container, 11-10 cleaning the 4 L waste container, 11-11 cleaning the sample station, 11-7 daily startup and shutdown, 11-1 daily startup and shutdown with plate loader, 11-5 Deep Clean, 11-8 liquid flow tubing inspection, 12-47 replacing the plate loader PEEK tubing, 12-32 replacing the sample peristaltic pump tubing for sample loading, 12-22 replacing the sheath fluid filter, 12-18 routine replacement/adjustment, 12-2 **RPTM** define, Abbreviations-2 running single positive control samples, 7-6 system startup program, 4-16 system startup program with plate loader, 4-19 running small sample volumes manual injection mode, 4-10

S

S/N define, Abbreviations-2 safety precautions, -xviii sample checks before running, 6-47 flow, description, 3-1 illuminated in the flow cell, 3-4 loading, automated, 3-1 sample acquisition position sample tube holder position, 1-22 sample carousel bar-code labels, 3-1 description, 3-1 See also carousel, sample and MCL sample flow description, 3-1 sample flow rate calibrating, 12-61 sample flow rate is unstable, 10-11 sample flow rate with plate loader calibrating, 12-65 sample injection control knob performance characteristics, C-1

sample injection mode selecting, 4-9 sample injection mode control kit components, C-2 sample is flowing, but no signal appears in the plot, 10-16 sample loading backup, 4-10 sample loading position sample tube holder position, 1-22 sample peristaltic pump tubing to replace, 12-22 sample peristaltic pump tubing for sample loading to replace, 12-22 sample peristaltic pump tubing for sample loading (replacing) routine maintenance, 12-22 sample probe to clean, 11-8 sample probe change from plate loader to single tube sample station, 12-43 from single tube sample station to plate loader, 12-38 sample probe for plate loader replacing, 12-32 sample probe is too low, 10-17 sample probe switch from plate loader to single tube sample station, C-5 from single tube sample station to plate loader, C-3 sample station, 1-21 to clean, 11-7 sample station (cleaning) routine maintenance, 11-7 sample tube holder cannot move up and down automatically, 10-11 sample tube holder position sample acquisition position, 1-22 sample loading position, 1-22 standby position, 1-22 sample wells with plate loader setting, 6-9 sampling data, 6-55 sampling flow rate is too fast, 10-12 saving

experiment, 6-83 screen acquisition, 2-3 analysis, 2-9, 8-1, 8-3 compensation experiment, 2-11 QC experiment, 2-12, 2-13 QC report, 2-12 software, 2-1 SDS (safety data sheets) how to order, 1-35 select conventions, xli selecting detector configuration, 6-48 proper sample injection mode, 4-9 service, contact information, ii setting channel, 6-16 channels, 6-16 collection conditions, 6-44 custom statistics, 6-34 customized parameters, 6-33 labels, 6-16 laser delay, 12-69 laser target power settings (CytoFLEX LX only), 6-40 plot display conditions, 6-46 retention period, B-21 sample wells with sample wells, 6-9 signature, B-22 the plots and statistics, 8-3 setting up violet side scatter channel (VSSC), 6-52 settings acquisition, 6-38 experiment, 2-45 gate, 2-47 language, 2-48 laser, 6-38 page setup, 2-47 plot, 2-46 software, 2-45 tube, 2-46 settings menu, 2-19 setup CytExpert API test client, 2-48 experiment directory (electronic record management), B-3 sheath fluid container, 1-17 to fill, 4-2

sheath fluid container 4 L (CytoFLEX) filling, 12-6 sheath fluid container/cubitainer, 1-18 sheath fluid cubitainer to replace, 4-4 sheath fluid cubitainer 10 L replacing, 12-7 sheath fluid filter to replace, 12-18 sheath fluid filter (replacing) routine maintenance, 12-18 sheath fluid filters fluidics module, 1-19 sheath fluid harness and sensor, 1-17, 1-18 sheath fluid in fluidic connection, 1-20, 4-3, 4-5 sheath fluid level sensor connector fluidic connection, 1-20, 4-3, 4-5 sheath return fluidic connection, 1-20, 4-3, 4-5 shut off the instrument, 9-1 shutdown automatic, 6-8, 9-2, 11-6 shutdown daily routine maintenance, 11-1 shutdown daily with plate loader routine maintenance, 11-5 side scatter (SS) cell illumination, 3-4 collection, 3-5 when to use, example, 3-4 sign experiment, B-17 signals processing of, operation principles, 3-6 signature setting, B-22 signature menu, 2-22 single positive control samples to run. 7-6 single-parameter plots, 2-34 SNR define, Abbreviations-2 software functions, 2-1 launching, 2-1 log in, **4**-6 main screen, 2-1 open, 4-6

to install, A-13 to start, A-24 software installation, A-12 installing CytExpert software, A-13 installing instrument configuration file, A-22 required materials, A-13 starting the software, A-24 software installation fails, 10-18 software menu, 2-14 acquisition and analysis screen menu, 2-15 electronic record management, B-1 software menu tree, 2-14 software operations create a new experiment, 2-2 software screen, 2-1 start page, 2-2 start page operations, 2-2 software settings, 2-45 specifications characteristics, 1-36 instrument, 1-36 specimen See sample SS See side scatter SSC define, Abbreviations-2 Standardization loading standardization sample, 5-29 Target value, 5-29 standardization, 5-28 applying in QC, 5-40, 5-43 standardization item adding, 5-36 deleting, 5-48 exporting, 5-47 importing, 5-46 standardization item parameters duplicating, 5-47 editing, 5-47 Standardization sample preparation, 5-28 standardization target library deleting standardization target settings, 5-46 duplicate standardization target settings, 5-46 editing standardization target settings, 5-46

exporting standardization target settings, 5-46 importing standardization target settings, 5-46 standby position sample tube holder position, 1-22 start page main software screen, 2-2 selecting experiments, 4-27 start page operations create a compensation experiment, 2-2 create a new experiment from a template, 2-2 exit the software, 2-2 main software screen, 2-2 open a compensation experiment, 2-2 open an experiment, 2-2 starting the software, A-24 startup automatic, 9-2 startup daily routine maintenance, 11-1 startup daily with plate loader routine maintenance, 11-5 station sample, 1-21 statistics setting, 3-8 to set, 8-3 status bar acquisition screen, 2-9 storing the instrument, A-1 support, Beckman Coulter customer, ii surface cleaning and disinfection, 11-13 suspending BitLocker drive encryption, G-21 switching sample probe from plate loader to single tube sample station, C-5 from single tube sample station to plate loader, C-3 symbols define, -xix system configuration, 1-31 system operation log electronic record management, B-15 system startup program to run, 4-16

system startup program with plate loader running, 4-19

Т

table troubleshooting, 10-9 target power settings (CytoFLEX LX only) laser, 6-40 target value Standardization, 5-29 temperature and humidity installation environment validation, A-4 test tubes acquisition screen, 2-7 the term press conventions, xli the term select conventions, xli threshold to adjust, 6-43 **TIME** parameter description, 3-7 transporting the instrument, A-1 troubleshooting, 10-1 alarm does not sound when the waste container is full or the sheath fluid container is low and the software status display is red, 10-10 calculation of the automatic compensation experiment is incorrect, 10-16 concentration calculation is incorrect, 10-17 connection indicator light in the lower left corner of the software screen is red and displays Disconnected and Error, 10-10 Cytometer cannot be turned on, 10-9 data populations are normal on one laser, but too low on another laser, 10-14 data populations are not where they are expected, 10-15 fluid status information displays red for Sheath and/or Waste even though the sheath fluid container is full and the waste container is empty, 10-11 instrument operations cannot be performed in the Acquisition screen, 10-18 laser delay values are out of range, 10-13 laser power is low, 10-12 mixer is not functioning, 10-18 no changes occurred after manually

adjusting compensation settings, 10-15 no data acquisition, 10-14 population amplitude is decreasing and CV values are increasing, 10-13 populations are drifting, 10-12 QC aborted due to low event rate, 10-19 OC failed, 10-20 sample flow rate is unstable, 10-11 sample is flowing, but no signal appears in the plot, 10-16 sample probe is too low, 10-17 sample tube holder cannot move up and down automatically, 10-11 sampling flow rate is too fast, 10-12 software installation fails, 10-18 table, 10-9 wash station drips during backflush, 10-17 Workstation cannot be turned on, 10-9 tube settings, 2-46 tube name to change, 6-38 turn off auto recalculate, 6-30 turn on auto recalculate, 6-30 turning the power on, 4-6

U

unlock user account, 2-27 unpacking inspection, A-4 check instrument configuration, A-4 installing the instrument and connecting the equipment, A-5, A-11 unpacking the instrument, A-4 unstained samples to define the negative population, 7-5 upgrade CytExpert software, A-24 USB define, Abbreviations-2 use your manual how to, xxxix user account unlock, 2-27 user management, 2-23 electronic record management, B-26 user management operation

log, 2-33 user management operation log electronic record management, B-16 user roles creating, 2-29 deleting, 2-30 modifying, 2-30 users creating, 2-25 deleting, 2-26 modifying, 2-26 using unstained samples, 7-5 UV define, Abbreviations-2

V

V define, Abbreviations-2 VA define, Abbreviations-2 VAC define, Abbreviations-2 ventilation and cleaning installation environment validation, A-2 verifying detector configuration, 6-48 vertex add to auto polygon gate, 6-30 vertical gates for single-parameter plots, 2-35 viewing user logs, 2-33 violet side scatter channel (VSSC) to set up, 6-52 VSSC define, Abbreviations-2

W

W define, Abbreviations-2 warning define, xvii wash station drips during backflush, 10-17 waste container, 1-17 waste container 4 L (CytoFLEX) emptying, 12-9 waste container/cubitainer, 1-18 waste cubitainer 10 L (CytoFLEX LX)

emptying, 12-10 waste disposal, A-4 waste fluid harness and sensor, 1-17, 1-18 waste harness to replace, 12-74 waste level sensor connector fluidic connection, 1-20, 4-3, 4-5 waste levels (10 L fluid cubitainers) check, 4-4 waste levels (4 L fluid containers) check. 4-2 waste out fluidic connection, 1-20, 4-3, 4-5 wavelength laser, 1-38 wavelength division multiplexer (WDM), 1-5 WDM define, Abbreviations-2 WDM beam splitter exchange detector configuration, F-4 well settings with plate loader modifying, 6-14 copying wells, 6-15 cutting wells, 6-15 modifying well settings copying wells, 6-15 cutting wells, 6-15 pasting wells, 6-15 pasting wells, 6-15 running samples, 6-58 sample, 6-58 sample injection mode selecting, 4-12 selecting proper sample injection mode, 4-12 Workstation, 1-2, 1-3, A-6 component, 1-2 Workstation cannot be turned on, 10-9 workstation connections inspection pre-boot, 4-5 worktable installation environment validation, A-2

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Related Documents

Instructions for Use

PN B49006

- Introduction
- System Overview
- Using the CytExpert Software
- Operation Principles
- Daily Startup
- Instrument Quality Control and Standardization
- Data Acquisition and Sample Analysis
- Compensation
- Data Review
- Daily Shutdown
- Troubleshooting
- Cleaning Procedures
- Replacement/Adjustment Procedures
- Instrument Installation
- CytExpert Electronic Record Management
- Sample Injection Mode Control Kit
- Deep Well Plate
- Custom Optical Filters
- WDM Beam Splitter
- Good Practices for Cyber Security
- Table of Hazardous Substances
- Abbreviations

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