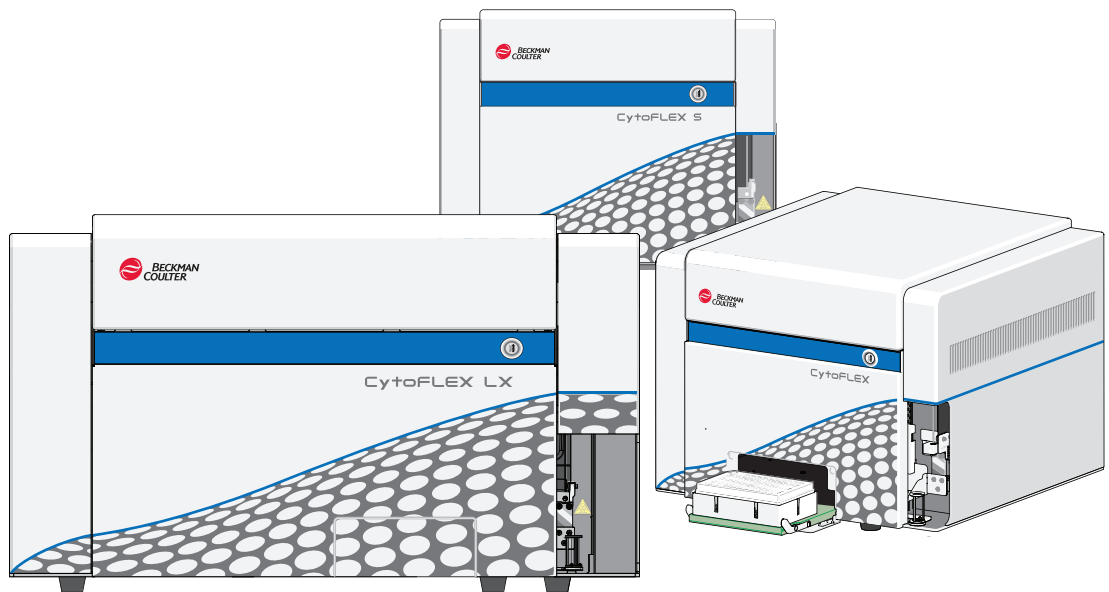


Instructions for Use

CytoFLEX Series

CytoFLEX, CytoFLEX S, and CytoFLEX LX Flow Cytometers

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



B49006AH
March 2017



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



CytoFLEX Series
Flow Cytometer
PN B49006AH (March2017)

© 2017 Beckman Coulter, Inc.
All rights reserved.

Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

Windows 7, Windows 8, and Windows 10 are registered trademarks or trademarks of Microsoft Corporation in the United States and/or other countries.

All other trademarks, service marks, products, or services are trademarks or registered trademarks of their respective holders.

Contact Us

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

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



Beckman Coulter Eurocenter S.A.
22, rue Juste-Olivier
Case Postale 1044
CH - 1260 Nyon 1, Switzerland
Tel: +41 (0) 22 365 36 11

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]

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

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

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

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

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

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](#)

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

CHAPTER 1, System Overview

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

CHAPTER 3, Daily Startup

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

CHAPTER 11, Replacement/Adjustment Procedures

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

APPENDIX A, Instrument Installation

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

Added the following Chapter:

APPENDIX C, Sample Injection Mode Control Kit

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

Safety Notices

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

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

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

Alerts for Danger, Warning, and Caution



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



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.

CAUTION

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

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

CAUTION









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

 **WARNING**

California Proposition 65:

This product may contain chemicals known to the State of California to cause cancer and birth defects or other reproductive harm.

Symbol Explanations

Symbol meanings	
	Caution / Warning
	Electrical shock hazard. Operate with caution!
	Possible laser radiation inside.
	Biohazard
	Potential pinch point. ^a
	Potential puncture hazard. ^a
	CE = Conformité Européenne A “CE” mark indicates that a product has been assessed before being placed on the market, and has been found to meet European Union safety, health, and/or environmental protection requirements. It is a mandatory marking for various product categories that are placed into service within the European Economic area.
	Consider all materials (specimens, reagents, controls, and monoclonal antibodies) and areas these materials come into contact with as being potentially infectious. Wear appropriate barrier protection and follow safe laboratory procedures when handling any material in the laboratory.

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

Contents

Revision History, iii

Safety Notices, ix

Alerts for Danger, Warning, and Caution, ix

Safety Precautions, x

Symbol Explanations, xi

Introduction, xxvii

Overview, xxvii

How to Use Your Manual, xxvii

About this Manual, xxvii

Conventions Used, xxix

Graphics, xxix

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, 1-9

Fluidics System, 1-10

Fluid Containers/Cubitainers, 1-11

Fluidics Module, 1-12

Sample Station, 1-14

Sample Tube Holder Positions, 1-15

Plate Loader Components, 1-16

Plate Holder Components, 1-18

System Configuration, 1-20

System Configuration [CytoFLEX], 1-20

System Configuration [CytoFLEX LX], 1-22

Consumables and Supplies, 1-24

Reagents, 1-24

Material Safety Data Sheets (SDS/MSDS), 1-24

Ordering Information, 1-24

- Instrument Specifications, 1-25
 - Dimensions [CytoFLEX], 1-25
 - Dimensions [CytoFLEX LX], 1-25
 - Installation Category, 1-25
 - Pollution Degree, 1-25
 - Acoustic Noise Level, 1-25
 - Electrical Ratings, 1-25
 - Cytometer, 1-26
- Performance Characteristics, 1-28
 - Performance Characteristics [CytoFLEX], 1-28
 - Performance Characteristics [CytoFLEX LX], 1-29
- Reagent Limitations, 1-29

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, 2-10
 - 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, 2-22
 - Creating, Deleting, and Modifying Users in User Manager, 2-24
 - Creating a New User in User Manager, 2-24
 - Deleting Users in User Manager, 2-25
 - Modifying Users in User Manager, 2-25
 - Unlocking a User Account, 2-26
 - Resetting a User Passwords, 2-26
 - Changing a User Password, 2-26
- Role Management, 2-26
 - Creating, Deleting, and Modifying User Roles in Role Manager, 2-28
 - Creating New User Roles in Role Manager, 2-28
 - Deleting User Roles in Role Manager, 2-29
 - Modifying User Roles in the Role Window, 2-29

- Account Policies, 2-30
- User Management Operation Log, 2-31
 - Viewing and Exporting User Logs, 2-31
- Graphic and Gating Styles, 2-32
 - Plots, 2-32
 - Gates, 2-33
- Software Settings, 2-34
 - Language Settings, 2-37
 - Setting Up CytExpert Application Programming Interface (API) Test Client, 2-37

CHAPTER 3: Daily Startup, 3-1

- Overview, 3-1
- Pre-Startup Inspection, 3-1
 - Check Waste and Reagent Levels [4 L Fluid Containers], 3-2
 - Check Waste and Reagent Levels [10 L Fluid Cubitainers], 3-4
 - Power Source Inspection, 3-5
 - Workstation Connections Inspection, 3-5
- Turning On the Instrument, 3-6
- Logging Into the Software, 3-6
 - Logging Out of the Software, 3-9
 - Selecting the Proper Sample Injection Mode, 3-9
 - Using Semi-Automatic Injection Mode, 3-10
 - Using the Manual Injection Mode, 3-11
 - Selecting the Plate Loader Sample Injection Mode [With Plate Loader], 3-12
 - Using Plate Loader Injection Mode, 3-12
 - Running the System Startup Program with the Single Tube Loader, 3-14
 - Running the System Startup Program [With Plate Loader], 3-17
 - Selecting Experiments from the Start Page, 3-24
- Initializing the Instrument, 3-25

CHAPTER 4: Instrument Quality Control and Standardization, 4-1

- Overview, 4-1
- Preparing the QC Sample, 4-3
 - Required Materials, 4-3
 - Preparation Process CytoFLEX Daily QC Fluorospheres, 4-3
 - Preparation Process CytoFLEX IR Daily QC Fluorospheres, 4-3
- Preparing the QC Sample [With Plate Loader], 4-4
 - Required Materials, 4-4
 - Preparation Process CytoFLEX Daily QC Fluorospheres, 4-4
 - Preparation Process CytoFLEX IR Daily QC Fluorospheres, 4-5
- Importing Lot-Specific Target Values, 4-6

- Collecting QC Data, 4-10
- Collecting QC Data [With Plate Loader], 4-12
- Confirming Results, 4-16
 - Creating Levey-Jennings Charts, 4-19
 - QC Result Manager, 4-21
- Standardization, 4-22
 - Adding, Editing, Importing, and Deleting Standardization Laser Target Values in the QC Experiment, 4-23
 - Adding a New Standardization Item, 4-24
 - Editing Standardization Item Parameters, 4-25
 - Importing a Standardization Item, 4-26
 - Deleting Standardization Items, 4-26
 - Applying the Standardization in QC, 4-26

CHAPTER 5: Data Acquisition and Sample Analysis, 5-1

- Overview, 5-1
- Creating an Experiment, 5-1
- Creating an Experiment [With Plate Loader], 5-3
 - Setting Sample Wells, 5-7
 - Modifying Well Settings, 5-11
 - Applying Existing Well Settings to Additional Wells, 5-12
 - Copying, Cutting, and Pasting Wells, 5-12
 - Moving the Location of a Well, 5-13
 - Running Samples, 5-13
 - Creating a Heat Map, 5-15
 - Refreshing a Heat Map, 5-21
 - Modifying Existing Heat Map Settings, 5-22
 - Deleting an Existing Heat Map, 5-23
 - Exporting a Heat Map, 5-23
- Load Sample and Record Data, 5-24
 - Before Running Samples, 5-24
 - Verifying, Selecting, Editing, and Creating Detector Configuration, 5-25
 - Setting Up Violet Side Scatter (VSSC) Channel, 5-29
 - Sampling and Collecting Data, 5-32
- Configuring Acquisition Settings, 5-36
 - Changing the Tube Name, 5-36
 - Setting the Channel and Label, 5-36
 - Creating Plots and Gates, 5-39
 - Creating and Adjusting Auto Gates, 5-48
 - Turning Auto Recalculate On/Off, 5-50
 - Adjusting Autogate Movement and Extent, 5-51
 - Laser Settings, 5-53
 - Setting Laser Target Power Settings [CytoFLEX LX Only], 5-54
 - Adjusting the Gain, 5-55
 - Adjusting the Threshold, 5-57

- Setting Collection Conditions, 5-58
- Setting Plot Display Conditions, 5-60
- Setting Customized Parameters, 5-61
- Setting Custom Statistics, 5-62
- Analyzing and Exporting Data, 5-64
 - Exporting FCS Files, 5-70
 - Exporting Single Tube Files, 5-70
 - Exporting Multiple FCS Files, 5-72
 - Exporting Plots or the Statistics Table of Multiple Tubes as Picture Files, 5-73
 - Importing and Exporting Instrument Settings, 5-73
 - Importing Instrument Settings, 5-74
 - Exporting Instrument Settings, 5-74
 - Importing and Exporting Compensation Settings, 5-75
 - Printing Graphics, 5-75
- Saving the Experiment, 5-78
 - Concluding the Experiment, 5-78
- CHAPTER 6: Compensation, 6-1**
 - Overview, 6-1
 - Creating a Compensation Experiment, 6-2
 - Preparing the Compensation Sample, 6-4
 - Using Control Samples to Generate the Compensation Matrix, 6-4
 - Defining the Negative Population Using Unstained Samples, 6-4
 - Running the Single Positive Control Samples, 6-6
 - Calculating Compensation Values, 6-8
 - Creating a Compensation Experiment [With Plate Loader], 6-10
 - Preparing the Compensation Sample, 6-13
 - Using Control Samples to Generate the Compensation Matrix, 6-13
 - Calculating Compensation Values, 6-13
 - Creating the Compensation Matrix from Previously Acquired Data, 6-14
 - Adjusting Compensation, 6-16
 - Manually Adjusting Compensation, 6-16
 - Importing and Exporting Compensation, 6-16
 - Importing Compensation Settings from Compensation Matrix Files, 6-16
 - Importing Compensation Settings from the Compensation Library, 6-17
 - Exporting Compensation Settings, 6-19
 - Managing the Compensation Library, 6-20
 - Adding Channels for Compensation, 6-21
- CHAPTER 7: Data Review, 7-1**
 - Overview, 7-1
 - Copying Experiments and Importing Data, 7-1

- Cleaning the Sample Station, 10-7
 - Cleaning the Sample Probe, 10-8
- Deep Clean Procedure, 10-8
- Cleaning the 4 L Sheath Fluid Container, 10-10
- Cleaning the 4 L Waste Container, 10-11

- Nonscheduled Cleaning, 10-13
 - Surface Cleaning and Disinfection, 10-13
 - Preparing the Instrument for Transport or Storage, 10-14
 - Lifting and Carrying Instructions, 10-18

CHAPTER 11: Replacement/Adjustment Procedures, 11-1

- Overview, 11-1

- Routine Replacement/Adjustment, 11-2
 - Front Cover Removal and Reinstallation, 11-2
 - Removal, 11-2
 - Reinstallation, 11-3
 - Right-Side Cover Removal and Reinstallation, 11-4
 - Removal, 11-4
 - Reinstallation, 11-5
 - Filling the 4 L Sheath Fluid Container [CytoFLEX], 11-6
 - Replacing the 10 L Sheath Fluid Cubitainer, 11-7
 - Emptying the 4 L Waste Container [CytoFLEX], 11-9
 - Emptying the 10 L Waste Cubitainer, 11-10
 - Managing the Maintenance Reminder, 11-14
 - Adding the Deep Clean Solution, 11-17
 - Replacing the Sheath Fluid Filter, 11-18
 - Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing, 11-22
 - Replacing the Sample Probe Assembly [With Plate Loader], 11-28
 - Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader [CytoFLEX With Plate Loader], 11-34
 - Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station [CytoFLEX With Plate Loader], 11-39
 - Inspecting the Liquid Flow Path for Leaks, 11-42
 - Priming the Flow Cell, 11-43
 - Replacing the Plate Holder [With Plate Loader], 11-45
 - Plate Loader Module Removal and Reinstallation [With Plate Loader], 11-46
 - Removal, 11-46
 - Installation, 11-50
 - Changing the Event Rate Setting, 11-55
- Nonscheduled Replacement/Adjustment, 11-56
 - Calibrating the Sample Flow Rate, 11-56
 - Calibrating the Sample Flow Rate [With Plate Loader], 11-59
 - Setting Laser Delay, 11-63
 - Replacing the Optical Filter, 11-64
 - Replacing the Fuse, 11-67
 - Replacing the Sheath Fluid Harness and/or Waste Harness, 11-69

Changing Sample Mixing and Backflush Settings, 11-71
Calibrating the Plate Position [With Plate Loader], 11-73

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], A-12
 Required Materials, A-13
 Installing the CytExpert Software, A-13
 Installing the Instrument Configuration File, A-21
 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
 Setting Up the Experiment Directory, B-3
 Folder Hierarchy Management, B-4
 Experiment Related Operations, B-5
 Importing an Experiment/Template, B-6
 Exporting an Experiment/Template, B-8
Log, B-11
 Experiment Operation Log, B-11
 System Operation Log, B-14
 User Management Operation Log, B-15
Electronic Signature, B-16

Signing an Experiment, B-16
Retracting an Experiment Signature, B-17

User Management, B-18
 User Administration, B-18
 Logging In and Out of the Software, B-18
 Role Management, B-18
 User Management, B-18
 Account Policies, B-18

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-2

Switching the Sample Probe from the Plate Loader to the Single Tube Sample
 Station [With Sample Injection Mode Control Knob] , C-4

APPENDIX D: Table of Hazardous Substances, D-1

Table of Hazardous Substances, D-1

Abbreviations

Index

Beckman Coulter, Inc.
Customer 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], 1-10
- 1.8 Fluid Cubitainers [CytoFLEX LX], 1-11
- 1.9 Fluidics Module View, 1-12
- 1.10 Fluidic Connections, 1-13
- 1.11 Sample Station, 1-14
- 1.12 Sample Tube Holder Positions, 1-15
- 1.13 Plate Loader [CytoFLEX Shown], 1-16
- 1.14 Plate Loader (Front Cover Removed), 1-17
- 1.15 Plate Holder, 1-18
- 1.16 Plate Holder Stage, 1-19
- 1.17 System Connections, 1-20
- 1.18 Back Cover Connections, 1-21
- 1.19 Front of Cytometer [CytoFLEX Without Plate Loader Shown], 1-21
- 1.20 System Connections, 1-22
- 1.21 Back Cover Connections, 1-23
- 1.22 Front of Cytometer, 1-23
- 2.1 Drawing Controls Toolbar (Top of Screen), 2-10
- 2.2 QC Report Screen [CytoFLEX LX Semi-Automatic Sample Injection Mode Shown], 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), 2-23
- 2.7 Role Manager, 2-27
- 2.8 Account Policies, 2-30
- 5.1 Plate Window [CytoFLEX LX Shown], 5-7

- 5.2 All Gates - Example Experiment, 5-46
- 5.3 Circular Gating Logic - Example Experiment, 5-46
- 5.4 Movement - Default Setting, 5-51
- 5.5 Movement - Max Setting, 5-52
- 5.6 Extent - Default Setting, 5-52
- 5.7 Extent - Maximum Setting, 5-53
- 5.8 Laser Setting Window [CytoFLEX LX shown], 5-53
- 6.1 Before Compensation, 6-1
- 6.2 After Compensation, 6-1
- 6.3 Positive Population Selected from the Unstained Sample, 6-7
- 6.4 Positive and Negative Populations Without an Unstained Sample, 6-8
- 9.1 Laser Warning Label on the Laser Optical Bench [CytoFLEX], 9-3
- 9.2 Laser Warning Label on the Laser Optical Bench [CytoFLEX LX], 9-3
- 9.3 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX], 9-4
- 9.4 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX LX], 9-4
- 9.5 Laser Warning Labels on the Cytometer Back Cover [CytoFLEX], 9-5
- 9.6 Laser Warning Labels on the Cytometer Back Cover [CytoFLEX LX], 9-5
- 9.7 Biohazard Label on the 4 L Fluid Containers, 9-6
- 9.8 Biohazard Label on the 10 L Fluid Cubitainers, 9-6
- 9.9 Biohazard Label Located in the Sample Station and on the Back of the Cytometer [CytoFLEX Shown], 9-7
- 9.10 Electrical Shock Hazard Label by the Power Switch [CytoFLEX Shown], 9-7
- 9.11 Caution Labels [CytoFLEX Shown], 9-7
- 9.12 Plate Loader Hazard Labels, 9-8
- 11.1 Plate Holder Stage, 11-45
- 11.2 Removing the Tubings from the Fluidics Module to the Plate Loader, 11-49
- 11.3 Plate Loader Module Securing Screws, 11-50
- 11.4 Removing the Plate Loader from the Cytometer, 11-50
- 11.5 Installing the Plate Loader in to the Cytometer, 11-51
- 11.6 Plate Loader Module Securing Screws, 11-51
- 11.7 Connecting the Tubings from the Fluidics Module to the Plate Loader, 11-52

B.1	Experiment Operation Log Window, B-12
B.2	Select Experiment Profile Window, B-13
B.3	Print and Export Preview Window, B-14
B.4	System Operation Log Window, B-15
B.5	User Management Operation Log Window, B-16
C.1	Sample Injection Mode Control Knob, C-2

Tables

1.1	WDM Optical Filter Mount Color Codes [CytoFLEX], 1-7
1.2	WDM Optical Filter Mount Color Codes [CytoFLEX LX], 1-7
9.1	Troubleshooting, 9-10
9.2	Troubleshooting [With Plate Loader], 9-22
D.1	Table of Hazardous Substances Name and Concentration [CytoFLEX], D-2
D.2	Table of Hazardous Substances Name and Concentration [CytoFLEX LX], D-3

Overview

This introduction contains the following information:

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

How to Use Your Manual

Your CytoFLEX flow cytometer system includes the manuals listed below:

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

About this Manual

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

CHAPTER 1, System Overview

Provides information regarding the individual components of the CytoFLEX flow cytometer and the corresponding functions of these components.

CHAPTER 2, Using the CytExpert Software

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

CHAPTER 3, Daily Startup

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

CHAPTER 4, Instrument Quality Control and Standardization

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

CHAPTER 5, Data Acquisition and Sample Analysis

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

CHAPTER 6, Compensation

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

CHAPTER 7, Data Review

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

CHAPTER 8, Daily Shutdown

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

CHAPTER 9, Troubleshooting

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

CHAPTER 10, Cleaning Procedures

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

CHAPTER 11, Replacement/Adjustment Procedures

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

APPENDIX A, Instrument Installation

Provides the instrument installation procedures for your CytoFLEX flow cytometer.

APPENDIX B, CytExpert Electronic Record Management

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

APPENDIX C, Sample Injection Mode Control Kit

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

APPENDIX D, Table of Hazardous Substances

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

Conventions Used

This document uses the following conventions:

- **Bold face** font indicates buttons or selections that appear on the workstation screen.
 - The term “select” is used to indicate the following action:
 - To click with a mouse.
- NOTE** The verb “press” is reserved for mechanical buttons, such as keys on the keyboard.
- The software path to a specific function or screen appears with the greater than (>) symbol between screen options.
 - Links to information in another part of the document for additional information are in blue and are underlined. To access the linked information, select the blue, underlined text.
 - The information in your Instructions for Use manual applies to the CytoFLEX and CytoFLEX S instruments equipped with and without a plate loader, and the CytoFLEX LX, unless otherwise specified.

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

- 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.

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 from a 1 laser, 4 colors, to a maximum of 3 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 three laser flow cytometer cannot be upgraded to a CytoFLEX S 4 laser flow cytometer.

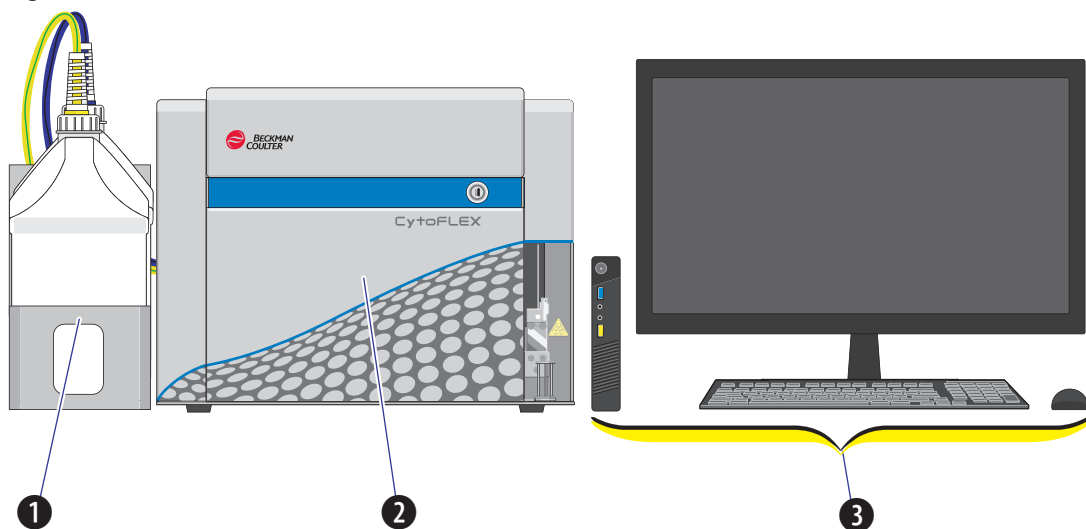
Main Components



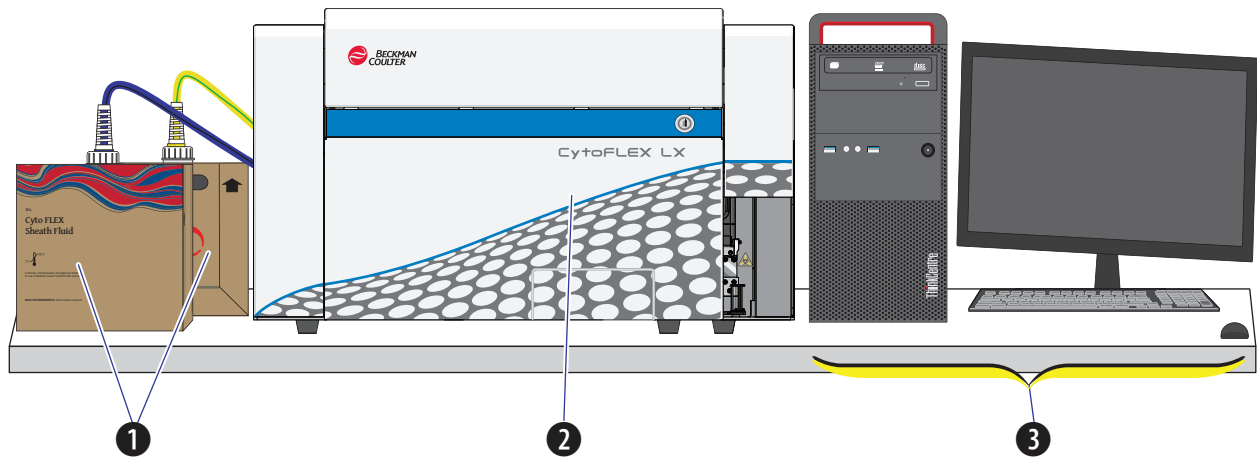
Risk of instrument damage and/or instrument stability. Do not place any objects on top of the instrument, as this could cause warping of the top cover or affect the stability of the optical path.

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 Containers.** Accommodates sheath fluid and waste liquids as required for operation of the instrument.

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

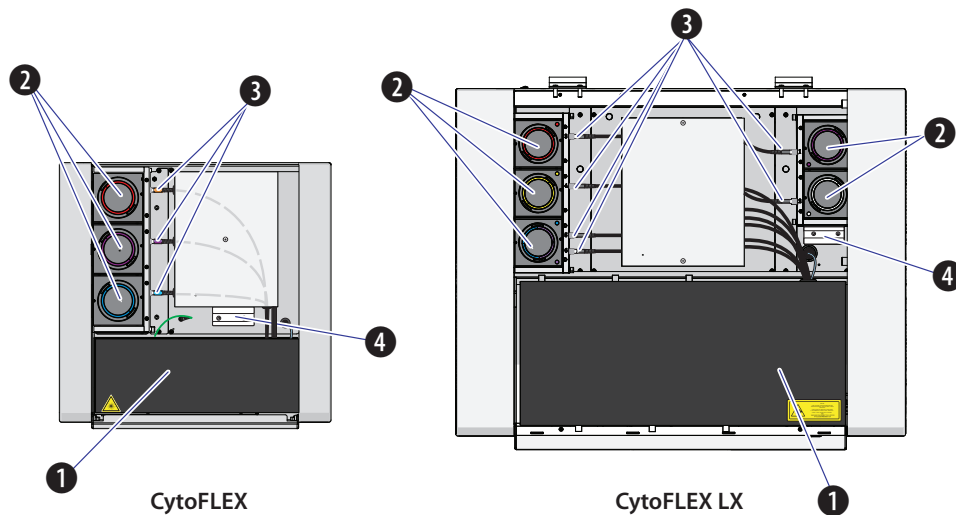
2. **Cytometer.** Provides signal generation and collection.
3. **Workstation.** Displays and manipulates the contents of the Workstation and displays data generated by the Cytometer.

Optical Components

CAUTION

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

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



- 1. Optical bench.** Includes laser light sources, an optical beam combiner, and an integrated optics flow cell assembly. The optical bench cover is equipped with a laser interlock that turns the lasers off unless the cover is tightly closed.
- 2. Wavelength division multiplexer (WDM).** Each WDM is a unique detector array that corresponds to a different laser, or in some cases two lasers. Each WDM contains optical filters and detectors for detecting channel fluorescence or scatter from a particular laser. It is necessary to ensure that the filter and software settings match for each channel.
- 3. Optical fiber.** Transmits emitted fluorescence to the specific WDM.

CAUTION

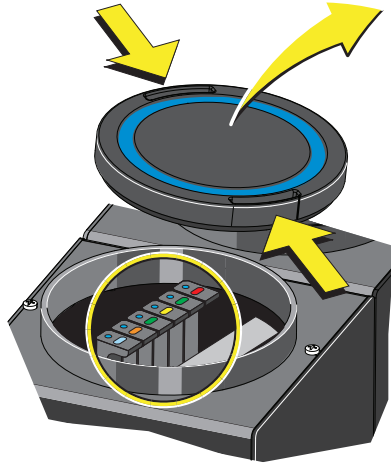
Risk of instrument damage. Do not place sample tubes in the optical filter holder. Liquid spills can damage instrument components. Use a tube rack to hold any sample tubes.

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

Wavelength Division Multiplexer (WDM)

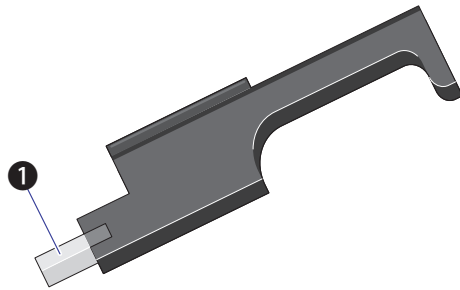
Each WDM corresponds to a different laser, or in some cases two lasers. The color of the ring on each cap corresponds to the color of the respective laser. Pressing the two release buttons on opposite edges of the cap allows you to open the unit and replace the filters inside. See [Figure 1.3](#). All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

Figure 1.3 Optical Filter Mounts



Each optical filter mount has an optical filter glass piece. See [Figure 1.4](#).

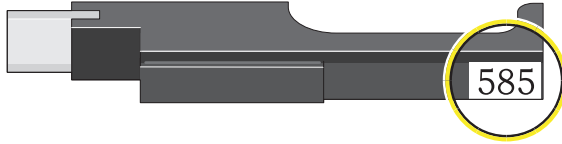
Figure 1.4 Optical Filter Mount with Optical Filter



1. Optical filter glass piece

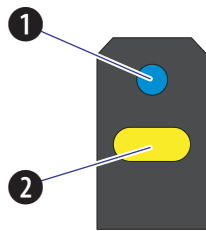
Each optical filter mount is labeled with the corresponding laser and band-pass information. See [Figure 1.5](#).



Figure 1.5 Optical Filter Mount Labeled with the Band-Pass Information



The top of each optical filter mount has two marks. The color of the dot (1) indicates the color of the laser. See [Figure 1.6](#). The color of the line (2) indicates the wavelength range of the optical band-pass filter. See [Figure 1.6](#).

Figure 1.6 Optical Filter Mount (Top)



1.  . Indicates corresponding laser color: Blue indicates a 488 nm laser; Red indicates a 638 nm laser; Violet indicates a 405 nm laser; Yellow indicates a 561 nm laser; Pink indicates a 808 nm laser; White indicates a 375 nm laser.
2.  . Indicates the band-pass wavelength ranges; the specific wavelength is indicated numerically on the lateral side of the mount.

Band-pass filters are used to transmit fluorescence at the specific wavelength ranges. These ranges are designed to measure fluorescence from the fluorochromes such as those listed in [Table 1.1](#). (with red and violet laser upgrades installed) that are excited by the lasers. You can change the optical filters according to your detector configuration. There is no need to realign the optical system when the filters are changed.

Table 1.1 WDM Optical Filter Mount Color Codes [CytoFLEX]

















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

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














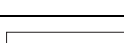













Lasers	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
 375 nm	 450/45 BP	NUV450	BUV395, DAPI
	 525/40 BP	NUV525	BUV496
	 675/30 BP	NUV675	Hoescht Red, BUV661

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

Lasers	Fluorescent Channel	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
 405 nm	 450/45 BP	V450-PB450	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
	 763/43BP	V763	BV785, Qdot® 800
 488 nm	 525/40 BP	B525-FITC	FITC, Alexa Fluor™ 488, CFSE, Fluo-3
	 690/50 BP	B690-PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI, DRAQ7™
	 610/20 BP	B610-ECD	ECD, PE-Texas Red®, PE-CF594, PI
 561 nm	 610/20 BP	Y610-MCherry	MCherry, ECD, PE-CF594
	 585/42BP	Y585-PE	PE, DsRed
	 675/30BP	Y675-PC5	PC5, mPlum
	 710/50BP	Y710-PC5.5	PC5.5, PE-AF680
	 763/43BP	Y763-PC7	PC7
 638 nm	 763/43BP	R763-APCA750	APC, Alexa Fluor™ 647, eFluor™ 660, Cy5
	 660/10 BP	R660-APC	APC-A700, Alexa Fluor™ 700, Cy5.5
	 712/25 BP	APC-A700	APC-A750, APC-Cy7, APC-H7, APC- eFluor™ 780, DRAQ7™
 808 nm	 840/20BP	IR840-A790	Alexa Fluor® 790
	 885/40BP	IR885	PromoFluor-840, IR fixable viability dye

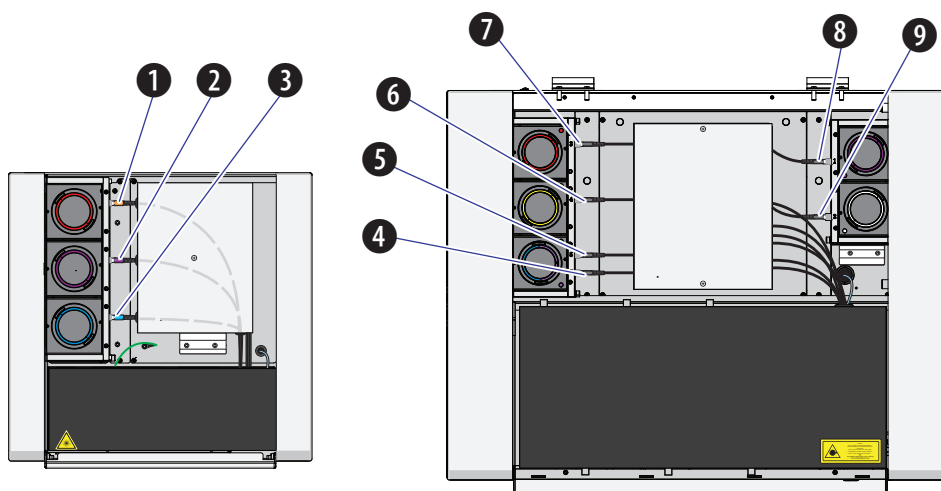
Optical Fiber

CAUTION

Risk of data integrity damage.

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

Fluorescence emitted by laser-excited fluorochromes is picked up and delivered by each optical fiber to the corresponding detector module. Each optical fiber has a colored ring on the end that connects to the WDM, indicating the color of the corresponding laser. Ensure that the correct fiber is properly connected to the corresponding WDM.



CytoFLEX

CytoFLEX LX

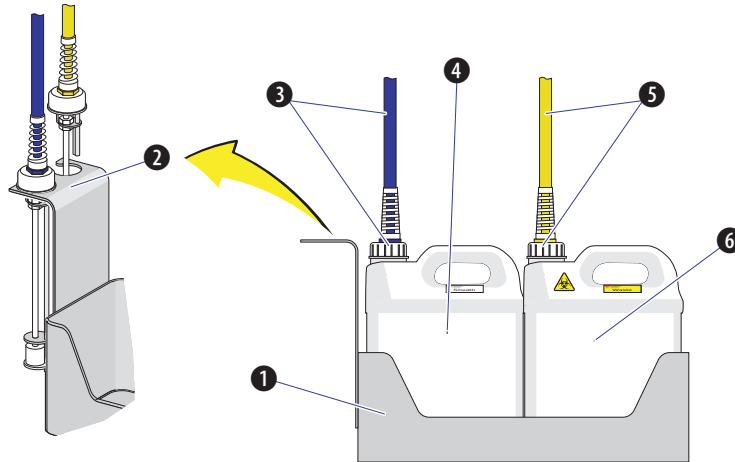
1. Red laser fiber
2. Violet laser fiber
3. Blue laser fiber
4. Infra Red laser fiber
5. Blue laser fiber
6. Yellow laser fiber
7. Red laser fiber
8. Violet laser fiber
9. NUV laser fiber

Fluidics System

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

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

Figure 1.7 Fluid Containers [CytoFLEX]

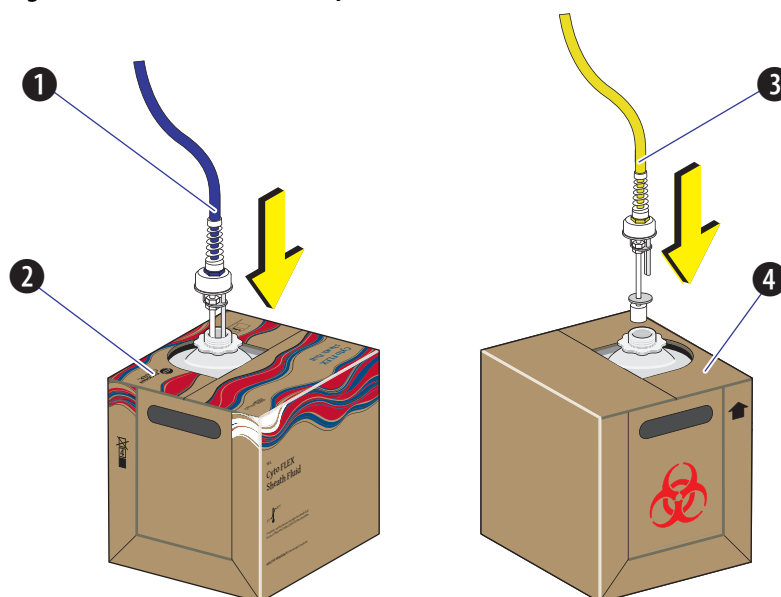


- 1. Fluid Container holder.** Holds the Fluid Containers.
- 2. Fluid sensor holder cutout.** Holds the sheath fluid harness and the waste harness when removed from their respective containers to protect the sensors from damage and/or contamination.
- 3. Sheath fluid harness.** Connects to the sheath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

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

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

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

Figure 1.8 Fluid Cubitainers [CytoFLEX LX]

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

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

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

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

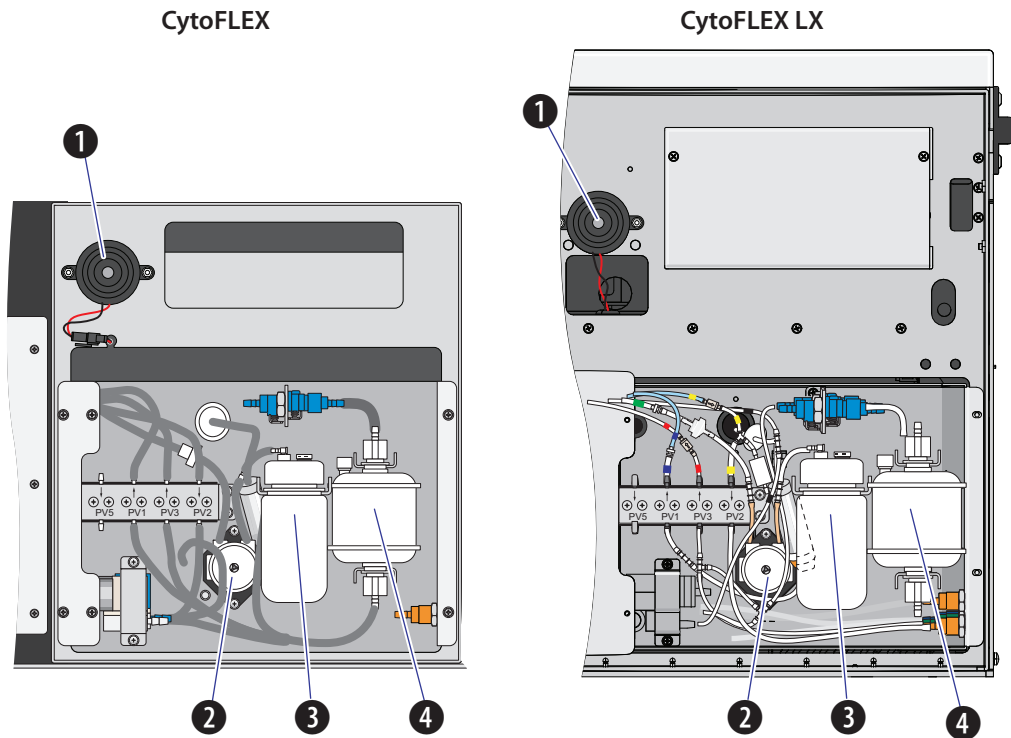
Fluid Containers/Cubitainers

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

Fluidics Module

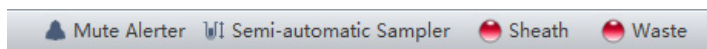
The Fluidics module is on the right side of the Cytometer. To access it, you must first open the right-side cover. Refer to [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#). Inside the module, in addition to working pumps, valves, and tubing, there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

Figure 1.9 Fluidics Module View

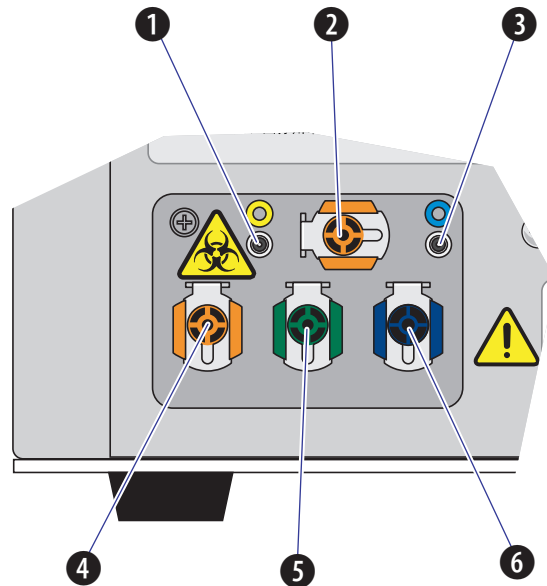


- 1. Alarm.** Emits a warning sound when there is a problem with the Fluid Container/Cubitainer capacity or with the performance of certain operations.

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



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

Figure 1.10 Fluidic Connections

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

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

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

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

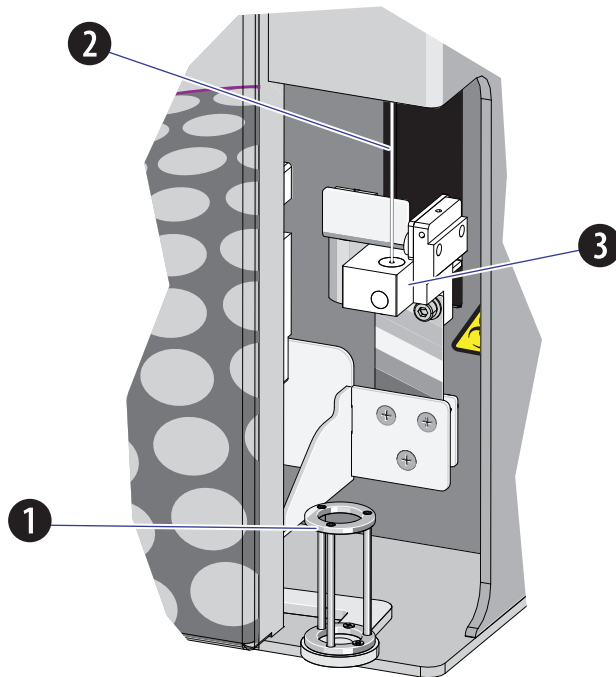
Sample Station



WARNING

Risk of biohazardous contamination and/or instrument damage. When running samples, it is important to insert the sample tube all the way down into the sample tube holder, until the bottom of the sample tube touches the base of the holder. Failing to do this could cause the sample probe to bend or break on entry. Sample tubes must not exceed 80 mm in height and the outside diameter must not exceed 13 mm.

Figure 1.11 Sample Station



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

Sample Tube Holder Positions

Three of the sample tube holder positions are shown in [Figure 1.12](#): sample loading position (1), standby position (2), and sample acquisition position (3). You can only distinguish the mixing position from the sample acquisition position if you are looking directly at the sample tube holder while the Cytometer is processing a sample. The mixing position is about 6 mm lower than the sample acquisition position.

Figure 1.12 Sample Tube Holder Positions

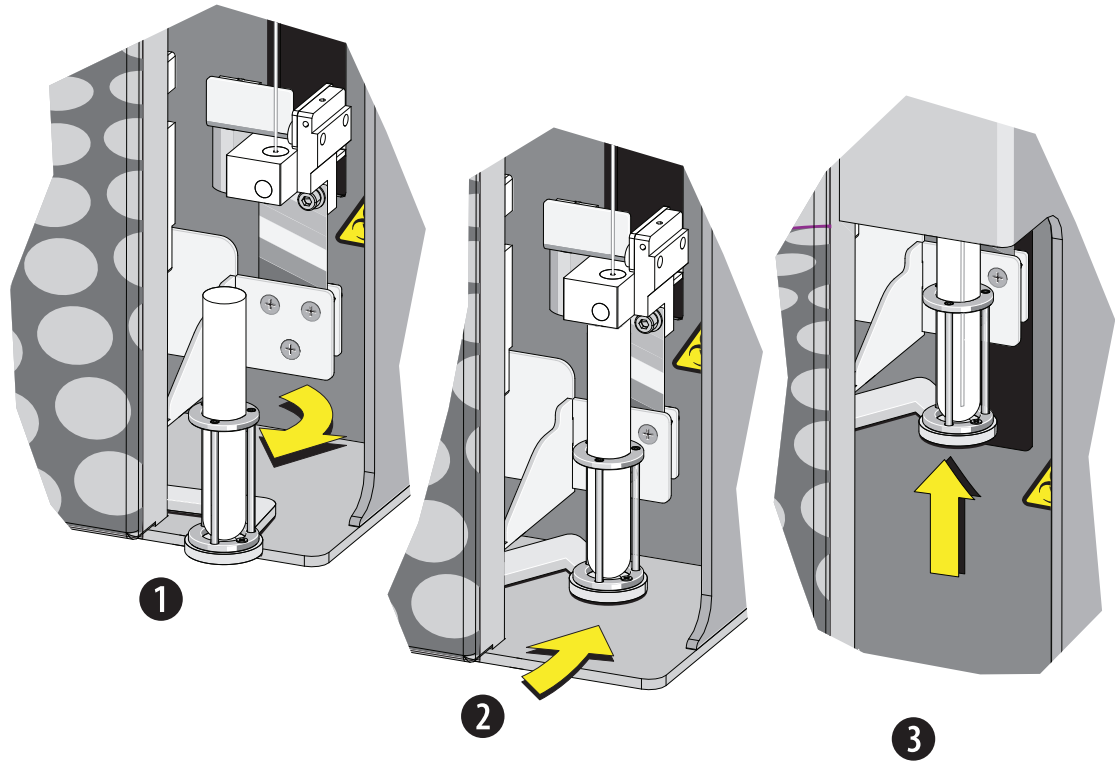
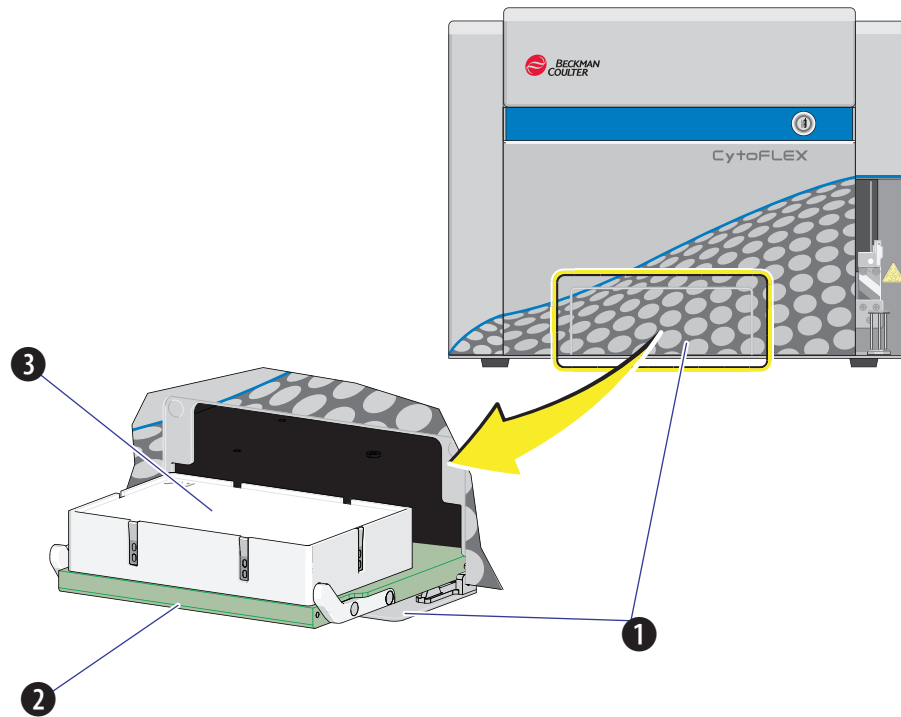


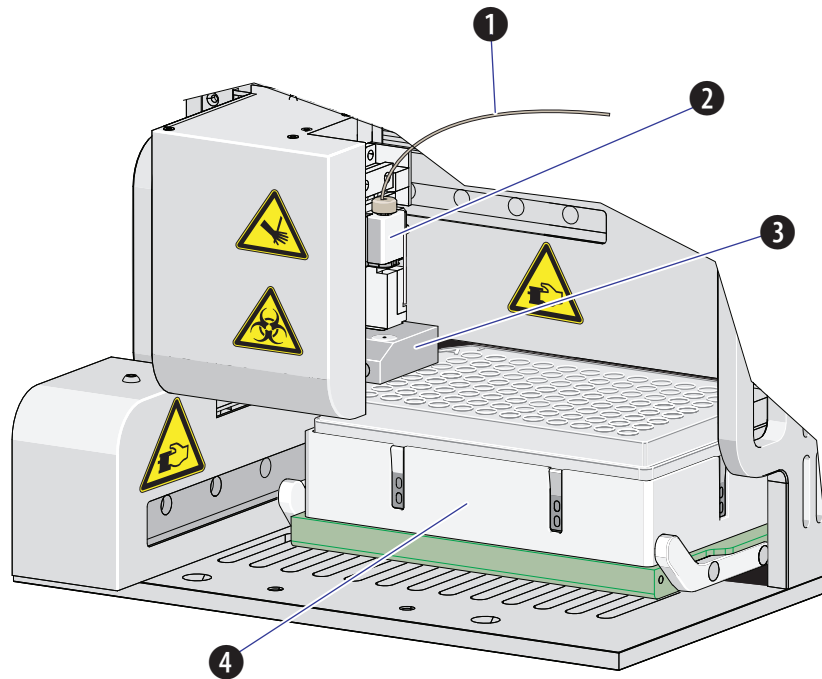
Plate Loader Components

Figure 1.13 Plate Loader [CytoFLEX Shown]



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

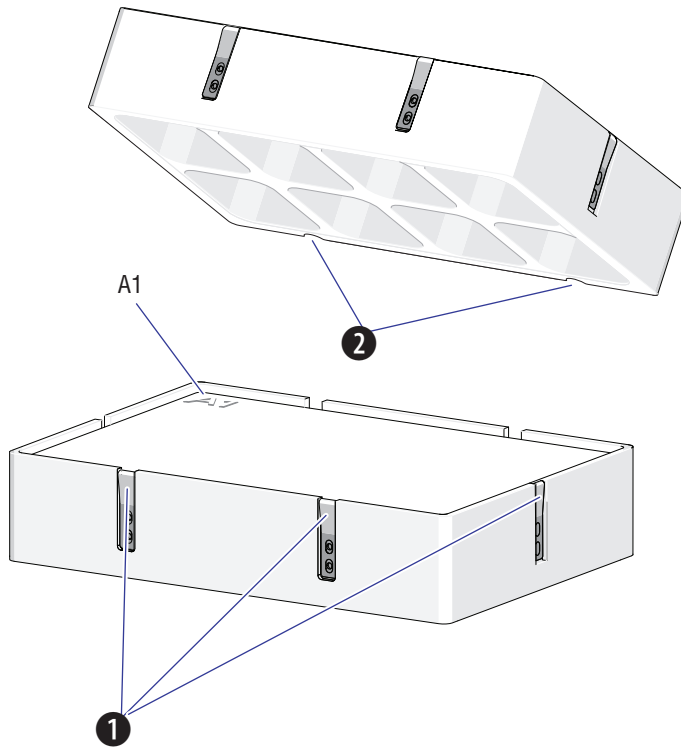
Figure 1.14 Plate Loader (Front Cover Removed)



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

Plate Holder Components

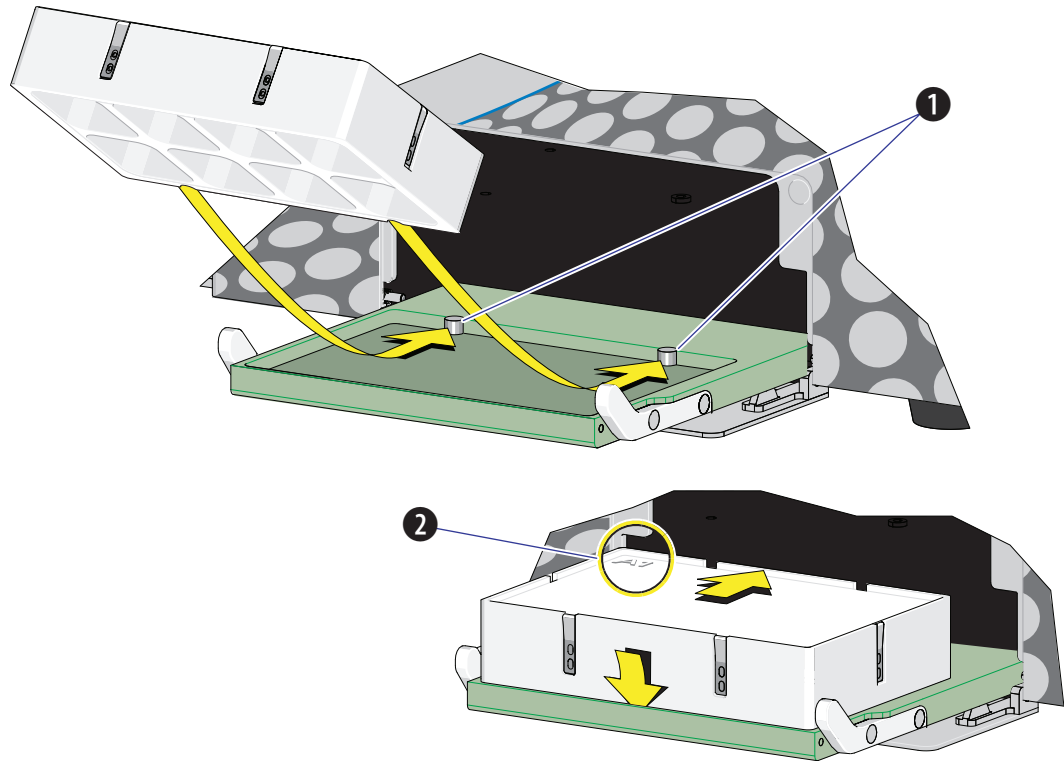
Figure 1.15 Plate Holder



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

NOTE The Plate holder is removable and replaceable. Refer to [Replacing the Plate Holder \[With Plate Loader\]](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

Figure 1.16 Plate Holder Stage



- 1. Pins
- 2. Position A1

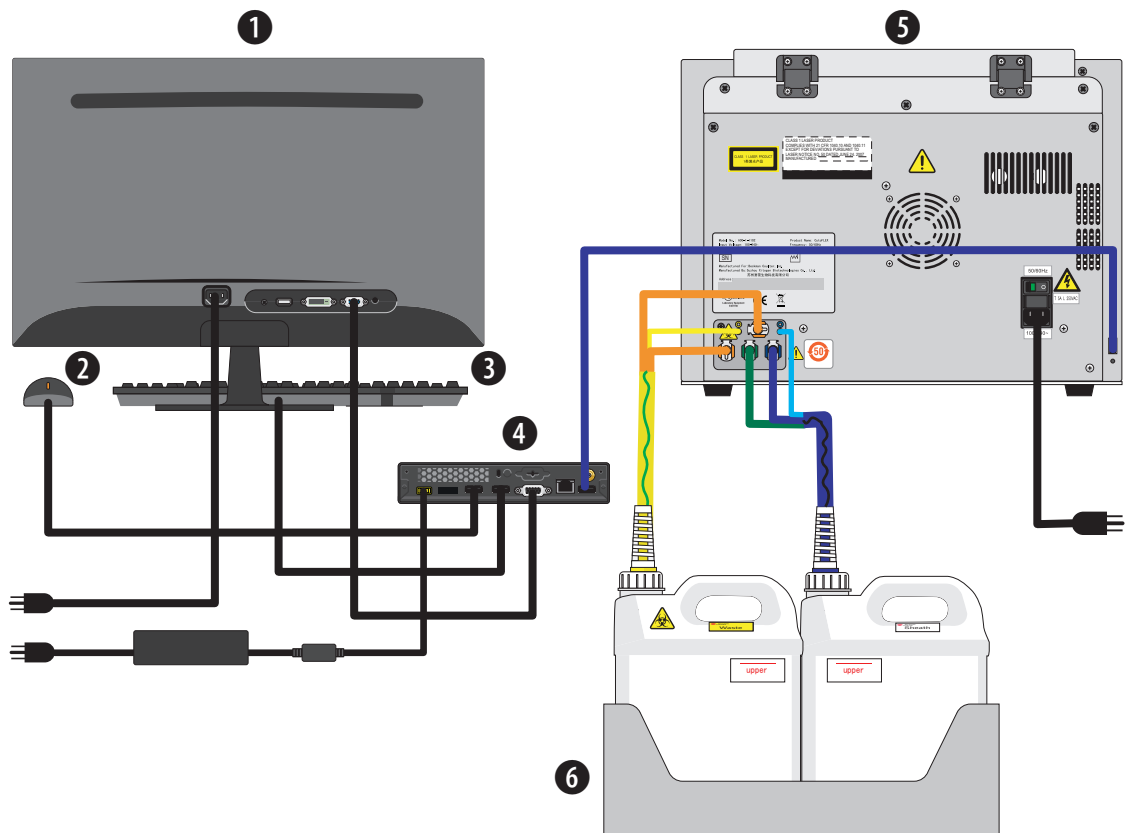
System Configuration



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.

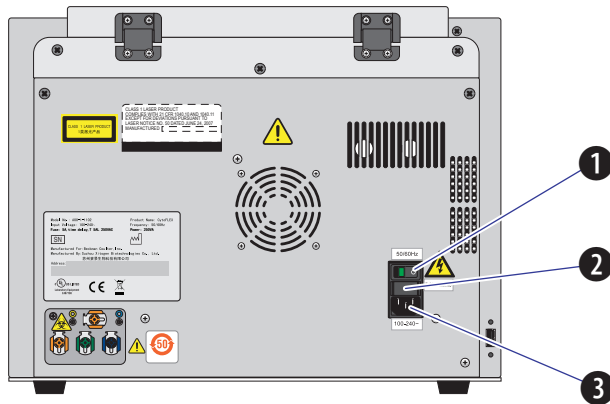
System Configuration [CytoFLEX]

Figure 1.17 System Connections



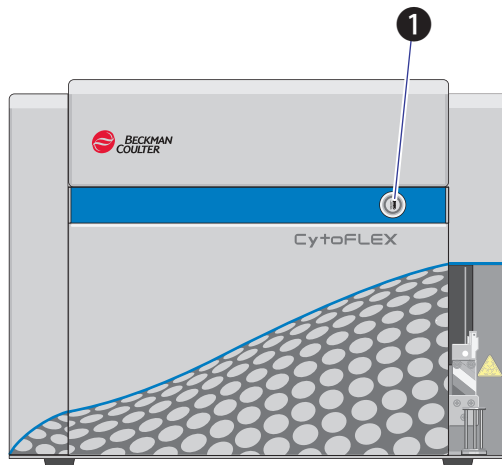
- | | |
|-------------|---------------------------|
| 1. Monitor | 4. Computer |
| 2. Mouse | 5. Cytometer |
| 3. Keyboard | 6. Fluid Container holder |

Figure 1.18 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 1.19 Front of Cytometer [CytoFLEX Without Plate Loader Shown]



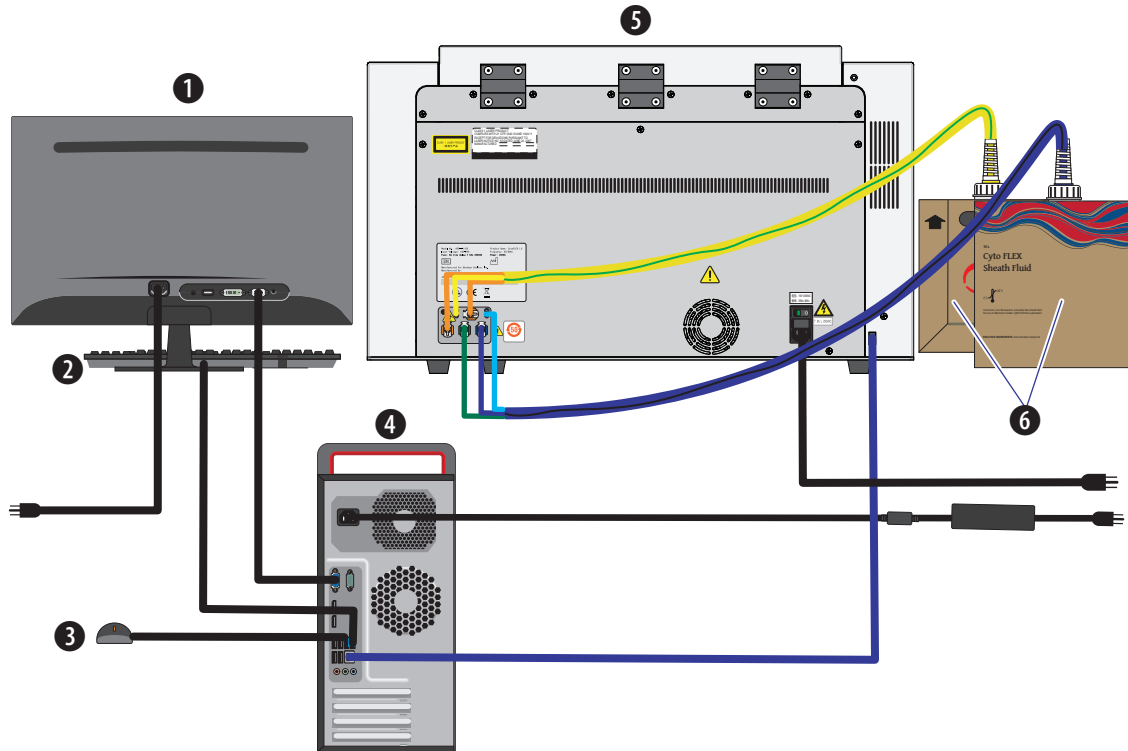
1. **Load button.** In addition to the software controls, this button can be used for automatic sample loading and data recording.

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

System Configuration [CytoFLEX LX]

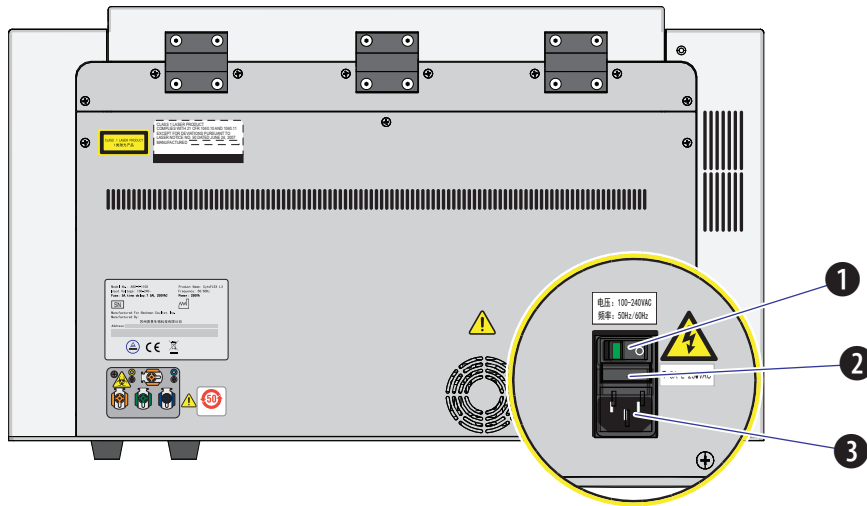
IMPORTANT The fluid cubitainers must be on the same level as the Cytometer. Do not place the fluid cubitainers on the floor.

Figure 1.20 System Connections



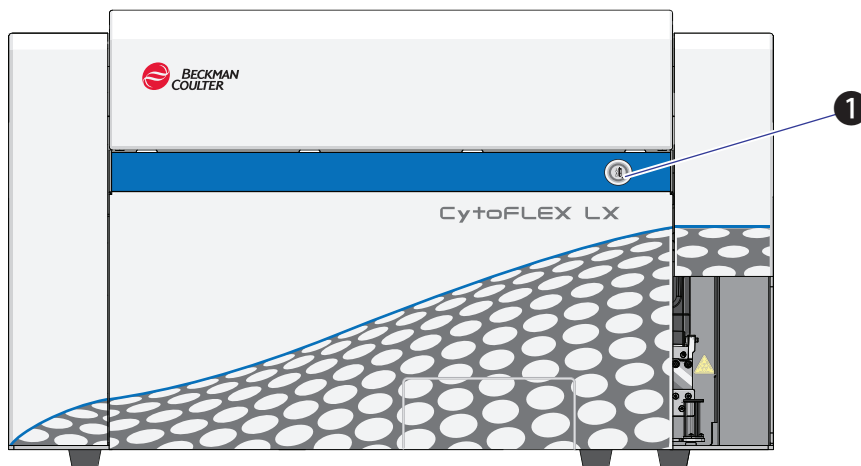
- | | |
|-------------|----------------------|
| 1. Monitor | 4. Computer |
| 2. Keyboard | 5. Cytometer |
| 3. Mouse | 6. Fluid Cubitainers |

Figure 1.21 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 1.22 Front of Cytometer



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

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

Consumables and Supplies

Reagents

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

CytoFLEX Daily QC Fluorospheres

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

CytoFLEX Sheath Fluid

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

Contrad® 70 Reagent

Diluted 1:1 with DI water for use in the Deep Clean solution bottle.

FlowClean

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

The following reagents are available for the IR laser QC:

CytoFLEX IR Daily QC Fluorospheres

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

Material Safety Data Sheets (SDS/MSDS)

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

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

Ordering Information

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

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

Otherwise, [contact us](#).

Instrument Specifications

Dimensions [CytoFLEX]

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

Dimensions [CytoFLEX LX]

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

Installation Category

Installation Category 2

Pollution Degree

Pollution Degree 2

Acoustic Noise Level

Measure Level: <65 dBA

Electrical Ratings

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

Cytometer

Optics	
Excitation Optics	<p>The CytoFLEX system can be configured with up to three spatially-separated lasers. The optical system is alignment free. The laser delays are automatically adjusted by the daily QC system, if required. No user intervention is required to ensure optimum system performance.</p> <p>The CytoFLEX LX system can be configured with up to six spatially-separated lasers. The optical system is alignment free. The laser delays are automatically adjusted by the daily QC system, if required. No user intervention is required to ensure optimum system performance.</p>
Emission Optics	<p>Patent-pending alignment-free integrated optics quartz flow cell design with >1.3 NA.</p> <p>Flow Cell dimensions: 420-μm x 180-μm internal diameter.</p>
Laser devices	<p>Standard wavelengths</p> <p>Blue laser</p> <ul style="list-style-type: none"> Wavelength: 488 nm, 50 mW Beam spot size: 5 μm x 80 μm <p>Red laser</p> <ul style="list-style-type: none"> Wavelength: 638 nm, 50 mW Beam spot size: 5 μm x 80 μm <p>Violet laser</p> <ul style="list-style-type: none"> Wavelength: 405 nm, 80 mW Beam spot size: 5 μm x 80 μm
	<p>Additional standard wavelengths [CytoFLEX S or CytoFLEX LX]</p> <p>Yellow laser</p> <ul style="list-style-type: none"> Wavelength: 561 nm, 30 mW Beam spot size: 5 μm x 80 μm <p>Near Ultraviolet (NUV) laser</p> <ul style="list-style-type: none"> Wavelength: 375 nm, 60 mW Beam spot size: 5 μm x 80 μm <p>Infrared (IR) laser</p> <ul style="list-style-type: none"> 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	<p>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.</p> <p>Reflective optics with a single transmission band-pass filter in front of each detector.</p>
Violet side scatter configuration (VSSC)	The system offers the ability to configure the violet laser detector to collect side scatter to better resolve nanoparticles from noise.

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

Fluidics System [With Plate Loader]		
Sample input formats	Plate Loader format	Flat/U/V bottom standard 96-well plate
Dead Volume	96-well flat bottom plate	20 µL
	96-well U bottom plate	10 µL
	96-well V bottom plate	10 µL
Minimum Sample Volume	45 µL/well	
Maximum Sample Volume	250 µL/well	

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

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

Data Management		
Minimum Workstation/ computer requirements [CytoFLEX LX]	Operating system	Windows® 7, 8, 10 Professional 64-bit
	Processor	6th Generation Intel Core i7 (8MB cache 4.0 ghz)
	Memory	8 GB RAM
	Storage	256 GB
	Port	1 GB Ethernet port
	USB	5 USB 2.0 and above ports
Compensation	Full matrix compensation, manual and automatic. Novel Compensation Library for storage of spillover values of dyes to easily determine the correct compensation matrix with new gain settings.	

Performance Characteristics

Performance Characteristics [CytoFLEX]

Performance		
Sensitivity	MESF	FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) from the 488-nm laser PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE) from the 561-nm laser
Fluorescence resolution	rCV <3% The CytoFLEX Flow Cytometer is capable of achieving <3% rCV only with calibration reagents that have the capability to achieve this specification.	
Blue Side scatter resolution	<300 nm	
Violet Side scatter resolution	<200 nm	
Forward and side scatter resolution	Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes as well as nanoparticles.	
Carryover	Single Tube Loader format	≤1.0%
Signal acquisition speed	30,000 particles/second with 15 parameters	

Performance [With Plate Loader]		
Carryover	Plate Loader format	<0.5%
Throughput [With Plate Loader] ¹	10 second acquisition without mixing or backflush: <32 min.	
	10 second acquisition with 3 second mixing and 3 second backflush: <45 min.	

1. This performance characteristic is different if you have the Sample Injection Mode Control Kit installed on your CytoFLEX flow cytometer. Refer to [APPENDIX C, Sample Injection Mode Control Kit](#).

Performance Characteristics [CytoFLEX LX]

Performance		
Sensitivity	MESF	FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE)
Fluorescence resolution	rCV <5% The CytoFLEX LX Flow Cytometer is capable of achieving <5% rCV only with calibration reagents that have the capability to achieve this specification.	
Blue Side scatter resolution	<300 nm	
Violet Side scatter resolution	<200 nm	
Forward and side scatter resolution	Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes as well as nanoparticles.	
Carryover	Single Tube Loader format	≤1.0%
Signal acquisition speed	30,000 particles/second with 23 parameters	

Performance [With Plate Loader]		
Carryover	Plate Loader format	<0.5%
Throughput [With Plate Loader]¹	10 second acquisition without mixing or backflush: <34 min.	
	10 second acquisition with 3 second mixing and 3 second backflush: < 47 min.	

1. 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](#).

Reagent Limitations

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

Using the CytExpert Software

Overview

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

This chapter contains information on:

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

Launching the Software

Select the desktop shortcut  to launch the CytExpert software.

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



> **All Programs** > **CytExpert**.

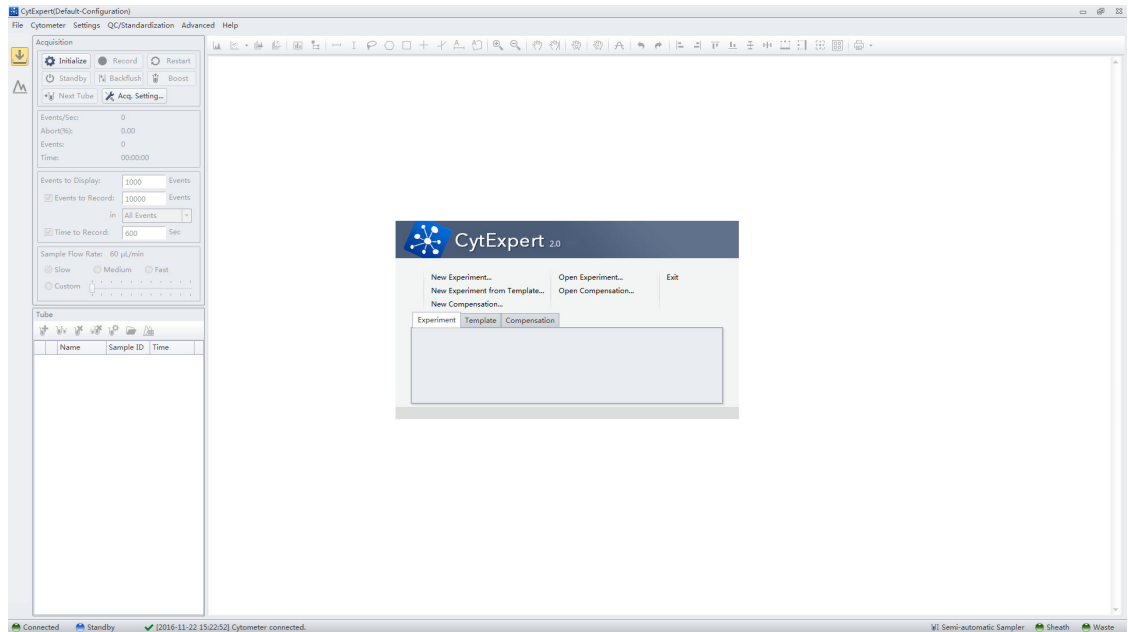
Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#), for detailed instructions on opening the software and confirming the connection status.

Main Software Screen

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

Start Page

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




The following operations can be selected from the start page:

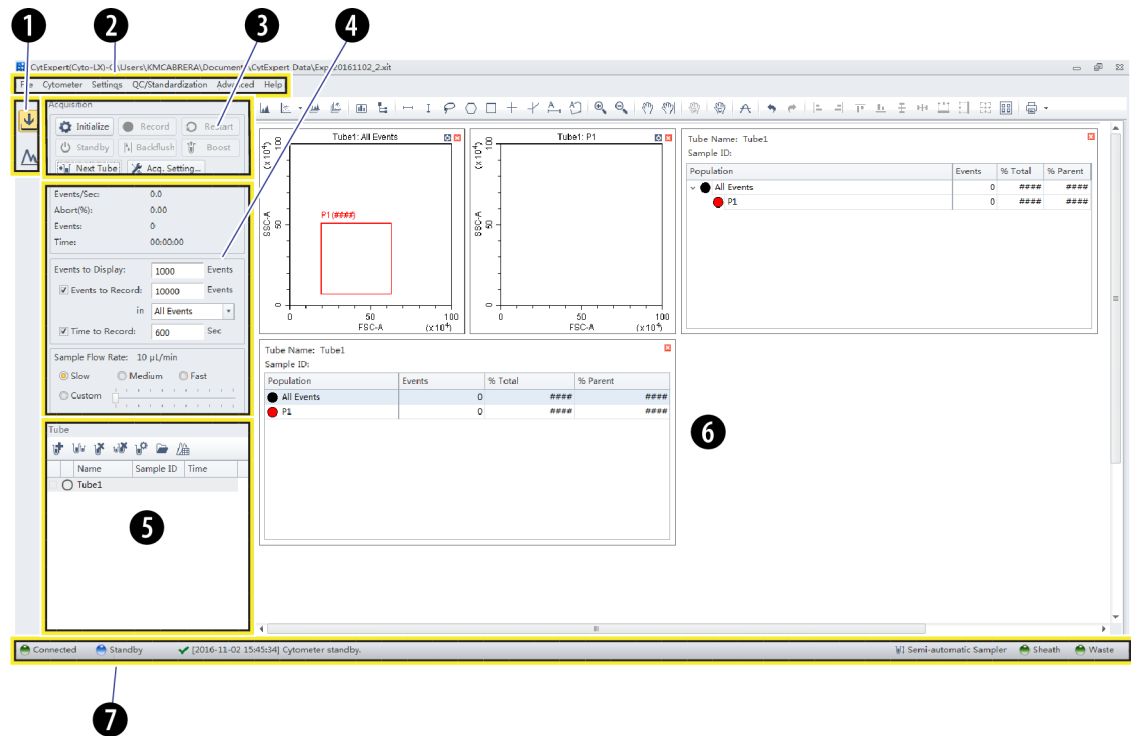


- **New Experiment.** For creating a new experiment. The process creates a file with the .xit extension and a folder with the same file name where the raw data (.fcs files) are kept.
- **New Experiment From Template.** For creating an experiment using a template saved from a previously saved experiment.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

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

Acquisition Screen

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



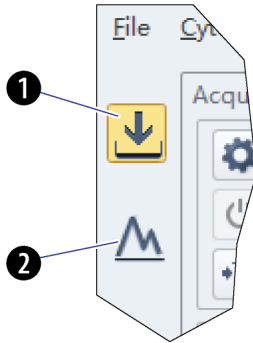
- 1. Navigation.** Gives the option of accessing the Acquisition screen or Analysis screen.
- 2. Menu.** Allows you to configure settings for sample acquisition, instrument operation, and software options.
- 3. Instrument Operation Controls.** Controls sample loading/unloading and data acquisition and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

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

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

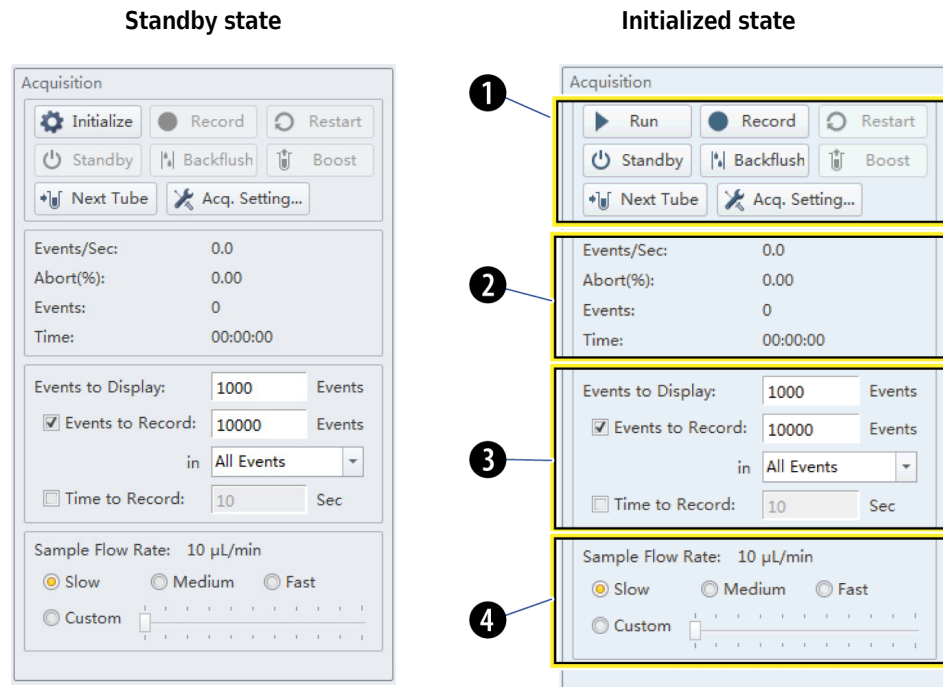
Acquisition Screen Navigation

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



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

Collection

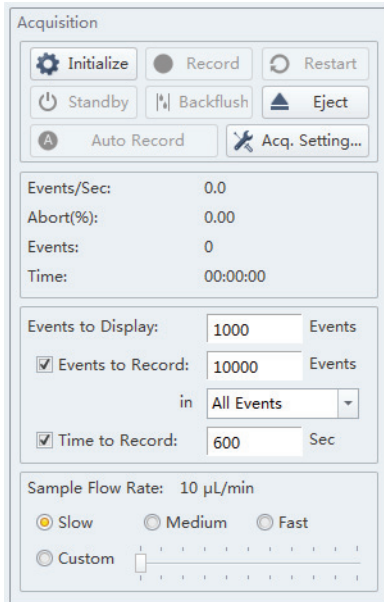


1. **Acquisition control.** Controls sample loading/unloading and data acquisition and recording.
2. **Acquisition status.** Displays such information as the acquisition rate (Events/Sec), event count, duration, and abort (%).
3. **Acquisition conditions.** Sets the necessary conditions for recording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

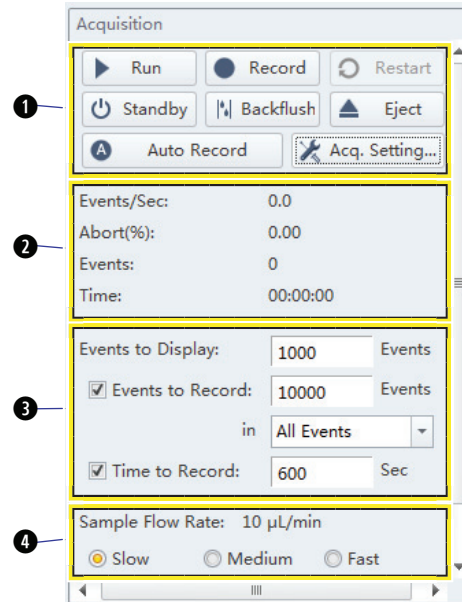
NOTE High acquisition rate may increase the abort rate and measurement CVs.

Collection [With Plate Loader]

Standby state



Initialized state



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

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

Test Tubes

Manual/Semi Automatic Sample Injection Mode

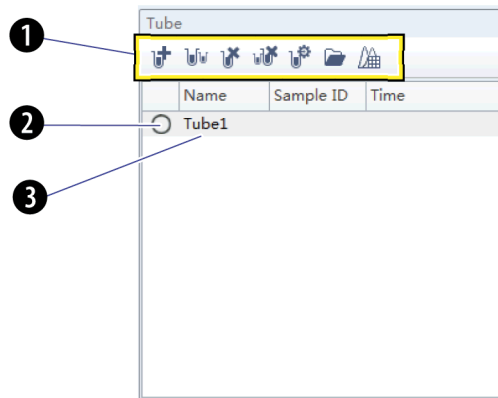
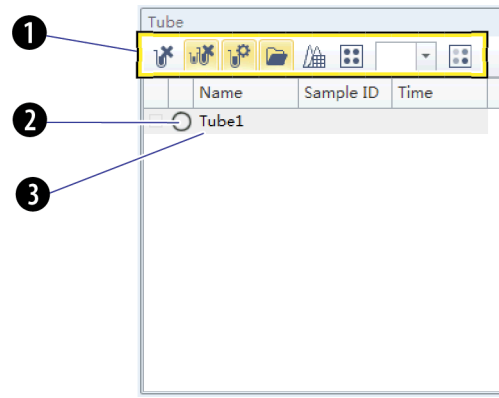
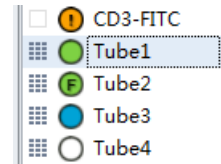


Plate Loader Sample Injection Mode



Test Tube Status



- 1. Tube management controls.** Manages sample tubes. Used to add, copy, or delete attributes, open the tube property, and open the compensation matrix.
- 2. Test tube status indication.** Displays a colored symbol in front of each tube indicating the status of the tube processing.

- indicates that the tube data was not collected.
- indicates that the tube data was acquired by selecting **Run** but can be overwritten.
- indicates that the tube data was saved by selecting **Record** or **Auto Record** and that this data cannot be overwritten.
- F indicates imported FCS data.

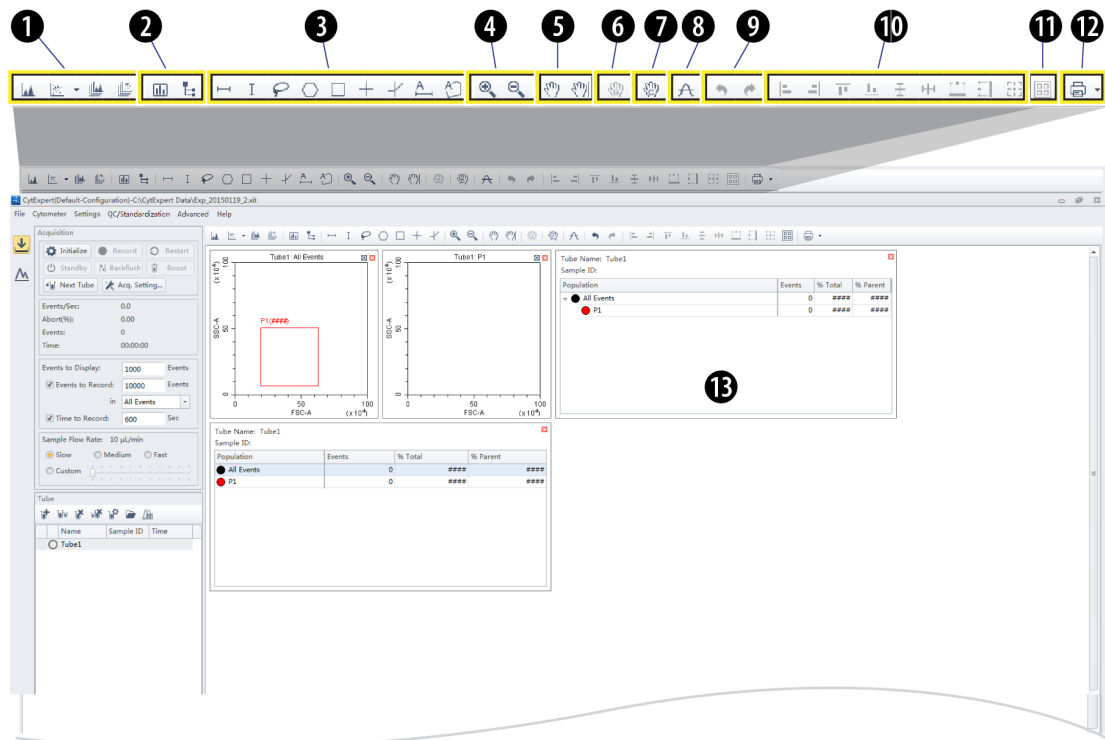
NOTE ■■■ to the left of the test tube status indication symbol indicates that the sample has been compensated.

- ⚠ indicates the data file is missing or there is an error in the data file.

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

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

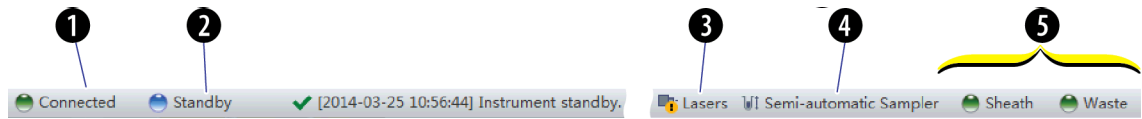
Plot area



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

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

Status Bar



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

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

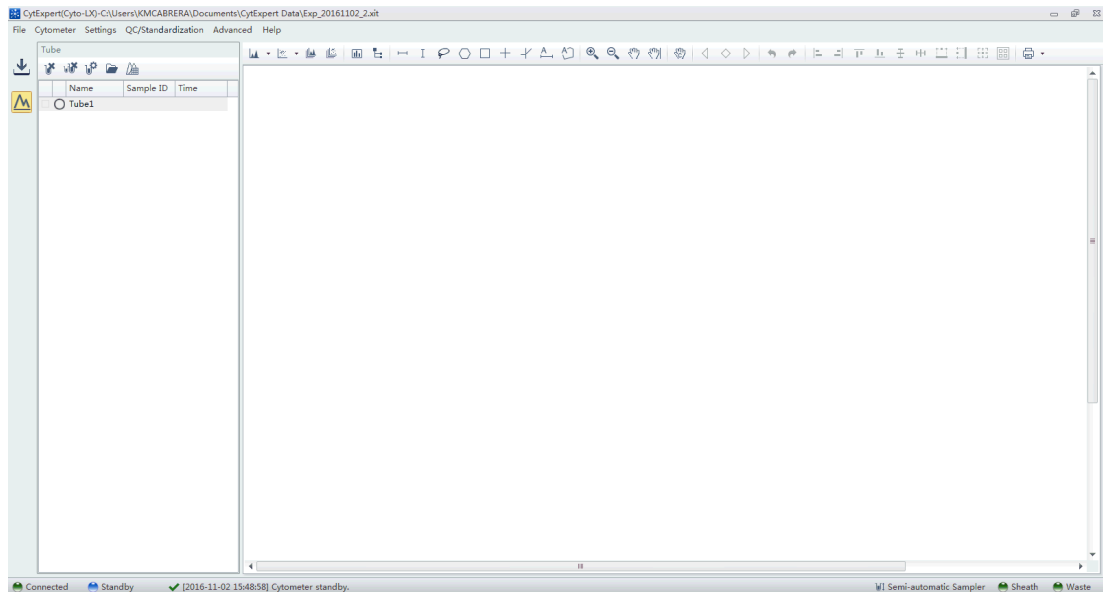
4. **Sampler status.** Displays the sample injection mode state. There are two sample injection modes: Semi-automatic sample injection mode and manual sample injection mode.

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

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

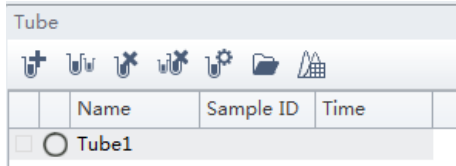
Analysis Screen

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



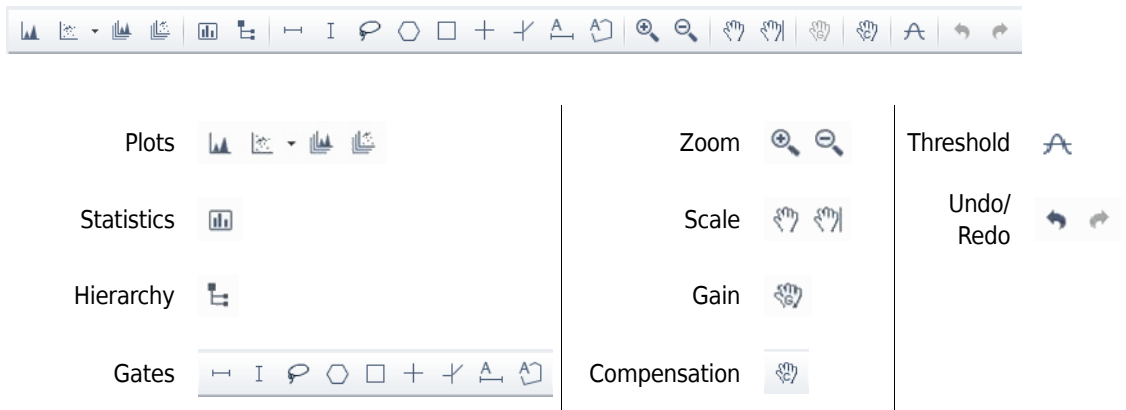
The Tube management module cannot add new sample tubes. Return to the Acquisition screen to add new sample tubes.

Semi Automatic Sample Injection Mode Shown



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

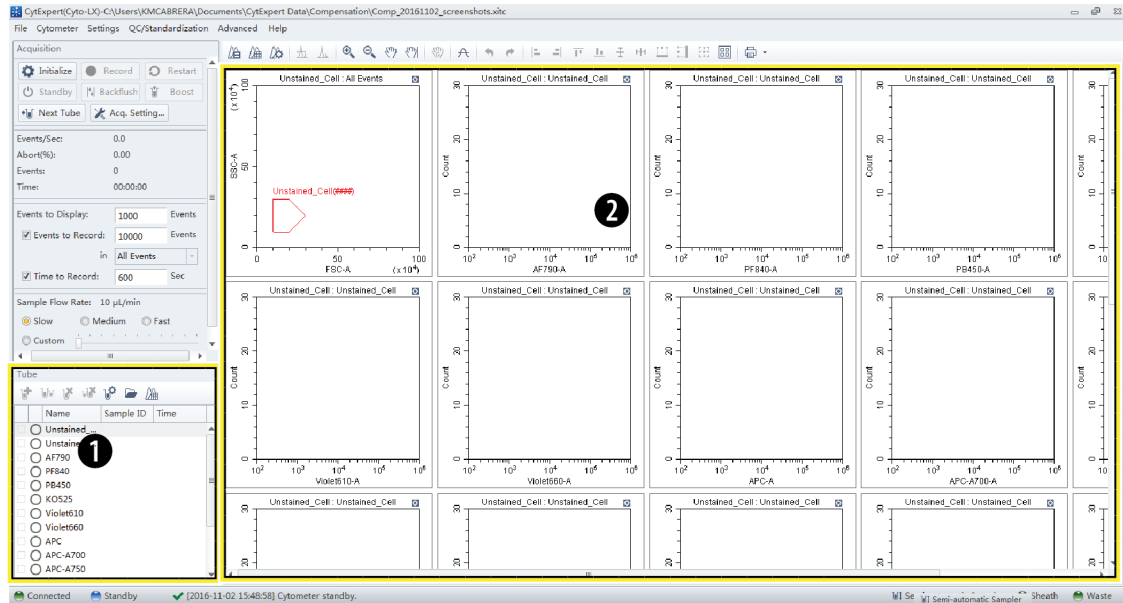
Figure 2.1 Drawing Controls Toolbar (Top of Screen)



Compensation Experiment Screen

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

Semi-Automatic Sample Injection Mode Shown



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

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

Compensation Controls

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



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

QC Experiment Screen

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

QC Report Screen

Before starting the QC routine, a Settings screen appears.

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

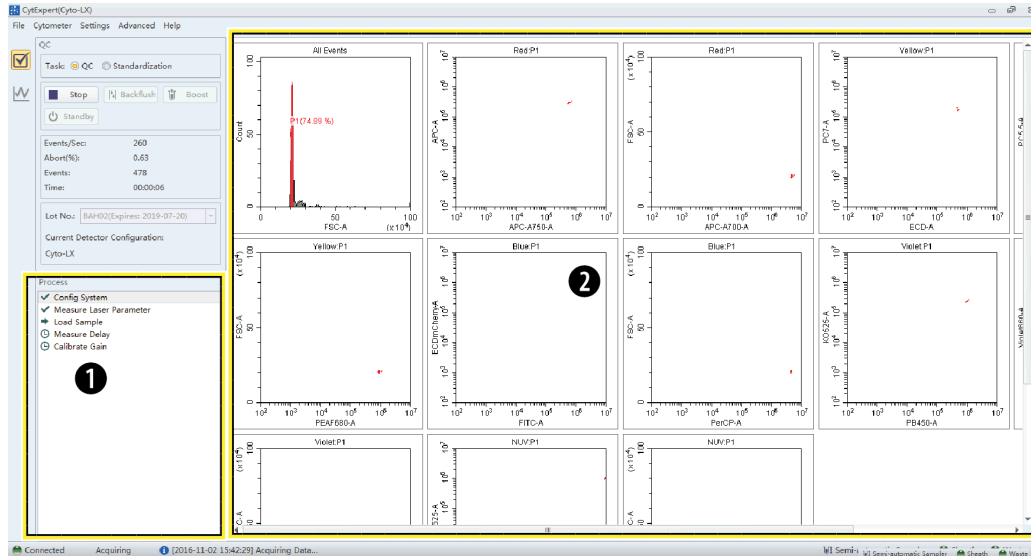
The screenshot shows the QC Report screen with five numbered callouts:

- 1. Menu:** Points to the top menu bar (File, Cytometer, Settings, Advance, Help).
- 2. Acquisition control:** Points to the 'Task' panel with 'Initialize', 'Backflush', and 'Boost' buttons.
- 3. Lot selection:** Points to the 'Lot No.' input field in the 'Report' panel.
- 4. QC results list area:** Points to the table of completed runs in the 'Report' panel.
- 5. QC reports area:** Points to the 'QC Report' summary box at the top of the main display area.

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

QC Experiment Screen

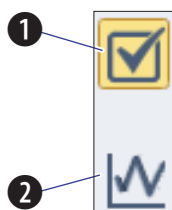
When acquiring QC samples, the software opens the QC screen.



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

QC Screen Navigation

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



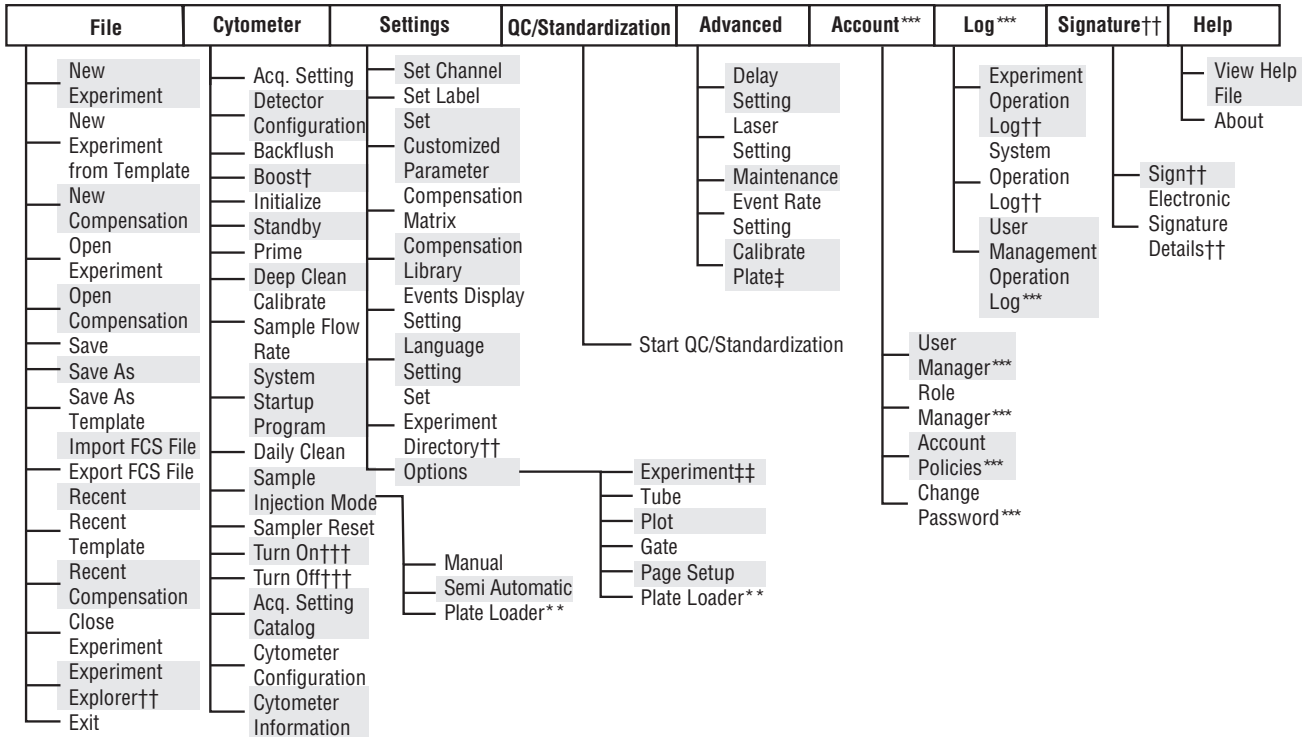
1. QC screen icon. Accesses the QC screen.
2. LJ screen icon. Accesses the Levey-Jennings (LJ) screen.

Software Menu

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

The CytExpert software contains the following selectable menu items:

Figure 2.3 Software Menu Tree*



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

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

‡ **Calibrate Plate** 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.

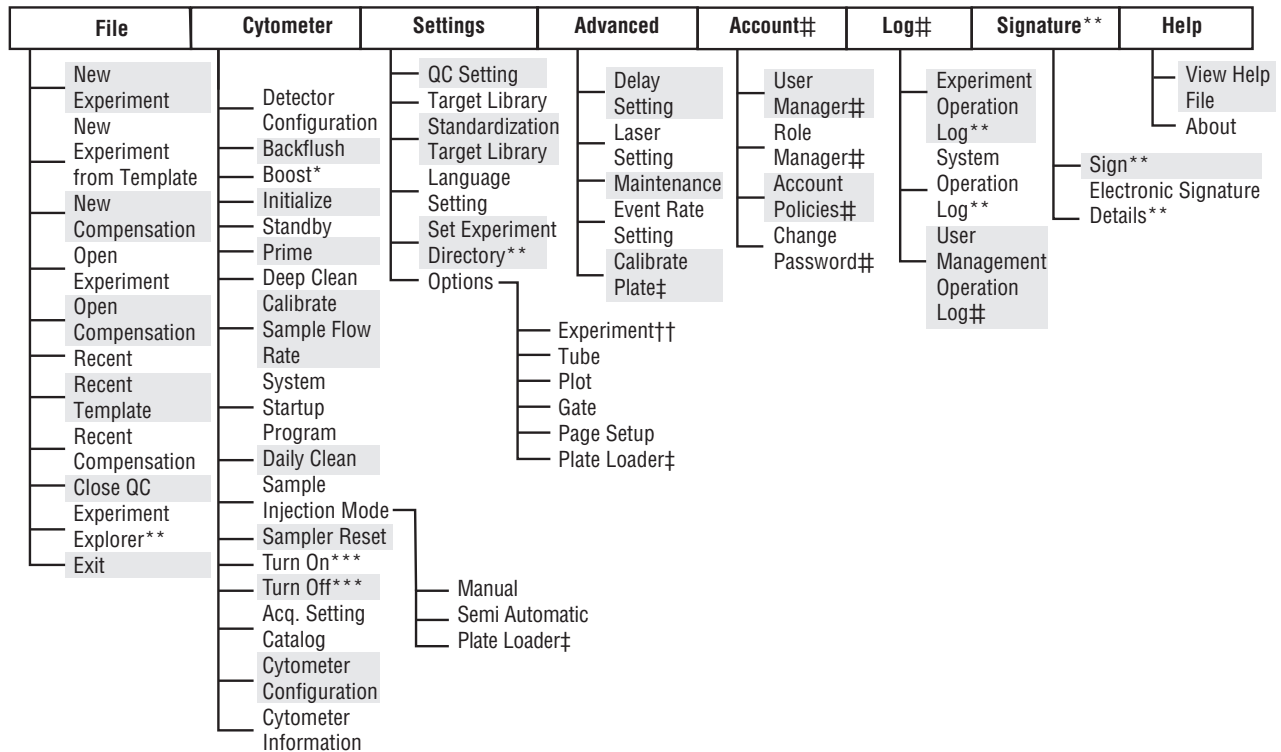
†† These options are only available if the CytExpert User Management and CytExpert Electronic Record Management software option is installed.

††† **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.

Figure 2.4 QC Software Menu Tree



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

† **Calibrate Plate** 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 and CytExpert Electronic Record Management software option is installed.

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

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

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

Acquisition and Analysis Screen Menu

CytExpert Default Software Option

File Cytometer Settings QC/Standardization Advanced Help

CytExpert User Management Software Option

File Cytometer Settings QC/Standardization Advanced Account Log Help

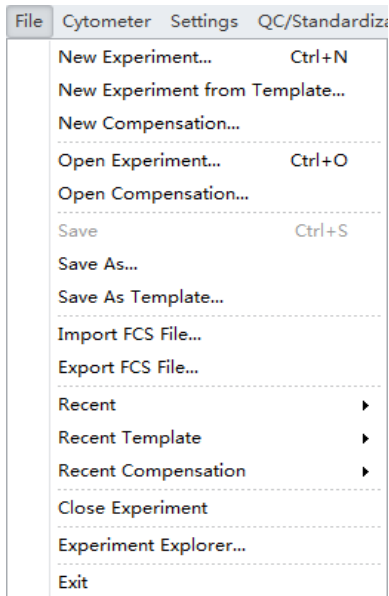
CytExpert Electronic Record Management Software Option

File Cytometer Settings QC/Standardization Advanced Account Log Signature Help

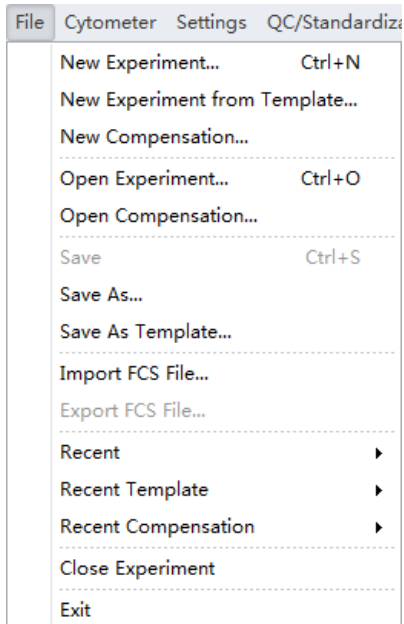
File Menu

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

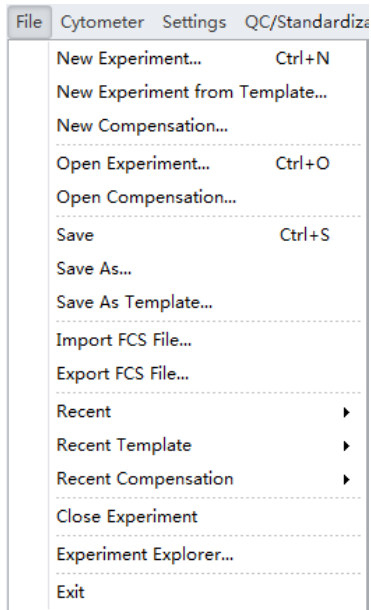
CytExpert Default Software Option



CytExpert User Management Software Option



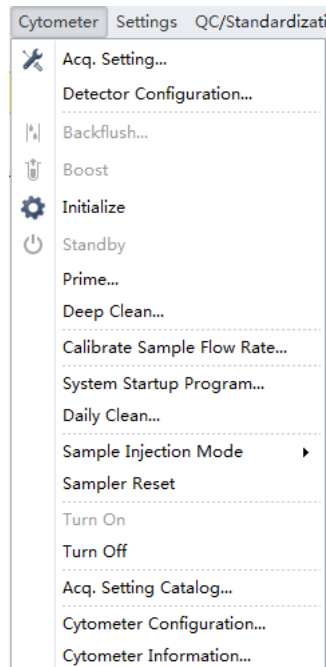
CytExpert Electronic Record Management Software Option



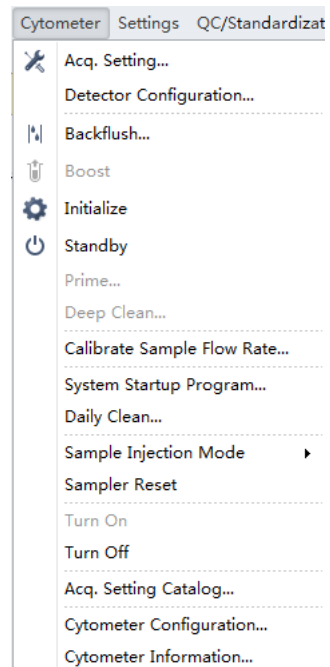
Cytometer Menu

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

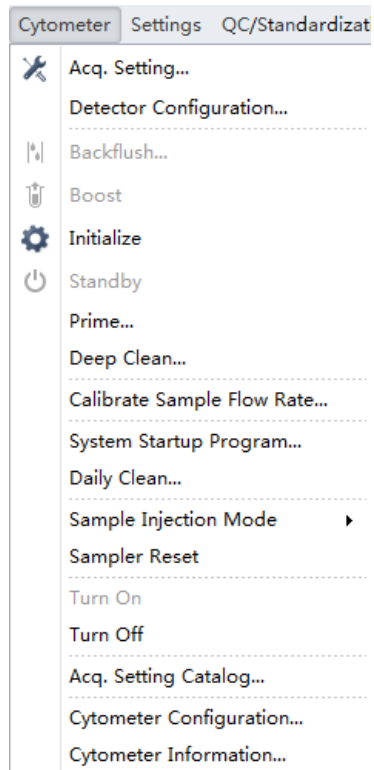
CytExpert Default Software Option - Standby state



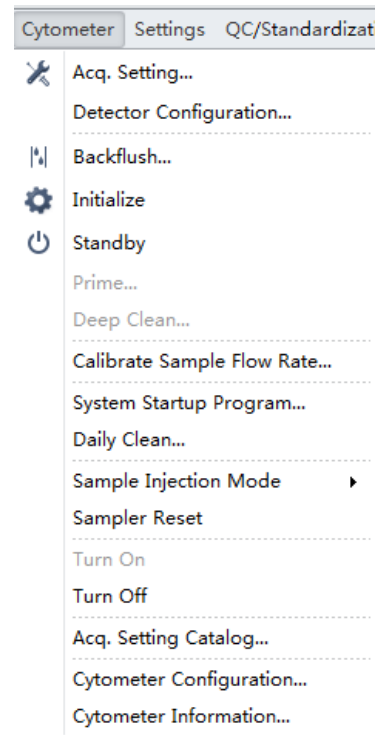
CytExpert Default Software Option - Initialized state



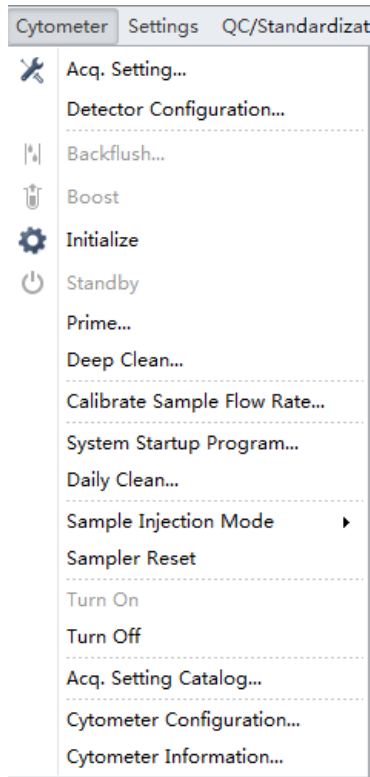
CytExpert User Management Software Option - Standby state



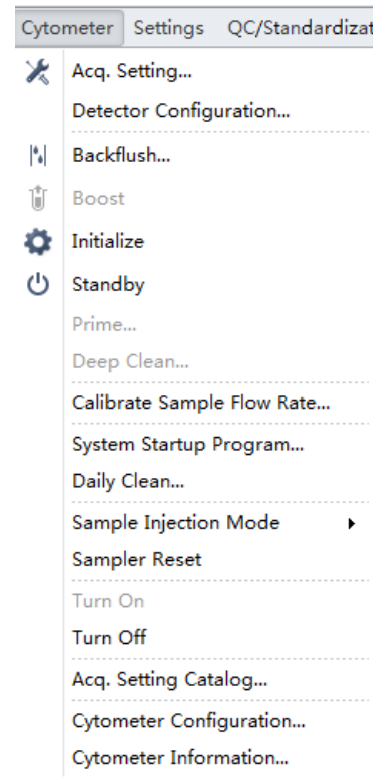
CytExpert User Management Software Option - Initialized state



**CytExpert Electronic Record Management
Software Option - Standby state**



**CytExpert Electronic Record Management
Software Option - Initialized state**

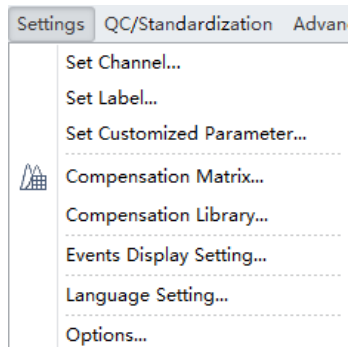


NOTE The **Turn On** and **Turn Off** selections are only available on the CytoFLEX LX.

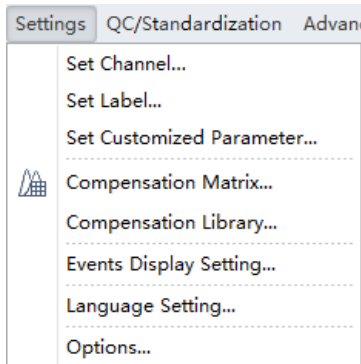
Settings Menu

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

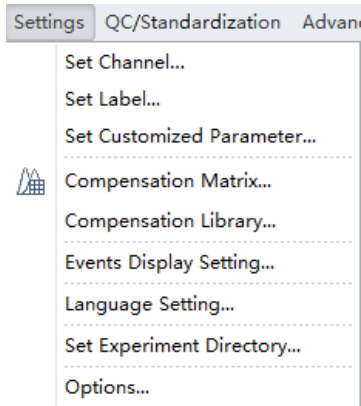
CytExpert Default Software Option



CytExpert User Management Software Option

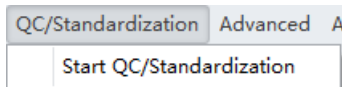


CytExpert Electronic Record Management Software Option



QC/Standardization Menu

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

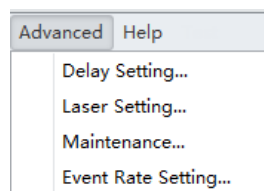


NOTE The QC/Standardization menu is the same for the CytExpert Default, CytExpert User Management, and the CytExpert Electronic Record Management software options.

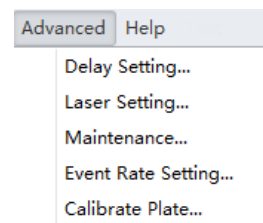
Advanced Menu

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

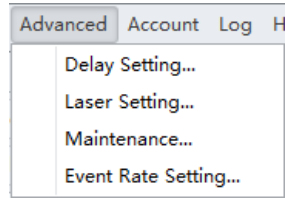
CytExpert Default Software Option - Semi-Automatic/Manual Sample Injection Mode



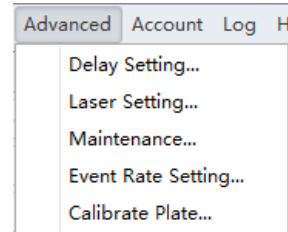
CytExpert Default Software Option - Plate Loader Sample Injection Mode



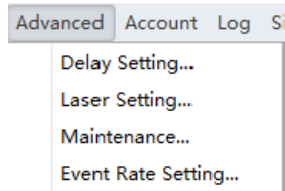
CytExpert User Management Option - Semi-Automatic/Manual Sample Injection Mode



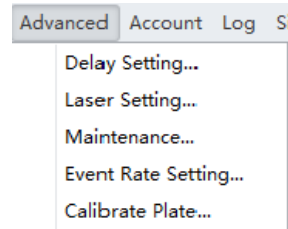
CytExpert User Management Software Option - Plate Loader Sample Injection Mode



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



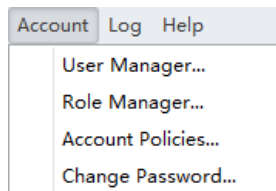
CytExpert Electronic Record Management Software Option - Plate Loader Sample Injection Mode



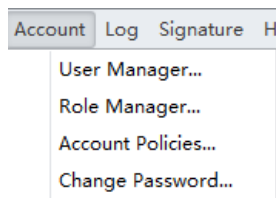
Account Menu

Used to for user account management settings.

CytExpert User Management Software Option



CytExpert Electronic Record Management Software Option

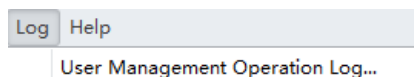


NOTE The Account menu is not available in the CytExpert Default software option.

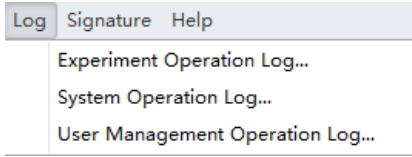
Log Menu

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

CytExpert User Management Software Option



CytExpert Electronic Record Management Software Option

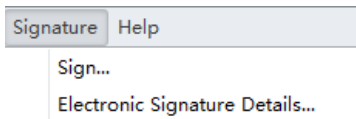


NOTE The Log menu is not available in the CytExpert Default software option.

Signature Menu

Used to sign experiment and view signature details.

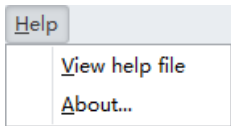
CytExpert Electronic Record Management Software Option



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

Help Menu

For displaying software version information and system Instructions for Use.



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

User Management

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

User Management is used to create and manage user accounts.

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

Figure 2.5 User Manager (Card View)

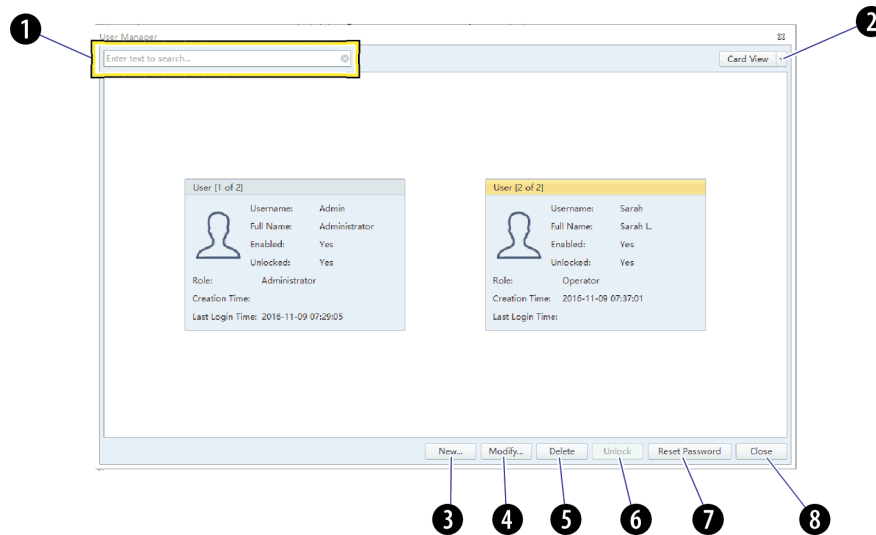
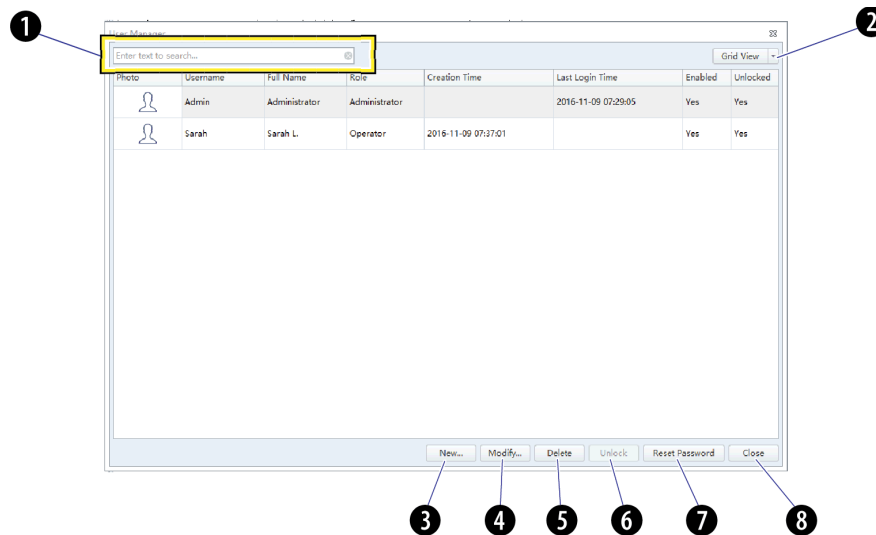


Figure 2.6 User Manager (Grid View)



- Search text box:** Filters users by username and display name.
- View drop-down:** Toggles between Card View (see Figure 2.5) and Grid View (see Figure 2.6).
- New:** Used to create a new user profile.
- Modify:** Used to modify an existing user profile.
- Delete:** Used to delete an existing user profile.

- Unlock:** Used to unlock an existing account that has been locked.

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

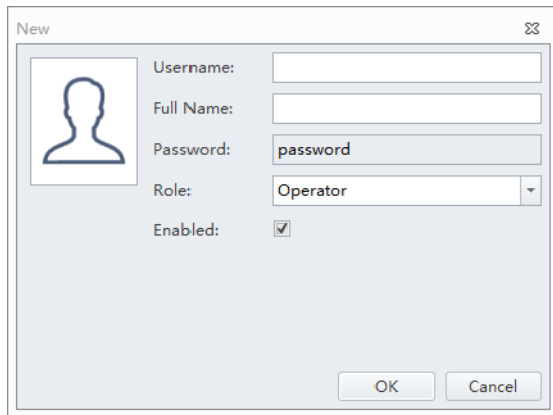
NOTE An account automatically unlocks after 30 minutes. The duration can be changed by the administrator. Refer to [Account Policies](#).

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

Creating, Deleting, and Modifying Users in User Manager

Creating a New User in User Manager

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



- 2 Fill in the new user information.
 - a. Enter the Username.
 - b. Enter the Full Name.
 - c. Select the user Role.
 - d. Select the Enabled checkbox to enable the user.

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

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

- 4 Select **Close**.
-

Deleting Users in User Manager

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

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

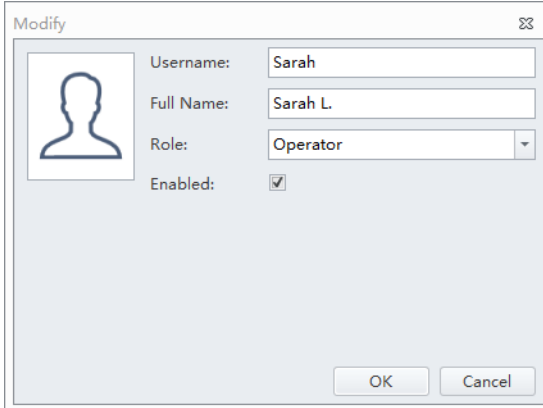
NOTE The user 'Admin' is a system default user and cannot be deleted.

- 2 Select .
-

Modifying Users in User Manager

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

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



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 .
-

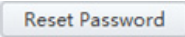
- 4 Select .
-

Unlocking a User Account

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

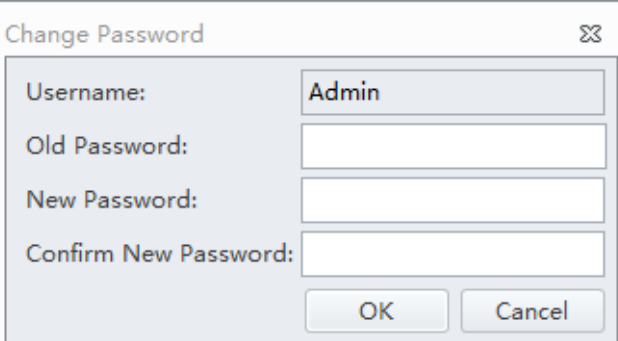
NOTE You cannot unlock an active user.

Resetting a User Passwords

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

Changing a User Password

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



The image shows a 'Change Password' dialog box with a close button (X) in the top right corner. It contains four text input fields: 'Username:' with the value 'Admin', 'Old Password:', 'New Password:', and 'Confirm New Password:'. At the bottom, there are two buttons: 'OK' and 'Cancel'.

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

- 3 Select .

Role Management

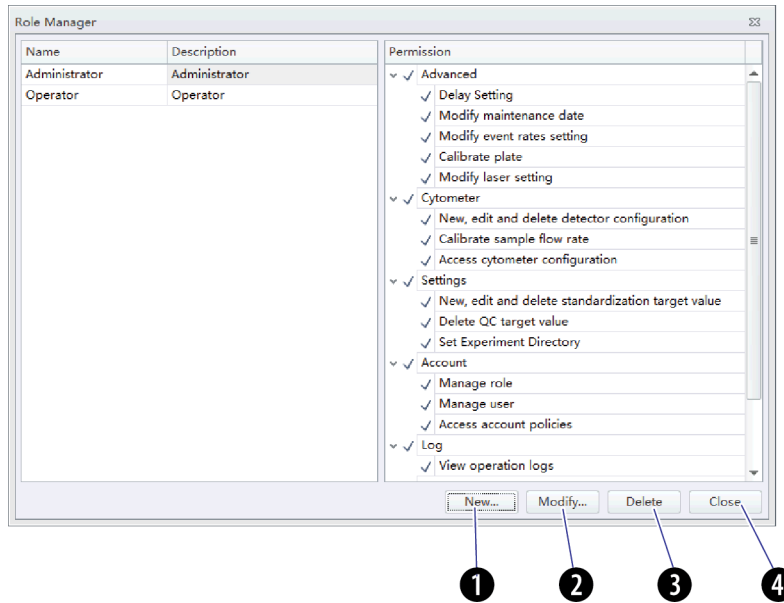
IMPORTANT Only an Administrator can manage users. You must have either the CytExpert User Management or CytExpert Electronic Record Management software option installed to use this feature. Refer to [CytExpert Software Installation Options](#) in [APPENDIX A, Instrument Installation](#).

Role Management is used to manage user account permissions.

NOTE Multiple users can be applied to the same role.

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

Figure 2.7 Role Manager

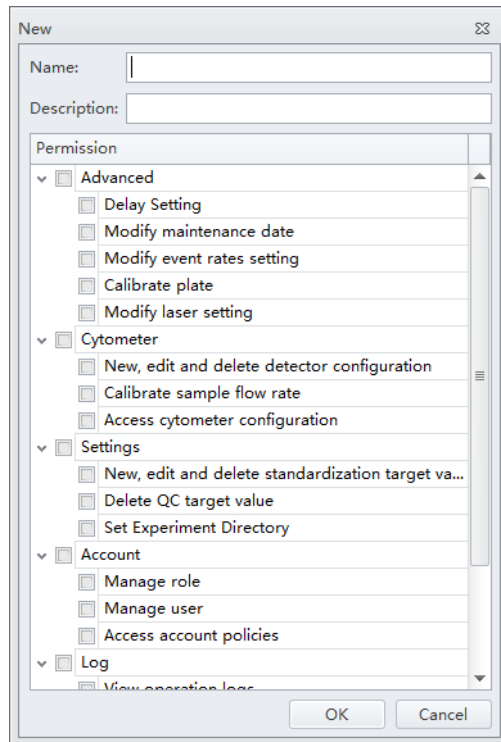


1. **New:** Used to create a new role profile.
2. **Modify:** Used to modify an existing role profile.
3. **Delete:** Used to delete an existing role profile.
4. **Close:** Closes the Role Manager window.

Creating, Deleting, and Modifying User Roles in Role Manager

Creating New User Roles in Role Manager

- 1 Select . The New window appears.



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

Deleting User Roles in Role Manager

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

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

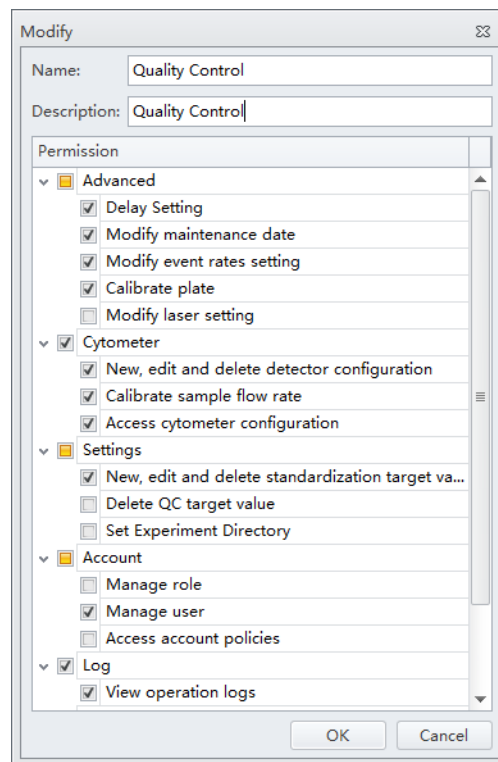
1 Select the Role to be deleted in Role Manager then select .

2 Select .

Modifying User Roles in the Role Window

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

1 Select . The Modify window appears.



2 Modify the role information as necessary.

3 Select .

4 Select .

Account Policies

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

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

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

Figure 2.8 Account Policies

The screenshot shows a dialog box titled "Account Policies" with a close button in the top right corner. It is divided into two main sections:

- Password Expiration Policy:**
 - Enforce password expiration
 - Password expires in days
 - Reminder for expiration before days
- Account Lockout Policy:**
 - Enforce account lockout
 - Invalid login attempts: times
 - Lockout time: minutes

At the bottom right of the dialog are "OK" and "Cancel" buttons.

NOTE The allowable range for each entry is as follows:

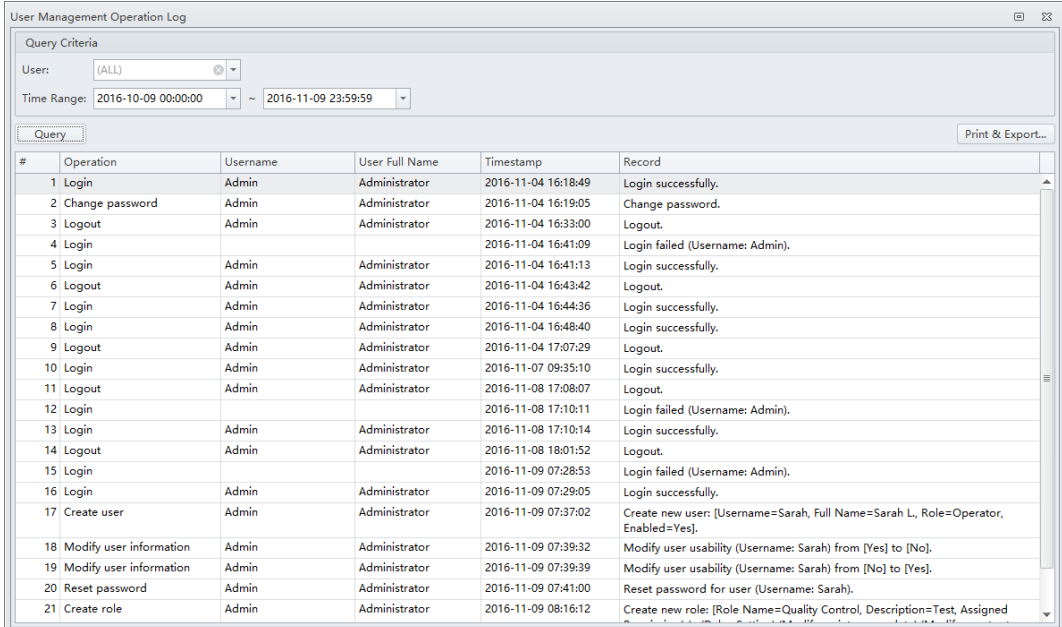
- Password Expiration: 1-999 days
- Reminder for Expiration: 1-90 days
- Invalid Login Attempts: 3-10 times
- Lockout Time: 15-1,440 minutes

User Management Operation Log

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

Viewing and Exporting User Logs

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



The screenshot shows the 'User Management Operation Log' window. At the top, there are 'Query Criteria' fields: 'User' set to '(ALL)' and 'Time Range' from '2016-10-09 00:00:00' to '2016-11-09 23:59:59'. Below these is a 'Query' button and a 'Print & Export...' button. The main area is a table with the following data:

#	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	Admin	Administrator	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	Admin	Administrator	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	Admin	Administrator	2016-11-09 07:28:53	Login failed (Username: Admin).
16	Login	Admin	Administrator	2016-11-09 07:29:05	Login successfully.
17	Create user	Admin	Administrator	2016-11-09 07:37:02	Create new user: (Username=Sarah, Full Name=Sarah L, Role=Operator, Enabled=Yes).
18	Modify user information	Admin	Administrator	2016-11-09 07:39:32	Modify user usability (Username: Sarah) from [Yes] to [No].
19	Modify user information	Admin	Administrator	2016-11-09 07:39:39	Modify user usability (Username: Sarah) from [No] to [Yes].
20	Reset password	Admin	Administrator	2016-11-09 07:41:00	Reset password for user (Username: Sarah).
21	Create role	Admin	Administrator	2016-11-09 08:16:12	Create new role: (Role Name=Quality Control, Description=Test, Assigned

- 2 Enter the filter conditions: User and Time Range.

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

NOTE User logs are exported as a .pdf file.

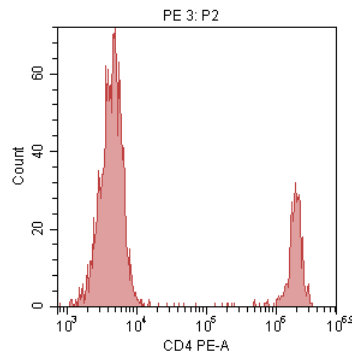
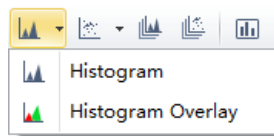
Graphic and Gating Styles

Plots

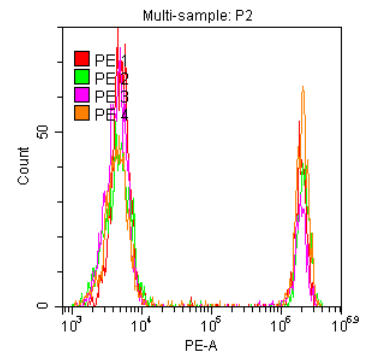
The CytExpert software offers a variety of plot formats including:

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

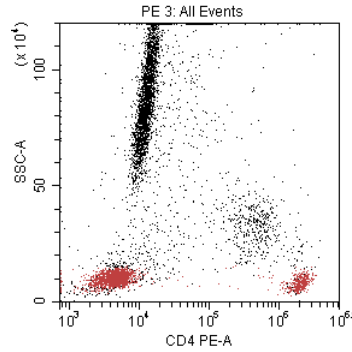
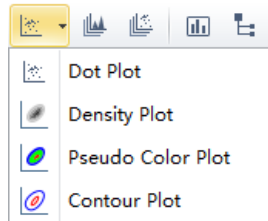
NOTE Histogram Overlays and Dot Plot Overlays can only be created from multiple samples in the Analysis screen. A maximum of 10 samples can be overlaid.



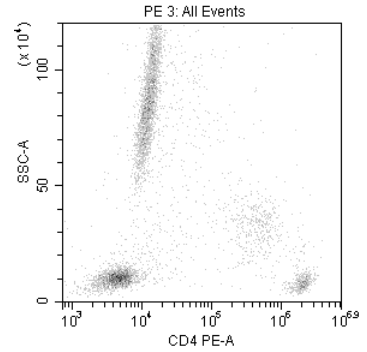
Histogram



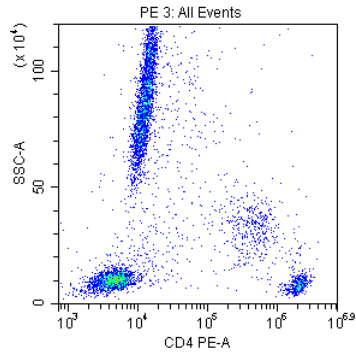
Multi-sample histogram overlays



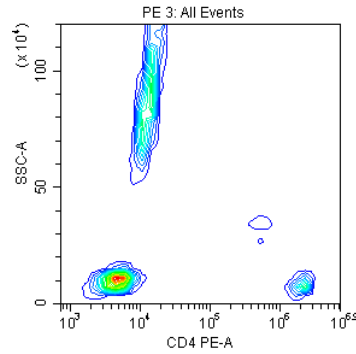
Dot plot



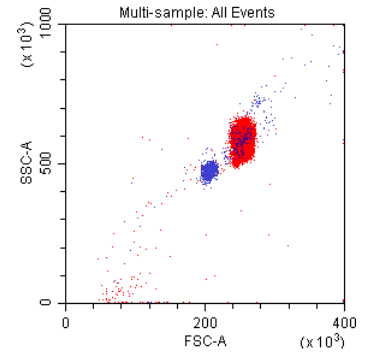
Density plot



Pseudo color plot



Contour plot



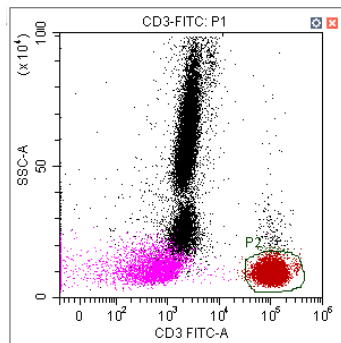
Dot plot overlay

Gates

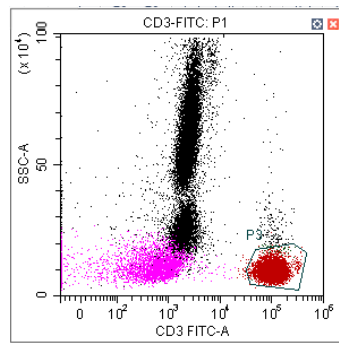
Various gating choices are available.

The software includes the following gate types:

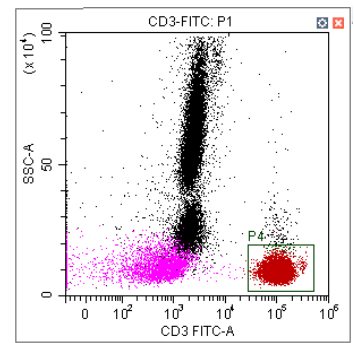
- For dual-parameter plots: lasso, polygon, rectangle, four-quadrant, hinged gates, and auto polygon
- For single-parameter plots: line-segment, vertical gates, and auto line segment



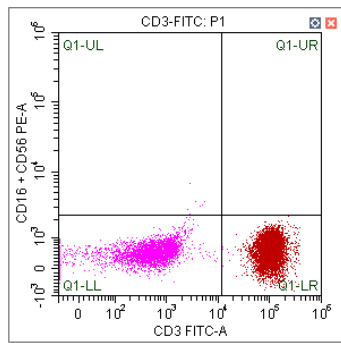
Lasso gate



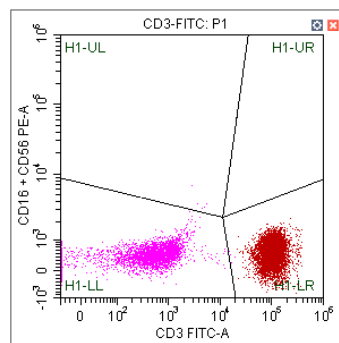
Polygon gate^a



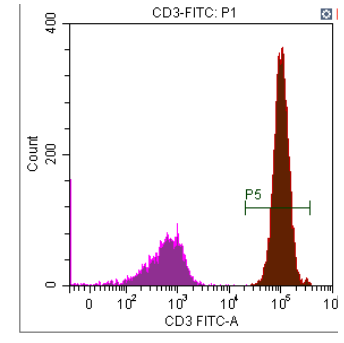
Rectangle gate



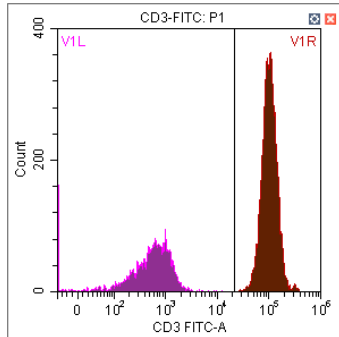
Four-Quadrant gate



Hinged gate



Line Segment gate^a



Vertical gate

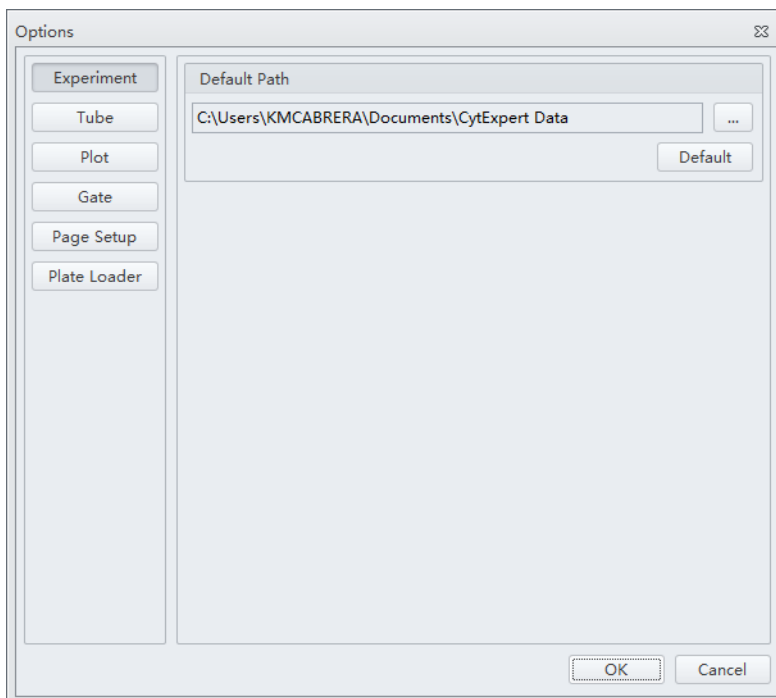
- a. This gate can be created using the autogate functionality. Refer to [Creating and Adjusting Auto Gates](#) in CHAPTER 5, [Data Acquisition and Sample Analysis](#)

Software Settings

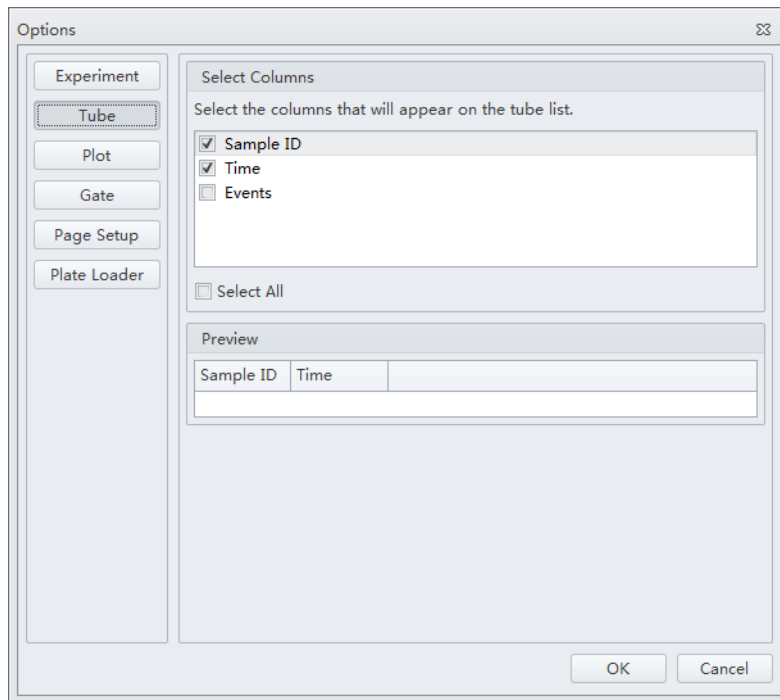
Select **Options** in the Settings menu to configure the software settings.

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

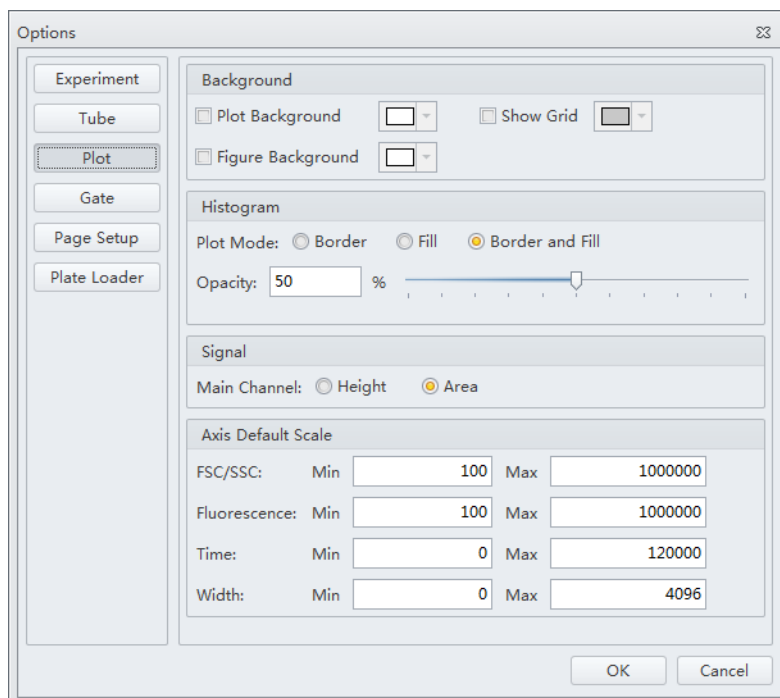
NOTE The Experiment setting is only available if either the CytExpert Default or the CytExpert User Management software option is installed.



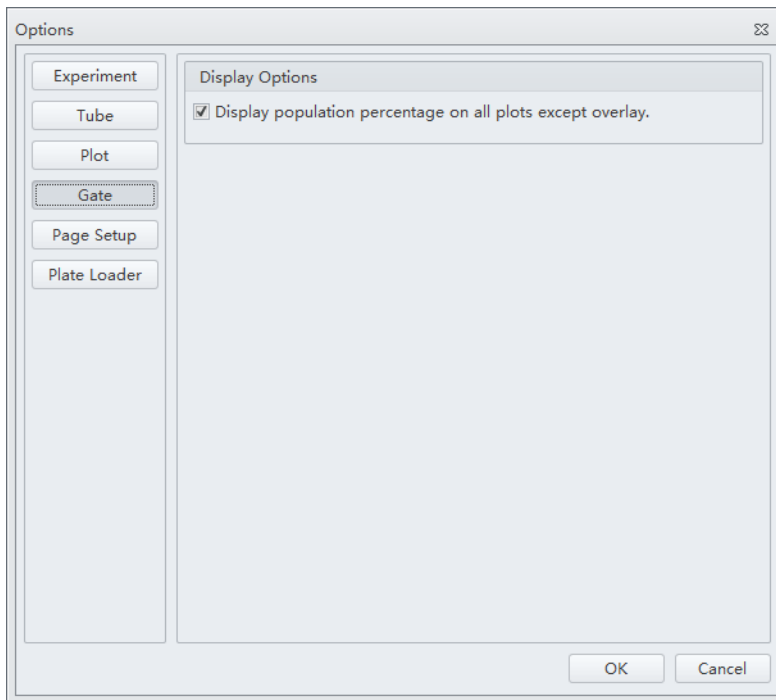
In the tube settings, you can select the columns that display in the tube section of the screen.



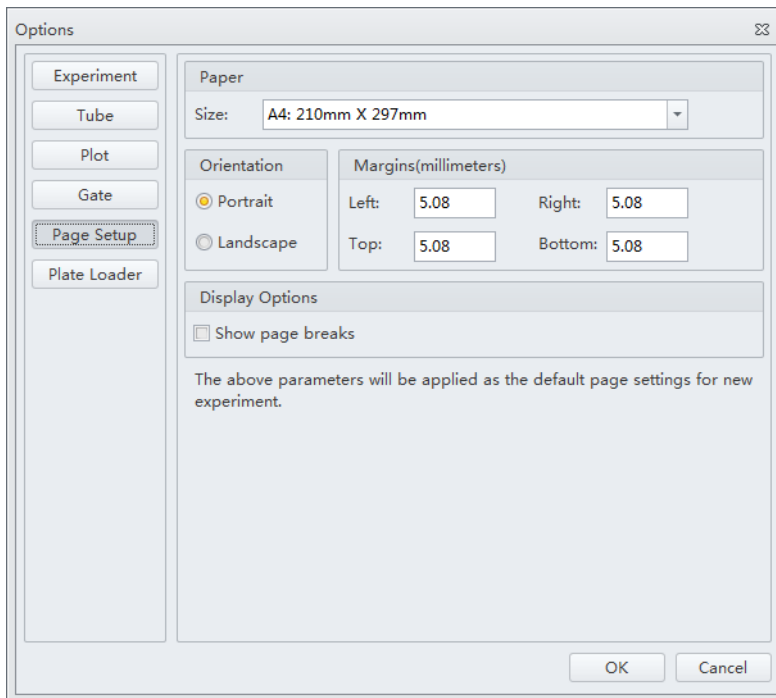
In the plot settings, you can define the background of the graphics display area, configure the histograms, and set the default signal parameters to either the channel's area or the channel's height. The default is area. You can also set the default axis display range.



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

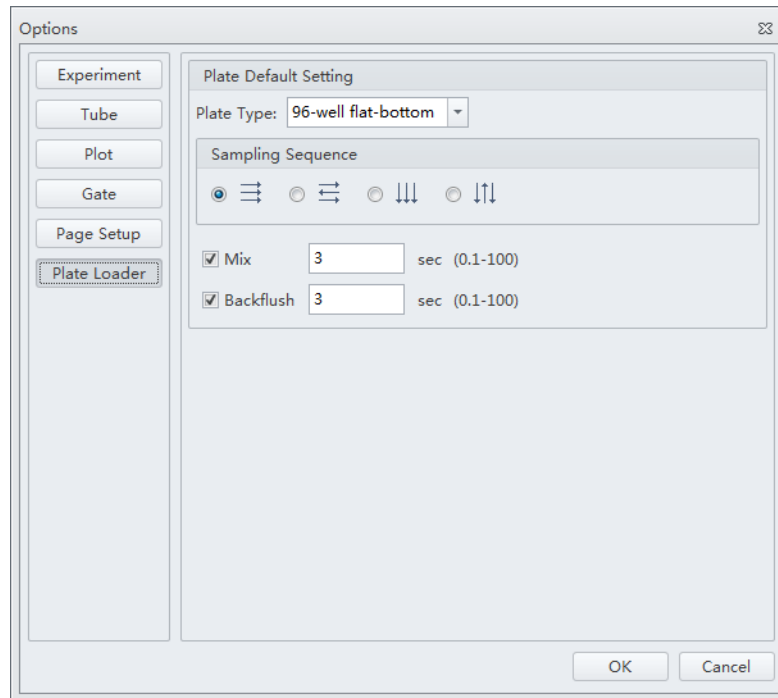


In the Page Setup settings, you can change the page size, orientation, margin size, and display options.



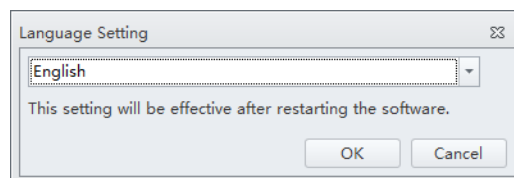
In the plate loader settings, you can select the plate type, sampling sequence, mix, and backflush settings for the plate loader.

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



Language Settings

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



Setting Up CytExpert Application Programming Interface (API) Test Client

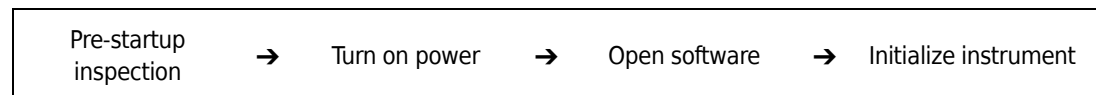
The CytExpert API is available for external software to control CytoFLEX series instruments. It allows external software to perform operations such as running methods and allows for basic control of the plate loader. It is also possible to report population statistics as each sample finishes. [Contact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

Overview

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

This chapter describes the instrument startup procedure.

Workflow:



This chapter contains information on:

- [Pre-Startup Inspection](#)
- [Turning On the Instrument](#)
- [Logging Into the Software](#)
- [Initializing the Instrument](#)

Pre-Startup Inspection

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

Check Waste and Reagent Levels [4 L Fluid Containers]

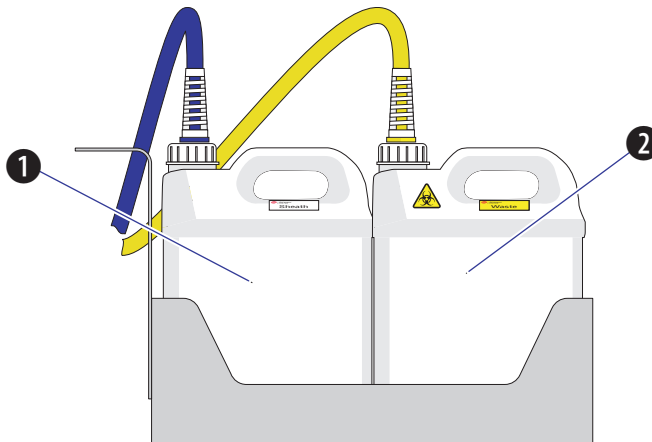


CAUTION

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

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

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



1. Sheath fluid container
2. Waste container

CAUTION

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

- 2 If necessary, fill the sheath fluid container with CytoFLEX Sheath Fluid or a similar nonionic sheath fluid while not exceeding the maximum volume indicated (4 L). Refer to [Filling the 4 L Sheath Fluid Container \[CytoFLEX\]](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

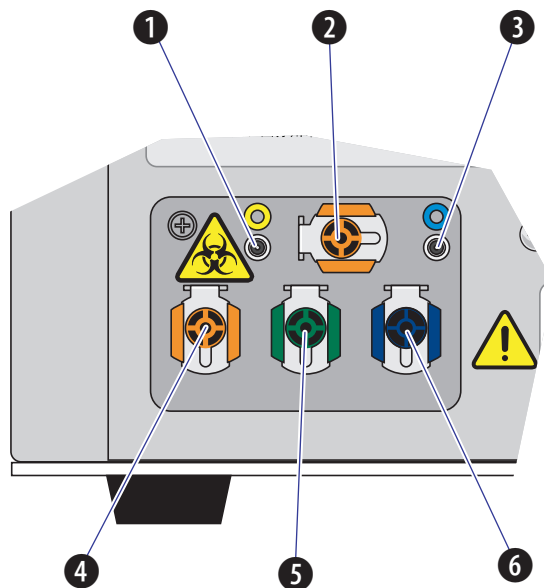
! WARNING

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

- 3** If necessary, empty all waste liquid from the waste container. If biohazardous samples are used for data collection, add 400 mL of 5 to 6% bleach to the waste container. Refer to [Emptying the 4 L Waste Container \[CytoFLEX\]](#) in [CHAPTER 11, 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.

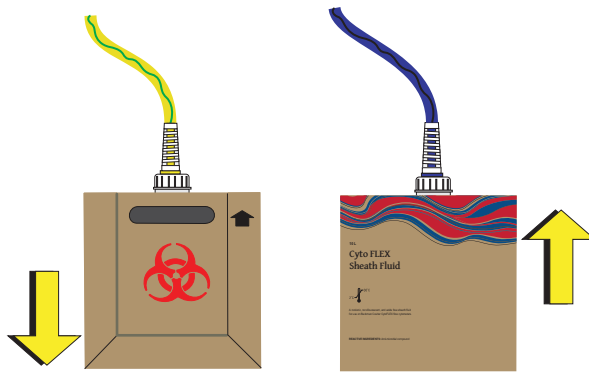
Check Waste and Reagent Levels [10 L Fluid Cubitainers]



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

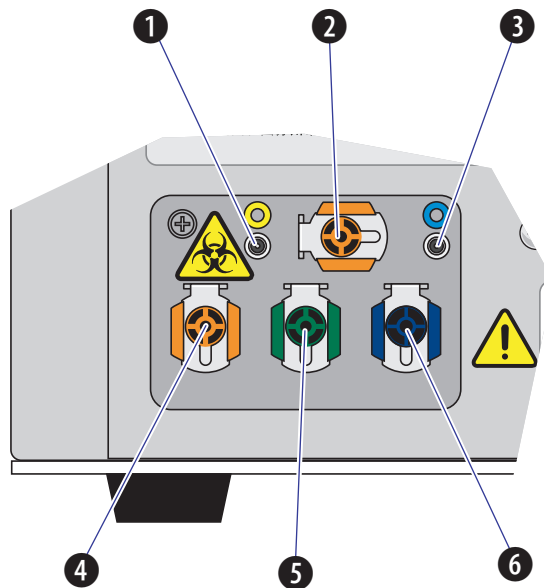
- 1 Examine the sheath fluid and waste cubitainers. Verify that there is sufficient sheath fluid in the sheath fluid container and that the waste container is empty.

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



- 2 Confirm that the instrument is in the standby state.
- 3 If necessary, replace the sheath fluid cubitainer with CytoFLEX Sheath Fluid or a similar nonionic sheath fluid. Refer to [Replacing the 10 L Sheath Fluid Cubitainer](#) in CHAPTER 11, Replacement/Adjustment Procedures.
- 4 If necessary, empty all waste liquid from the waste container. Refer to [Emptying the 10 L Waste Cubitainer](#) in CHAPTER 11, 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 1.17](#).


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  to open the software.

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

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

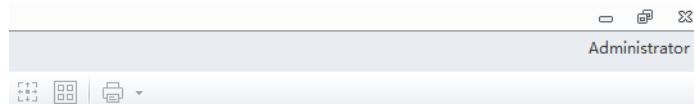


NOTE The default software shortcut appears on the desktop. If you do not see the icon, the default installation path is under C:/Program Files/CytExpert. Double-click CytExpert.exe to run the software.

2 Enter your username and password.

3 Select .

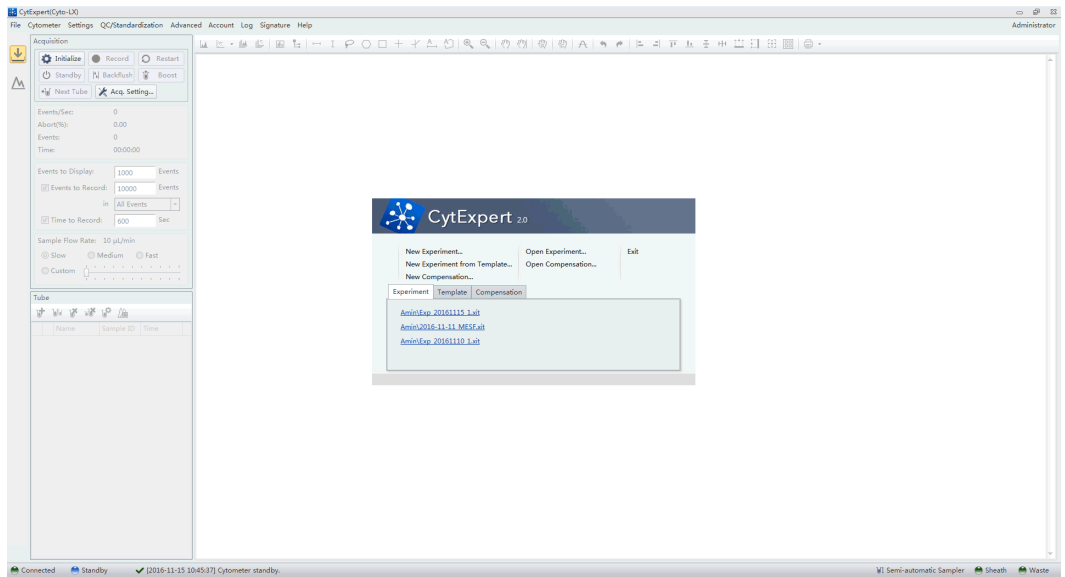
NOTE The display name of the user that is currently logged in displays in the top, right corner of the software screen.



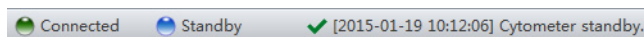
4 Confirm that the software and the Cytometer are properly connected.

a. Open the software. The Startup screen appears.

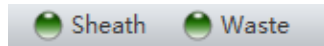
[CytExpert Electronic Record Management Software Option Shown]



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

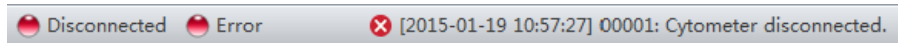


- c. Verify that the *Sheath* and *Waste* flow indicators in the lower right corner of the software screen are green indicating that the fluidics system is normal.

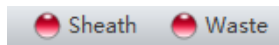


NOTE

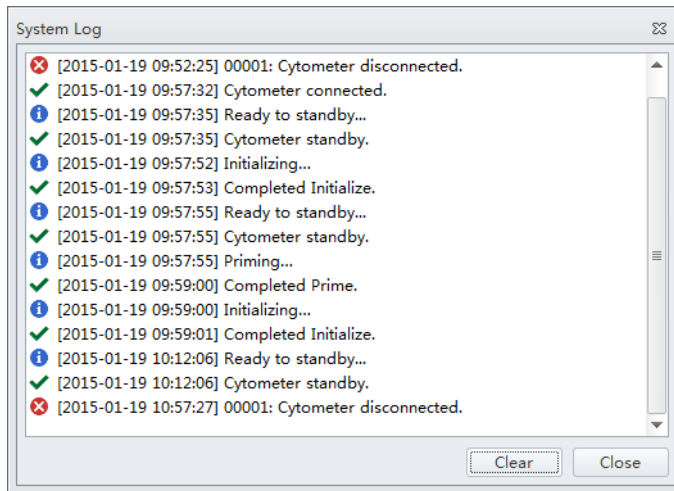
- A red connection indicator light indicates that there is a faulty connection. Ensure that the instrument is properly turned on and connected. If necessary, restart both the Cytometer and the Workstation.



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

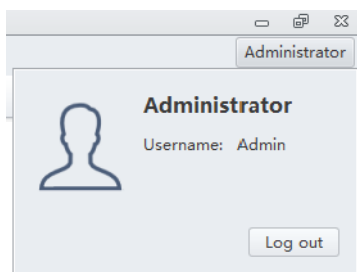


- When the waste fluid sensor is disconnected, the waste flow indicator shows that the waste container is full or nearly full.
- Select the status information in the lower left to open the system log. Send a copy of the system log to your Beckman Coulter Representative for support if a service call is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



Logging Out of the Software

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

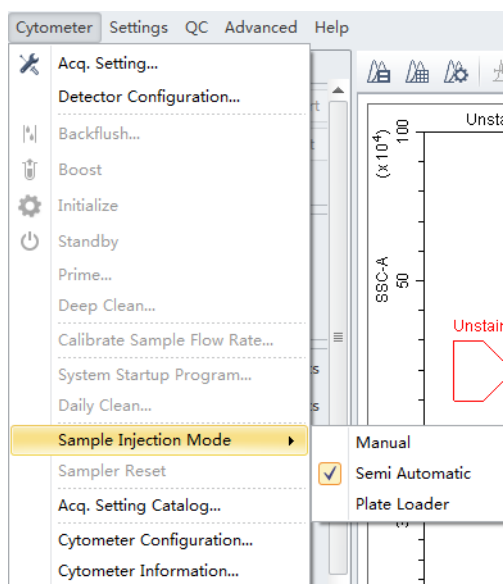


If you have the CytExpert Default software option installed, log out is not required. Select **File > Exit** to close the CytExpert software.

Selecting the Proper Sample Injection Mode

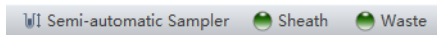


Select **Sample injection Mode** in the Cytometer Menu to change between the Semi-Automatic Injection mode and the Manual Injection mode. The Semi-Automatic Injection mode is recommended under most circumstances. The Manual Injection mode can be used for two purposes: running 1.5-mL and 2-mL microcentrifuge sample tubes and a backup mode that allows you to continue to collect data if the Semi-Automatic Injection mode is not working correctly.



Using Semi-Automatic Injection Mode

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



- 2 Select **Initialize**. The sample tube holder swings out from the standby position to the sample loading position (see [Figure 1.12](#)) 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*.



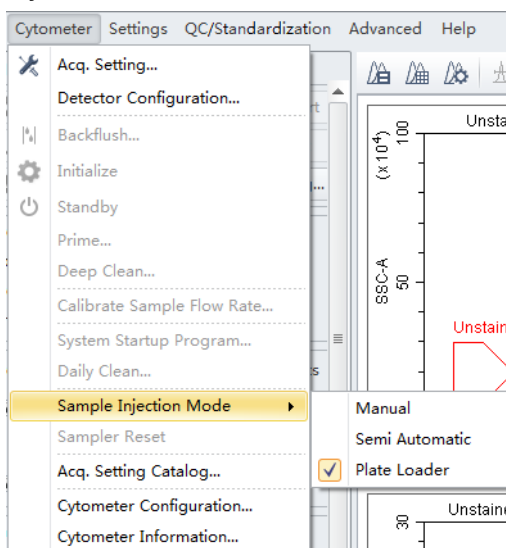
- 2 Manually swing the sample tube holder out from the standby position to the sample loading position (see [Figure 1.12](#)).
- 3 Select **Initialize**.
- 4 Load the sample tube.
NOTE The sample tube holder accommodates 1.5-mL, 2.0-mL, and 12 x 75 mm sample tubes.
- 5 Manually swing the sample tube 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]

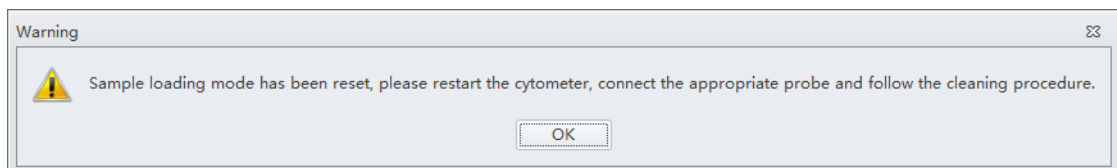
Select **Sample injection Mode** in the Cytometer Menu to change between the Semi-Automatic Injection mode, the Manual Injection mode, and the Plate Loader Injection mode. The Plate Loader Injection mode can be used for running small volumes using the following plates: 96-well flat-bottom, 96-well V-bottom,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

[CytoFLEX Shown]



Using Plate Loader Injection Mode

- 1 Select **Sample Injection Mode > Plate Loader** in the Cytometer menu to change the Sample Injection mode selection.
- 2 The restart warning prompt appears on screen. Select **OK**.



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

- 3 Turn the Cytometer's main power switch off.

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

IMPORTANT If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, refer to [APPENDIX 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 11, 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*.

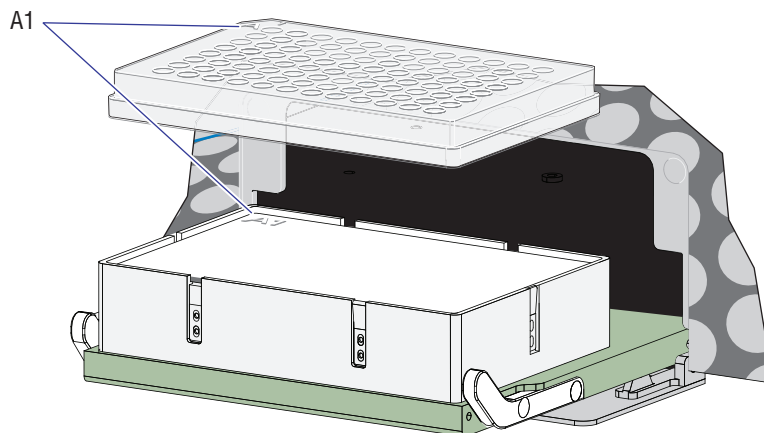


NOTE If you have a CytoFLEX LX instrument, you can turn the Cytometer's power on by selecting **Cytometer > Turn On**.

- 6 Select **Initialize**.

- 7 Select **Eject**.

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



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

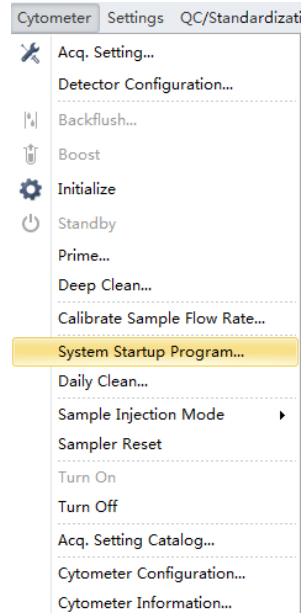
IMPORTANT Instructions on the software window vary depending on whether you are in semi-automatic injection mode or manual injection mode.

The system startup program takes approximately 8 minutes.

-
- 1** Select **Initialize**.

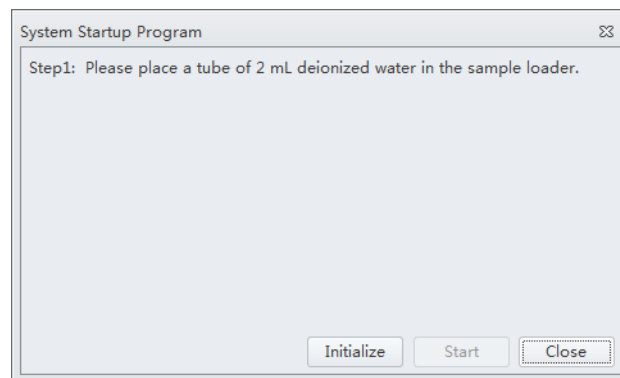
- 2 Select **System Startup Program** in the Cytometer menu.

[CytoFLEX LX Shown]

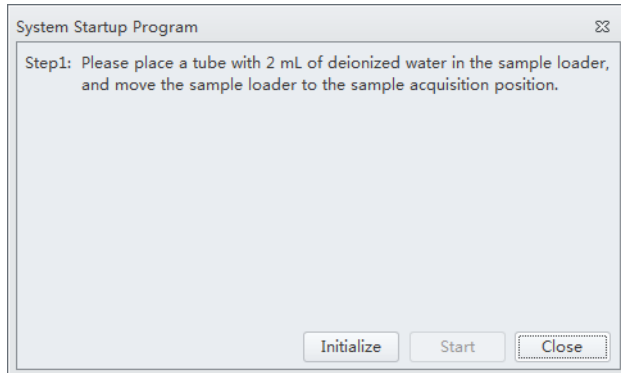


- 3 The System Startup Program window appears. Select **Initialize**.

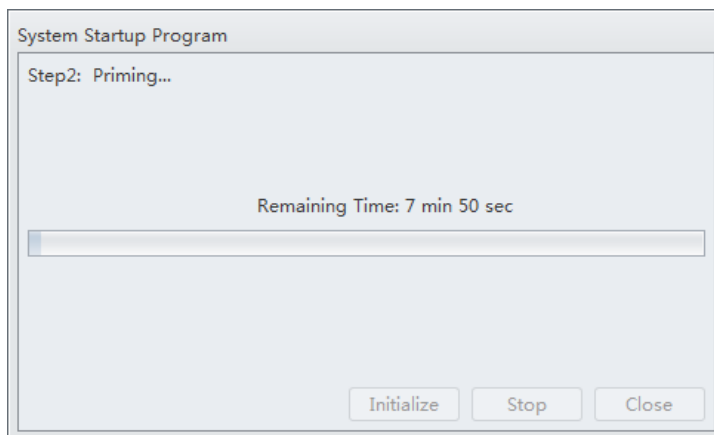
System Startup Program Window in Semi-Automatic Injection Mode



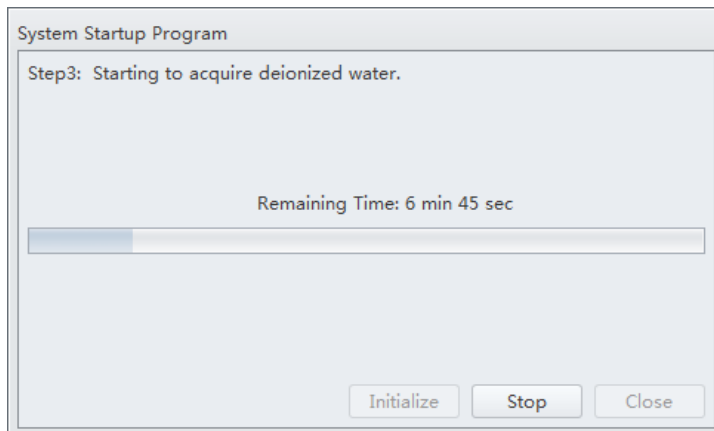
System Startup Program Window in Manual Injection Mode



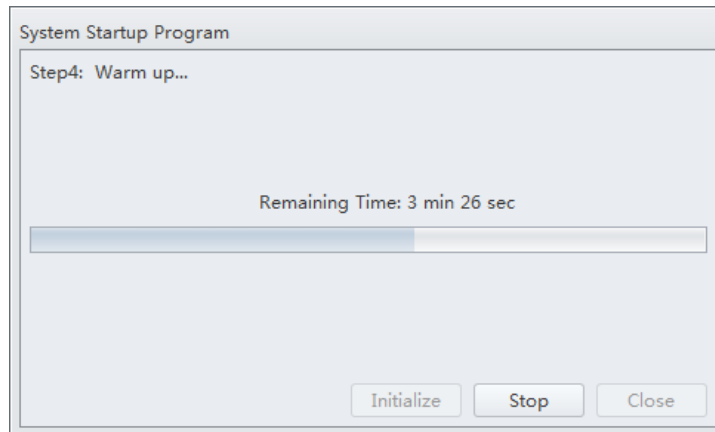
- 4 Wait for the system to initialize. Follow the on screen software prompts, then select **Start**. The instrument begins to prime. This process takes about 1 minute.



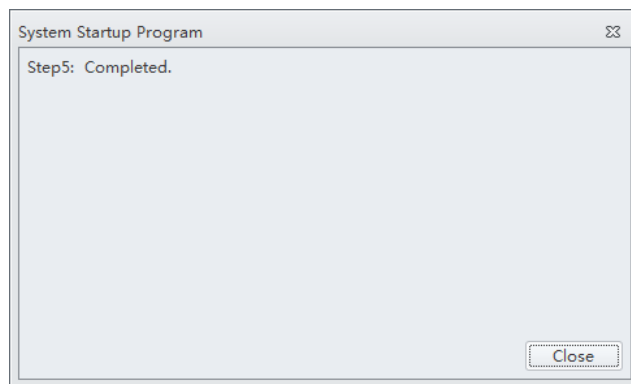
After priming, the system initializes again. The sample is loaded automatically. This process takes about 3 minutes.



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



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



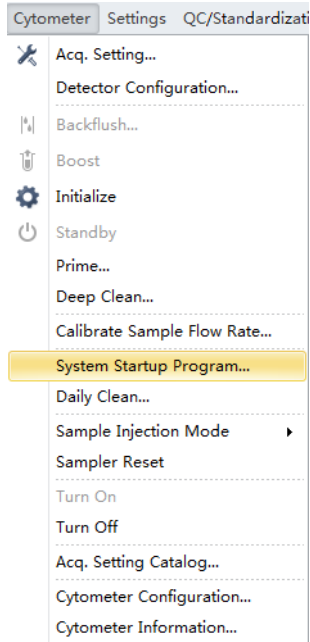
Running the System Startup Program [With Plate Loader]

The system startup program takes approximately 8 minutes.

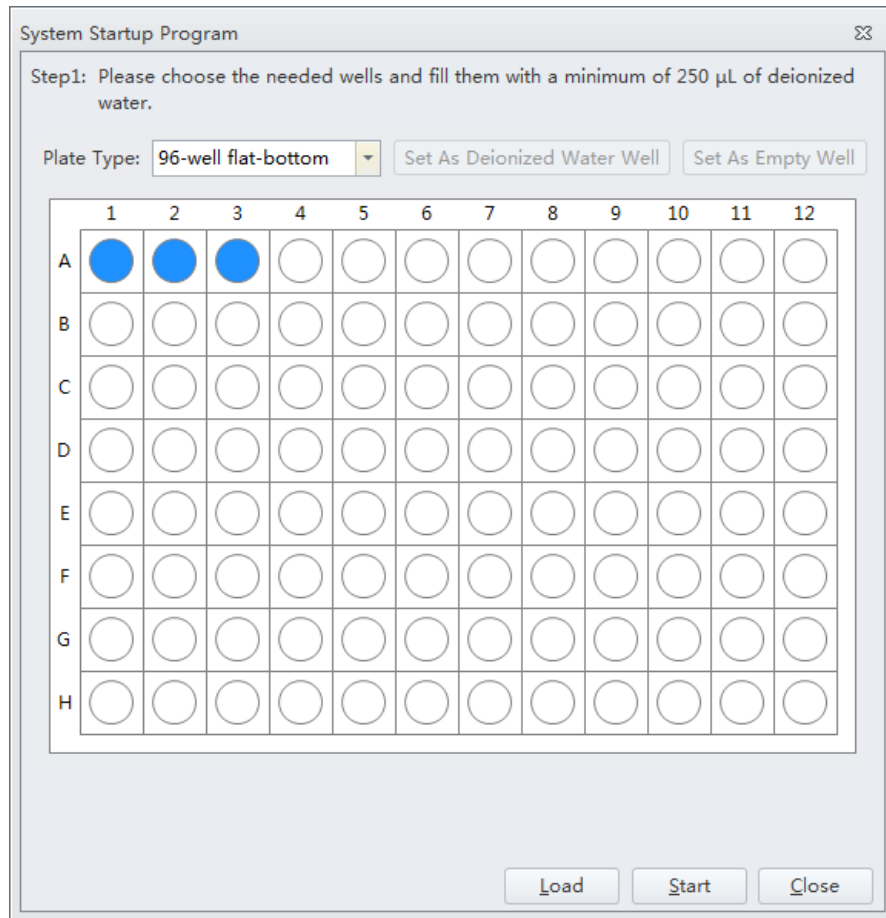
- 1 Select **Initialize**.

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

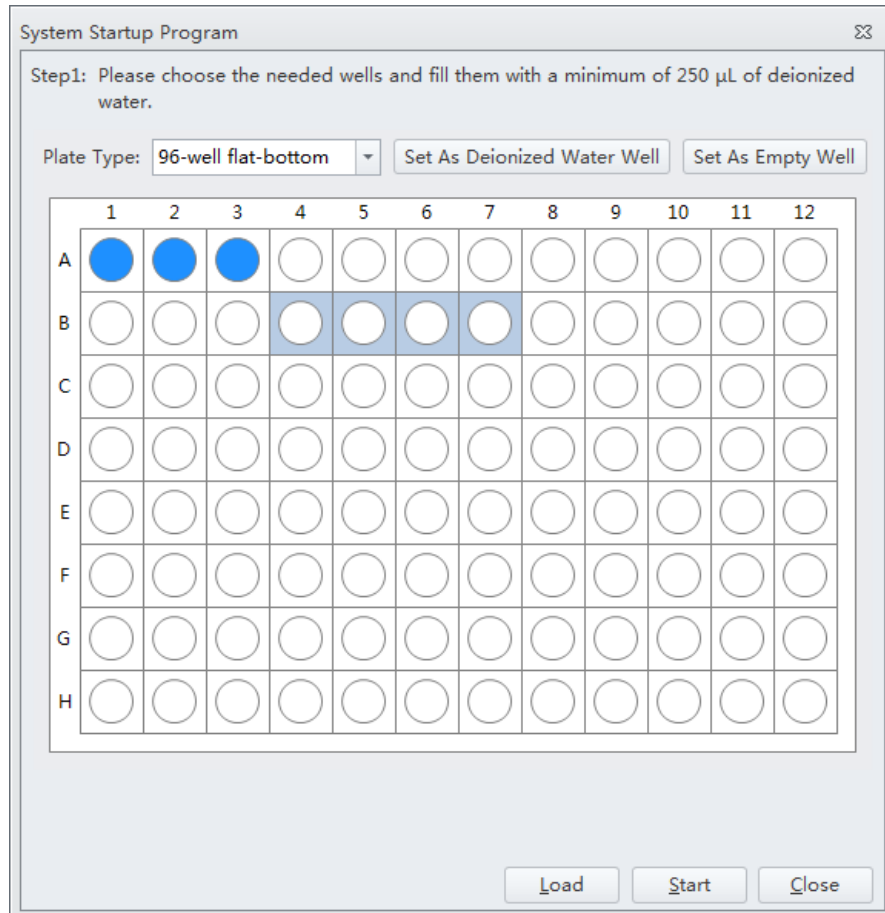
[CytoFLEX LX Shown]



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



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

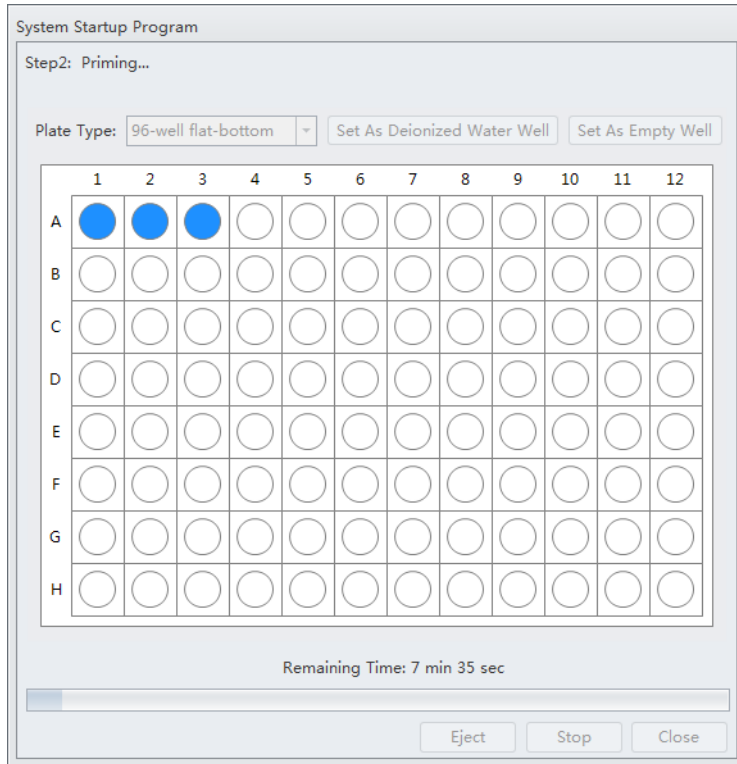


NOTE To deselect water wells, select the desired well and select **Set As Empty Well**.

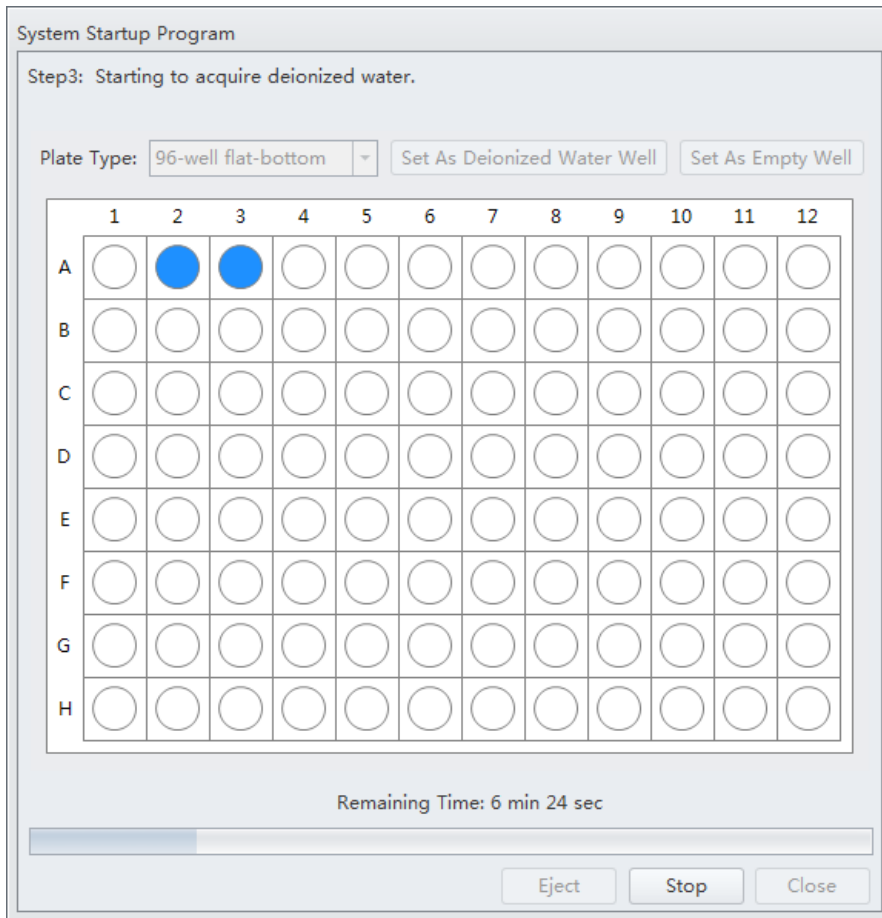
NOTE Prepare two to six sample wells with deionized water.

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

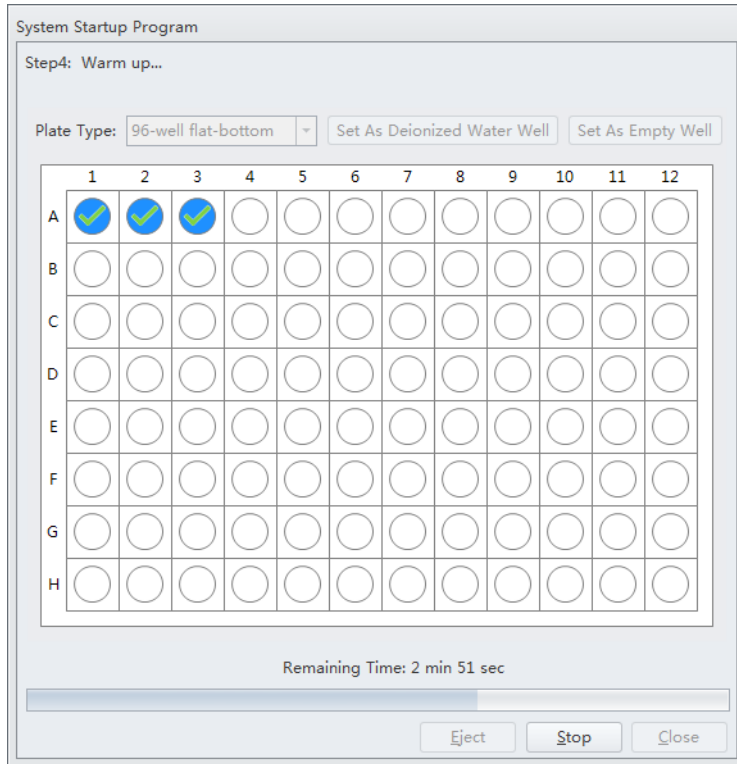
Wait for the system to initialize. The instrument begins to prime. This process takes about 1 minute.



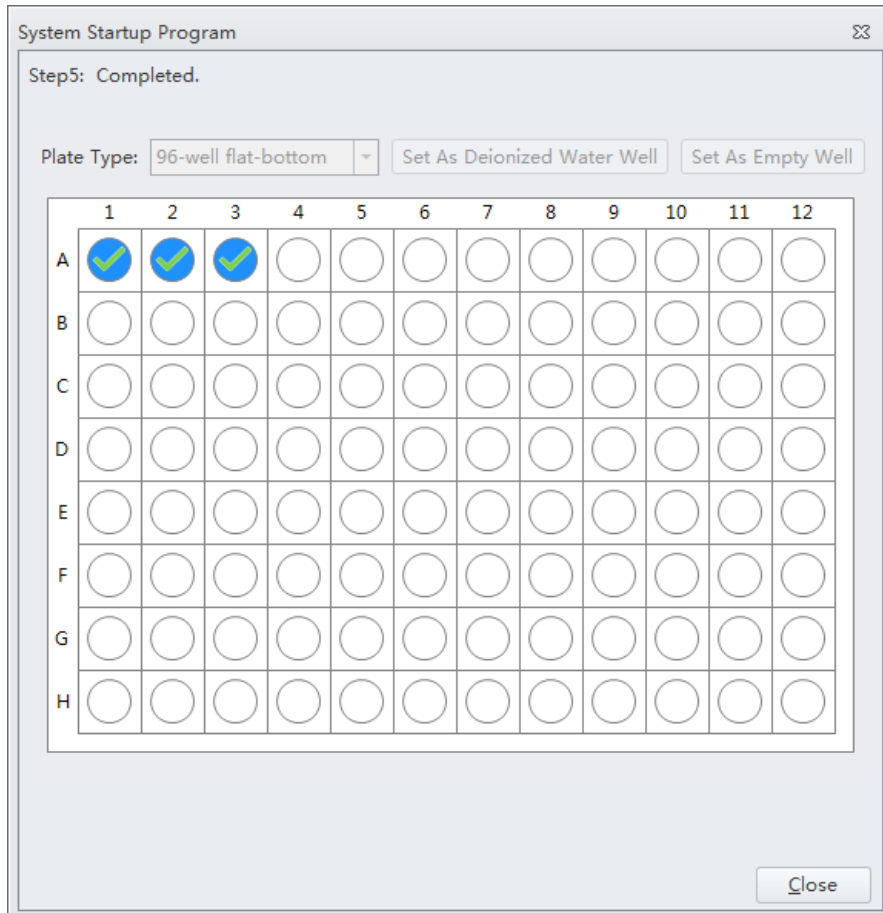
After priming, the system initializes again. The sample is loaded automatically. This process takes about 1 minute per well.



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



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

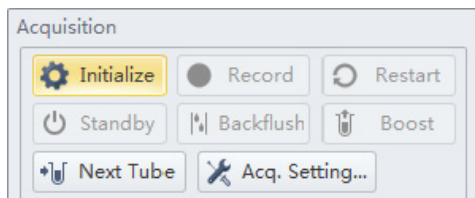


Selecting Experiments from the Start Page

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

Initializing the Instrument

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



NOTE 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](#)).

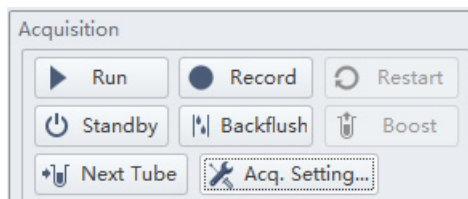
- 2 Wait for the beep indicating that the instrument properly initialized.

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 5, Data Acquisition and Sample Analysis](#).

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

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

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



Instrument Quality Control and Standardization

Overview

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

This chapter also provides information on performing standardization. CytoFLEX Daily QC Fluorospheres or any other reference material that is relevant for your application(s) may be used as the standardization sample(s).

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

The QC process verifies important system functions. The system:

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

OR

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

6. Verifies and calibrates the gain settings. If any of these parameters are outside of the operating limits, the system automatically adjusts these parameters. If the system is unable to adjust these parameters to fall within the operating limits, the system notifies you.

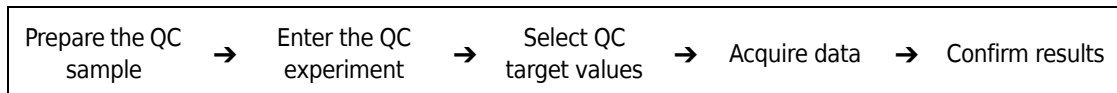
NOTE Beckman Coulter recommends performing QC on a daily basis.

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

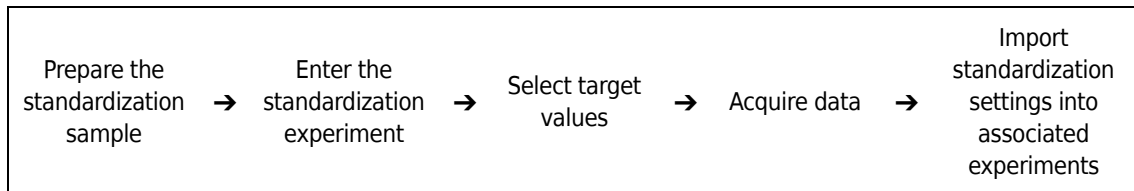
NOTE CytExpert QC includes auto daily QC routine with Levey-Jennings (LJ) charts tracking and logging.

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

QC Workflow:



Standardization Workflow:



This chapter contains information on:

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

Preparing the QC Sample

Required Materials

The following materials are required to complete the QC process:

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

Preparation Process CytoFLEX Daily QC Fluorospheres

- 1 Take one sample tube and label it as the QC sample tube.
- 2 Add approximately 1 mL of deionized water to the sample tube.
- 3 Use the vortexer or shake vigorously to thoroughly mix the bottle of CytoFLEX Daily QC Fluorospheres.
- 4 Add three drops of CytoFLEX Daily QC Fluorospheres to the sample tube.
- 5 Vortex the sample tube until a uniform mixture is achieved.
- 6 Place the sample tube in a dark location at 2-8 °C until ready to load the tube into the instrument for QC.

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

Preparation Process CytoFLEX IR Daily QC Fluorospheres

- 1 Take one sample tube and label it as the QC/IR 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 IR Daily QC Fluorospheres.
- 4** Pipette 100 μ L of CytoFLEX IR Daily QC Fluorospheres into the sample tube.
- 5** Vortex the sample tube until a uniform mixture is achieved.
- 6** Place the sample tube in a dark location at 2-8 $^{\circ}$ C until ready to load the tube into the instrument for QC.

NOTE Tubes containing diluted CytoFLEX IR Daily QC Fluorospheres should immediately be stored and sealed in a dark location at 2-8 $^{\circ}$ C for up to 5 days.

Preparing the QC Sample [With Plate Loader]

Required Materials

The following materials are required to complete the QC process:

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

Preparation Process CytoFLEX Daily QC Fluorospheres

- 1** Take one 96-well plate and label one well as the QC sample well.
- 2** Use the vortexer or shake vigorously to thoroughly mix the bottle of CytoFLEX Daily QC Fluorospheres.

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

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

5 Place the well plate in a dark location at 2-8 $^{\circ}\text{C}$ 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 $^{\circ}\text{C}$ for up to 5 days.

Preparation Process CytoFLEX IR Daily QC Fluorospheres

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

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

3 Pipette 25 μL of CytoFLEX IR Daily QC Fluorospheres to the sample well.

4 Add approximately 225 μL of deionized water to the sample well.

5 Place the well plate in a dark location at 2-8 $^{\circ}\text{C}$ until ready to load the well plate into the instrument for QC.

NOTE Well plates containing diluted CytoFLEX IR Daily QC Fluorospheres should immediately be stored and sealed in a dark location at 2-8 $^{\circ}\text{C}$ for up to 5 days.

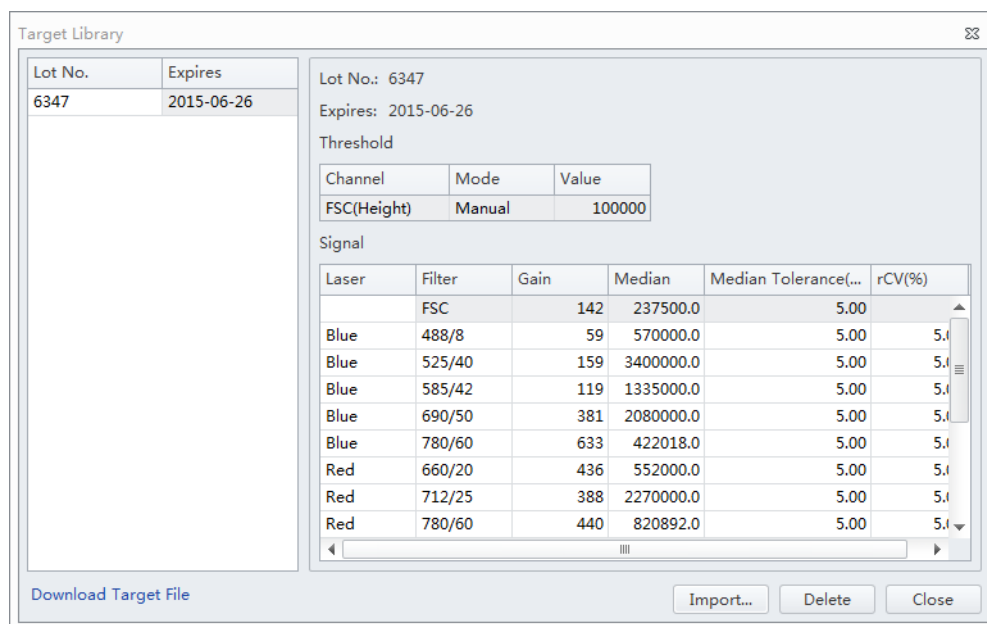
Importing Lot-Specific Target Values

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



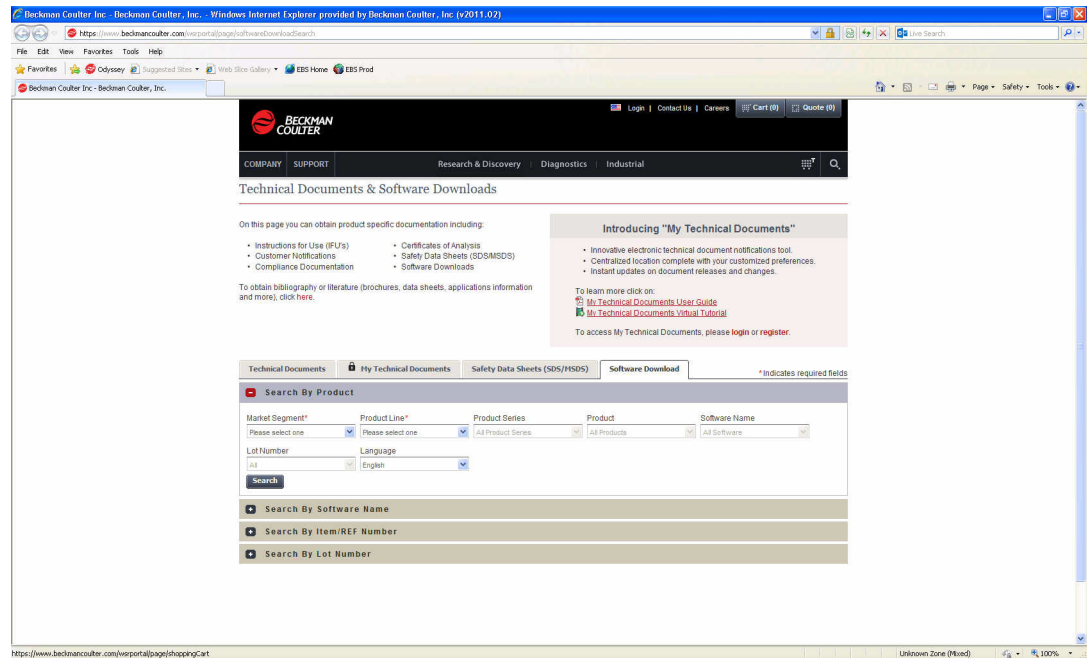
Risk of erroneous QC results. Different target value information correspond to different lot numbers. Selecting the wrong lot number will lead to erroneous QC results.

- 1 Open the CytExpert QC screen.
- 2 Select **Target Library** from the Settings menu. The Target Library window appears.



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

3 Select **Download Target File**. The Beckman Coulter Technical Documents and Software Downloads page appears.



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

4 In the Search By Product section of the screen, select the following:

- a. Select **Research & Discovery** from the Market Segment drop-down menu.
- b. Select **Flow Cytometry** from the Product Line drop-down menu.
- c. Select **Instruments** from the Product Series drop-down menu.
- d. Select **CytoFLEX** from the Product drop-down menu.
- e. Select **CytoFLEX QC Fluorospheres Target** 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.

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

Search By Product

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

Lot Number | Language
 All | English

Search

+ Search By Software Name

+ Search By Item/REF Number

+ Search By Lot Number

5 Select **Search**.

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

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

Search By Product

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

Lot Number | Language
 All | English

Search

+ Search By Software Name

+ Search By Item/REF Number

+ **Search By Lot Number**

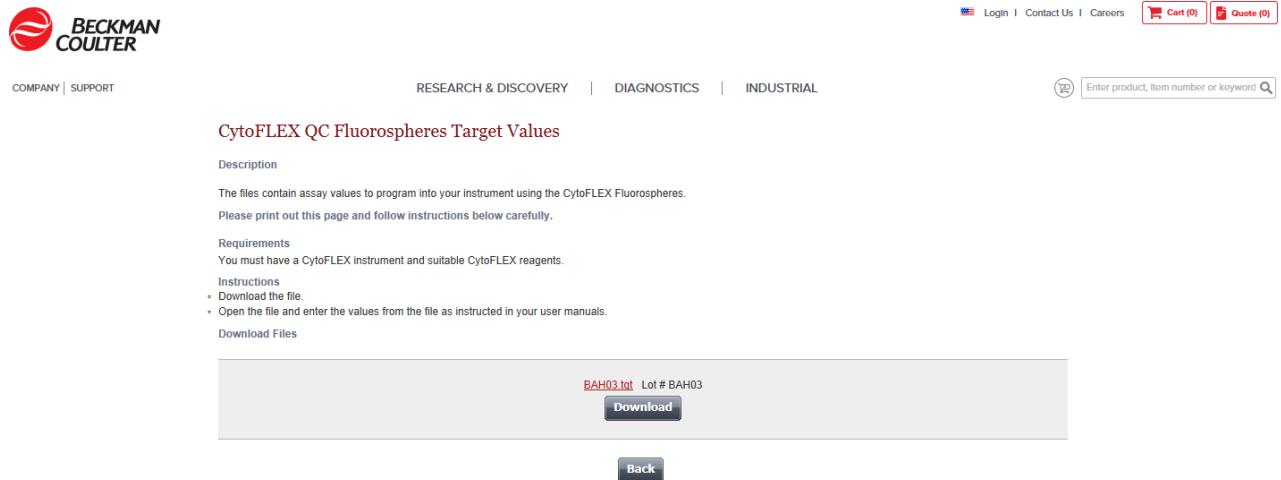
Software Download Search Results

First Prev 1 Next Last View 25 per page

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

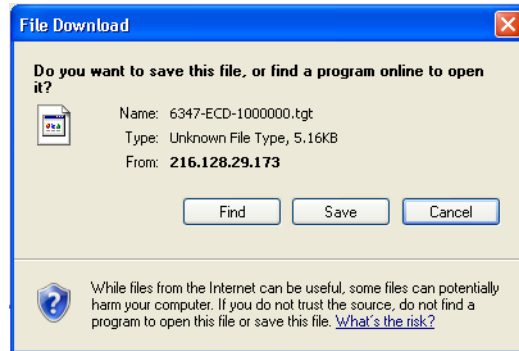
First Prev 1 Next Last Displaying 1 to 1 of 1 software found.

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



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

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



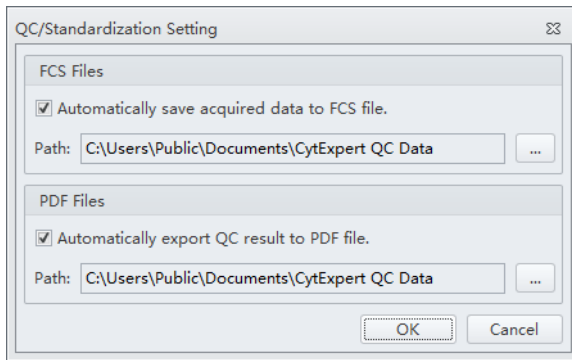
- 10 Select **Import** from the Target Library window in the CytExpert software.


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

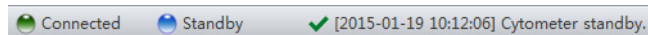
- 12 Select **Close** to exit the Target Library window.

Collecting QC Data

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



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



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

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

-
- 3 Verify the laser settings. Refer to [Laser Settings](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#).

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

[CytoFLEX LX Shown]

QC Report

Bead Lot No.: BAH02_SE
 Bead Expires: 2019-07-20
 Cytometer Name: Cyto-LX
 Detector Configuration: Cyto-LX
 Loader Type: Semi Automatic
 QC Date: 2016-11-02 15:07
 Cytometer SN:

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

Laser

Laser	Delay(us)	Default Delay(us)	Difference Delay(us)	Power(mW)	Target Power(mW)	Result
NUV	72.80	72.96	-0.16	60	50-70	✓
Blue	0.00	0.00	0.00	49	40-60	✓
Red	36.16	36.32	-0.16	49	40-60	✓
Yellow	-35.68	-35.84	0.16	30	20-40	✓
Violet	-70.24	-70.40	0.16	88	70-120	✓

Signal Value

Parameter	Gain	Target Gain	%Difference Target Gain	Median	Target Median	%Difference Target Median	rCV(%)	Target rCV(%)	Width	Result
FSC	252	252	0.00	206408.8	209181.4	-1.33	-	-	802.0	✓
SSC	81	81	0.00	604528.9	585960.0	3.17	-	-	883.2	✓
PB450	56	56	0.00	910944.4	920622.8	-1.05	2.13	5.00	1316.2	✓
K0525	34	34	0.00	234576.1	233571.7	0.43	2.03	5.00	1315.0	✓
Violet610	202	202	0.00	413936.1	416223.8	-0.55	2.23	5.00	1318.8	✓
Violet660	185	185	0.00	108776.3	108938.6	-0.15	2.32	5.00	1312.5	✓
Violet780	62	62	0.00	63120.5	62851.0	0.43	2.03	5.00	1294.6	✓
DAPI	69	69	0.00	8750280.0	8804270.0	-0.61	0.80	7.00	1604.2	✓
NUV525	21	21	0.00	951872.7	937573.0	1.53	0.78	7.00	802.9	✓
APC	341	341	0.00	326824.1	328142.5	-0.40	3.55	5.00	1063.2	✓
APC-A700	329	329	0.00	545977.0	5340872.0	2.23	3.74	5.00	1087.4	✓
APC-A750	44	44	0.00	666992.6	649742.0	2.65	3.78	5.00	1070.0	✓

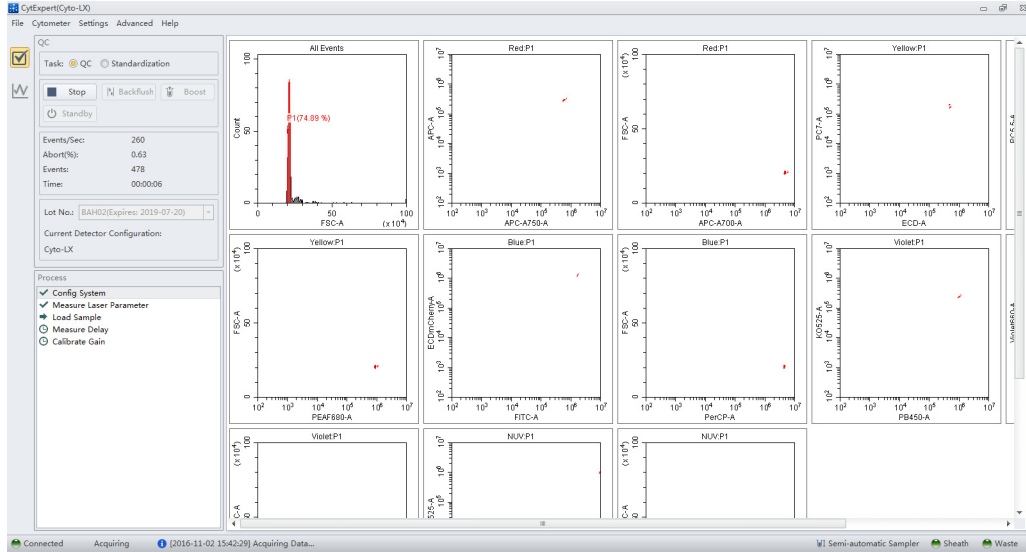
Ensure that the QC bead lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, refer to [Importing Lot-Specific Target Values](#), then select the proper lot number.

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

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

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

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

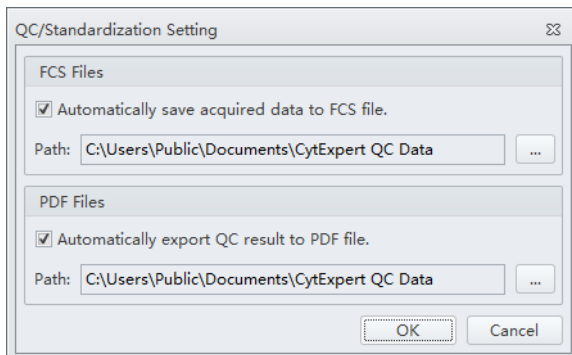



8 If the sampling rate is too low, the Cytometer stops the QC run and displays a prompt alerting you that the QC run failed to reach the required event flow rate. This is not considered a QC failure. If this situation occurs, increase the sample concentration by adding one drop of CytoFLEX Daily QC Fluorospheres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

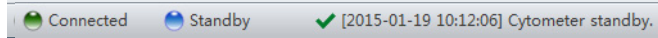
9 Run Daily Clean to remove any residual fluorosphere particles. Refer to [Daily Clean](#) in [CHAPTER 10, Cleaning Procedures](#).

Collecting QC Data [With Plate Loader]

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



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



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

- 2 Verify the detector configuration. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#).

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

- 3 Verify the laser settings. Refer to [Laser Settings](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#).

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

[CytoFLEX LX Shown]

QC Report

Bead Lot No.: BAH02-SF
 Bead Expir: 2019-09-20
 QC Date: 2016-11-18 15:30
 Cytometer Name:
 Detector Configuration: Cyto-LX
 Cytometer SN:
 Loader Type: Semi Automatic

Threshold
 Channel: FSC(H) Mode: Manual Value: 50000

Laser	Delay(µs)	Default Delay(µs)	Difference Delay(µs)	Power(mW)	Target Power(mW)	Result
NIUV	80.80	80.80	0.00	65	50-70	✔
Blue	0.00	0.00	0.00	51	40-60	✔
Red	34.72	34.72	0.00	50	40-60	✔
Yellow	-46.24	-46.24	0.00	30	20-40	✔
Violet	-49.12	-49.12	0.00	90	70-120	✔

Signal Value

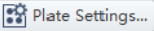
Parameter	Gain	Target Gain	%Difference Target Gain	Median	Target Median	%Difference Target Median	σCV(%)	Target σCV(%)	Width	Result
FSC	149	149	0.00	256788.2	237089.0	-0.12	-	-	961.7	✔
SSC	321	321	0.00	1632097.0	1653480.0	-1.27	-	-	1086.9	✔
PR450	35	35	0.00	769325.1	763724.0	0.73	1.97	5.00	1376.7	✔
KD525	86	86	0.00	833732.9	827384.0	0.77	2.15	5.00	1377.9	✔
Violet610	220	220	0.00	646996.1	648212.0	-0.19	2.34	5.00	1380.4	✔
Violet650	223	223	0.00	144385.5	142294.0	1.49	2.43	5.00	1381.6	✔
Violet710	270	270	0.00	505566.8	505794.0	-0.04	2.67	5.00	1402.8	✔
DAPI	43	43	0.00	6076546.0	5884821.0	3.26	1.90	7.00	1137.6	✔
NIUV525	114	114	0.00	4584568.0	4576957.0	0.19	1.82	7.00	921.7	✔
HoectestRed	150	150	0.00	1356143.0	139745.0	-0.27	1.71	7.00	942.5	✔
APC	72	72	0.00	81376.7	81698.0	-0.38	2.19	5.00	1401.0	✔
APC-A700	109	109	0.00	2055745.0	2065561.0	-0.48	2.35	5.00	1415.8	✔
APC-A750	64	64	0.00	729272.6	739500.0	-1.38	2.34	5.00	1414.0	✔
PE	72	72	0.00	294380.6	291292.0	0.36	2.79	5.00	1325.2	✔
ECD	95	95	0.00	31092.6	31334.0	-0.60	2.79	5.00	1323.0	✔
PC5.5	162	162	0.00	379588.9	381424.0	-3.02	3.18	5.00	1324.6	✔
PEAF80	122	122	0.00	907208.3	907040.0	0.02	3.41	5.00	1330.6	✔
PC7	153	153	0.00	449256.5	453590.0	-0.93	3.02	5.00	1331.7	✔
FTC	107	107	0.00	165840.0	165956.0	-0.21	3.11	5.00	1096.1	✔
ECDmChe...	158	158	0.00	1113664.0	1120281.0	-0.59	1.24	5.00	1078.6	✔
PerCP	85	85	0.00	1084768.0	1106620.0	-1.97	1.65	5.00	1092.7	✔

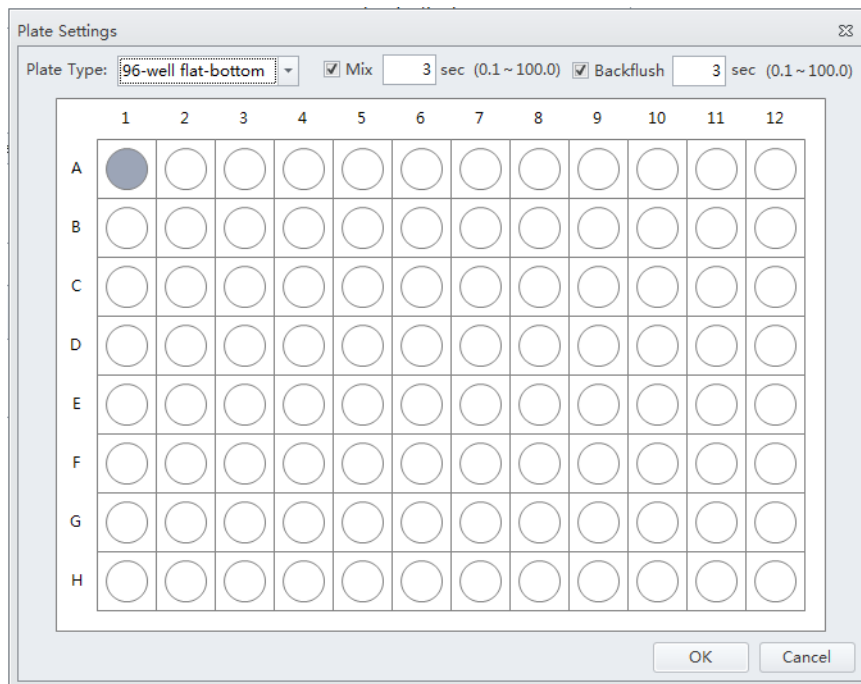
Ensure that the QC bead lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, refer to [Importing Lot-Specific Target Values](#) in [CHAPTER 4, Instrument Quality Control and Standardization](#), then select the proper lot number.

-
- 5 Select **Initialize**.

 - 6 Select **Eject**.

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

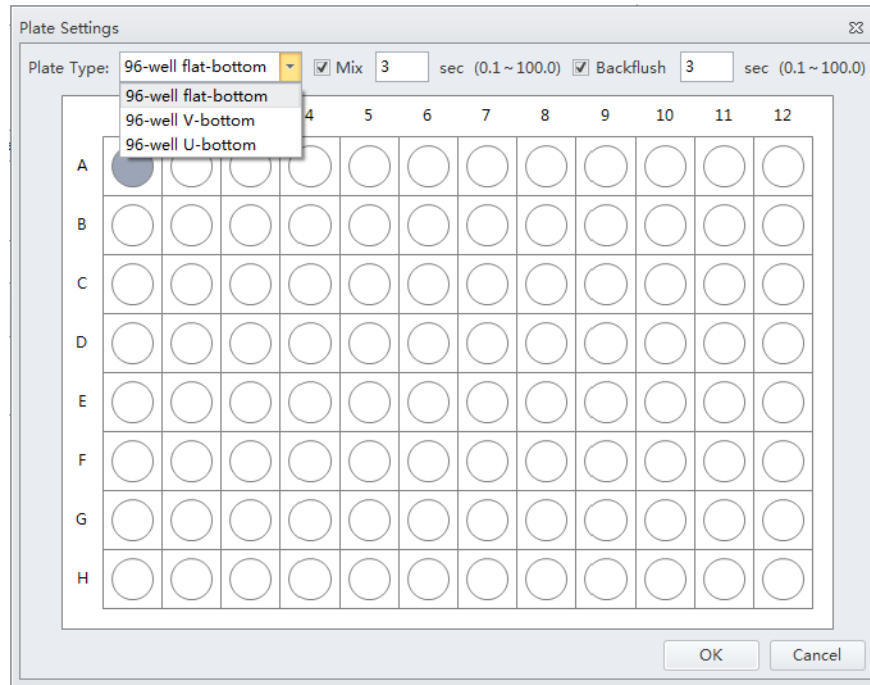
 - 8 Select  **Plate Settings...**. The Plate Settings window appears.



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

- 9 Select the appropriate QC well.

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

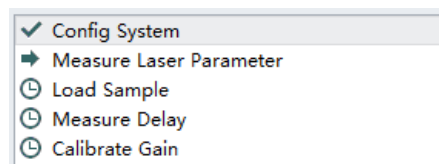


- 11 Select the Mix and Backflush settings in the top of the Plate Settings window.

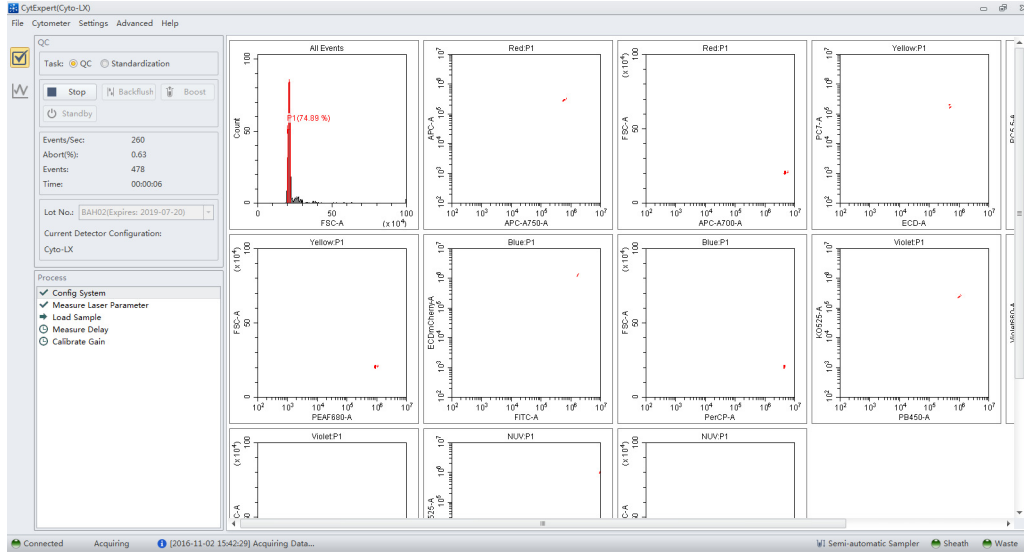
- 12 Select **OK**.

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

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



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



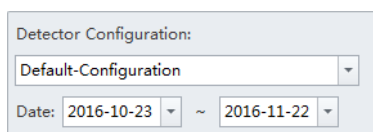
14 If the sampling rate is too low, the Cytometer stops the QC run and displays a prompt that the QC run fails to reach the required event flow rate. This is not considered a QC failure. If this situation occurs, increase the sample concentration by adding one drop of CytoFLEX Daily QC Fluorospheres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

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

Confirming Results

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

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



NOTE At least one date range must be specified.

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



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

NOTE The results column indicates a passing QC result with a ✔ and a failed QC result with ✘.

QC results must meet the following criteria to pass:

- The gain differences must be $\leq 20\%$ from the target gain.
- The median fluorescence intensity (MFI) differences must be $\leq 5\%$ from the target MFI.
- The rCV must be $\leq 5\%$.

NOTE The CytoFLEX Daily QC Fluorospheres rCV pass QC criteria is $\leq 5\%$.

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



QC Report

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







Threshold

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

Laser

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

Signal Value

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

Specifications

Delay: $-5.00\mu\text{s} \leq \text{Difference Delay} \leq 5.00\mu\text{s}$.
 Gain: $-20.00\% \leq \% \text{Difference Target Gain} \leq 20.00\%$.
 Median: $-5.00\% \leq \% \text{Difference Target Median} \leq 5.00\%$.
 rCV: $\text{rCV}(\%) \leq \text{Target rCV}(\%)$.

Result

QC Failed.

Comment



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

If QC fails, follow the procedure below:

- Verify whether the beads used were within their shelf life and stored in accordance with the appropriate instruction manual.
- Verify whether the allocated sample tube was prepared as required and correctly positioned.
- Run [Priming the Flow Cell](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#), and retest.

- d. Run [Daily Clean](#) in [CHAPTER 10, Cleaning Procedures](#), and retest.
- e. Run [Deep Clean Procedure](#) in [CHAPTER 10, Cleaning Procedures](#), and retest.
- f. Repeat Steps [c-d](#).


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

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


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

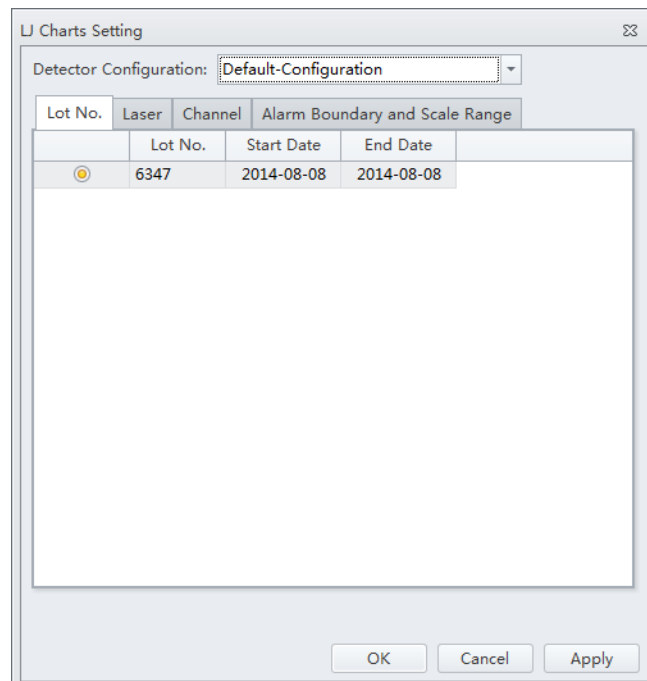
Creating Levey-Jennings Charts

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

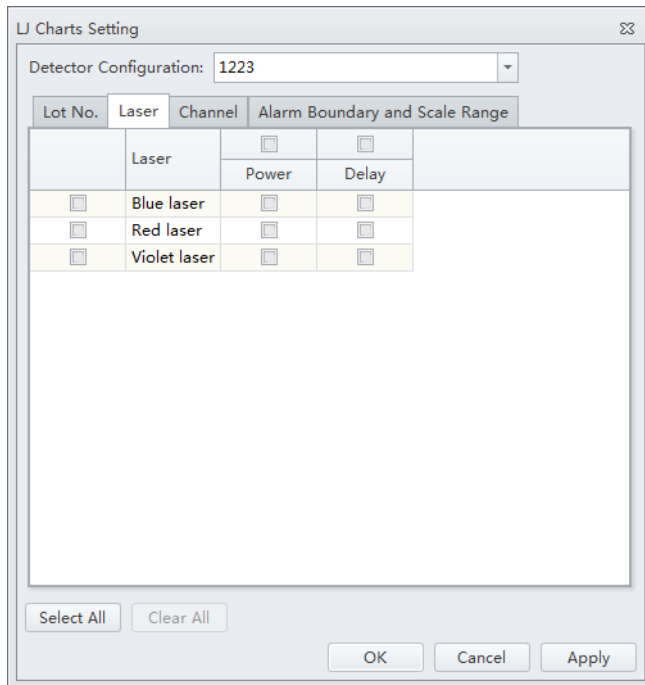
2 Select LJ chart  on the left side of the screen.

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

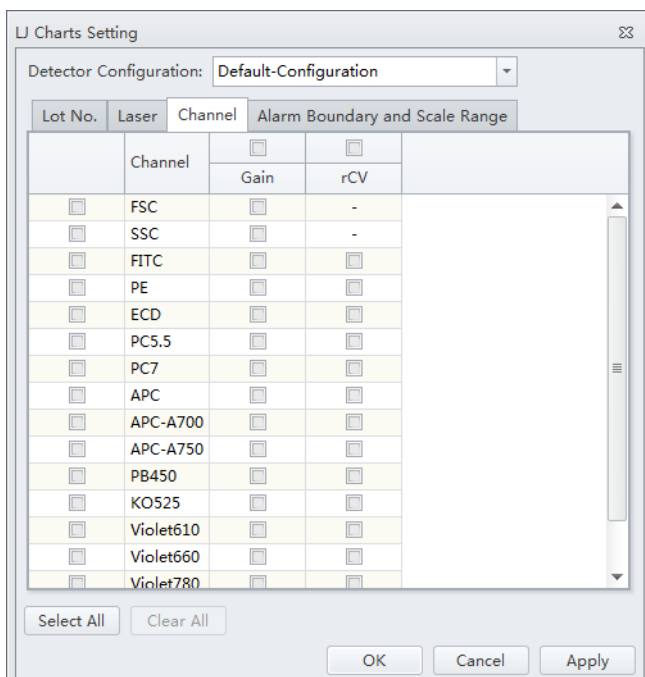
3 Select LJ Chart Settings  on the top of the LJ Chart screen. The LJ Chart Settings screen appears.



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



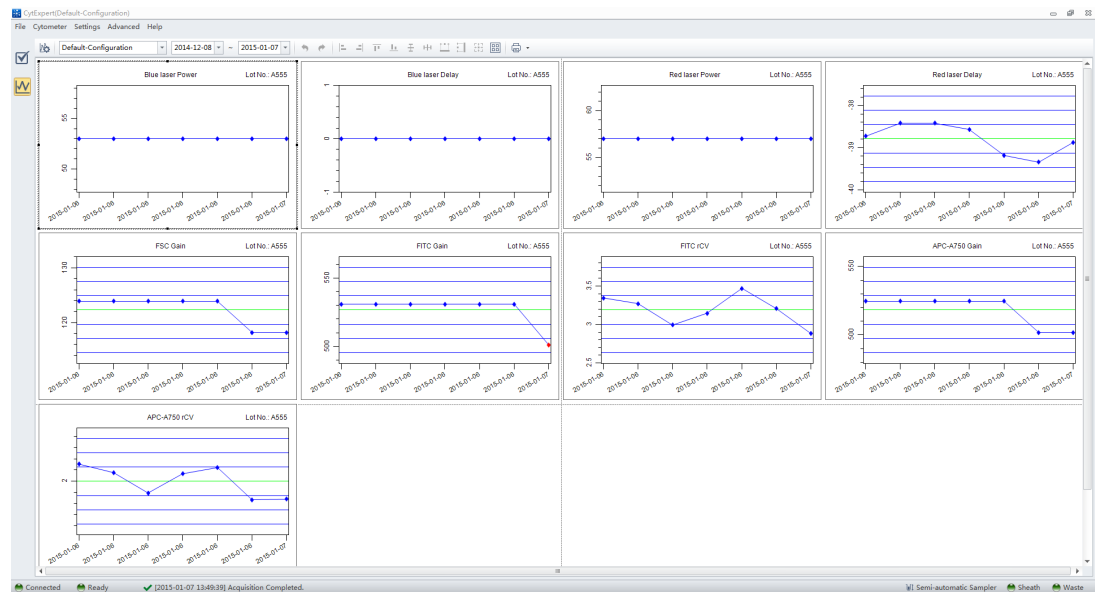
- 5 Select the **Channels** tab, and select each channel checkbox as needed.



6 Select **Apply**.

7 Select **OK**.

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



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

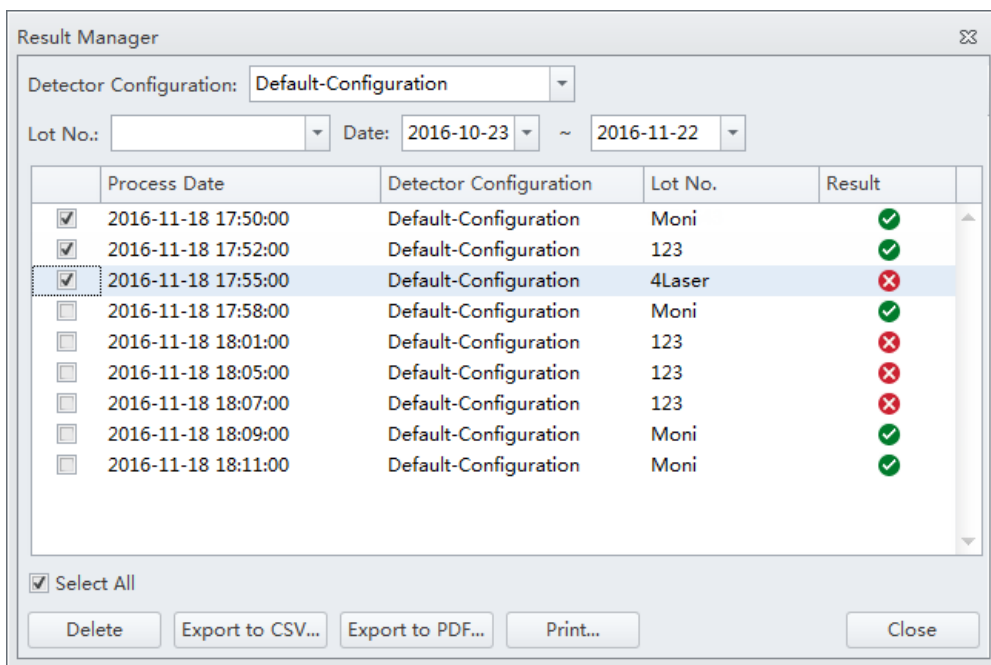
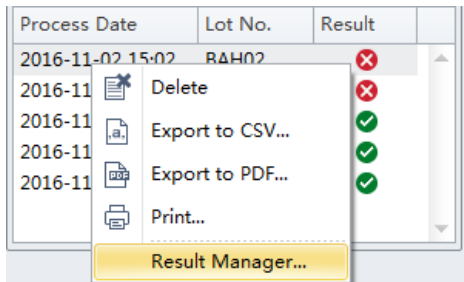


9 Select **Close QC/Standardization** in the File menu to exit the QC screen.

QC Result Manager

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

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



Standardization

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

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

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

Item	Lot No.	Expires
------	---------	---------

Lot No.:

Expires:

Threshold

Type	Channel	Mode	Value
------	---------	------	-------

Signal

Channel	Median	Median Tolerance(%)
---------	--------	---------------------

Add... Delete Edit... Duplicate... Import... Export... Close

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

Adding a New Standardization Item

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

Channel	Median	Median Tolerance(%)
<input checked="" type="checkbox"/> FSC	0.00	5.00
<input type="checkbox"/> SSC	0.00	5.00
<input type="checkbox"/> Fitc	0.00	5.00
<input type="checkbox"/> mCherry	0.00	5.00
<input type="checkbox"/> PerCP	0.00	5.00
<input type="checkbox"/> APC	0.00	5.00
<input type="checkbox"/> APC-A700	0.00	5.00
<input type="checkbox"/> APC-A750	0.00	5.00
<input type="checkbox"/> PE	0.00	5.00
<input type="checkbox"/> ECD	0.00	5.00
<input type="checkbox"/> PC5.5	0.00	5.00
<input type="checkbox"/> PE-AF680	0.00	5.00
<input type="checkbox"/> PC7	0.00	5.00
<input type="checkbox"/> PB450	0.00	5.00
<input type="checkbox"/> KO525	0.00	5.00
<input type="checkbox"/> Violet610	0.00	5.00
<input type="checkbox"/> Violet660	0.00	5.00
<input type="checkbox"/> VC1-300	0.00	5.00

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

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

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

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

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

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

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

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

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

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

NOTE FSC is a required channel.

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

The saved results display in the Standardization Target Library window.

Standardization Target Library

Item	Lot No.	Expires
6C STD.	123456	2016-12-01

Lot No.: 123456
Expires: 2016-12-01

Threshold

Type	Channel	Mode	Value
Standardization Test Setting	FSC(Height)	Manual	50000
Acquisition Setting	FSC(Height)	Manual	50000

Signal

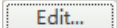
Channel	Median	Median Tolerance(%)
FSC	10000000.00	5.00
SSC	10000000.00	5.00
V450-PB	5000000.00	5.00
V525-KrO	956025.00	5.00
R660-APC	300000.00	5.00
R763-APCA750	652000.00	5.00
Y585-PE	80000.00	5.00
B525-FITC	100000.00	5.00

Add... Delete Edit... Duplicate... Import... Export... Close

- 6 Select  to exit the Standardization Target Library window.

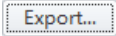
Editing Standardization Item Parameters

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

 .


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

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

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

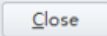
-
- 4 Select  to exit the Standardization Target Library window.

Importing a Standardization Item

-
- 1 Select  on the Standardization Target Library window.

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

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

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

Deleting Standardization Items

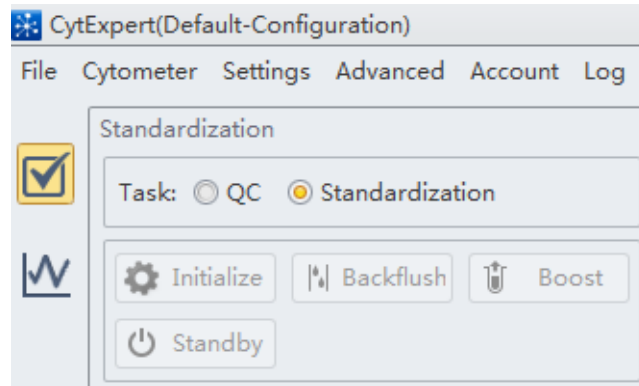
Select an item from the Item column on the Standardization Target Library window and select

. Select  to exit the Standardization Target Library window.

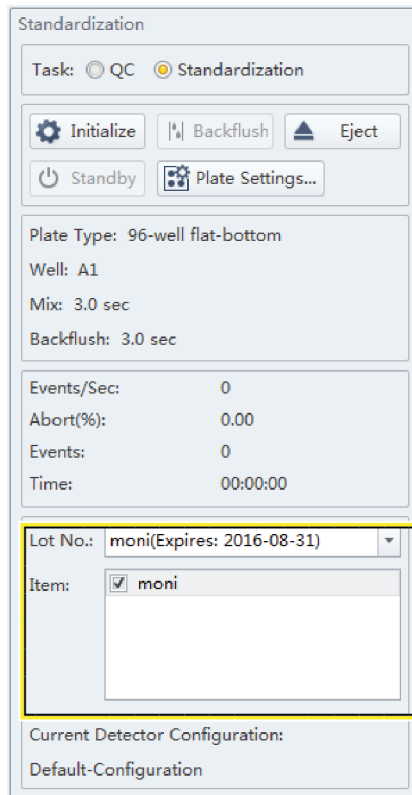
Applying the Standardization in QC

-
- 1 Open the CytExpert QC screen.

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

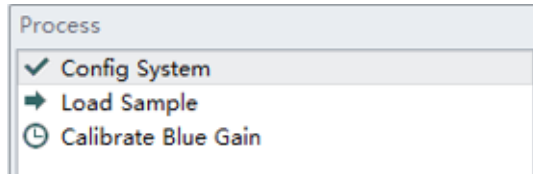


- 3 Select the Lot No. and the Items to be applied.

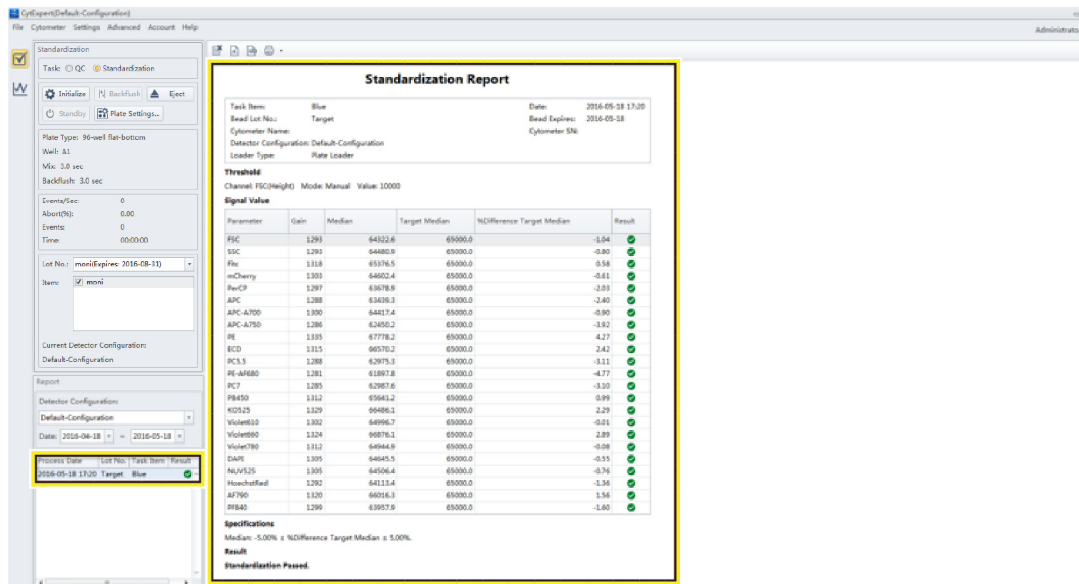


4 Select  .

The Process section of the screen displays the process details.



Once the process is complete, the Custom Setting Calibration Report displays.



Standardization Report

Task Item: Blue Date: 2016-05-18 17:30
 Bead Lot No.: Target Bead Expires: 2016-05-18
 Cytometer Name: Cytometer SN:
 Detector Configuration: Default Configuration
 Loader Type: Rate Loader

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

Parameter	Gain	Median	Target Median	%Difference Target Median	Result
FSC	1293	44323.6	65000.0	-1.04	✔
SSC	1293	94485.9	65000.0	-0.90	✔
Flx	1318	45376.5	65000.0	0.58	✔
mCherry	1303	44602.4	65000.0	-0.61	✔
Flu-C9	1297	43078.9	65000.0	-2.03	✔
APC	1288	63439.3	65000.0	-2.40	✔
APC-A700	1300	44417.4	65000.0	-0.90	✔
APC-A750	1286	62450.2	65000.0	-3.92	✔
PE	1305	67778.2	65000.0	4.27	✔
ECD	1115	66079.2	65000.0	2.42	✔
PC3.3	1288	42975.3	65000.0	-3.11	✔
PE-AF680	1281	41887.8	65000.0	-4.77	✔
PC7	1295	62967.6	65000.0	-3.10	✔
PE450	1312	65643.2	65000.0	0.99	✔
KO525	1329	94486.1	65000.0	2.29	✔
Vol4803	1302	64986.7	65000.0	-0.61	✔
Vol4890	1324	98876.1	65000.0	2.89	✔
Vol4790	1312	64944.9	65000.0	-0.68	✔
DAR1	1305	44645.5	65000.0	-0.55	✔
NLV325	1305	44566.4	65000.0	-0.74	✔
Household	1290	64113.4	65000.0	-1.56	✔
AF760	1320	64056.3	65000.0	1.54	✔
PE840	1299	63957.8	65000.0	-1.60	✔

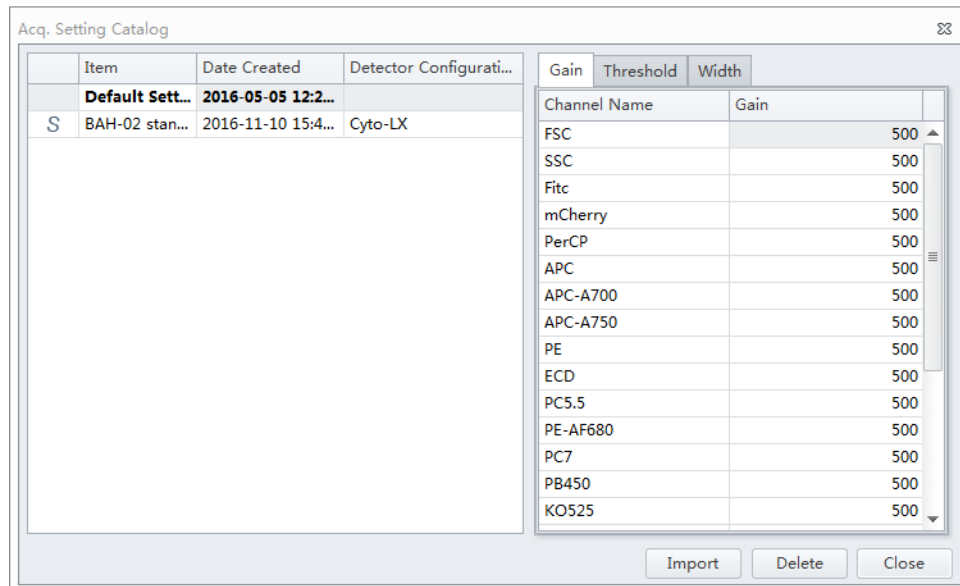
Specifications
 Median: -5.00% ± %Difference Target Median ± 5.00%

Result
Standardization Passed.

Process Date: 2016-05-18 17:30 Task Item: Target Bead Lot No.: Blue

5 Verify the gain settings.

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



NOTE S designates test items from Standardization.

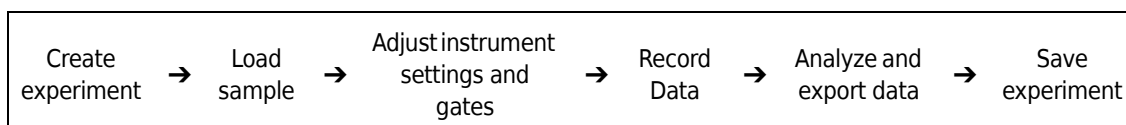
- b. Select the desired Test Item, from the list in the Gain, Threshold, and Width tabs.

Data Acquisition and Sample Analysis

Overview

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

Workflow:



This chapter contains information on:

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

Creating an Experiment

 **CAUTION**

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

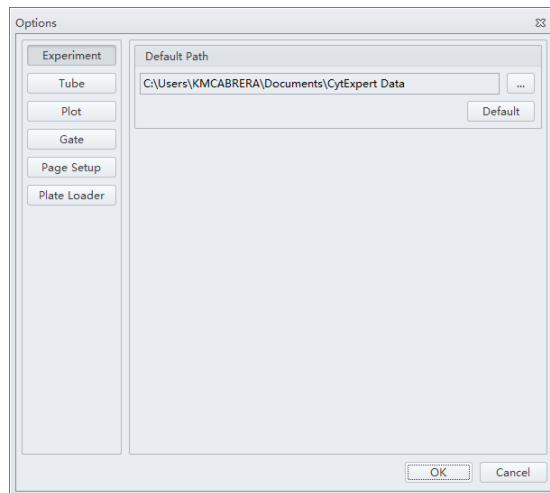
- 1 Open the CytExpert software and confirm that the instrument is connected. Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#).
- 2 Verify the detector configuration. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#).

-
- 3** Verify the laser settings. Refer to [Laser Settings](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#).
-
- 4** Create or open an experiment using one of the following methods:
- Create a new experiment:
 - Select **New Experiment** on the Start page, specify the file path, and save the experiment.
Or
 - Select **New Experiment** in the File menu, specify the file path, and save the experiment.
 - Create a new experiment from a template:
 - Select **New Experiment from Template** on the Start page. Select **Browse** next to New Experiment and specify the file path for the new experiment, then select **Browse** next to Template and specify the file path to the existing template.
Or
 - Select **New Experiment from Template** in the File menu, specify the file path and save the experiment.
Or
 - Select the Template tab on the Start page and select the template from the list of recently used templates. Specify the file path and save the experiment.
 - Open an existing experiment:
 - **If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option:** Select **Open Experiment** on the Start page, specify the file path and save the experiment.
If you are using the CytExpert Electronic Record Management Software Option: Select **Open Experiment** on the Start page, specify the experiment file and open the experiment.
Or
 - **If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option:** Select **Open Experiment** in the File menu, specify the path and save the experiment.
If you are using the CytExpert Electronic Record Management Software Option: Select **Open Experiment** in the File menu, specify the experiment file and open the experiment.
Or
 - **If you are using either the CytExpert Default Software Option or the CytExpert User Management Software Option:** Select the Experiment tab on the Start page and select the experiment from the list of recently opened experiments. Specify the file path and save the experiment.
If you are using the CytExpert Electronic Record Management Software Option: Select the Experiment tab on the Start page and select the experiment from the list of recently opened experiments. Specify the experiment file and open the experiment.

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

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

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



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

Creating an Experiment [With Plate Loader]

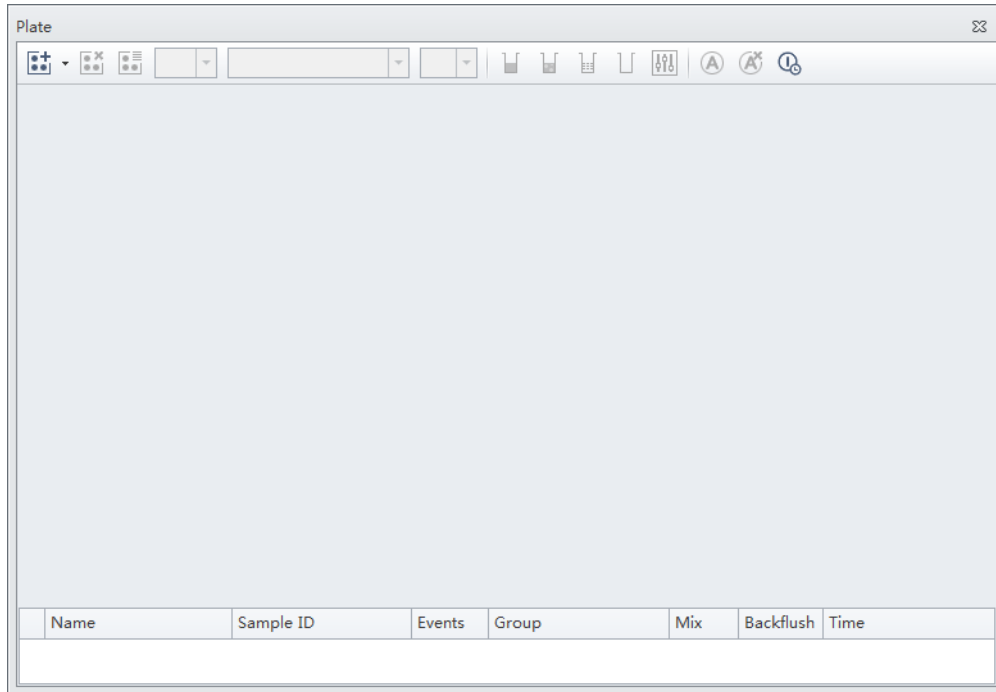
 **CAUTION**

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

- 1 Create an experiment. Refer to [Creating an Experiment](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#).

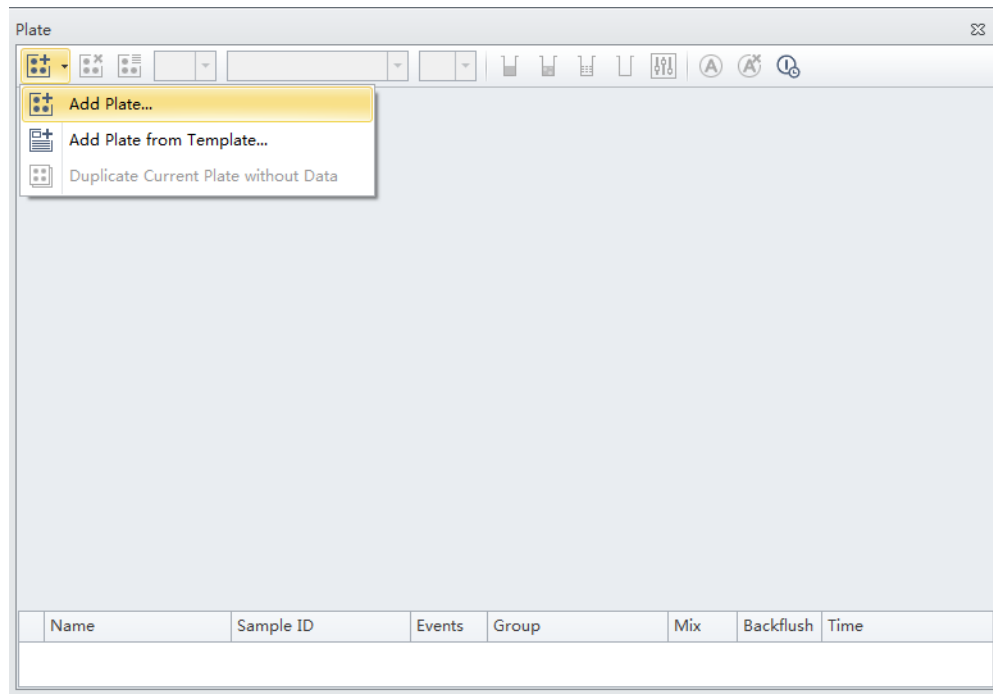
- 2 Select . The Plate window appears.

[CytoFLEX LX Shown]

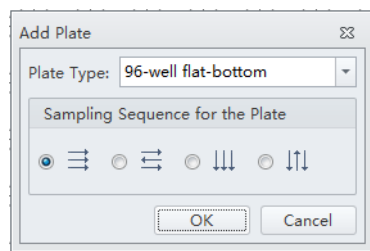


- 3 Select the Add Plate dropdown and select **Add Plate**.

[CytoFLEX LX Shown]



The Add Plate window appears.



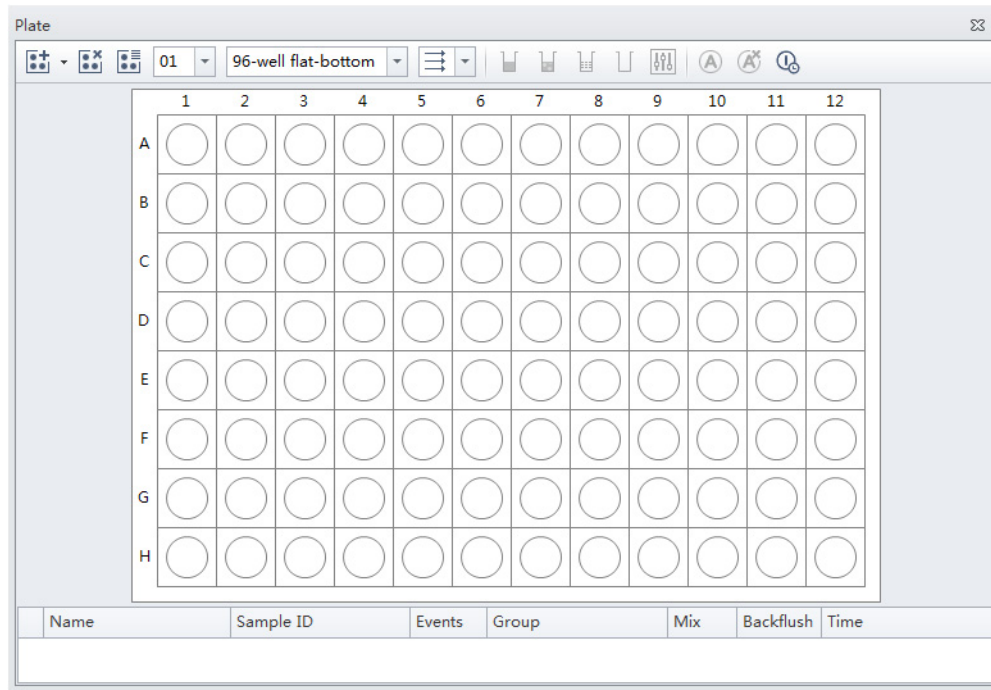
NOTE Select **Add Plate from Template** to add a plate template with preset plate settings.

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

- 4 Select the Plate type from the dropdown.



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

[CytoFLEX LX Shown]



- 6 If you need to automatically turn off the cytometer after auto acquisition is complete [CytoFLEX LX Only]:
 - a. Ensure you set the last sample wells with the appropriate number of cleaning agent wells and deionized water wells.

- b. Select .

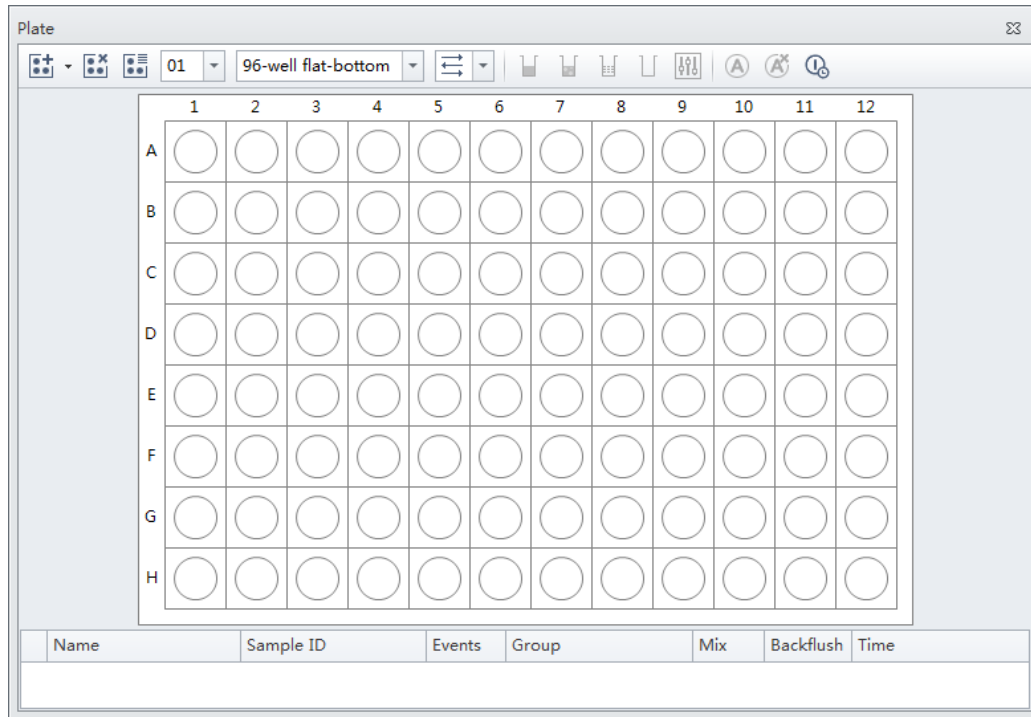
NOTE A gray background () indicates autoshutdown is disabled. A yellow background () indicates autoshutdown is enabled.

- 7 Select **OK**.


Setting Sample Wells

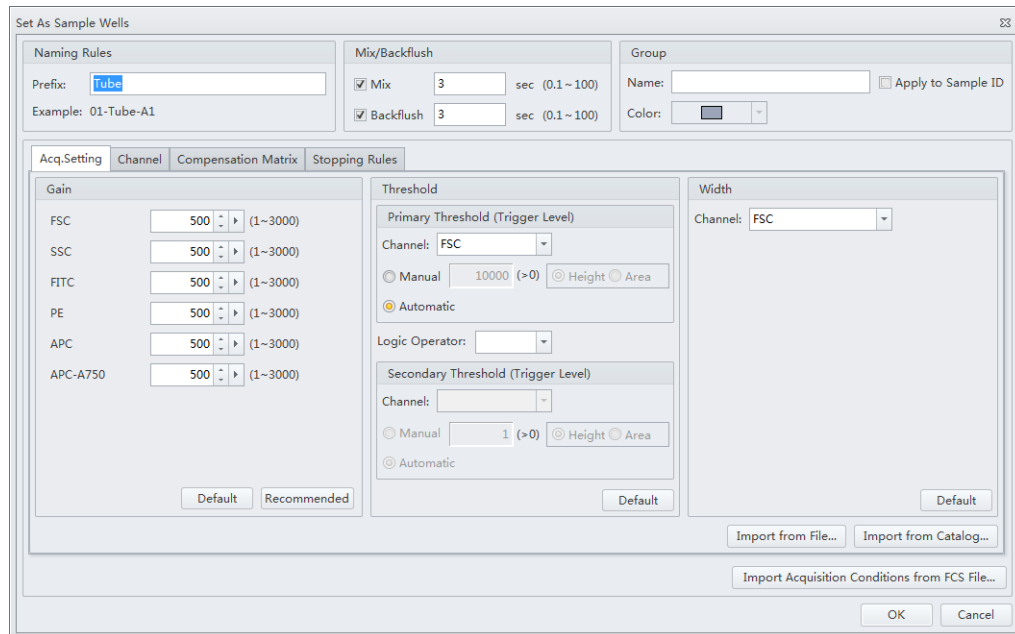
Once the plate protocol is created, the plate window appears. Refer to [Figure 5.1](#).

Figure 5.1 Plate Window [CytoFLEX LX Shown]



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

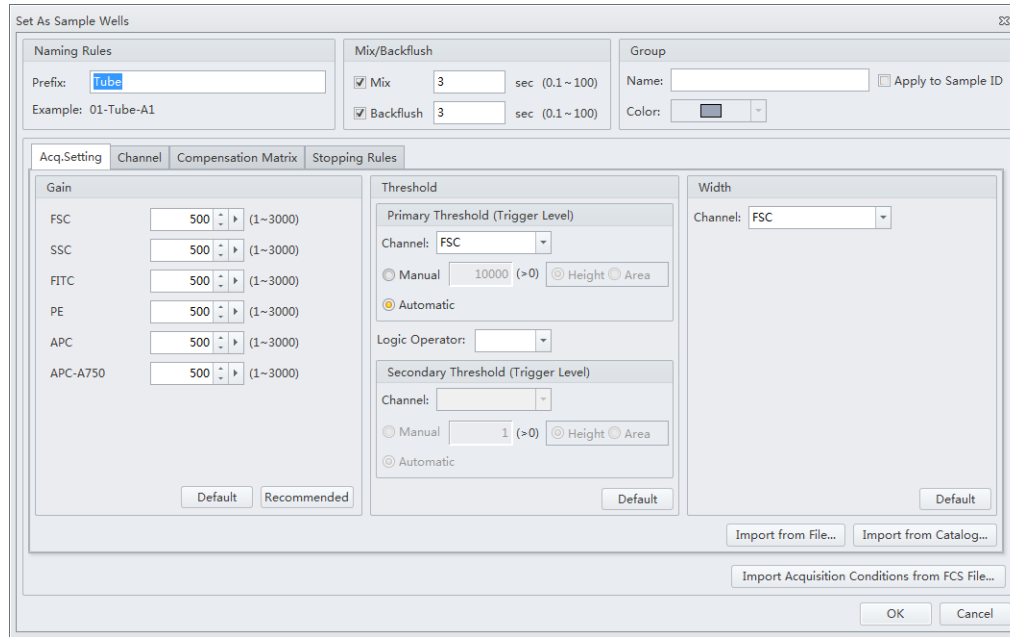
- 1 Left-click and drag your mouse to highlight the desired wells or hold the Control key and select each desired well.
- 2 Select  or right-click the selected wells and select **Set As Sample Wells**. The Set As Sample Wells window appears.



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

- 3 Enter the name in the Prefix box in the Naming Rules section of the window.
- 4 Select the desired Mix and Backflush settings from the Mix/Backflush section of the window.
- 5 Enter the Group Name in the Name box in the Group section of the window.
- 6 Select the sample well color using the color dropdown under the Group section of the window.

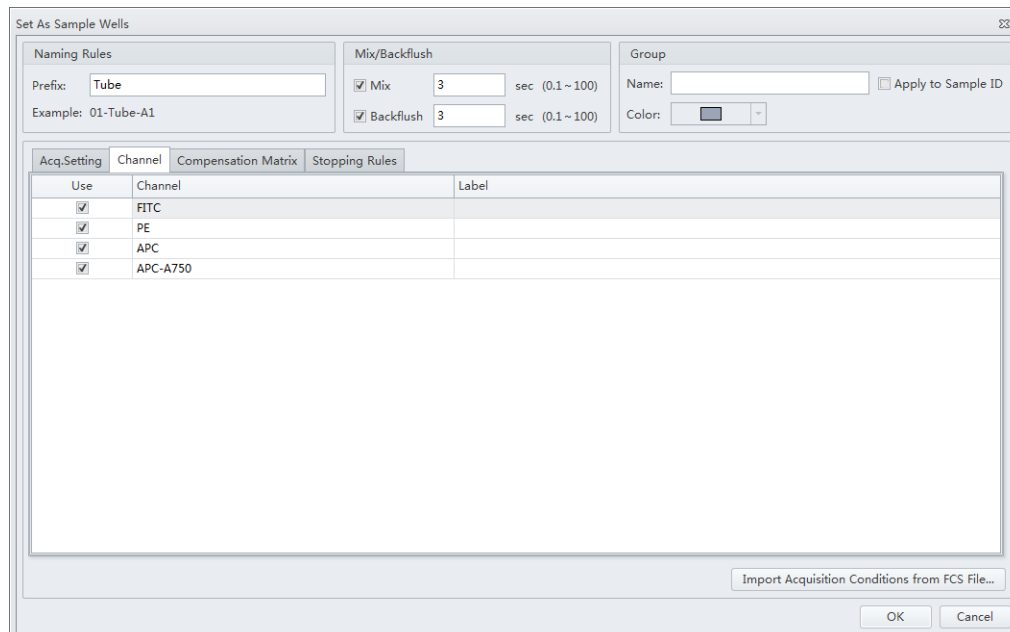
7 Select the desired acquisition settings under the Acq. Setting tab.



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

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

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



- 9 Set compensation under the Compensation Matrix tab. Refer to [CHAPTER 6, Compensation](#) for detailed instructions on setting compensation.

The screenshot shows the 'Set As Sample Wells' dialog box with the 'Compensation Matrix' tab selected. The 'Naming Rules' section has 'Prefix: Tube' and 'Example: 01-Tube-A1'. The 'Mix/Backflush' section has 'Mix' and 'Backflush' checked, both with a value of 3 seconds. The 'Group' section has 'Name' and 'Color' fields. The 'Compensation Matrix' table is as follows:

Autofl.	Channel	-FITC%	-PE%	-APC%	-APC-A750%
0.00	FITC		0.00	0.00	0.00
0.00	PE	0.00		0.00	0.00
0.00	APC	0.00	0.00		0.00
0.00	APC-A750	0.00	0.00	0.00	

Buttons at the bottom include 'Import from Library...', 'Import...', 'Import Acquisition Conditions from FCS File...', 'OK', and 'Cancel'.

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

The screenshot shows the 'Set As Sample Wells' dialog box with the 'Stopping Rules' tab selected. The 'Naming Rules' and 'Mix/Backflush' sections are the same as in the previous screenshot. The 'Stopping Rules' section has 'Events to Record' checked with a value of 10000 and 'Time to Record' checked with a value of 600. The 'in' dropdown is set to 'All Events'. Buttons at the bottom include 'Import Acquisition Conditions from FCS File...', 'OK', and 'Cancel'.

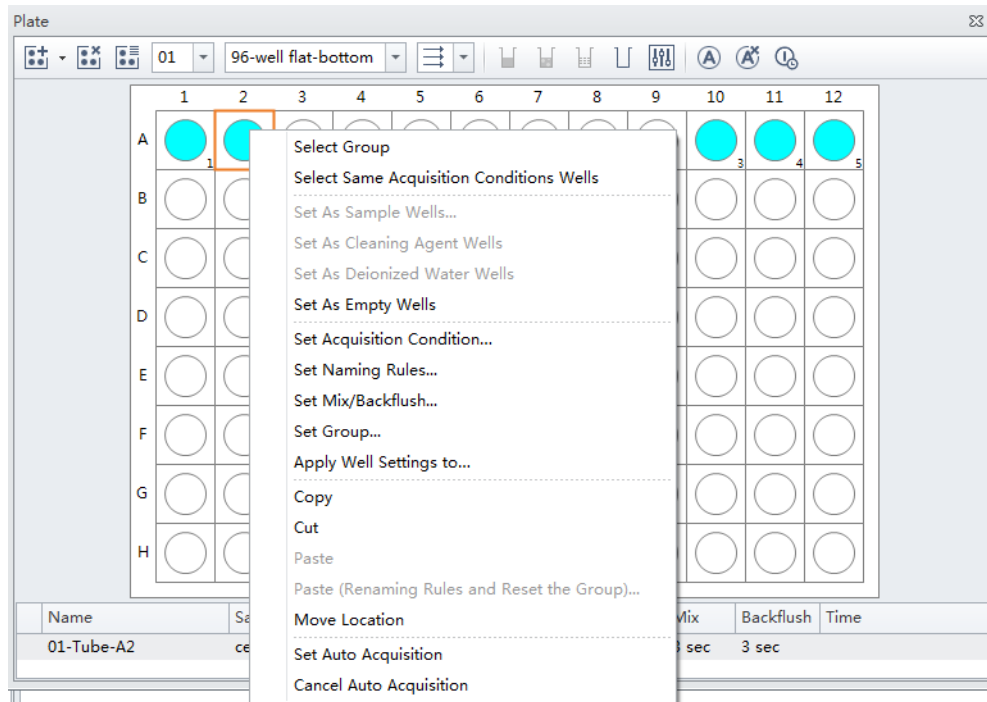
NOTE Beckman Coulter recommends setting an acquisition time limit, so the acquisition will not continue if the event limit cannot be reached.

11 Select **OK**.

Modifying Well Settings

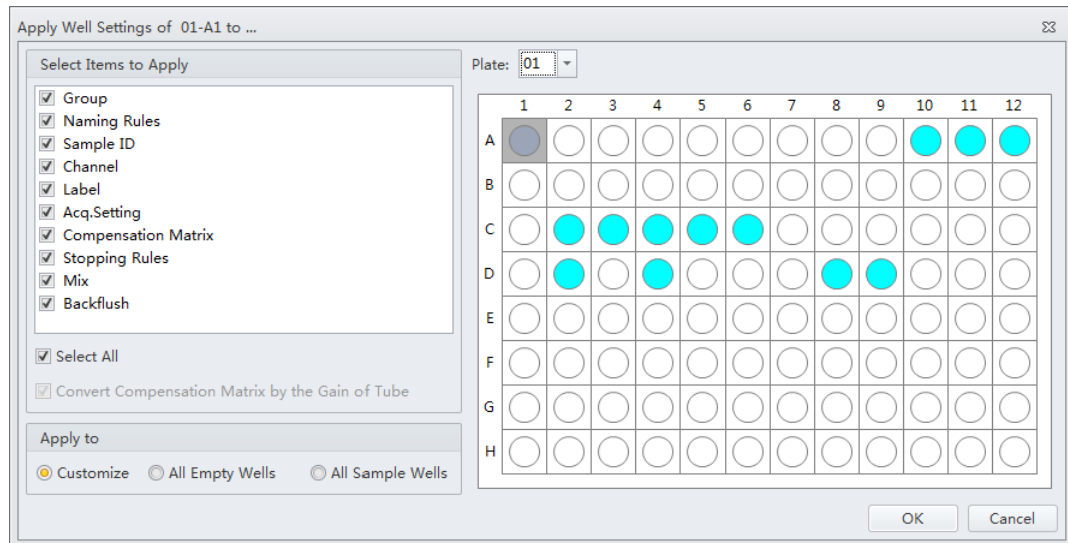
If any well settings require modification, right-click the sample well and select the setting to change.

[CytoFLEX LX Shown]



Applying Existing Well Settings to Additional Wells

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



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

- 2 Select the wells to apply the well settings to.

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

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

NOTE

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

Copying, Cutting, and Pasting Wells

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

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

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



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

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

Moving the Location of a Well

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

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



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

Running Samples

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


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

NOTE Acquisition settings can be adjusted during acquisition.

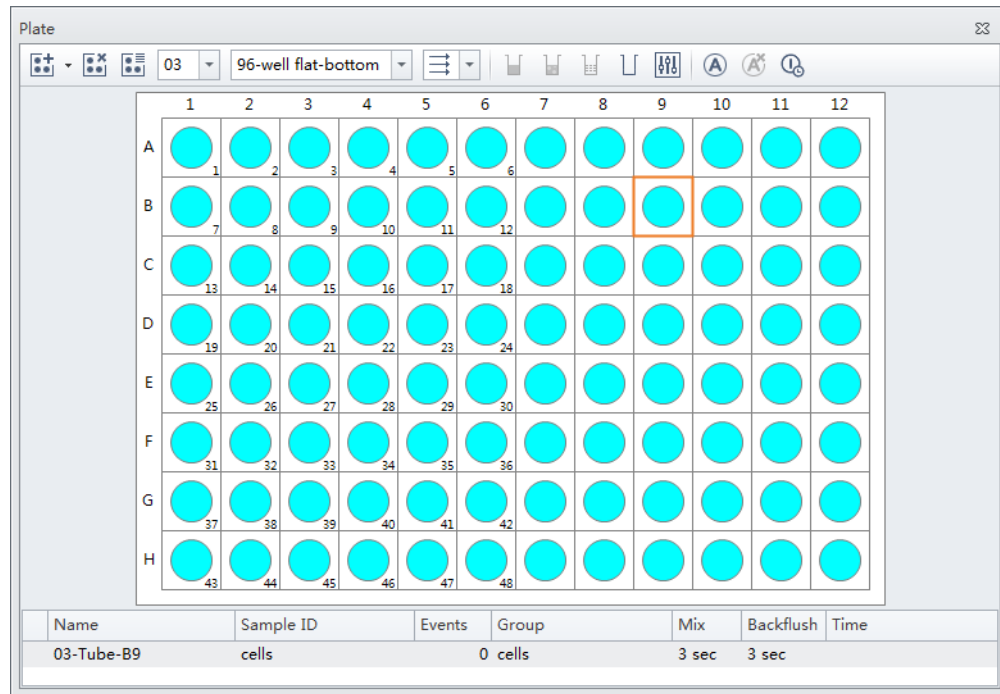
- c. Select **Record** to save the data.
- d. Select **Eject** to prompt the plate loader to eject.


To run a set of wells:

- a. Select the desired wells.

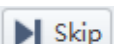
- b. Select  or right-click and select **Auto Record** to set the selected wells for auto record. Number labels appear in the bottom right corner of each well set for auto record. Sample acquisition occurs in the order indicated by the numbers.

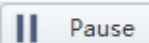
[CytoFLEX LX Shown]

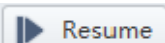


NOTE Select  to remove the auto record setting from the selected wells.


- 2 Select  **Auto Record** to begin sample acquisition.

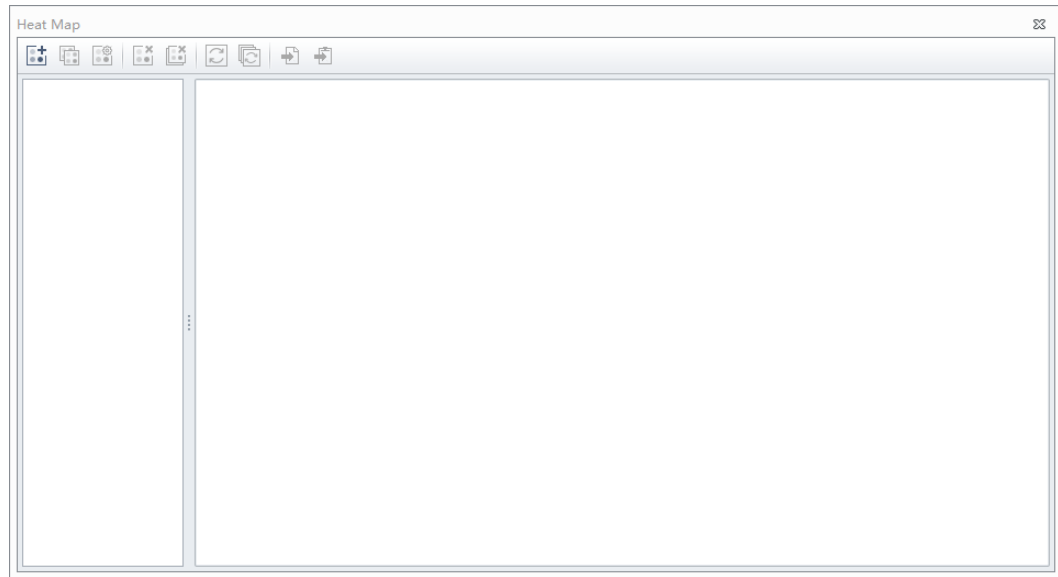
NOTE Select  **Skip** to skip the current well and move to the next well for acquisition.


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.

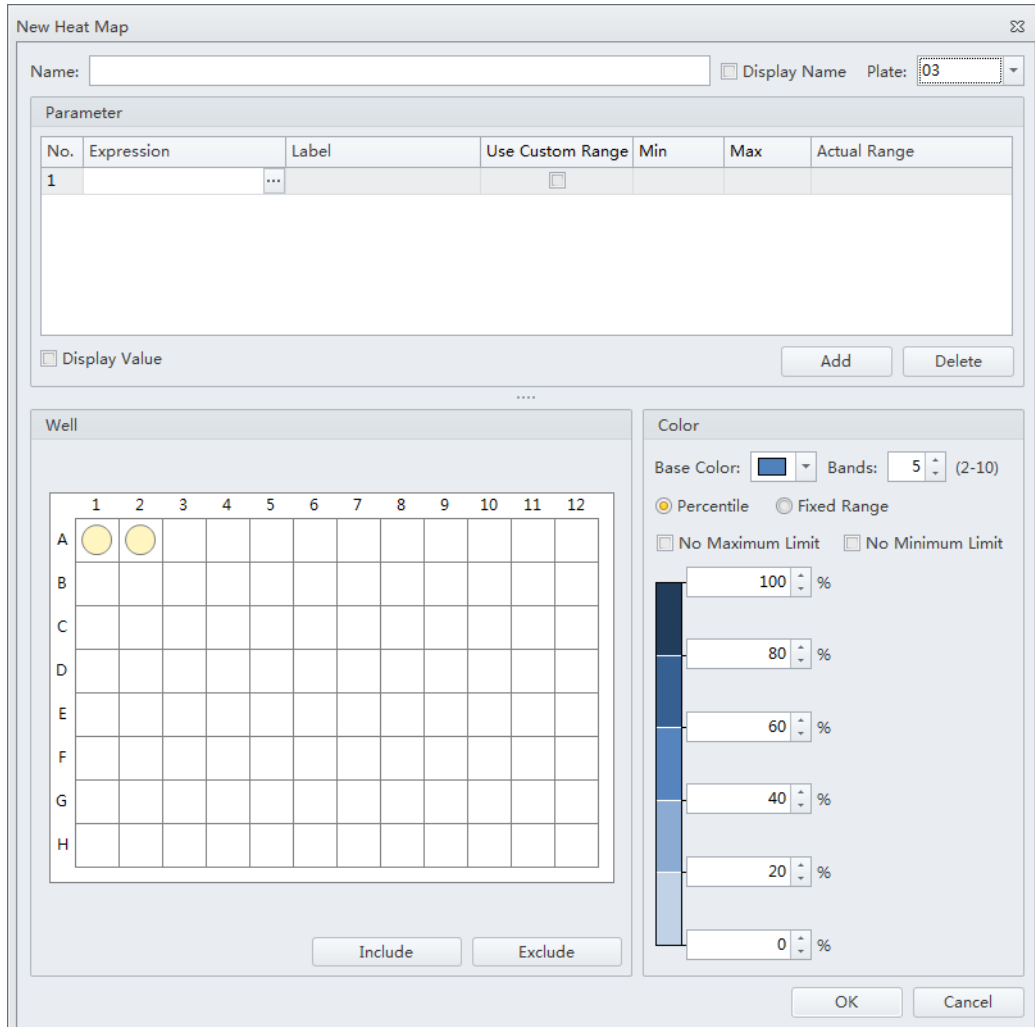
NOTE Select  **Resume** to continue the acquisition

Creating a Heat Map

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



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




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

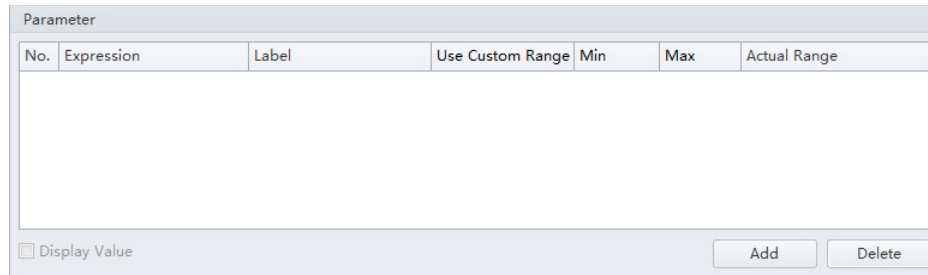
NOTE Select **Display Value** to display the value within the heat map well on the Heat Map window. Display Value is only selectable when using a single parameter.

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

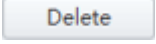


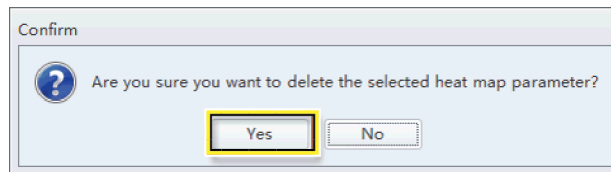
NOTE The plate drop down menu only displays plates that contain data.


- 4 Select  to add a parameter item to the Parameter section of the New Heat Map window.

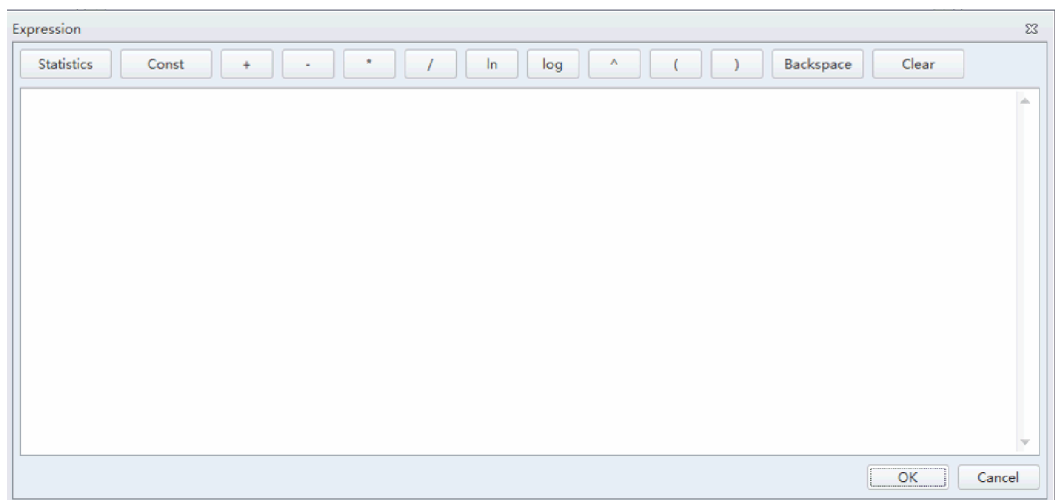


NOTE A maximum of 6 parameters can be added to a single heat map.

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



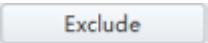
- 5 Change the Parameter elements in the Parameter section of the New Heat Map window.
- Change the Label name if needed.
 - Select  from the Statistic Expression section of the Parameter list. The Expression window appears.

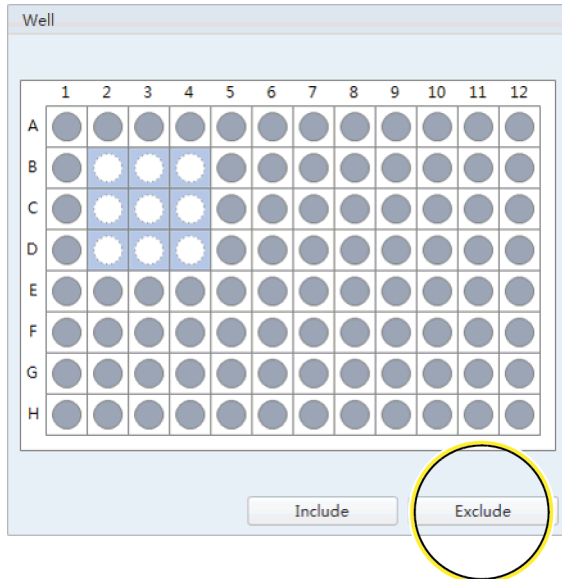



- Enter the desired expression for the selected parameter then select **OK**. The Actual Range displays in the Parameter section of the Heat Map window.


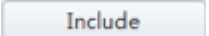
d. Select the **Use Custom Ranges** checkbox if needed and enter the Min and Max ranges.

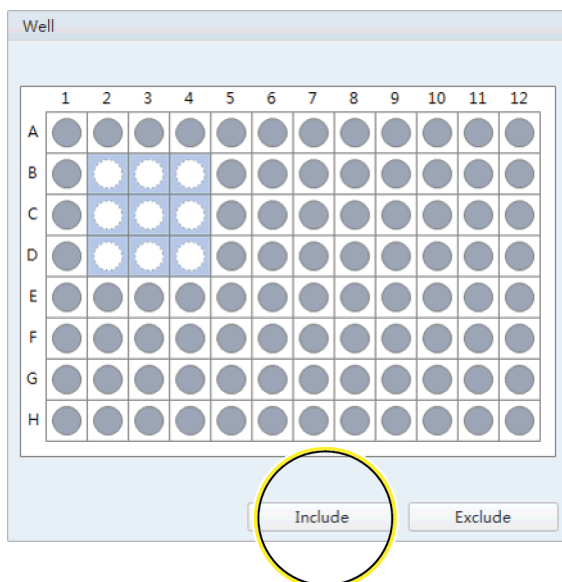
NOTE The Actual Range displays when the statistics parameter can be calculated.

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



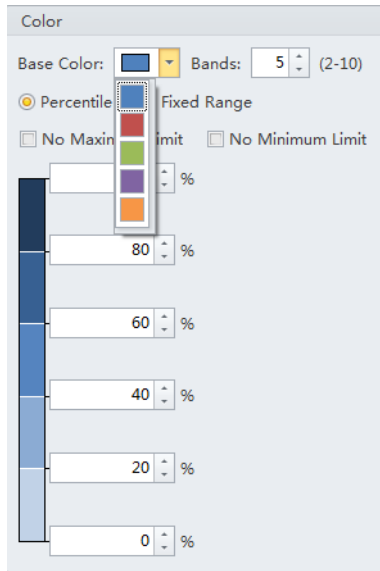
NOTE  designates that a well is included. All wells are included by default.

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

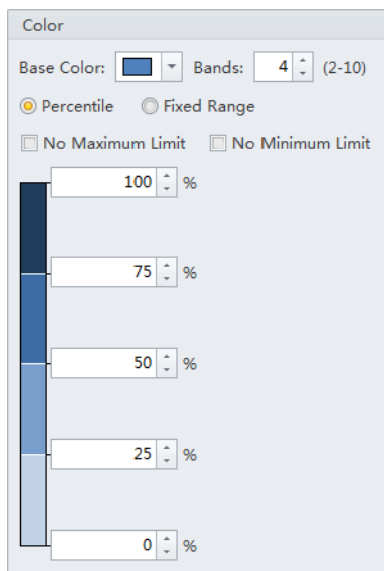


7 Edit the color elements in the Color section of the New Heat Map window.

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



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



NOTE You can choose between 2 and 10 color bands.

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

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

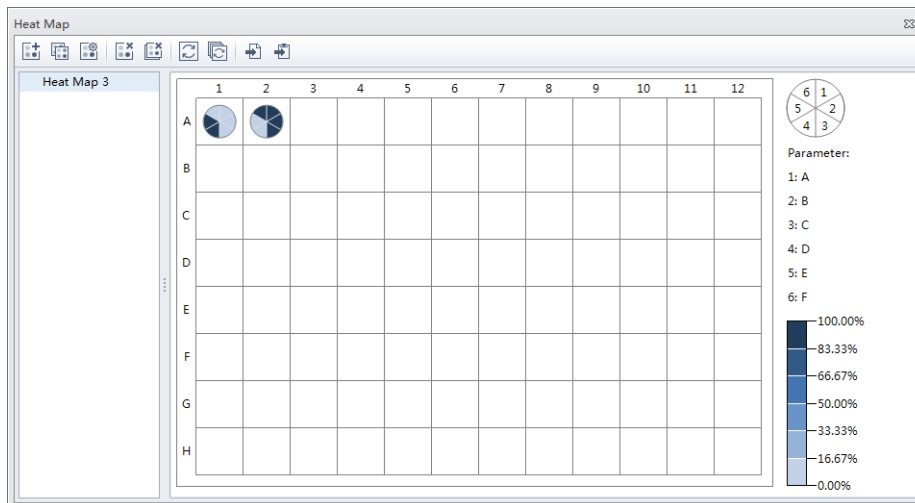
Or

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

NOTE Fixed Range can only be used with one parameter.

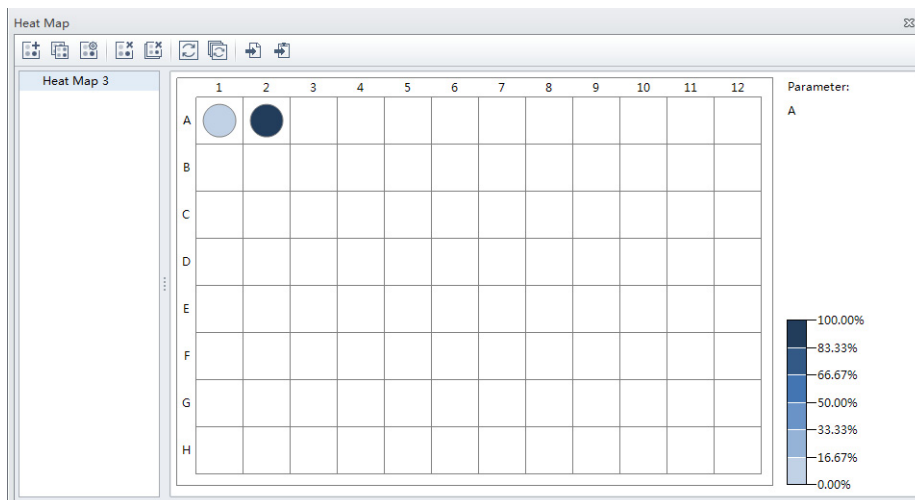
- 8 Select **OK**. The New Heat Map window closes to display the Heat Map window.

Heat Map with 6 Parameters




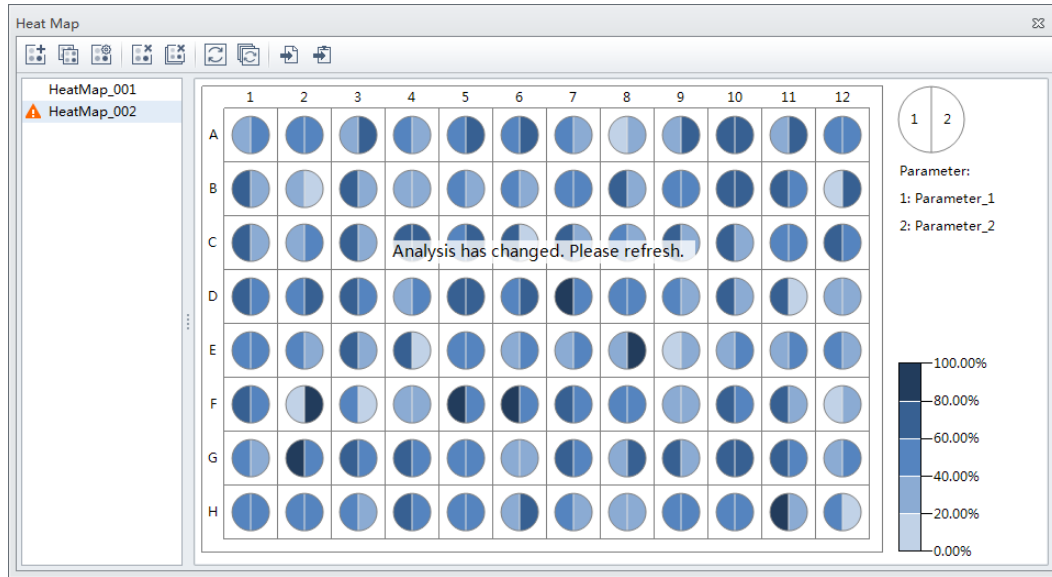
NOTE When viewing more than one parameter, the parameter location relative to the pie chart is visible in the top, right corner of the Heat Map screen.


Heat Map with 1 Parameter




Refreshing a Heat Map

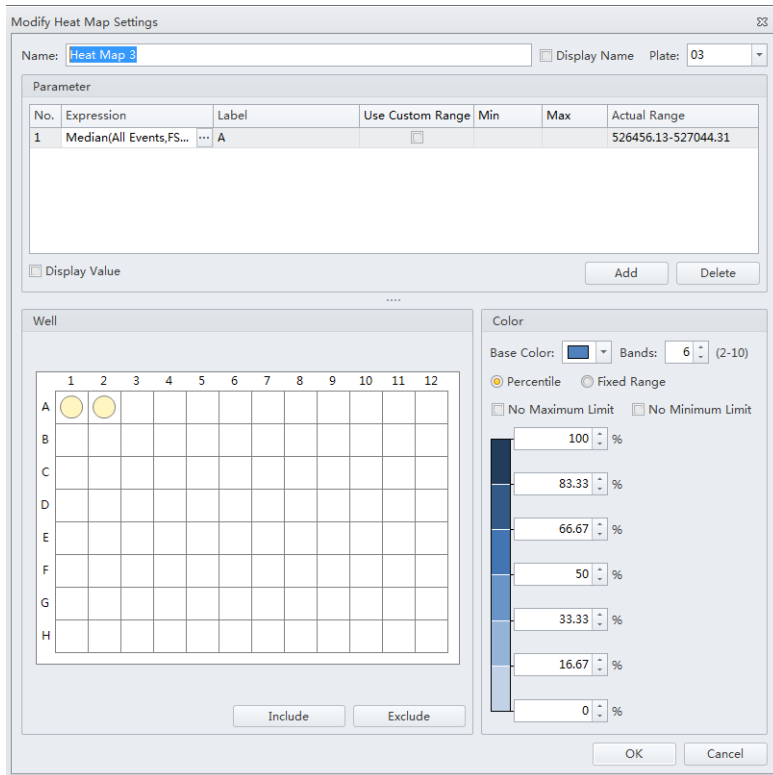
When data displayed in a heat map is no longer current, the  symbol appears next to the heat map name and the message *Analysis changed. Please refresh.* appears on the Heat Map window.




Select  from the heat map toolbar to refresh the analysis.


Modifying Existing Heat Map Settings

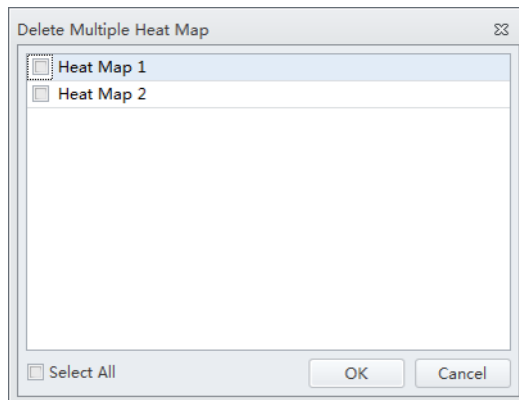
Select  from the heat map toolbar to modify existing heat map settings. The Modify Heat Map Settings window appears.



Deleting an Existing Heat Map

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

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





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

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

Exporting a Heat Map

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

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

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

Load Sample and Record Data

Before Running Samples



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

- 1 Run the [Daily Startup](#) procedure.
 - 2 Run the [Instrument Quality Control and Standardization](#) procedure.
 - 3 Create an experiment. Refer to [Creating an Experiment](#).
 - 4 Verify mixer settings. Refer to [Changing Sample Mixing and Backflush Settings](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).
 - 5 Ensure that there is sufficient space on your hard drive for sample processing and data acquisition.
 - 6 Verify the detector configuration. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#).
 - 7 Verify the laser settings. Refer to [Laser Settings](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#).
-

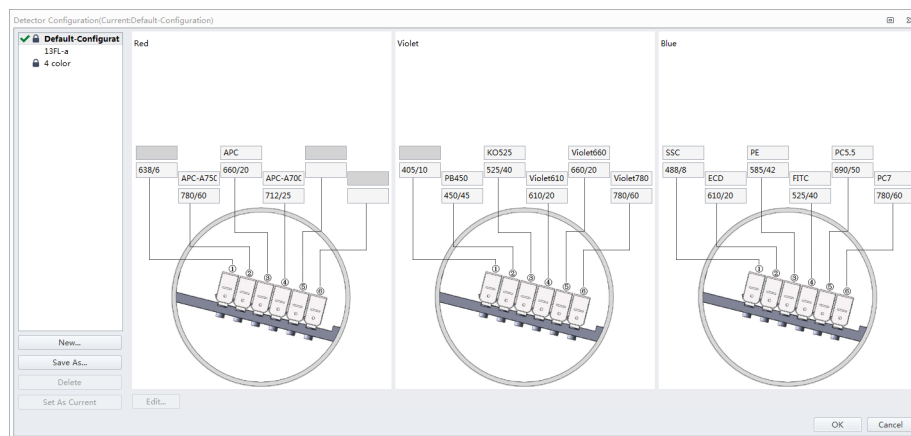
Verifying, Selecting, Editing, and Creating Detector Configuration


CAUTION

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

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

[CytoFLEX Shown]



NOTE A configuration is locked when  appears to the left of a configuration. A configuration locks for two reasons:

- QC was run using the configuration.
- The compensation library contains data for the configuration.

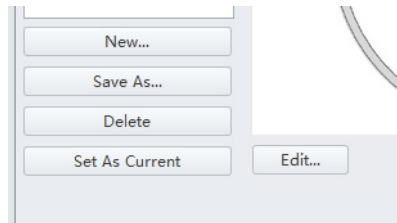
Locked configurations can be deleted, but not edited.

- 2 Select **OK** to close the Detector Configuration screen.
- 3 Proceed to Step 4 if you need to edit the Detector Configuration settings, or skip to Step 5 if you need to create a new Detector Configuration, or skip to Step 12 if you need to delete a Detector configuration.

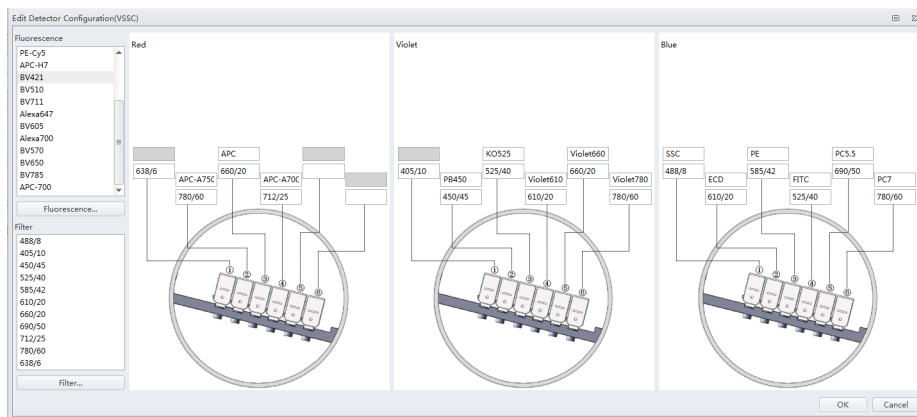
4 If a saved configuration requires changes, edit that configuration.

NOTE The factory configuration is in bold and cannot be edited.

- a. Select the configuration, then select **Edit** to access the Edit Detector Configuration screen.



- b. Channels with a white background can be edited. Drag the names of the appropriate fluorescence channels and optical filters on the left to the correct channels.



- c. Continue to Step 6.

5 If an appropriate configuration is not saved, create a new configuration.

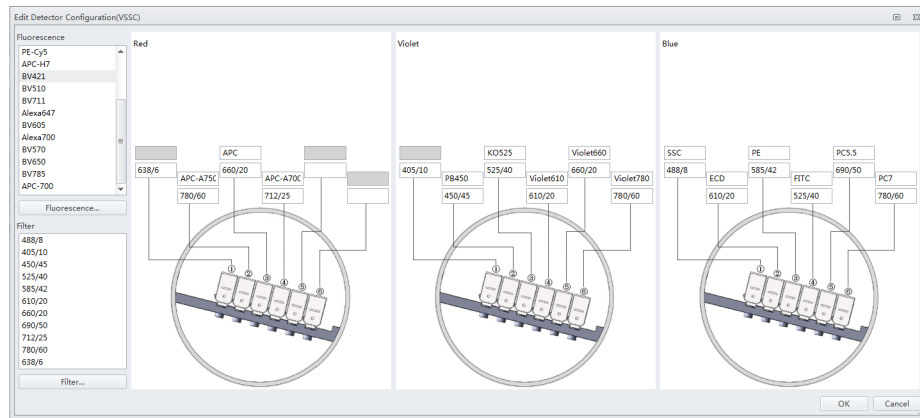
- a. Select **Detector Configuration...** in the Cytometer menu.

- b. Select **New...** and name your configuration.

You can also select a previously saved configuration and select **Save As** to create a copy.

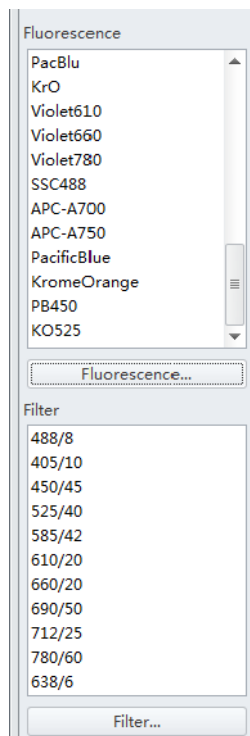
- c. Select **OK**.

- d. Ensure the new configuration is highlighted, then select **Edit**. The Edit Detector Configuration window appears.

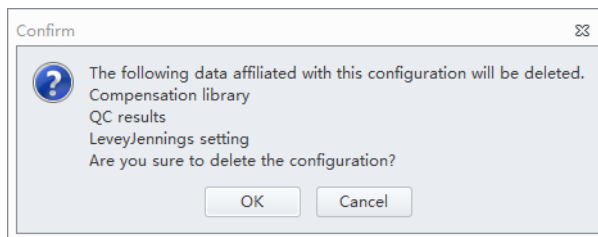


- e. Customize the new configuration. Channels with a white background can be edited. Drag the names of the appropriate fluorescence channels and optical filters on the left to the correct channels.
- f. Continue to Step 6.

- 6 If a required channel name or filter is not listed on the left, select **Fluorescence** or **Filter** to add or modify the channel name or the filter.



-
- 7 When finished, select **OK**.
-
- 8 Select the appropriate configuration.
-
- 9 Verify that the correct optical filters are installed in the Cytometer and match the newly created configuration.
-
- 10 Select **Set As Current**.
-
- 11 Select **OK**.
-
- 12 To delete a configuration created in error, select **Delete**. The following confirmation message appears. Select **OK**.



Setting Up Violet Side Scatter (VSSC) Channel

For microparticles, a VSSC option can be added to better separate side scatter signals from noise. Beckman Coulter recommends using this channel to detect particles smaller than 500 nm.

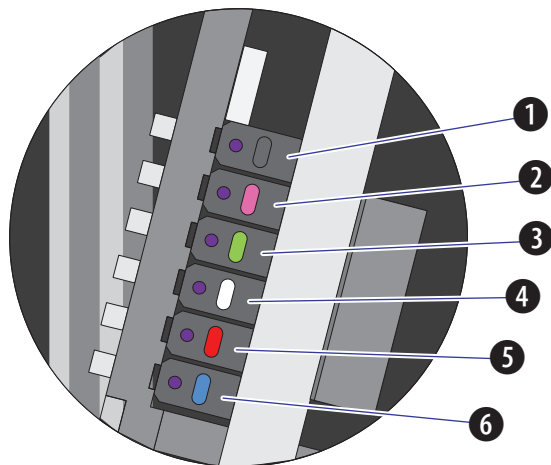
NOTE Since the total available channel numbers remain the same when the VSSC channel is used, the number of fluorescent channels in the violet WDM is reduced by 1 channel.

CAUTION

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

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

NOTE Refer to [Table 1.1](#) to identify the WDM filter mount color codes.

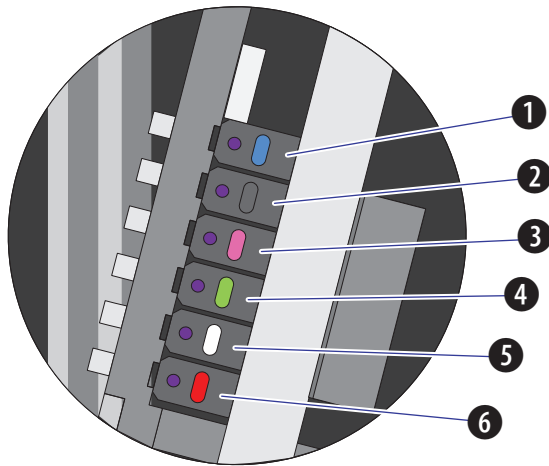


- | | |
|---------------|---------------|
| 1. Position 1 | 4. Position 4 |
| 2. Position 2 | 5. Position 5 |
| 3. Position 3 | 6. Position 6 |

NOTE The orientation for position 1 through 6 starts with position 1 located closest to the back of the Cytometer, and position 6 located closest to the front of the Cytometer.

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

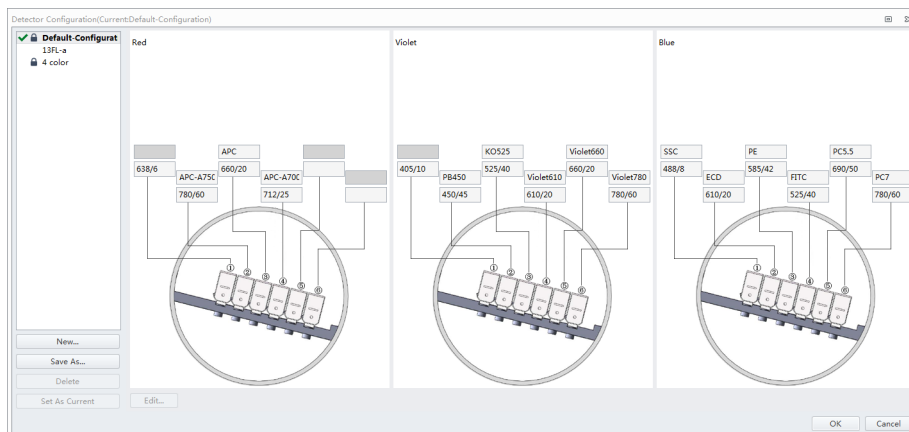
NOTE For the Violet WDM, Beckman Coulter recommends placing the filters in sequential order from the shortest wavelength to the longest wavelength in positions 2 to 6. Position 1 will always contain the unused filter.



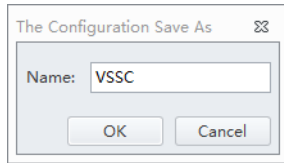
- | | |
|---------------|---------------|
| 1. Position 1 | 4. Position 4 |
| 2. Position 2 | 5. Position 5 |
| 3. Position 3 | 6. Position 6 |

- 3 Start the CytExpert software. Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#).

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

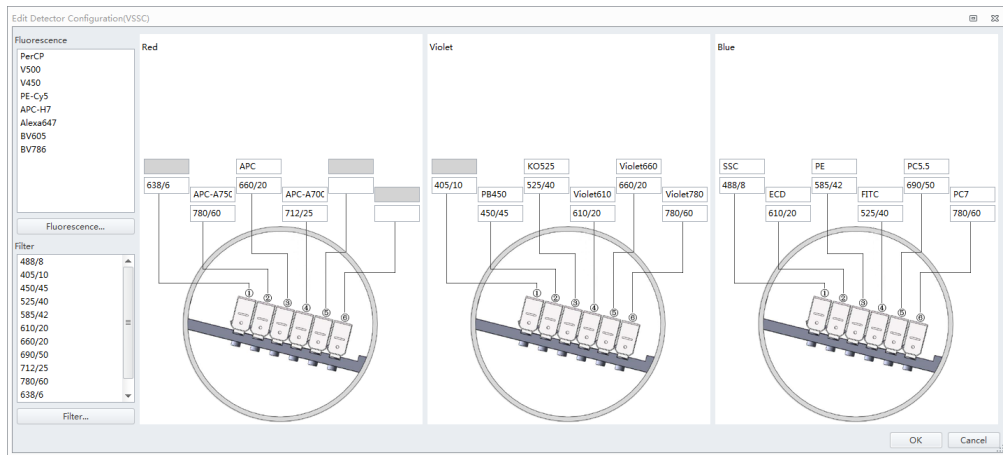


- Select the Default Configuration and select **Save As**. The Configuration Save As window appears.



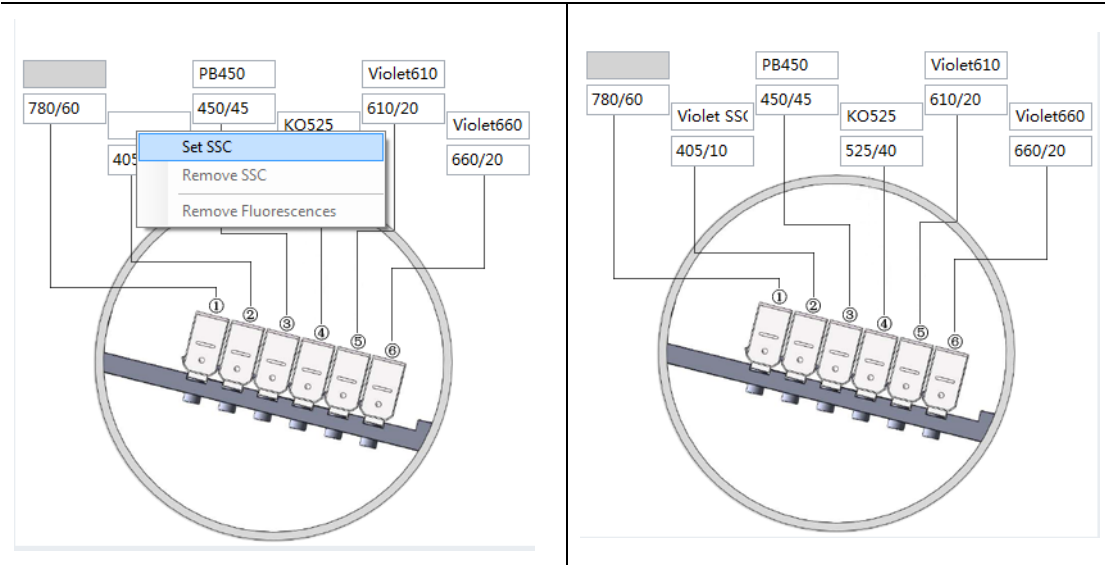
- Name the new configuration VSSC and select **OK**.

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



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

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



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

11 Select **Set as Current**.

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


13 Create a new experiment using the VSSC configuration. Refer to [Creating an Experiment](#).

Sampling and Collecting Data



NOTE Settings can be imported from the Acquisition Settings Catalog. Refer to [Importing and Exporting Instrument Settings](#).

If compensation settings are desired, import the compensation from the Compensation Library or import the compensation file. Refer to [Importing and Exporting Compensation](#) in [CHAPTER 6, Compensation](#).

1 Select  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**.

WARNING

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


- 5 Place the sample tube in the sample tube holder.

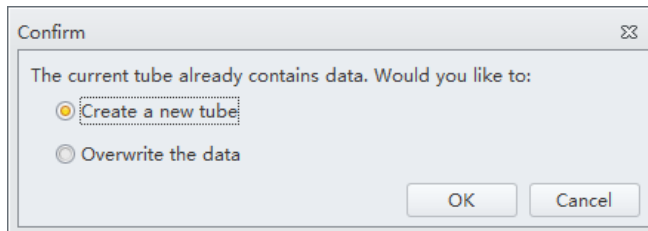
- 6 Select the desired acquisition parameters (Events/Time to Record and Sample Flow Rate) on the left side of the screen.

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



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

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



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

8 View the plots and establish the gates. Refer to [Creating Plots and Gates](#). Adjust the gate and instrument settings as necessary. Refer to [Configuring Acquisition Settings](#).


9 Adjust the gain settings. Refer to [Adjusting the Gain](#).

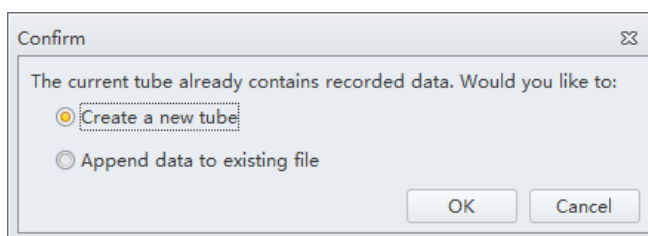
10 Adjust the threshold settings. Refer to [Adjusting the Threshold](#).

11 Adjust the Acquisition conditions. Refer to [Setting Collection Conditions](#).

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


Wait for the saving process to finish. The sample tube holder returns to the sample loading position (see [Figure 1.12](#)).

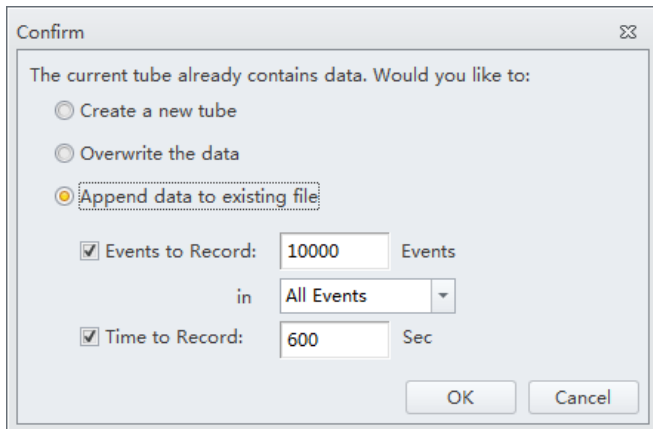
NOTE When you select a tube that contains recorded data, as indicated by the green tube  in the test tube section of the screen, the following message appears:



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

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

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



The image shows a 'Confirm' dialog box with the following content:

Confirm

The current tube already contains data. Would you like to:

- Create a new tube
- Overwrite the data
- Append data to existing file:

Events to Record: 10000 Events

in All Events

Time to Record: 600 Sec

OK Cancel

- **Create new tube.** Creates a new tube in the test tube section of the screen for the data.
- **Overwrite existing data.** Overwrites the current tube data with new data.
- **Append data to existing file.** Adds new data to the existing data.

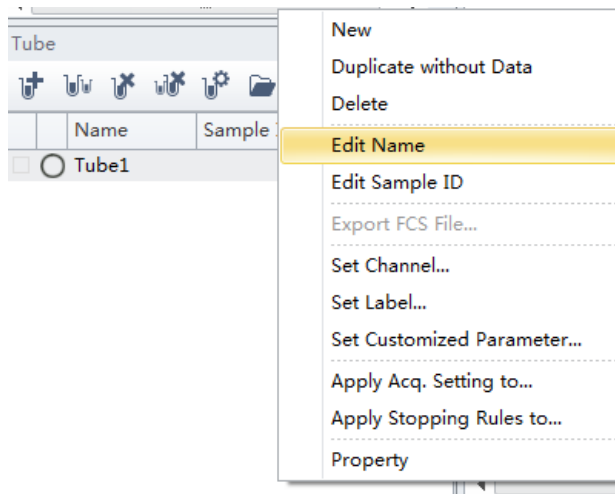
13 Repeat steps 1-12 until all sample tube data required for testing has been collected.

NOTE If the rate suddenly appears to drop, check to see if the sample has run dry or the sample probe is clogged. Any time the sample probe becomes clogged, immediately select **Stop** to unload the sample. Then select **Backflush** to clean the sample probe. Refer to [Daily Clean](#) in [CHAPTER 10, Cleaning Procedures](#), to flush out the sample probe. If you are still unable to clear the sample probe, [contact us](#).

Configuring Acquisition Settings

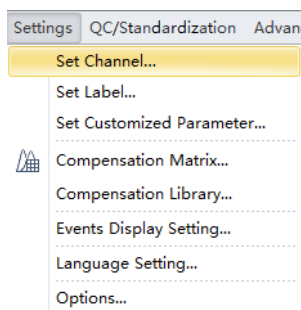
Changing the Tube Name

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

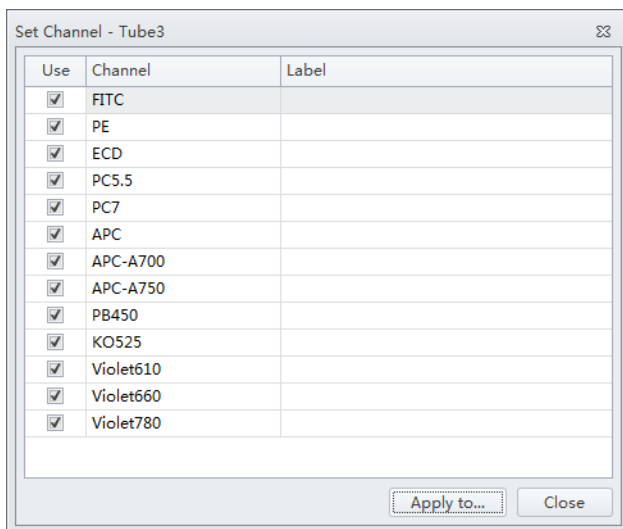


Setting the Channel and Label

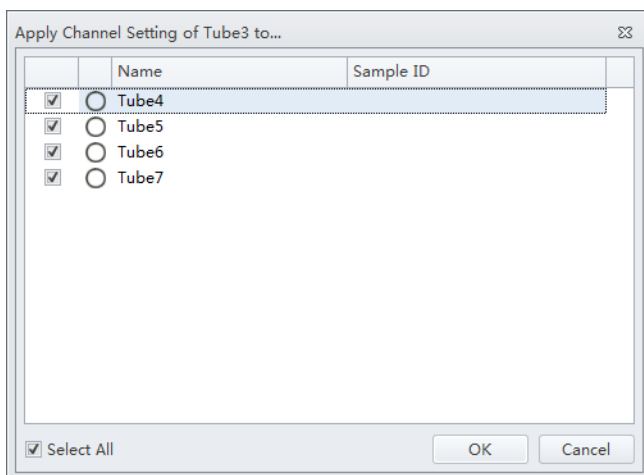
- 1 Select **Set Channel** in the Settings menu. The Set Channel window appears.



- 2 In the Set Channel window, modify which channels are used and how they are displayed.
- Select the channel signal checkbox, 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.

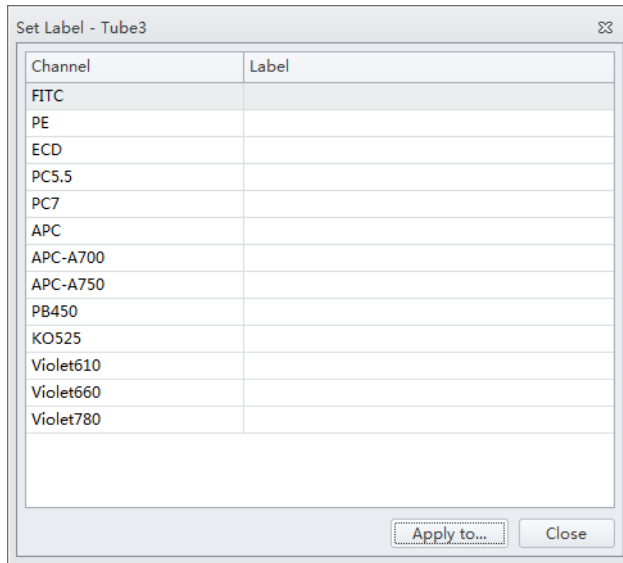


- Select **Apply to**. The Apply Channel Setting window appears.

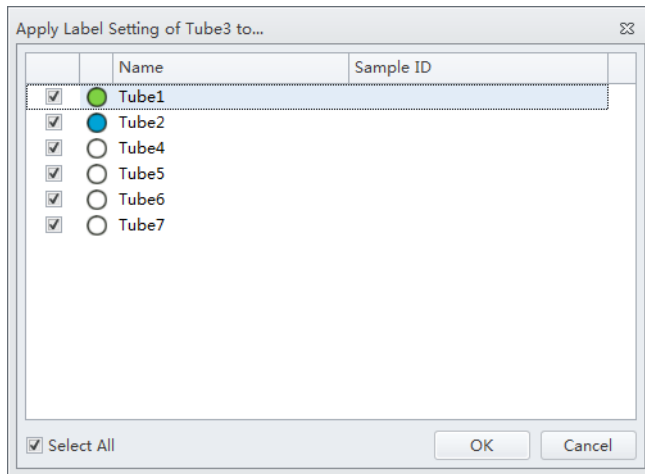


- Select the tubes to apply the channel settings to and select **OK**.

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





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



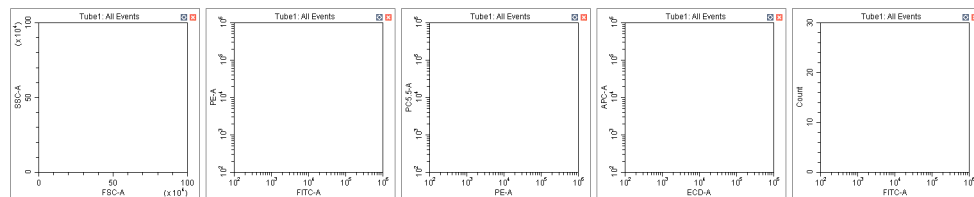
- f. Select the tubes to apply the label settings to and select **OK**.

Creating Plots and Gates

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

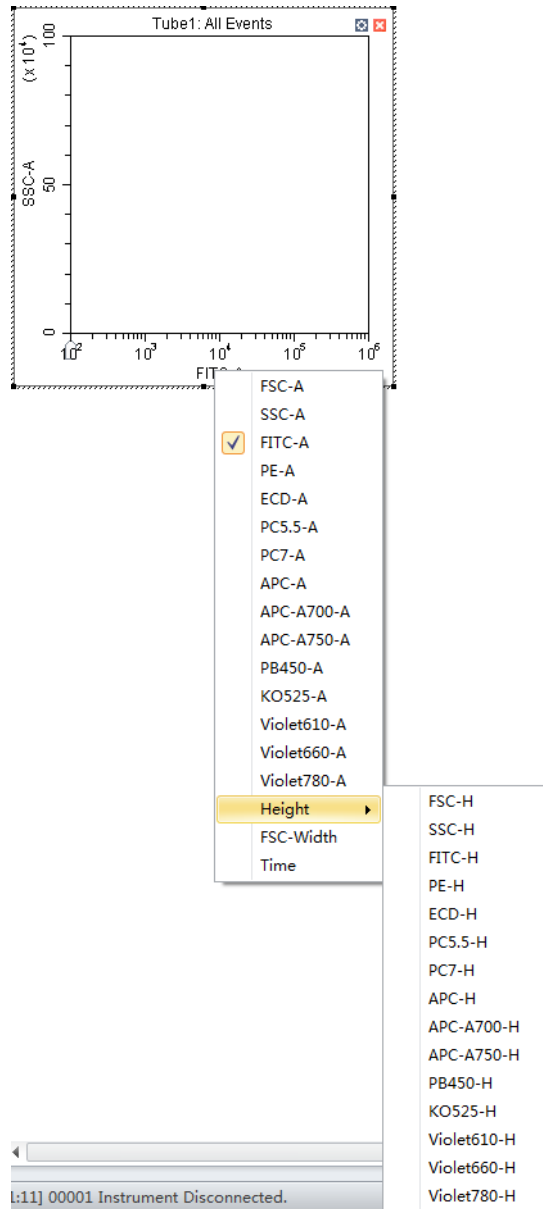
Use the   icons to generate histograms, dot plots, density plots, pseudo color plots, and contour plot.

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



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

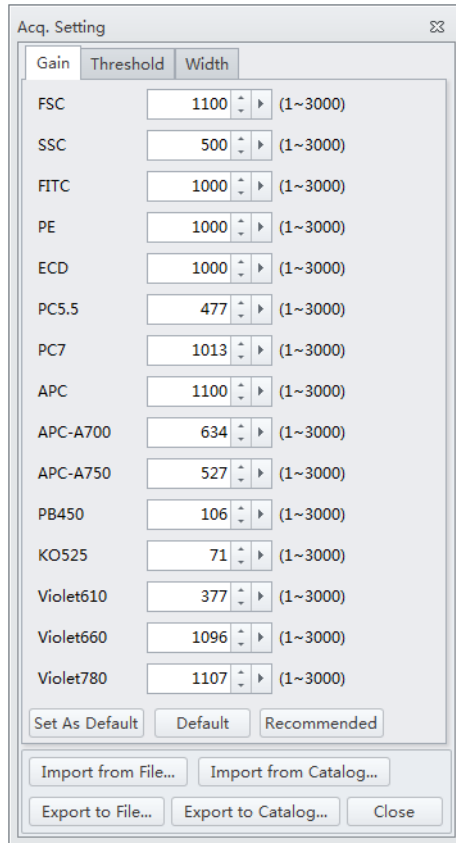
- b. Select an axis name to change which channel is displayed. An “A” after the channel name indicates signal pulse area, while an “H” indicates height. The default setting is "A".



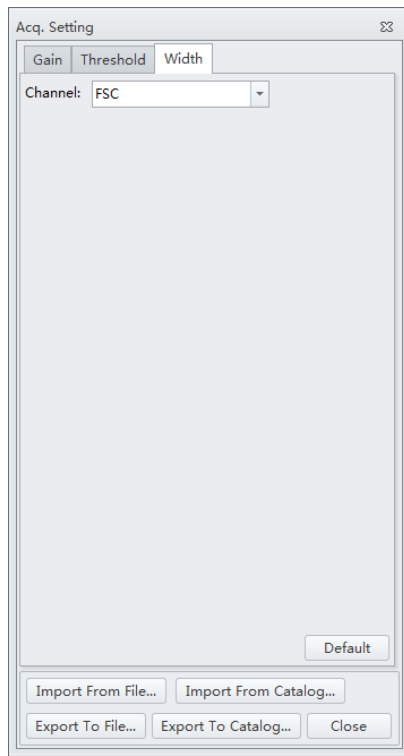
NOTE To modify the default settings, select **Options** in the Settings menu. The Options window appears. Select **Plot** on the left side of the Options window. Under the Signal section of the window, change the Main Channel default by selecting the **Height** or **Area**.

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

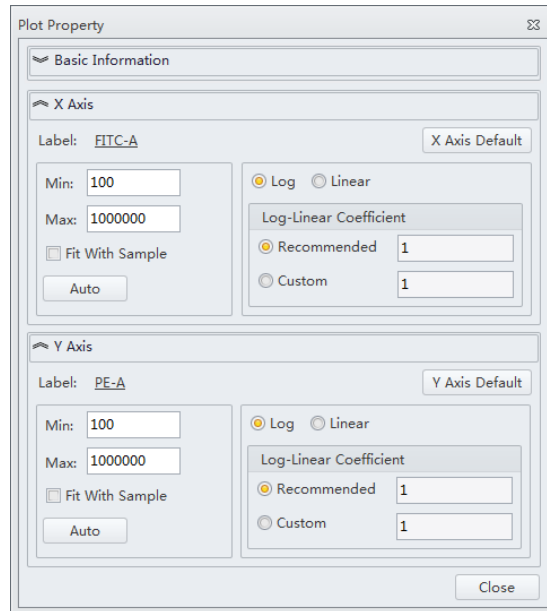
- c. Signal width can be used as a tool for doublet discrimination and to differentiate somatic cell adhesion. If necessary, select  to open the Acq. Setting window.



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



- e. Plot properties can be configured to display axes in Log, Log-Linear, or Linear format.
 - 1) Double-click the plot or right-click the plot and select **Property** from the drop-down menu. The Plot Property screen appears.



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

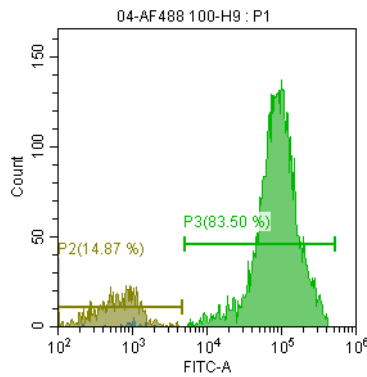
Or

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

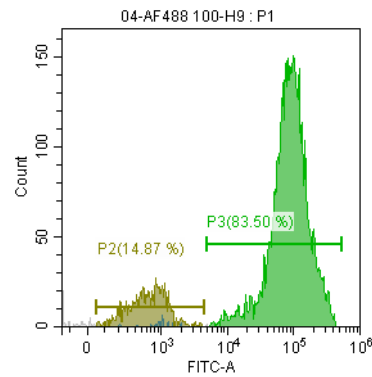
NOTE The log-linear slider is also available during data acquisition.

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

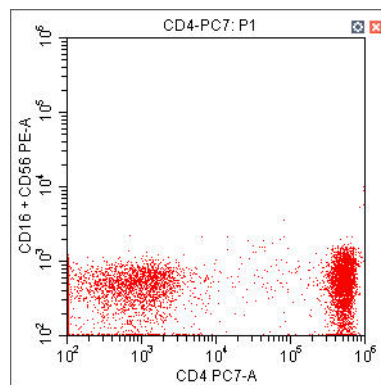
Histogram with logarithmic X-axis



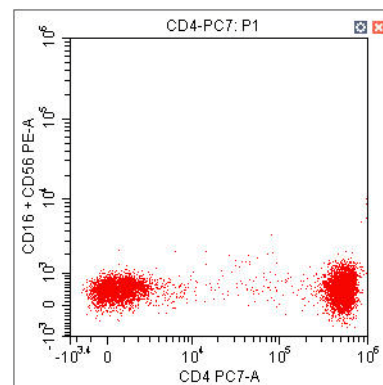
Histogram with log-linear X-axis






Dot plot with logarithmic X-axis

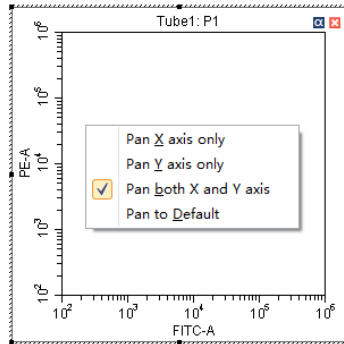



Dot plot with log-linear X-axis



- f. You can adjust axis ranges using the pan axis display controls located at the top of the screen.
 - Select , to zoom-in and define which area of a plot to enlarge. The selected area can be magnified to fill the entire graph. By selecting the zoom-out function, you can click on the graph and restore the plot to its original appearance before magnification.

- Select  to shift the axes. The mouse pointer appears as a hand. It allows you to drag the graph to reveal the axis segment you need.
 -  Pan: Modifies the axis display range dimensions when panning both axes. When the pan control is selected, you can right-click the graph and select which axis you need to adjust when dragging. You can also pan directly to the default axis range.



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


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

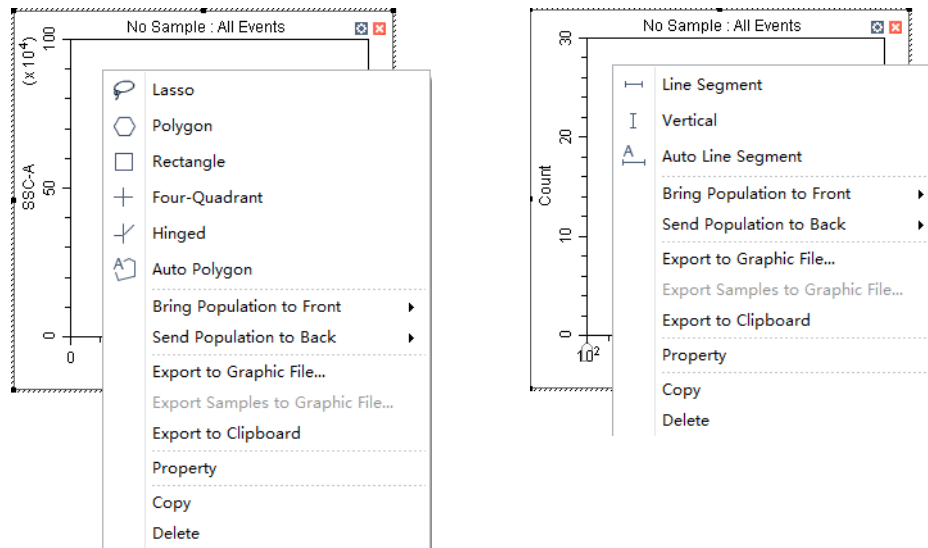
- Double-click the border area of the plot to open the Plot Property window, or right-click the plot, then select **Property** to open the same Plot Property window.
- In the Plot Property window, manually enter the minimum and maximum display values for the X- and Y-axes. You can also select **Fit With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

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

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

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

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

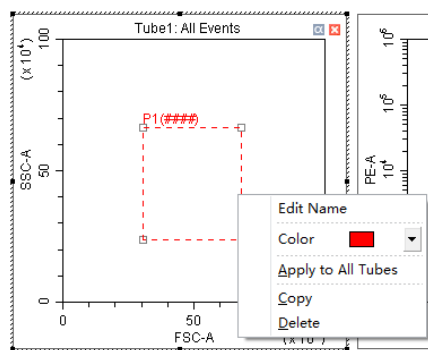


NOTE To add a vertex to a polygon gate:

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

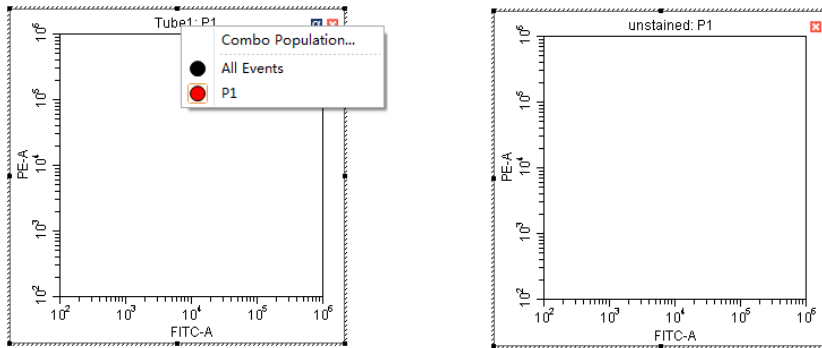
NOTE A newly created gate becomes a subset of the plot where it appears. The relationship between parent and progeny/daughter gates can be changed when a displayed gate is subsequently modified.

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



3 Select the gates to display.

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



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

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

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

Figure 5.2 All Gates - Example Experiment

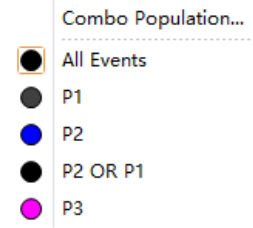
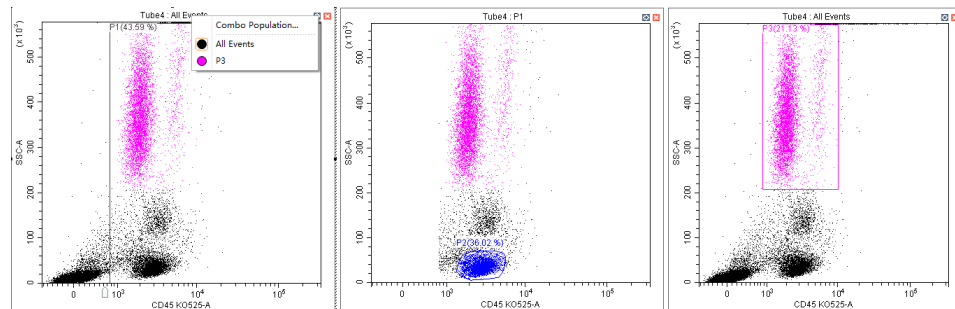
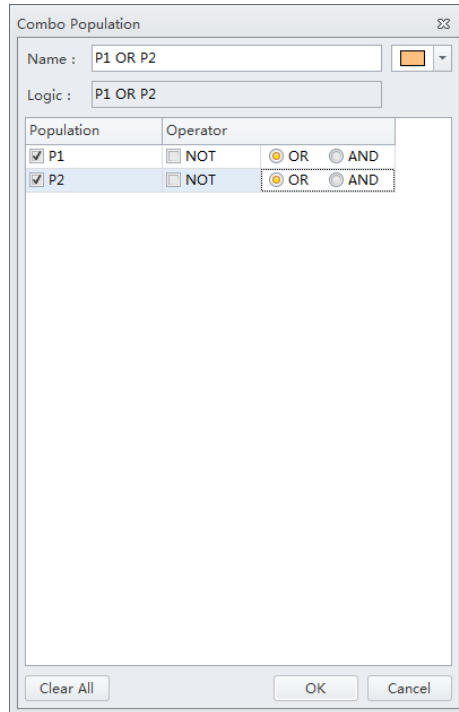



Figure 5.3 Circular Gating Logic - Example Experiment



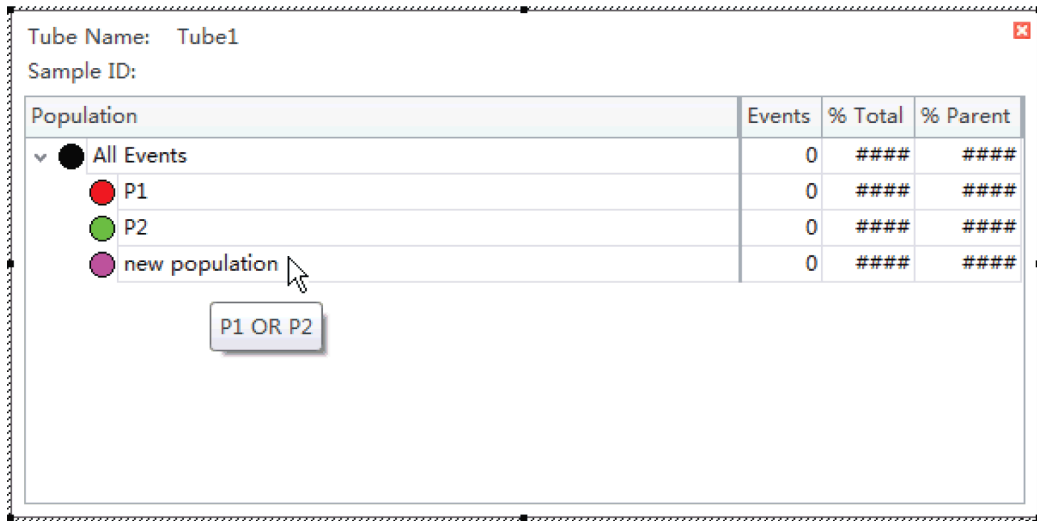
- b. If necessary, you can select the **Combo Population** option from the drop-down menu to create a combination gate, using the boolean relationships “and”, “or”, and “not” to produce a new gate. You can also select the population color or change the gate name.



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


- 4 Select  to display the population hierarchy.

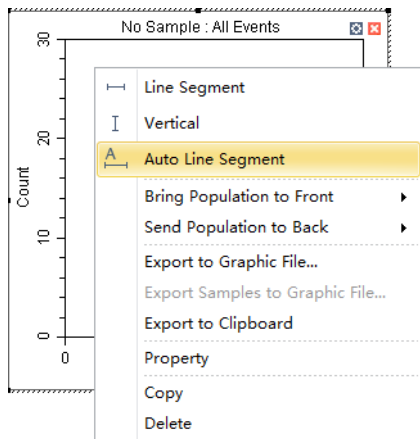
The Population Hierarchy function allows you to view how gates rank in relation to one another. To change the display color, double-click the default color and select the desired color from the drop-down color palette. To change the name of each gate, double-click the name of the desired gate. By hovering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



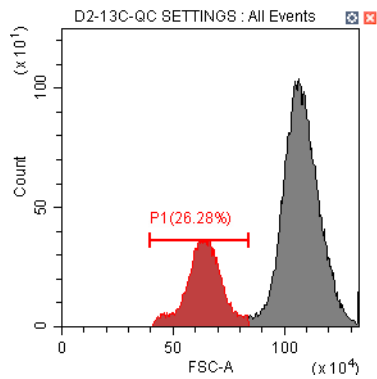
Creating and Adjusting Auto Gates


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

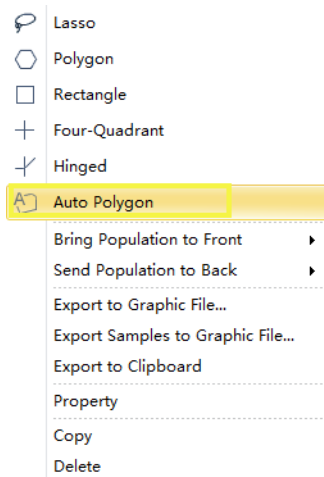
To create an auto line segment gate, select  from the toolbar or right-click on the histogram and select **Auto Line Segment** from the drop down menu.



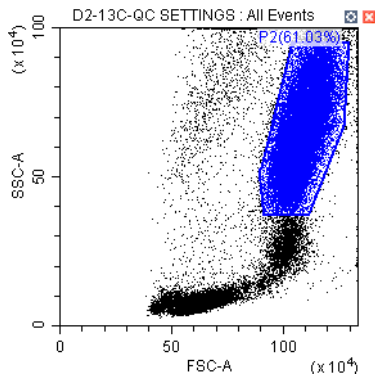
Select the population you want to gate in the histogram to automatically gate that population.



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



Select the population you want to gate in the 2-D plot. The gate will automatically be drawn to fit the population.



NOTE To add a vertex to an auto polygon gate:

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

Turning Auto Recalculate On/Off

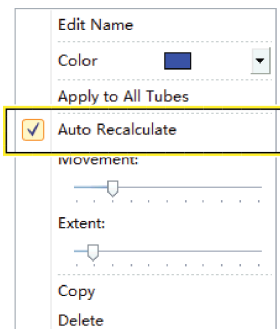
When auto recalculate is turned on, all autogates will recalculate when:

- The current tube is changed
- Compensation is changed
- Gating is changed
- Collection stops
- An FCS file is imported to the tube or well

Auto recalculate turns off after a gate is moved or the size of a gate is altered. You must select **Auto Recalculate** from the auto gate menu again to turn auto recalculate back on.

NOTE Auto recalculate turns on after adjusting movement or extent.

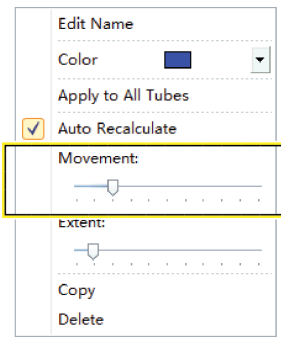
Right-click an autogate and select **Auto Recalculate** from the auto gate menu to toggle auto recalculate on and off.



Adjusting Autogate Movement and Extent

Movement — The distance an autogate can move to find the target population.

To adjust movement, right-click an autogate and drag the Movement handle in the auto gate menu left and right.



NOTE The default value setting for movement is 20 units. The minimum value setting for movement is 0 units and the maximum value setting for movement is 100 units.

If a target population is consistently in the same location, movement is not needed. However, if a target population is periodically missing from some samples, or events are rare, movement can be used to move the gate within a certain percentage of its axis to capture the correct population. Refer to [Figure 5.4](#) for an example of the default movement setting. Refer to [Figure 5.5](#) for an example of the maximum movement setting.

Figure 5.4 Movement - Default Setting

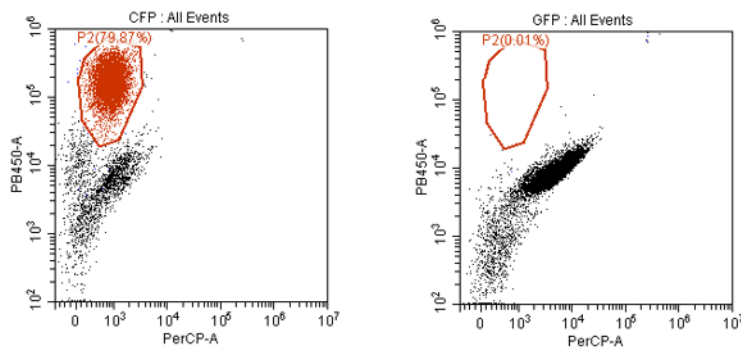
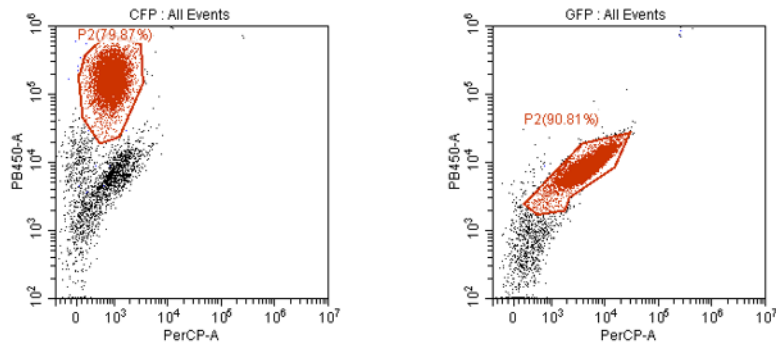
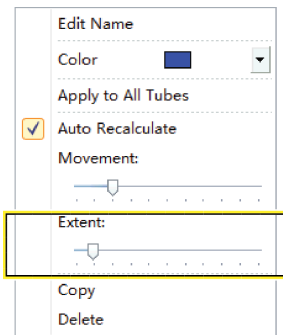


Figure 5.5 Movement - Max Setting



Extent — Shrinks or expands the gate around the population.

To adjust extent, right-click an autogate and drag the Extent handle in the auto gate menu left and right.



NOTE The default value setting for extent is 20 units. The minimum value setting for extent is 0 units and the maximum value setting for extent is 100 units.

Refer to [Figure 5.6](#) for an example of the default extent setting. Refer to [Figure 5.7](#) for an example of the maximum extent setting.

Figure 5.6 Extent - Default Setting

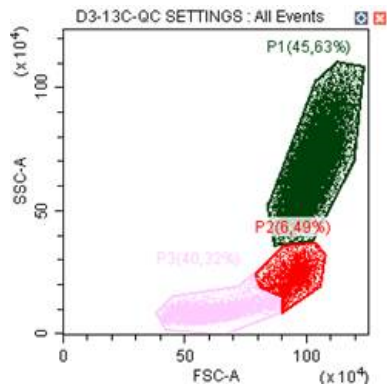
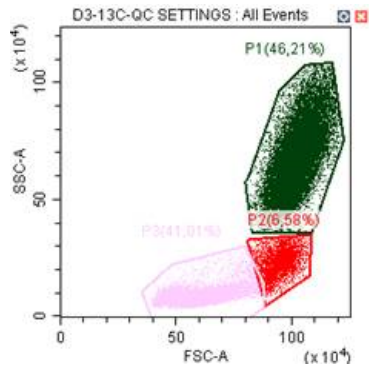


Figure 5.7 Extent - Maximum Setting

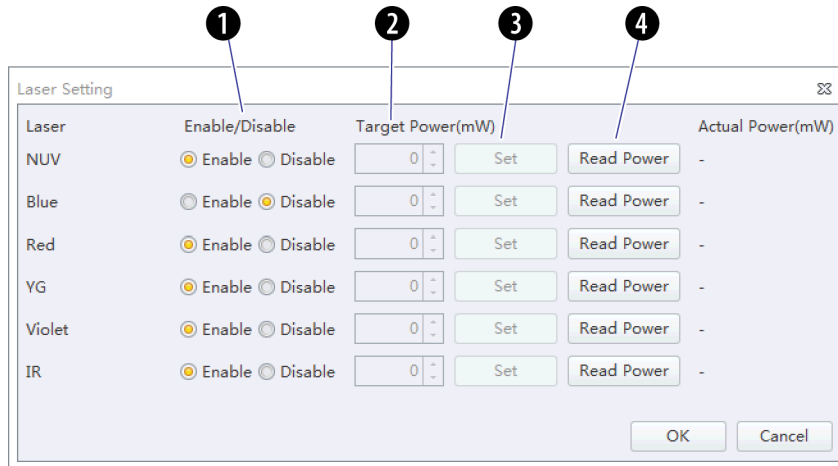


Laser Settings

To access the Laser Setting window, select **Advanced > Laser Setting**. The Laser Setting window appears. Refer to [Figure 5.8](#).

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

Figure 5.8 Laser Setting Window [CytoFLEX LX shown]



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

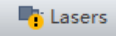
NOTE Laser target power can only be adjusted on the CytoFLEX LX system.

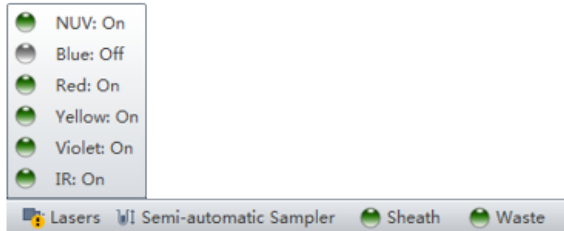
NOTE Refer to the target powers listed for each laser in the QC reports area of the QC Report Screen (refer to [Figure 2.2](#)) for range limits.

- 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.

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  to display details for each laser.



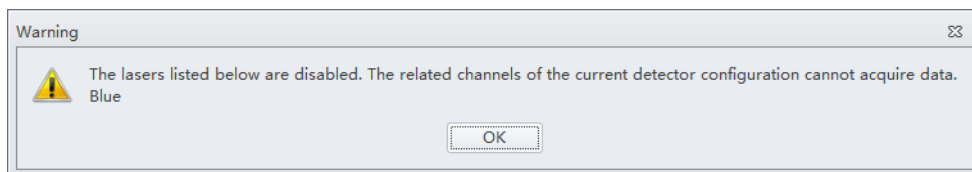
NOTE Lasers can only be enabled and disabled when the system is in standby mode.

Setting Laser Target Power Settings [CytoFLEX LX Only]

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

- 1 To access the Laser Setting window, select **Advanced > Laser Setting**. The Laser Setting window appears.
- 2 Ensure that all desired lasers are enabled.

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



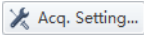
- 3 Select **Read Power** to view the real-time laser power reading.
The laser power displays under the **Actual Power (mW)** column located in the far right section of the Laser Setting window.
- 4 Adjust the target power for each laser as needed.
- 5 Select **Set** to set the device power.

-
- 6 Standardize your laser target values in the QC Experiment. Refer to [Standardization](#) in [CHAPTER 4, Instrument Quality Control and Standardization](#).

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

Adjusting the Gain

While the instrument is in use, the signal value can be increased or decreased by adjusting the instrument's gain configuration.

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

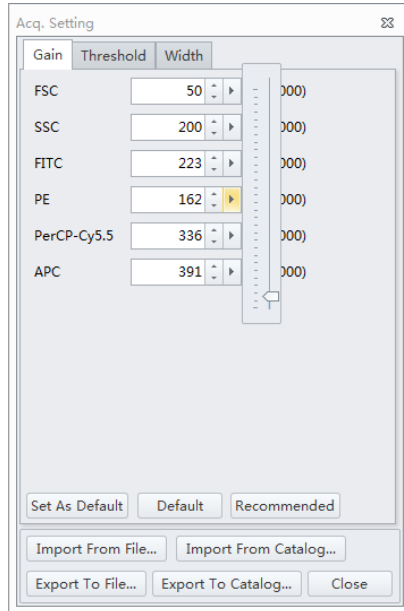
- 2 Select the **Gain** tab in the Acq. Setting window.

Select or edit the instrument's default gain settings using one of the following methods:

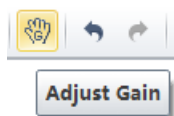
- Edit the gain settings and select **Set as Default** to create a new default setting.
- Select **Default** to return to your saved default settings.
- Select **Recommended** to use the instrument's QC settings.

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

- 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.



Another option is to use the **Gain Control** button  on the toolbar 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.

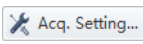
- If necessary, change the coordinate display range and the plot type.

Adjusting the Threshold

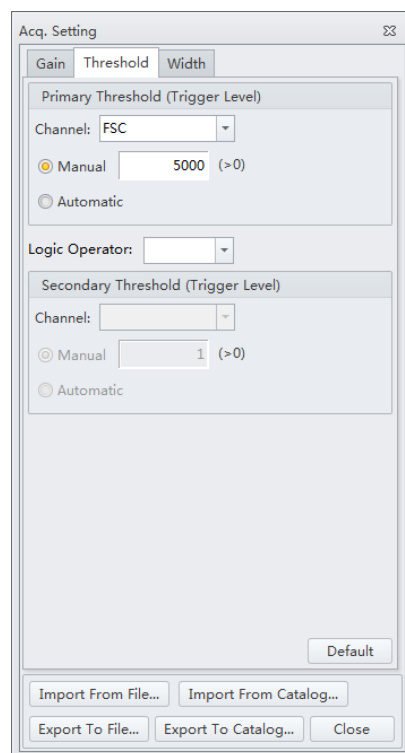
By adjusting the threshold, the user can remove unnecessary signal noise to ensure that most of the data collected consists of desired signal data. After the threshold settings have been configured for a given channel, the acquisition of data from this channel will only be triggered by signals that exceed the established threshold. Threshold settings have considerable bearing on whether the appropriate events can be acquired.

- 1 Create a plot to view the channels where the threshold will occur. Generally, a bivariate plot showing FSC and SSC is used.

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

- 2 Select  on the left side of the screen.

- 3 Select the **Threshold** tab in the Acq. Setting window.



4 Set the desired threshold using one of the following methods:

- Choose the channel that is used for setting the threshold. Manually enter the threshold value in the Threshold tab.


NOTE For dual-parameter plots, you can right-click the plot and select both parameters if desired. Then, select the desired threshold boundary for the second parameter.

- Select **Automatic** in the Primary Threshold Trigger Level section of the Acq. Setting screen to seek the target signal based on the background signal. It can quickly help find the target population if the signal-to-noise ratio (SNR) of the channel is comparatively good. The threshold can be set to either “H” (signal height) or “A” (signal area).

NOTE The automatic threshold value is based on the relative signal difference. When adjusting gain, you do not need to update the threshold settings. For channels with a low SNR or an excessively impure signal, manually setting the threshold parameters is recommended.

Moreover, “and” as well as “or” can be applied to as many as two channels, so as to allow these boolean logical operators to be used in setting the threshold value.

- “and”: Data is displayed and collected only when two threshold conditions are met simultaneously.
- “or”: Data is displayed and collected when at least one of two threshold conditions are met.

- Select  from the plot control area. Move your mouse pointer to the desired threshold position in the desired plot and select once.

5 Select **Close**.

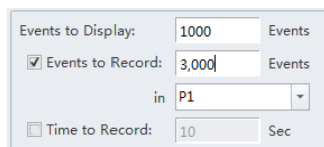
Setting Collection Conditions

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

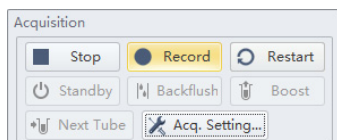
Two stop count collection conditions are available for sample recording:

- **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.

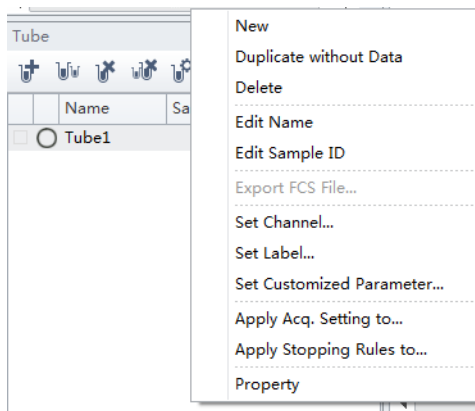
For example, if the event to record is set to record 1,000 P1 events, the software automatically stops recording when P1 events reach 1,000 events. However, the software saves all data acquired, including events outside of P1, when 1,000 P1 events is reached. You can also specify the time to store if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



- 2 Select **Record** and wait for the software to complete collecting the data, at which time the sample tube holder returns to the sample loading position (see [Figure 1.12](#)).

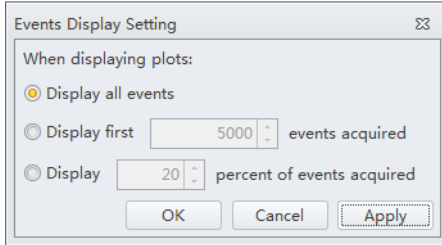


- 3 If you made changes to the data acquisition conditions and need to apply these changes to an established sample tube, right-click the sample tube and select **Apply Acq. Settings To**, to apply the conditions accordingly.



Setting Plot Display Conditions

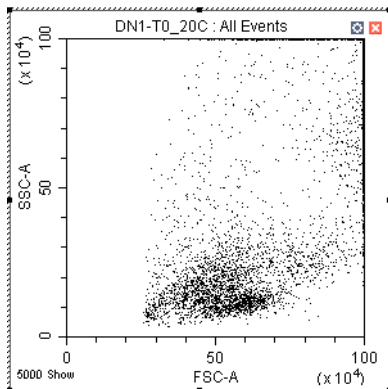
Select **Events Display Setting** in the Settings menu. The Events Display Setting window appears.



Three display options are available:

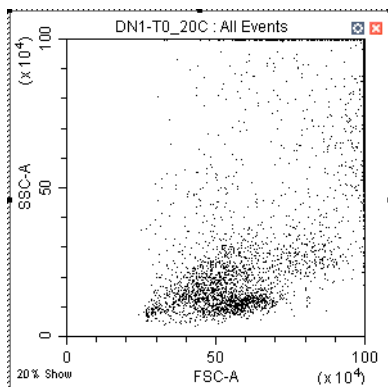
- **Display all events.** Used to view all events on the plot.
- **Display first XXXX events.** Used to set the set number of events to display.

NOTE The selected number of events displays in the bottom, left corner of the plot. For example if you choose to show 5000 events, the bottom, left corner of the plot displays *5000 Show*.



- **Display XX percent of events acquired.** Used to set the percentage of events to display.

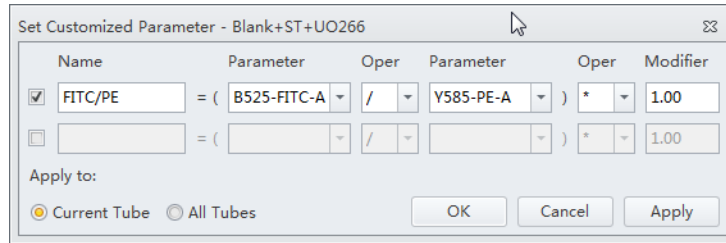
NOTE The selected percentage of events displays in the bottom, left corner of the plot. For example if you choose to show 20 percent of events acquired, the bottom, left corner of the plot displays *20% Show*.



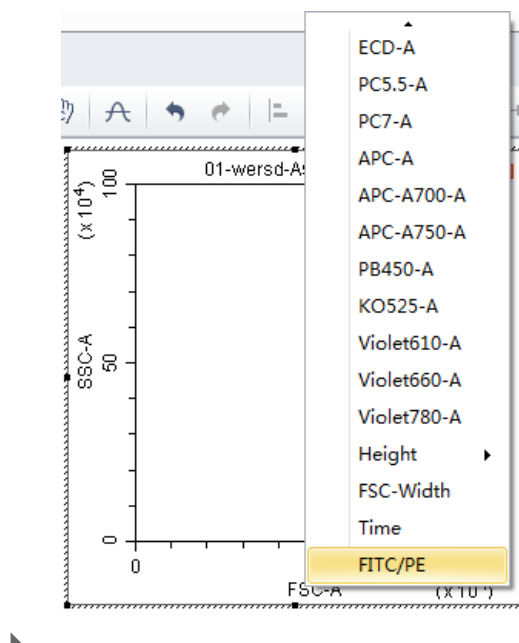
Setting Customized Parameters

Set custom parameter to create fluorescence calculations.

- 1 Select **Set Customized Parameter** from the Settings menu. Or, right-click a test tube from the test tube menu and select **Set Customized Parameter**. The Set Customized Parameter window appears.



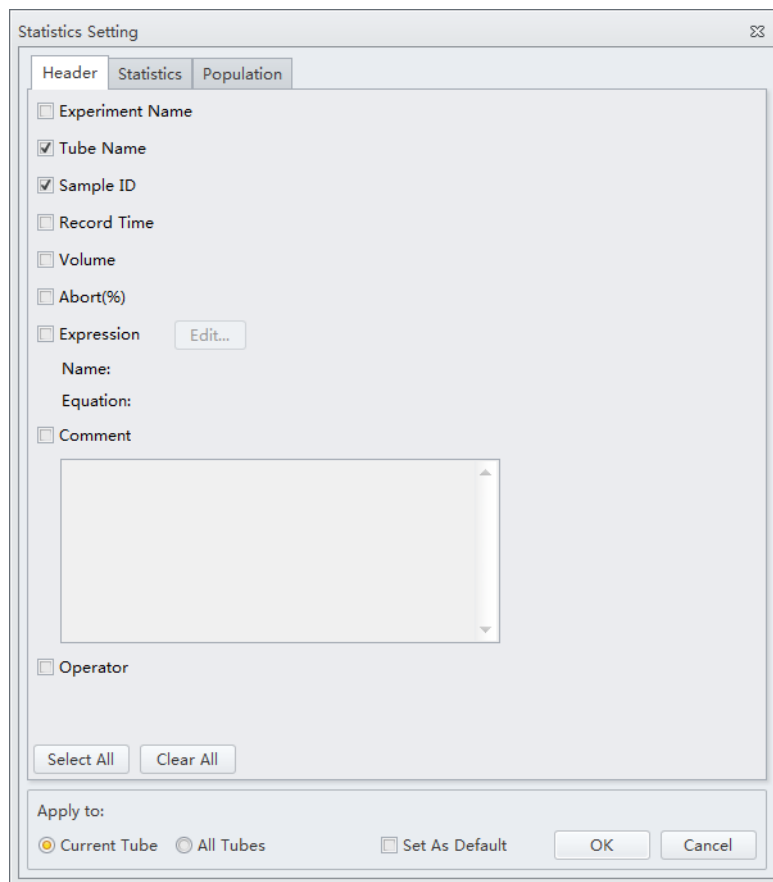
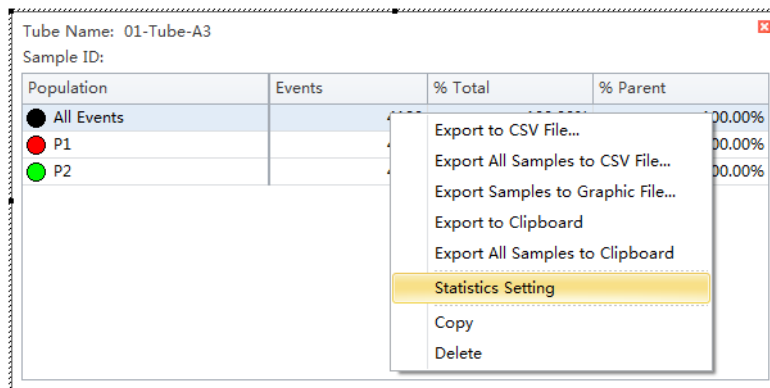
- 2 Enter a name for the parameter in the Name section.
- 3 Select the parameters for calculation in the Parameter dropdowns.
- 4 Select the equation operations from the Open dropdown menu.
The new parameter name is displayed in the list of parameters and statistic items.



Setting Custom Statistics

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

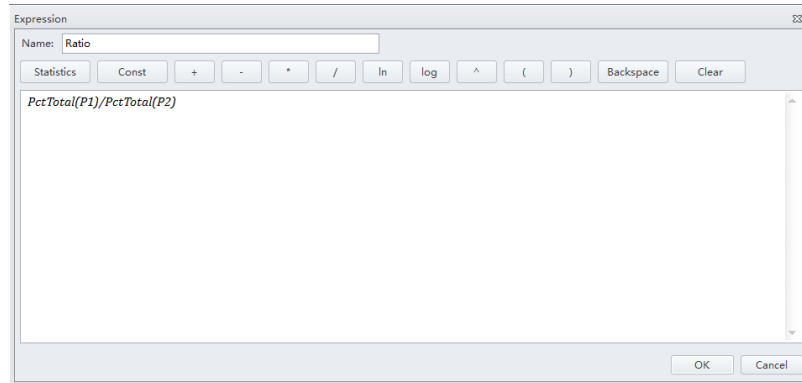
- 1 Right-click the statistics table and select **Statistics Setting**. The Statistics Setting window appears.



- 2 Select **Expression**.

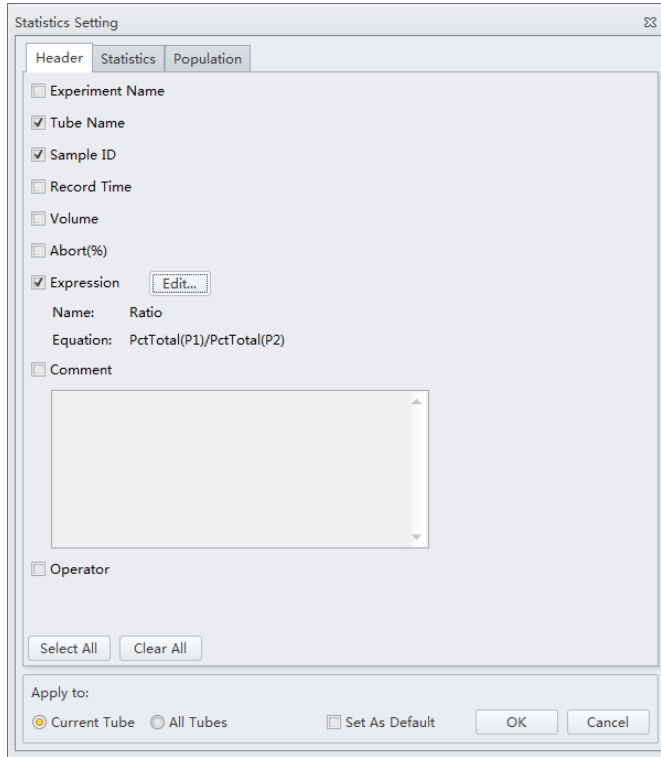
3 Select **Edit**. The Expression window appears.

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



5 Select OK.

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



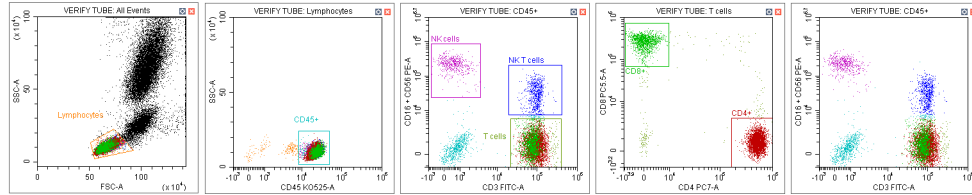
Tube Name: Admin Run
Sample ID:
Ratio: 0.92

Population	Events	% Total	% Parent
All Events	7803	100.00 %	100.00 %
P1	6799	87.13 %	87.13 %
P2	7370	94.45 %	94.45 %
P3	6904	88.48 %	88.48 %

Analyzing and Exporting Data

1 Select the sample tube to be analyzed.

- Establish new gates or adjust the position of existing gates. Refer to [Creating Plots and Gates](#).

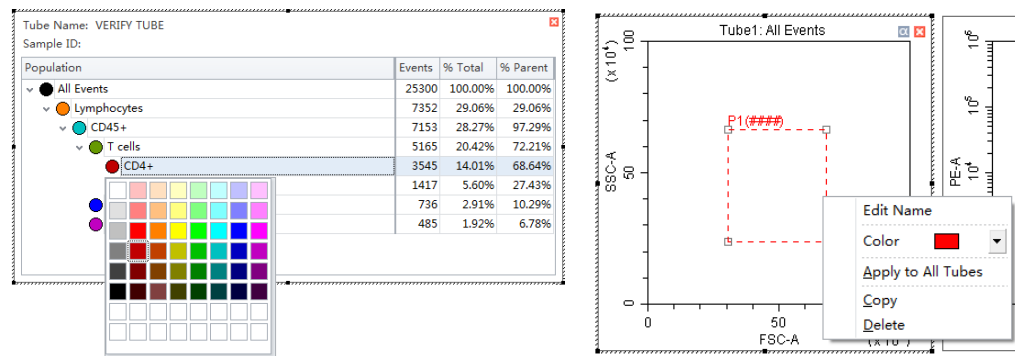


NOTE Changing a gate’s position does not affect the positions of other gates already established on a given sample tube. Each test tube individually records the positions of its associated gates. If you need to make a change that concerns all the tubes, you must select the gate, then right-click the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

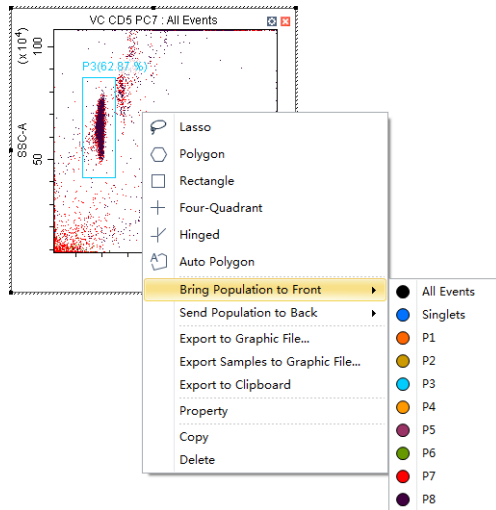
- Select . The Gate Hierarchy screen appears.


- 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.



- 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.



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

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

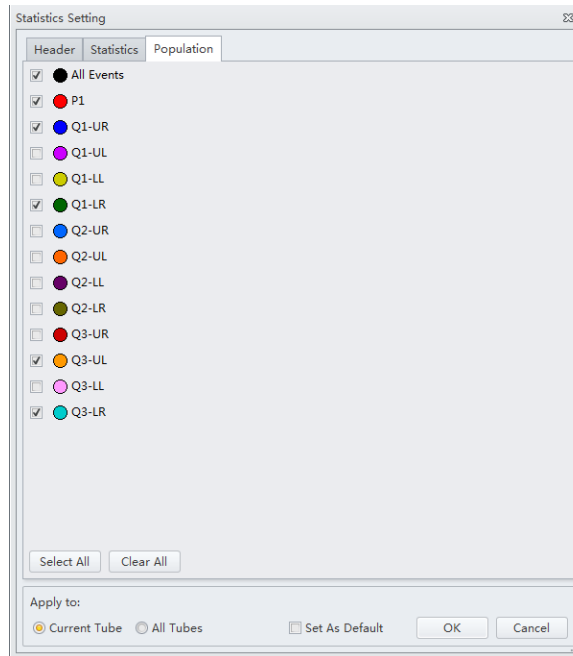
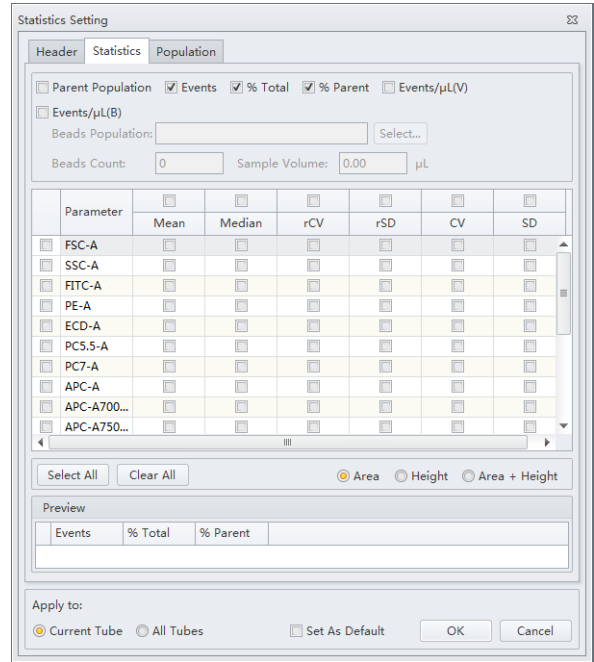
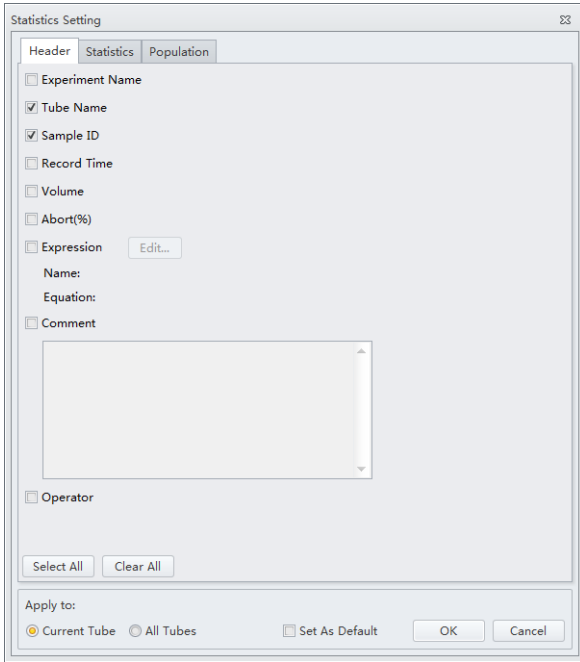
Tube Name: 4 color

Sample ID:

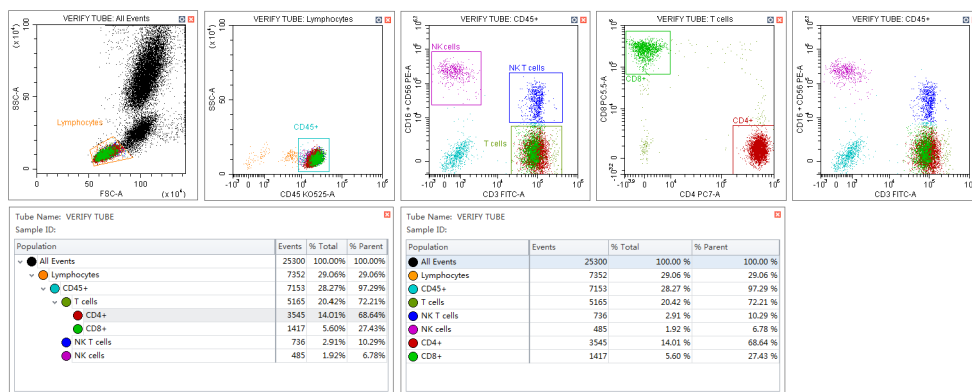
Population	Event	Parent
All Events		####
P1		####
Q1-UR		####
Q1-UL		####
Q1-LL		####
Q1-LR		####

A context menu is open over the table, listing options: Export to CSV File..., Export All Samples to CSV File..., Export Samples to Graphic File..., Export to Clipboard, Export All Samples to Clipboard, Statistics Setting, Copy, and Delete.

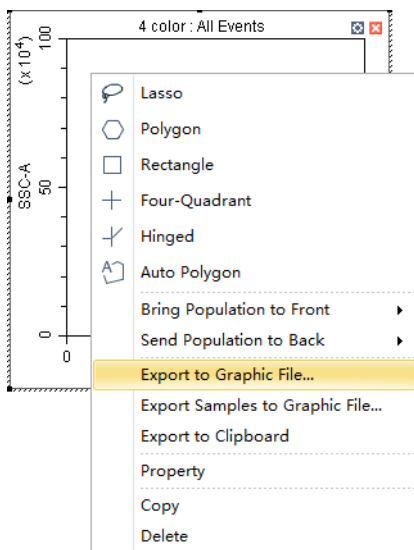
The Statistics Setting window allows you to change the display of the header, statistical elements and cell populations included.



The final generated plots appear as below.



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



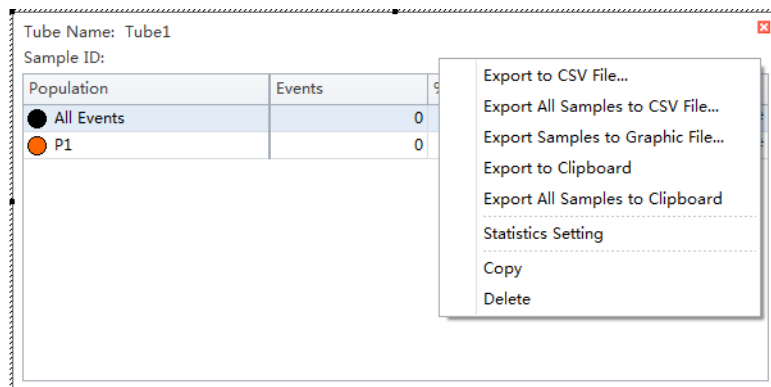
- **Export to Clipboard** copies the plot to the clipboard, allowing you to paste it directly into documents in common file formats.

NOTE Multiple plots can only be copied and pasted into Microsoft® Word. If a single plot is copied, this can be pasted into both Microsoft® Word or Microsoft® PowerPoint.

- **Export to File** saves the plot as an image file.

NOTE **Export to 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 File** exports individual tube statistics as a single CSV file.
- **Export All Samples to File** exports all tube statistics as a single CSV file.
- **Export to Clipboard** copies the statistics of an individual sample to the clipboard, allowing you to paste them directly into a Microsoft® Excel file or other file formats.
- **Export All Samples to Clipboard** assembles the statistics for all the sample tubes of an experiment and copies them together to the clipboard. From there they can be pasted as a group into a Microsoft® Excel file or other file formats.
- **Copy** converts a statistical table into an image format that can be pasted into documents.

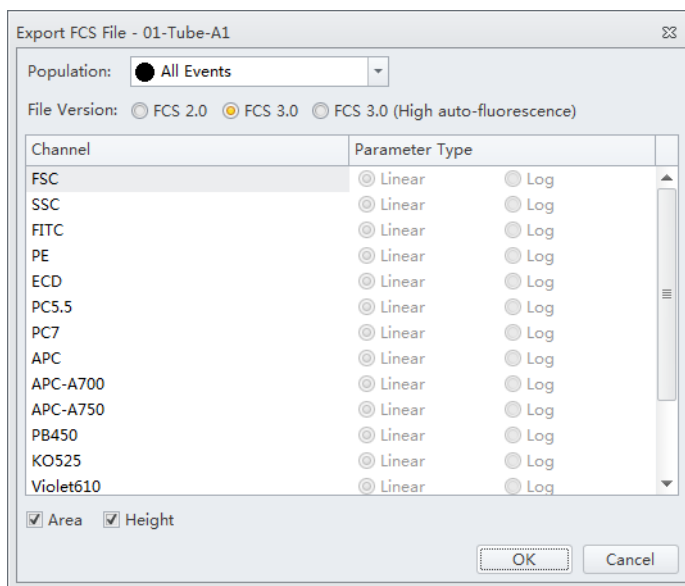
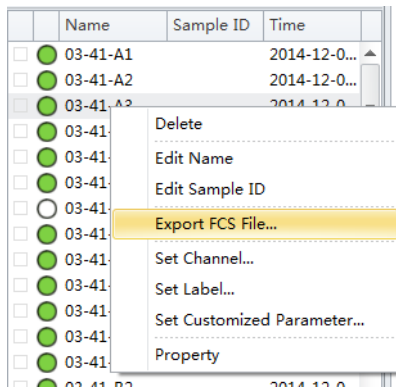
- 10 Export the FCS file if necessary. Refer to [Exporting FCS Files](#).

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

Exporting FCS Files

Exporting Single Tube Files

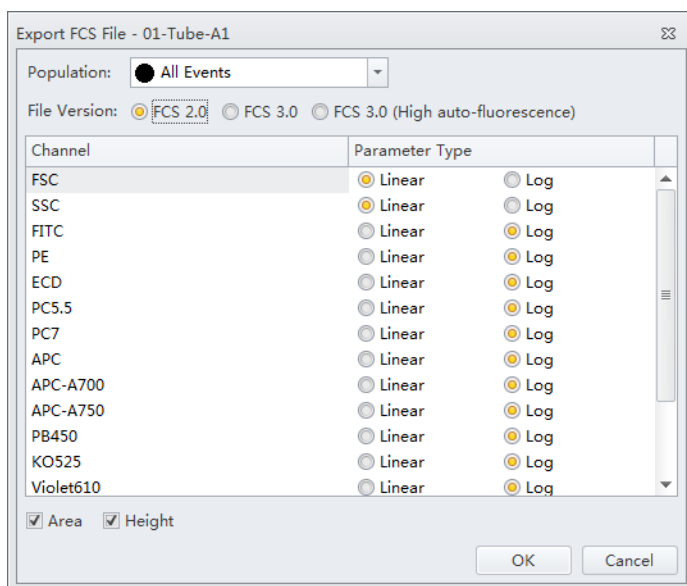
- 1 Right-click the desired tube from the test tube section of the screen and select **Export FCS File**. The Export FCS File window appears.



- 2 Select the population from the Population dropdown menu.
- 3 Select either **Area** or **Height**.

- 4 Select the FCS format next to File Version.

NOTE The default setting is FCS 3.0. If FCS 2.0 is selected, select the parameter type (linear or log) from the parameter type section of the window.



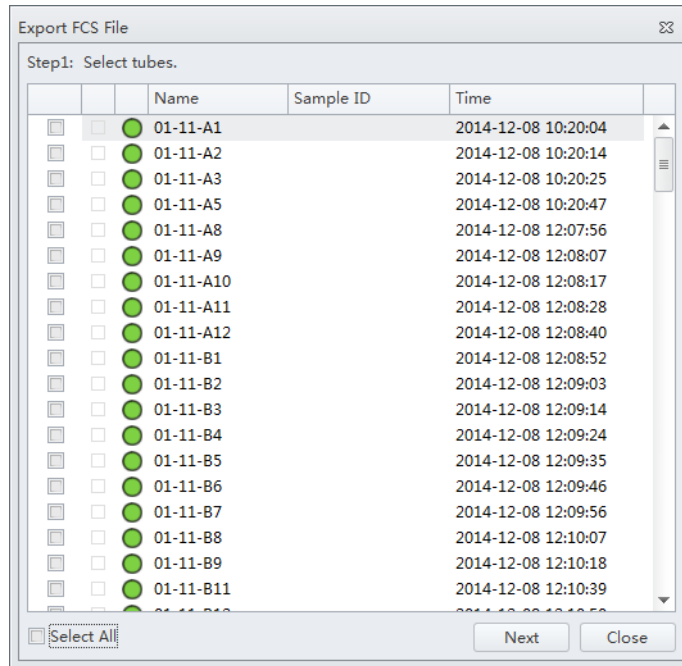
NOTE The default CytExpert FCS file contains high auto-fluorescence vector values that may not be recognized by third party software. Therefore, the data displays differently in third part software packages than in CytExpert. Auto-fluorescence values are added for the FCS 3.0 (High auto-fluorescence) export option to accommodate the use of third party software. Since both FCS 3.0 options have the same .fcs file extension, ensure that you save the FCS 3.0 (High auto-fluorescence) files to a different folder than the FCS 3.0 files.

- 5 Select the path to save the FCS file to from the Path section of the window.

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

Exporting Multiple FCS Files

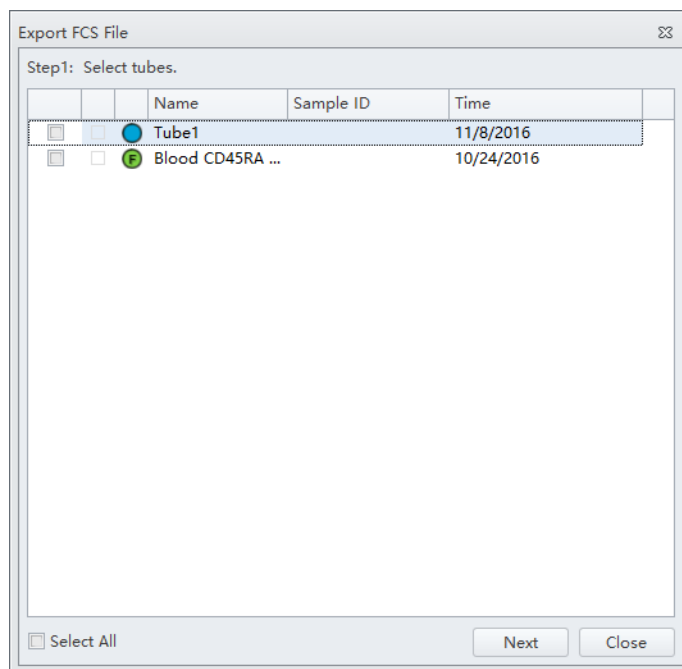
- 1 Select **Export FCS File** from the File menu. The Export FCS File window appears.



- 2 Select the tubes to export.
- 3 Repeat Steps 2-6 from [Exporting Single Tube Files](#).

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

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



- 2 Select the tubes to export.

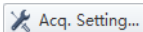
- 3 Select the path.

- 4 Select **OK**.

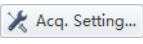
NOTE The plots of the selected tubes save as .bmp file.

Importing and Exporting Instrument Settings

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

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

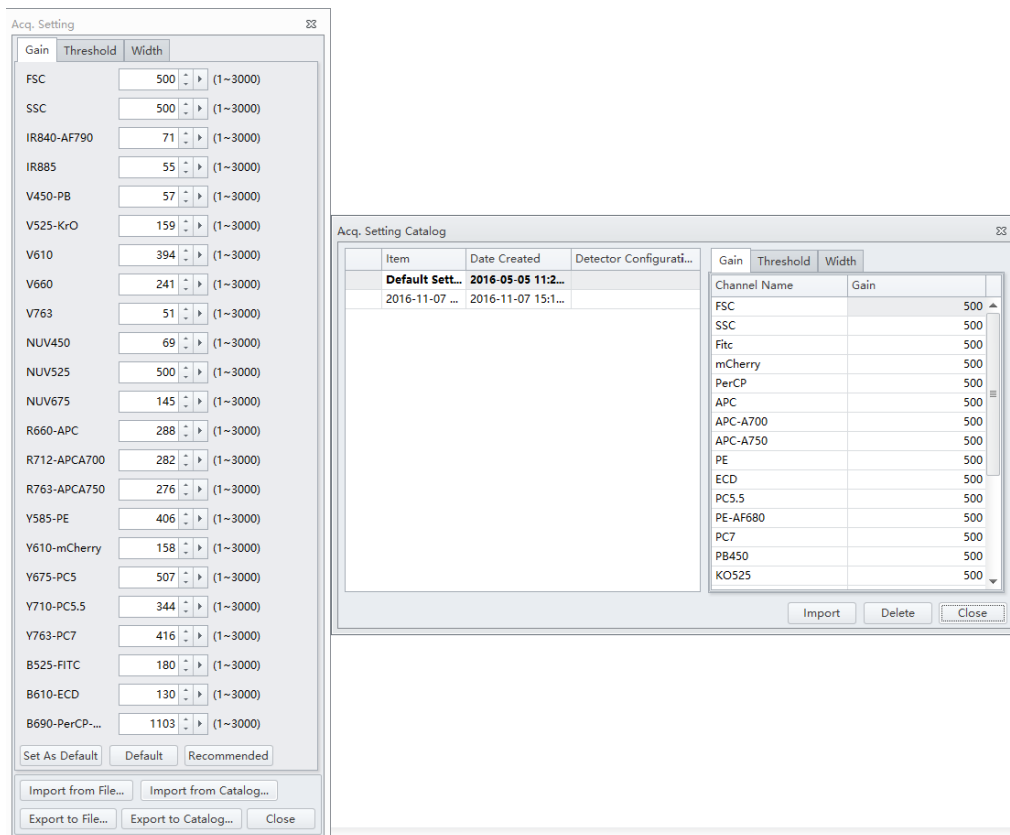
Importing Instrument Settings

- 1 Select the desired sample tube to import. Then select .

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

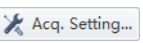
- 2 Select **Import From File**, locate the file with the required instrument settings, or select **Import From Catalog** to import the instrument settings.

[CytoFLEX LX Shown]



- 3 Select **Close**.

Exporting Instrument Settings

- 1 Select the desired sample tube to export. Then select .


-
- 2 Select **Export To File** to export a current set of instrument settings, stored in a file ending in .acq.
Or
Select **Export To Catalog**, give a name to the settings to be exported, and export the file to the software's Acquisition Setting Catalog, then select **OK**.
-
- 3 Select **Close**.
-

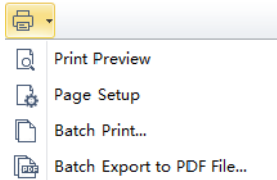
Importing and Exporting Compensation Settings


The software supports unrestricted importing and exporting of compensation data, regardless of whether the sample tube data has already been acquired. Imported compensation values only cover channels identical with the current instrument configuration. The software automatically adjusts compensation values according to differences in the gain level. Refer to [Importing and Exporting Compensation](#) in [CHAPTER 6, Compensation](#).

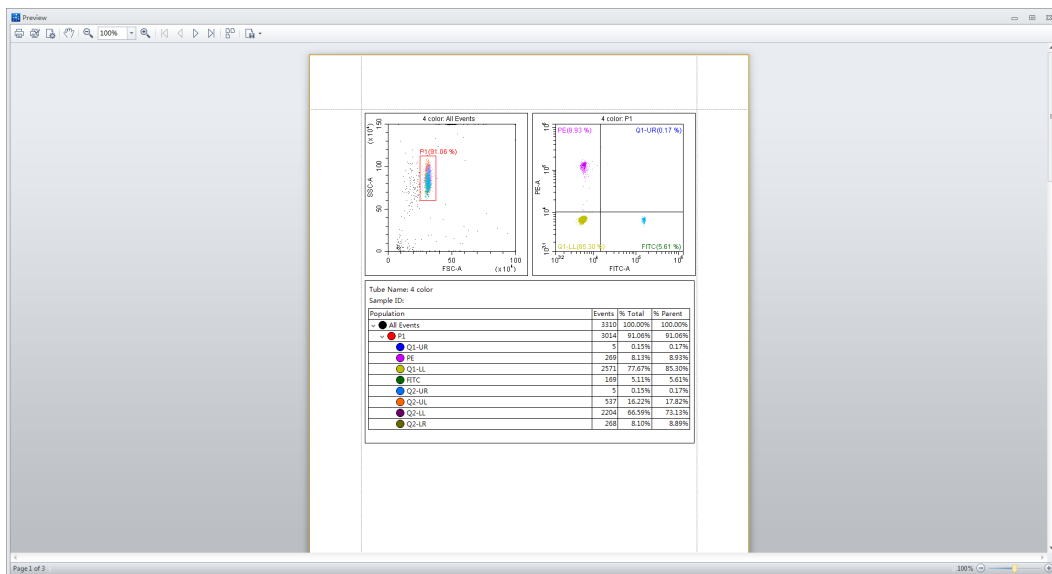
Printing Graphics


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

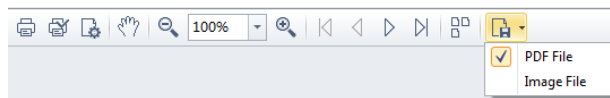
Select  in the printer control area to print directly. Or, select the print drop-down arrow for the following options:



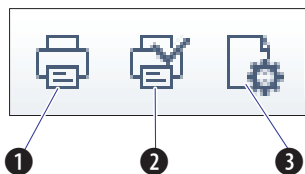
-  **Print Preview.** Used to access the Preview screen.





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

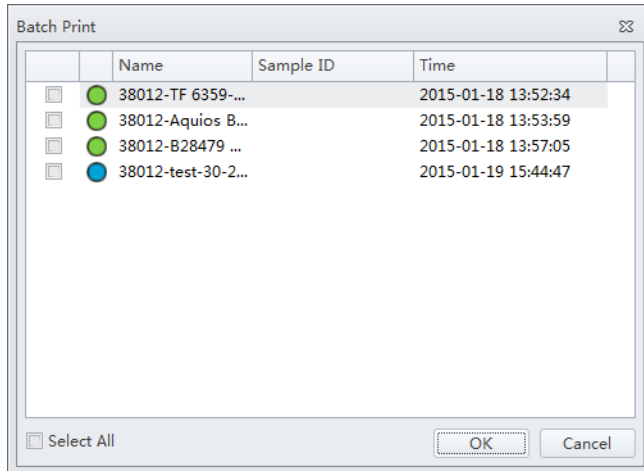


- Print preview also lets you choose between printing directly (1), modifying the printer configuration (2), or adjusting the page settings (3).




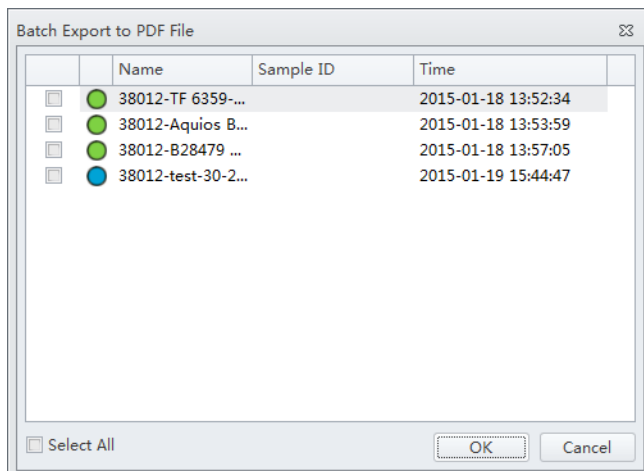
-  **Page Setup.** Used to adjust the page settings.

-  **Batch Print.** Used to print data for multiple tubes.
 1. Select **Batch Print**. The Batch Print window appears.



2. Select the tubes to print.
3. Select **OK**.

-  **Batch Export to PDF File.** Used to print a PDF of the data for multiple tubes.
 1. Select **Batch Export to PDF File**. The Batch Export to PDF File window appears.



2. Select the tube to print to PDF.
3. Select **OK**.

Saving the Experiment

Selecting **Save** in the File menu allows you to save the experiment.

Selecting **Save As** and saving the experiment under a different name allows you to create a backup.

Selecting **Save As Template** in the File menu allows you to save the experiment as a template.

Concluding the Experiment

Conclude the experiment as follows:

- Select **Standby** to return the instrument to the standby state.
- Select **File > Close** Experiment to clear the experiment and return to the Start Page.

NOTE If changes were made to the experiment, the software prompts you to save the latest changes in the experiment before returning to the Start Page.

- Shut down the system. Refer to [CHAPTER 8, Daily Shutdown](#).

Overview

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

Compensation involves correction for fluorescence spillover emitted by the primary fluorochrome that is detected by the secondary fluorescent channels. For example, the excitation and the resulting fluorescence emission for the PE fluorochrome leads to the spillover fluorescence detected in the ECD, PC5.5, and PC7 channel. Compensation reduces the spillover fluorescence of the PE-positive population to match the background of the PE-negative population in the secondary channels. Compensation requires a single positive and a negative population for every single color sample.

Properly configured compensation minimizes false data interpretation caused by spillover fluorescence from another fluorochrome. Refer to [Figure 6.1](#) and [Figure 6.2](#) for an example of plots before and after compensation. Compensation adjustments can be completed during the data acquisition process or after the data acquisition process is complete.

Figure 6.1 Before Compensation

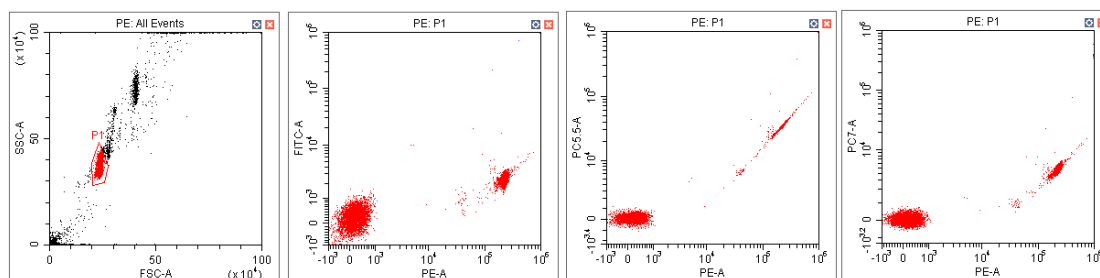
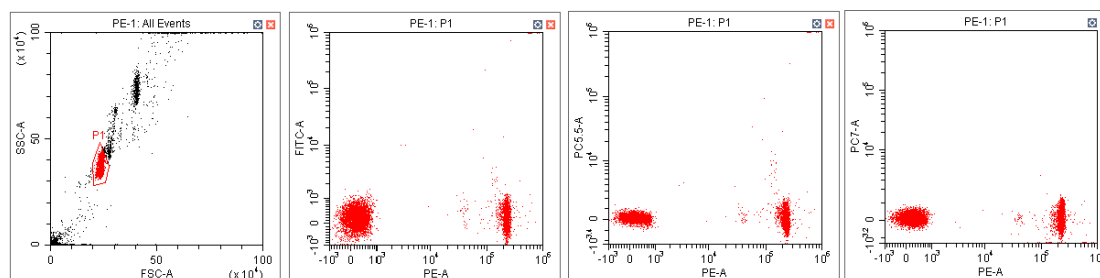


Figure 6.2 After Compensation



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

CytExpert compensation also includes a novel Compensation Library for storage of spillover values of dyes to easily determine the correct compensation matrix with new gain settings.

Workflow:



This chapter contains information on:

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

Creating a Compensation Experiment

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

- 1 Select **New Compensation** in the File menu or on the start page to create a new compensation experiment.

NOTE The file name of the newly created compensation experiment has a ".xipc" suffix.

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



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.

Compensation Setup

Compensation on:

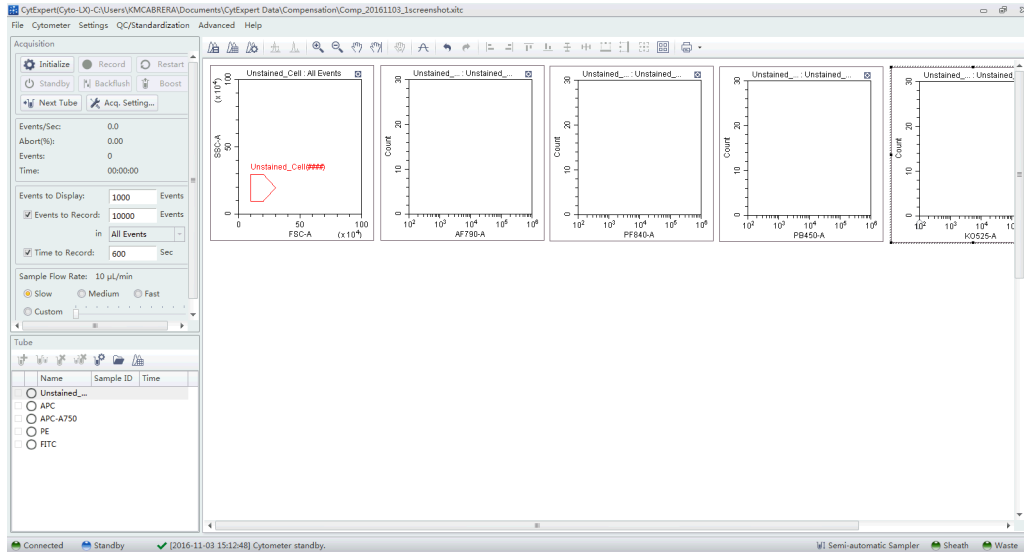
Area Height

Use	Tube	Label	Lot No.	Sample Type
<input checked="" type="checkbox"/>	Unstained_Cell			<input type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	Unstained_Bead			<input type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	FITC			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PE			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	ECD			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PC5,5			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PC7			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC-A700			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	APC-A750			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	PB450			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	KO525			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	Violet610			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	Violet660			<input checked="" type="radio"/> Cell <input type="radio"/> Bead
<input checked="" type="checkbox"/>	Violet780			<input checked="" type="radio"/> Cell <input type="radio"/> Bead

OK Cancel

4 Select OK.

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



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

Preparing the Compensation Sample

To perform a compensation experiment, prepare:

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

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

For the negative control sample and single positive control sample, you can use blood, cells, or dedicated compensation beads such as VersaComp Antibody Capture Beads. For details, refer to the appropriate reagent instructions for use. The negative control tube is used to determine the autofluorescence of the sample.

Using Control Samples to Generate the Compensation Matrix

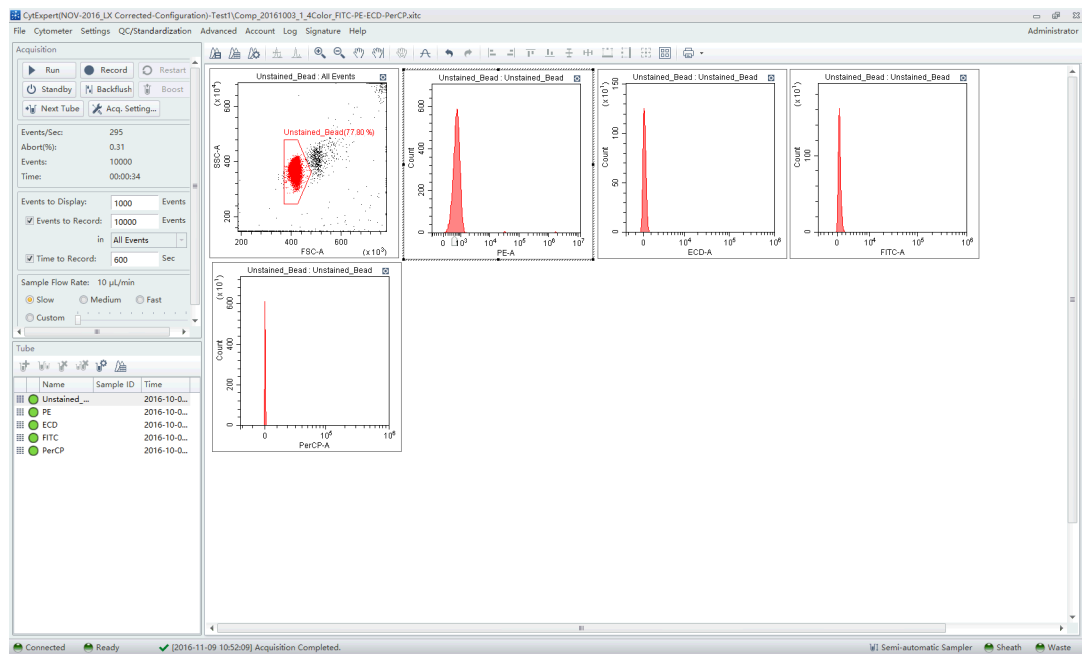
Defining the Negative Population Using Unstained Samples

- 1 Confirm that the instrument has been initialized. Refer to [Initializing the Instrument in CHAPTER 3, Daily Startup](#).

CAUTION

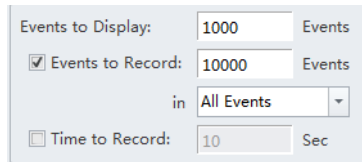
Risk of erroneous results. Calculations based on excessively small volumes of sampled data can be inaccurate. Ensure that more than 1,000 positive events and more than 1,000 negative events are sampled. If the ratio of positive cells is comparatively low, increase the number of acquisition events to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

- 2 Import the gain setting and apply the setting to all tubes. Refer to [Adjusting the Gain](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#). Use the pan tool to adjust the axis scale so that the sample signal appears in a suitable position. Adjust the gate so that it encloses the target cell population (see [Creating Plots and Gates](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#)).



- 3 Place the negative control tube in the sample tube holder.
- 4 Select the unstained tube.
- 5 Select **Run** to load the sample.

- 6** Set an appropriate number of cells to save in Events to Record located on the left side of the screen.



The screenshot shows a dialog box with the following settings:

- Events to Display: 1000 Events
- Events to Record: 10000 Events
- in All Events (dropdown menu)
- Time to Record: 10 Sec

- 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.
-

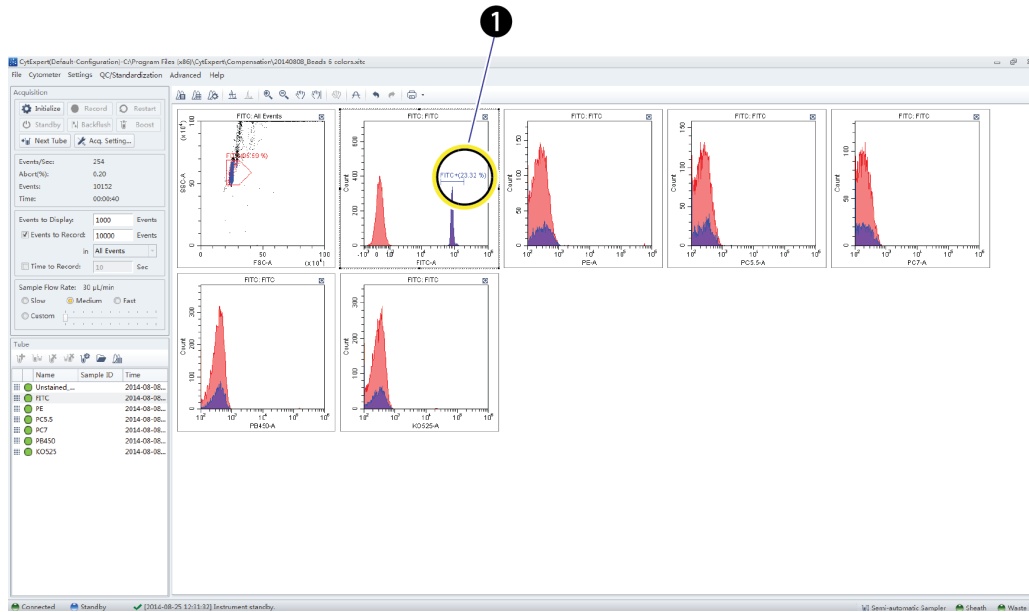
CAUTION

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

- 4 Move the gate in the FSC/SSC plot so that it encloses the desired population. Move the positive gate in the plot so that it encloses the positive population. If necessary, move the positive gate so that it encloses the positive population.

NOTE Figure 6.3 shows an example of selecting the positive population when the negative population is defined by the unstained sample.

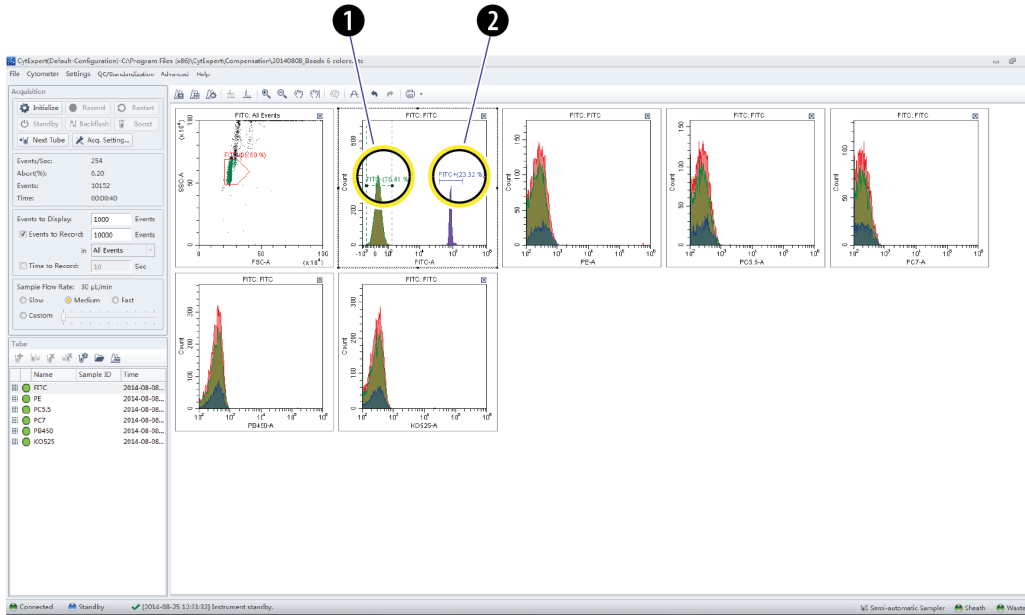
Figure 6.3 Positive Population Selected from the Unstained Sample



1. Positive population

NOTE Figure 6.4 shows an example of selecting both the positive and negative populations without an unstained sample.

Figure 6.4 Positive and Negative Populations Without an Unstained Sample



1. Negative population
2. Positive population

5 Select **Record**.

6 Repeat steps 1-5 to acquire data from subsequent single positive sample tubes.

CAUTION

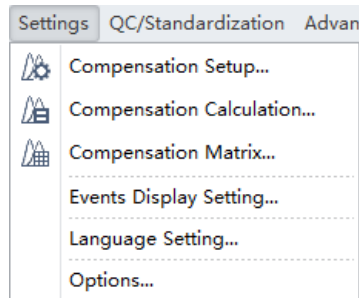
Risk of erroneous results. While the software automatically adjusts the compensation calculation according to gain, excessive manual adjustment of the fluorescence gain could lead to inaccurate results.

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

Calculating Compensation Values

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

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



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

Channel	-FITC%	-PE%	-ECD%	-PC5.5%	-PC7%	-APC%	-APC-A700...	-APC-A750...	-PB450%	-KOS25%	-Violet610%	-Violet660%	-Violet780%
FITC		0.66	0.11	0.02	1.43	0.00	0.08	0.00	0.00	0.91	0.11	0.00	0.02
PE	35.08		18.23	1.13	2.39	0.00	0.10	0.00	0.00	1.14	4.10	0.07	0.01
ECD	12.83	41.79		0.55	1.75	0.00	0.09	0.00	0.00	0.80	9.47	0.34	0.02
PC5.5	4.96	18.15	65.09		1.64	0.52	0.88	0.03	0.00	0.49	9.15	2.04	0.07
PC7	1.36	4.32	19.07	71.65		0.17	1.04	1.39	0.00	0.05	3.28	0.58	7.29
APC	0.02	0.03	1.12	2.67	0.22		9.25	6.07	0.00	0.16	1.10	75.01	0.08
APC-A700	0.03	0.15	0.45	43.95	0.00	28.94		2.48	0.00	0.20	0.50	36.76	0.36
APC-A750	0.10	0.21	0.20	21.48	13.85	13.34	56.58		0.00	0.13	0.23	14.52	23.06
PB450	0.10	0.22	0.00	0.04	0.00	0.00	0.15	0.00		8.26	8.62	6.12	2.94
KOS25	1.89	0.15	0.00	0.03	0.33	0.00	0.06	0.00	7.67		0.55	0.35	0.19
Violet610	0.47	3.11	6.79	0.02	1.14	0.01	0.05	0.00	0.58	81.36		13.97	0.08
Violet660	0.26	1.48	3.56	0.22	0.00	2.03	0.19	0.10	0.00	45.01	92.28		0.12
Violet780	0.13	0.23	0.78	4.62	8.40	0.46	2.22	3.72	0.00	12.20	30.20	27.58	

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

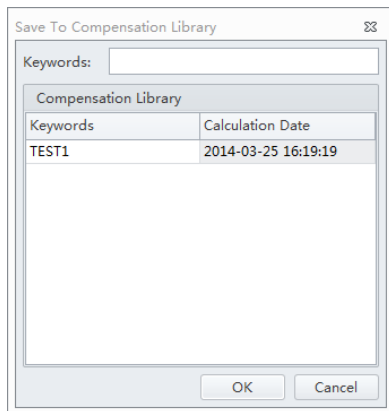
NOTE In the Compensation Matrix window:

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

- 3 Select **Save As** to export the compensation matrix as a .comp file and specify where to save it.

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

- 4 Select **Save To Compensation Library** to save the single color compensation values in the compensation library.

5 Specify the key words and select **OK**.

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

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

6 Select **Close**.

Creating a Compensation Experiment [With Plate Loader]

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

- 1** Select **New Compensation** in the File menu or on the start page to create a new compensation experiment.

NOTE The file name of the newly created compensation experiment has a ".xipc" suffix.

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

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

CAUTION

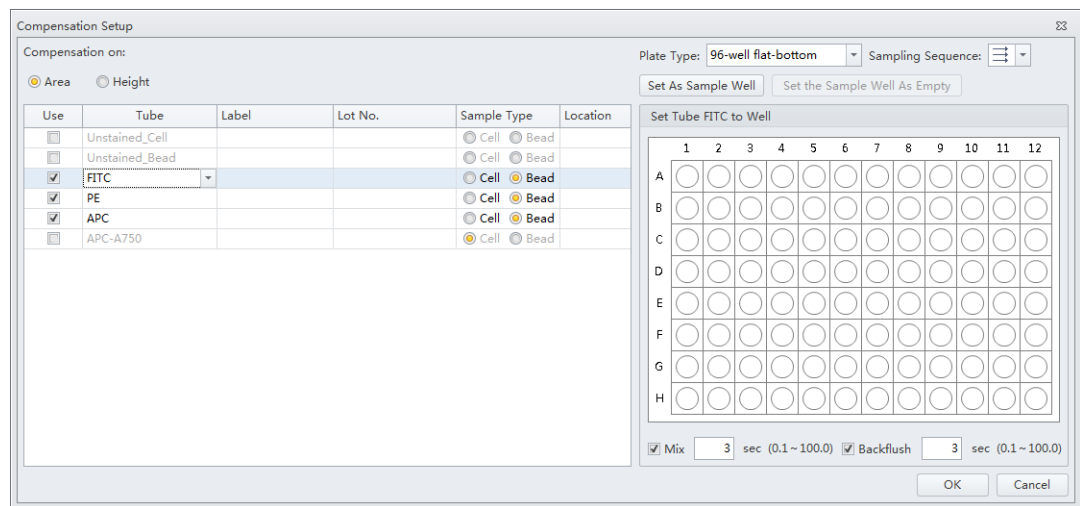
Risk of erroneous results. Select an unstained tube, according to which the fluorescence background will be set. If there is not an unstained tube, then each single marker tube must have a negative population.

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

- 4 Select the channel requiring compensation calculation, and the sample type.
If a negative population is not present in each single color well, then an unstained control well is recommended.

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

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



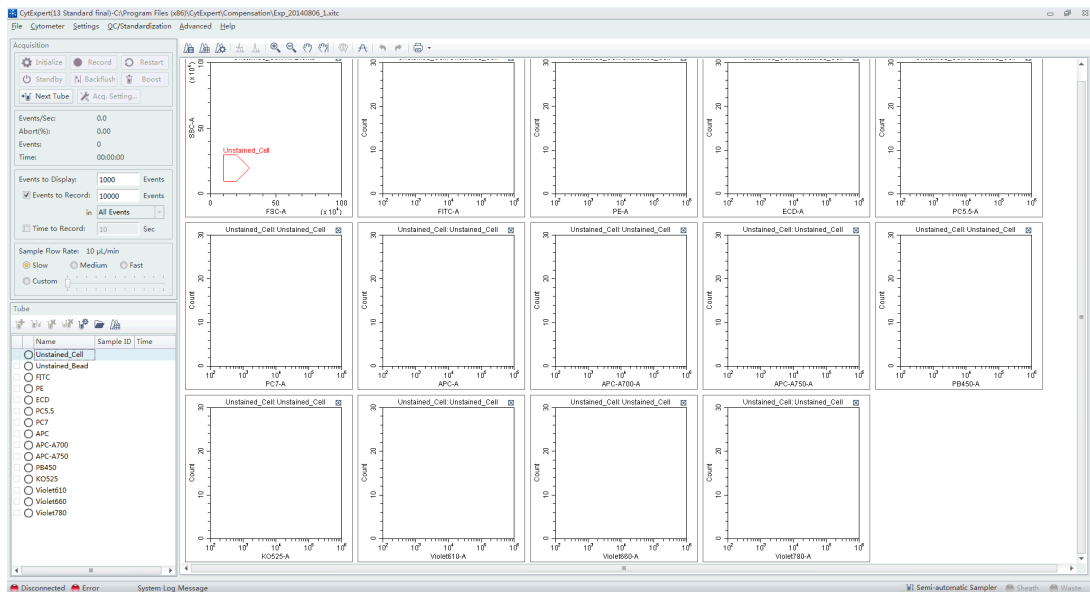
- 5 Select the Mix and Backflush settings in the bottom, right of the Compensation Setup window.

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

NOTE The well location populates in the location column.
 - d. Repeat Steps a-c for each fluorochrome.

7 Select **OK**.

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



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

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

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

Preparing the Compensation Sample

To perform a compensation experiment, prepare:

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

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


For the negative control sample and single positive control sample, you can use blood, cell lines, or dedicated compensation beads such as VersaComp Antibody Capture Beads. For details, refer to the appropriate reagent instructions for use. The negative control tube is used to determine the autofluorescence of the sample.

Using Control Samples to Generate the Compensation Matrix

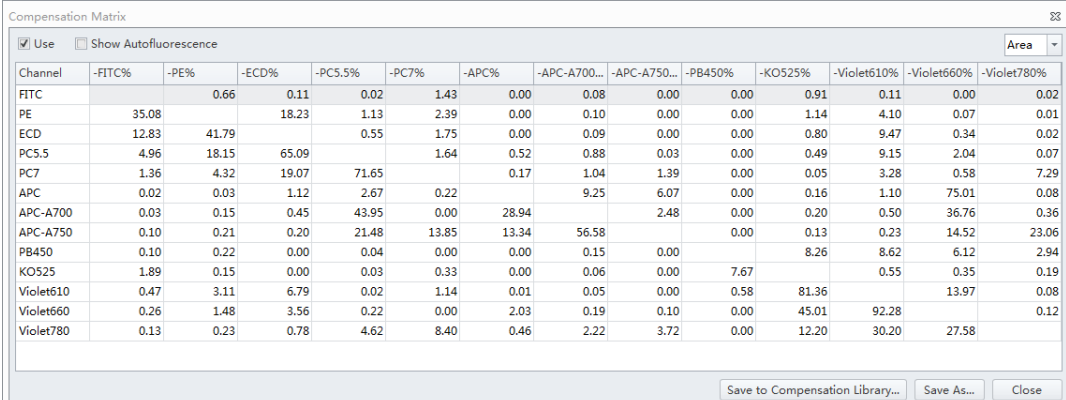
Refer to [Defining the Negative Population Using Unstained Samples](#) and [Running the Single Positive Control Samples](#) in CHAPTER 6, Compensation.

Calculating Compensation Values

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

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

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

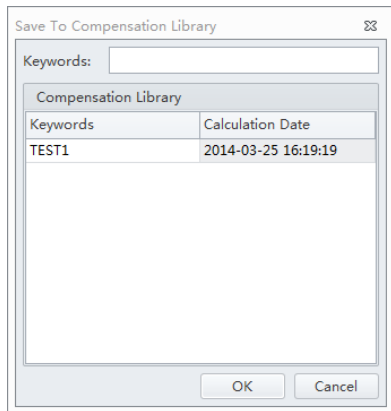


Channel	-FITC%	-PE%	-ECD%	-PC5.5%	-PC7%	-APC%	-APC-A700...	-APC-A750...	-PB450%	-KO525%	-Violet610%	-Violet660%	-Violet780%
FITC		0.66	0.11	0.02	1.43	0.00	0.08	0.00	0.00	0.91	0.11	0.00	0.02
PE	35.08		18.23	1.13	2.39	0.00	0.10	0.00	0.00	1.14	4.10	0.07	0.01
ECD	12.83	41.79		0.55	1.75	0.00	0.09	0.00	0.00	0.80	9.47	0.34	0.02
PC5.5	4.96	18.15	65.09		1.64	0.52	0.88	0.03	0.00	0.49	9.15	2.04	0.07
PC7	1.36	4.32	19.07	71.65		0.17	1.04	1.39	0.00	0.05	3.28	0.58	7.29
APC	0.02	0.03	1.12	2.67	0.22		9.25	6.07	0.00	0.16	1.10	75.01	0.08
APC-A700	0.03	0.15	0.45	43.95	0.00	28.94		2.48	0.00	0.20	0.50	36.76	0.36
APC-A750	0.10	0.21	0.20	21.48	13.85	13.34	56.58		0.00	0.13	0.23	14.52	23.06
PB450	0.10	0.22	0.00	0.04	0.00	0.00	0.15	0.00		8.26	8.62	6.12	2.94
KO525	1.89	0.15	0.00	0.03	0.33	0.00	0.06	0.00	7.67		0.55	0.35	0.19
Violet610	0.47	3.11	6.79	0.02	1.14	0.01	0.05	0.00	0.58	81.36		13.97	0.08
Violet660	0.26	1.48	3.56	0.22	0.00	2.03	0.19	0.10	0.00	45.01	92.28		0.12
Violet780	0.13	0.23	0.78	4.62	8.40	0.46	2.22	3.72	0.00	12.20	30.20	27.58	

3 Select **Save As** to export the compensation matrix as a .comp file and specify where to save it.

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

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



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

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

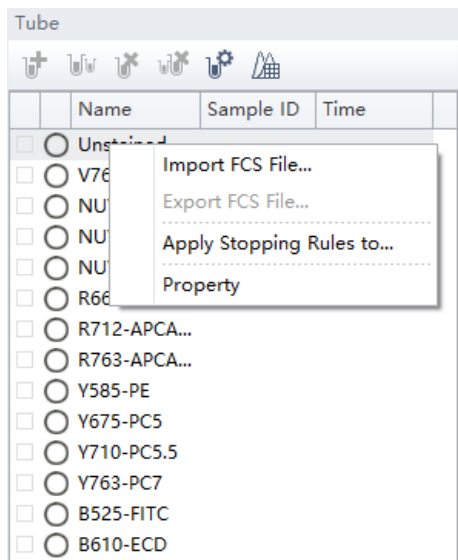
-
- 6 Select **Close**.
-

Creating the Compensation Matrix from Previously Acquired Data

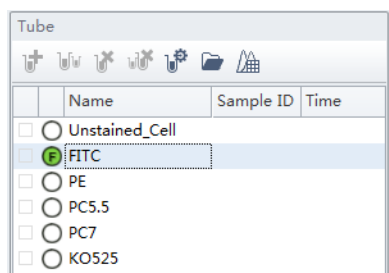
The software supports importing single color data acquired from other experiments into a compensation experiment to perform compensation calculations. The data to be imported must match the active detector configuration at the time that the compensation experiment was created. Otherwise, the data cannot be imported. It is important to ensure that imported data comes from the same instrument and uses the same configuration and channels. Data originating from a different instrument will cause erroneous calculations.

-
- 1 Select **New Compensation** from the File menu or the start page.
-
- 2 To create a compensation experiment, select the required channels. Refer to [Setting the Channel and Label](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#).

- 3 Right-click on the appropriate test tube and select **Import FCS File**. Locate the corresponding data file and import the file. Only files compatible with the detector configuration are supported by the software for importing.



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




- 4 After importing the data, adjust the gates to properly identify the positive population and the negative population for each single-color samples.
- 5 Calculate the compensation values and export them. Refer to [Calculating Compensation Values](#).

Adjusting Compensation

Manually Adjusting Compensation

The compensation can be manually adjusted in an experiment in two ways:

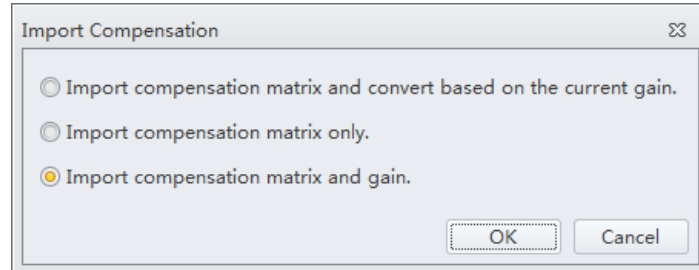
- Select the bivariate plot which the compensation needs to be adjusted. Select  from the graphic control area, then click and drag the mouse pointer up and down or left and right inside the plot to adjust compensation.
- Select **Compensation Matrix** in the Setting menu to open the compensation matrix. Adjust the compensation value between the primary channel and the secondary channel.

Importing and Exporting Compensation

Importing Compensation Settings from Compensation Matrix Files

- 1 Select the desired sample tube for importing compensation values.
- 2 Select **Compensation Matrix** in the Setting menu.
- 3 Select **Import** and locate the path where compensation matrix files are saved. Select the corresponding compensation matrix file (.comp) to import the compensation values.
You can also select **Import from Library** to import compensation values from the compensation library. The Import from Compensation Library window appears. Refer to [Importing Compensation Settings from the Compensation Library](#).
Both methods allow you to import the compensation values with or without the adjustment based on the gain settings.

- 4 After opening the desired compensation file, the Import Compensation window appears. Select one of the following:
- Import compensation matrix and convert it with current gains.
 - Import compensation matrix.
 - Import compensation matrix and gain.

**NOTE**

- If the tube does not have any data when importing compensation values calculated from other instrument settings, the software prompts you to select whether the gain settings must be imported as well. Select **Yes** to import fluorescence channel gains settings along with the rest of the data. Select **No** to allow the CytExpert software to adjust the compensation matrix values based on the current gain settings.
- If the tube does have data when importing compensation values from other instrument settings, the software prompts you to select whether the compensation values are adjusted based on the current gain settings.
- It is important to note that automatic adjustments to compensation values calculated from other instrument gain settings could result in incorrect compensation. Always review the data after importing compensation values to ensure the sample is compensated properly.

- 5 Select **OK**.

- 6 If necessary, select **Apply to** to apply the compensation values to the selected test tubes.

- 7 Select **Close**.

Importing Compensation Settings from the Compensation Library

You can choose which single color data to include from the compensation library. Only single color data in the compensation library from the same detector configuration can be imported into the compensation matrix.

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

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

[CytoFLEX LX Shown]

- 2 In the Keywords column, the corresponding compensation values can be selected for each channel. The compensation values of the same keyword can also be selected using the drop-down menus in the Keywords column.

- 3 Select **OK** to import the compensation values.

Exporting Compensation Settings

- 1 Select the desired sample tube to export.
- 2 Select **Compensation Matrix** in the Setting menu.
- 3 Select **Export** to specify a path and filename for the compensation file you are saving.

[CytoFLEX LX Shown]

Autofl.	Channel	-AF79 0%	-PF840...	-PB45 0%	-KOS2 5%	-Violet 610%	-Violet 660%	-Violet 780%	-DAPI%	-NUV5 25%	-HoechstRe...	-APC%	-APC- A700%	-APC- A750%	-PE%	-ECD%	-PC5.5%	-PEAF 680%	-PC7%	-FITC%	-ECDm Cherry...	-PerCP...
0.00	AF790		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PB440	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PB450	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	KOS25	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	Violet610	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	Violet660	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	Violet780	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	DAPI	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	NUV525	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	HoechstR...	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC-A700	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	APC-A750	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	PE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	ECD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00
0.00	PC5.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
0.00	PEAF680	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00
0.00	PC7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
0.00	FITC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00
0.00	ECDmCh...	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00
0.00	PerCP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

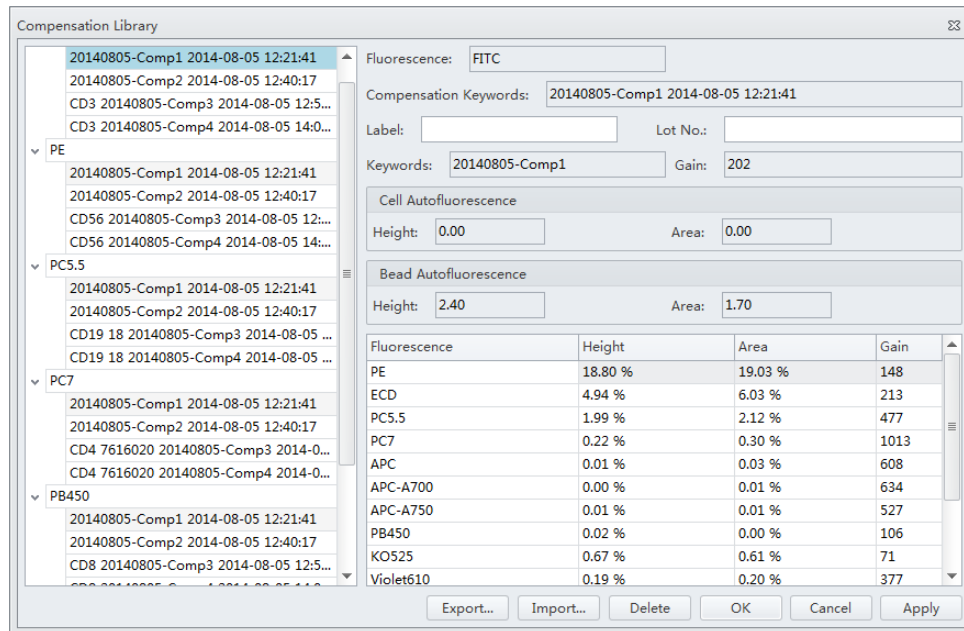
- 4 Select **Save**.

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

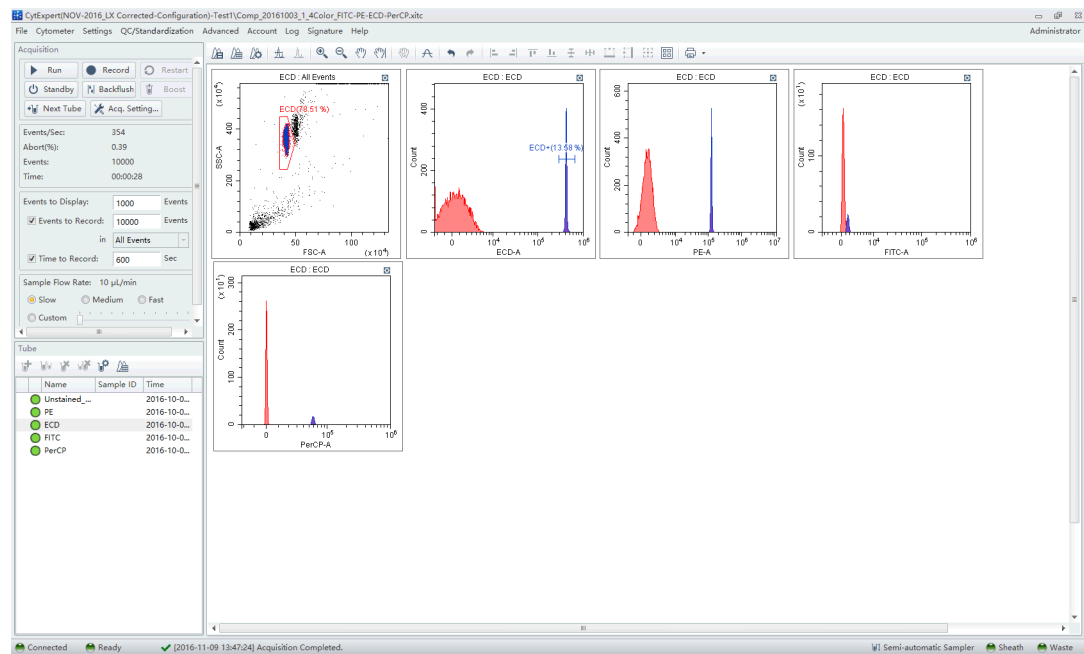
Channels requiring compensation calculations that have not been previously acquired can be added to the compensation experiment by acquiring the necessary positive tubes.

- 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.

Overview

This chapter discusses how to use the Analysis screen to analyze data. Data can be analyzed using any computer equipped with the CytExpert software. No online connection is required.

Workflow:

Import experiment or data → Plot and configure statistics → Export results

This chapter contains information on:

- [Copying Experiments and Importing Data](#)
- [Setting the Plots and Statistics](#)
- [Calculating Sample Volume and Concentration](#)
- [Adjusting Compensation Settings](#)
- [Exporting Results](#)

Copying Experiments and Importing Data

Copying a Previously Acquired Experiment

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

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

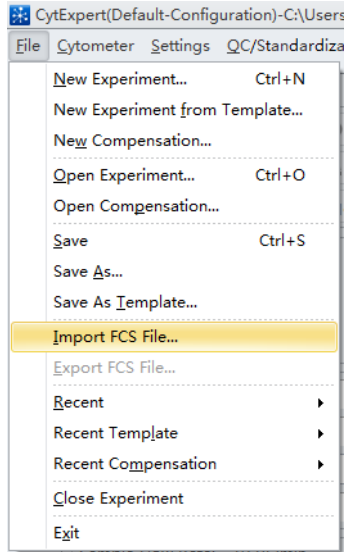
NOTE The .xit and data folder must be stored in the same path.

Importing Previously Acquired Data

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

- 1 Create a new experiment or open a saved experiment. Refer to [Creating an Experiment](#) in [CHAPTER 5, Data Acquisition and Sample Analysis](#).

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




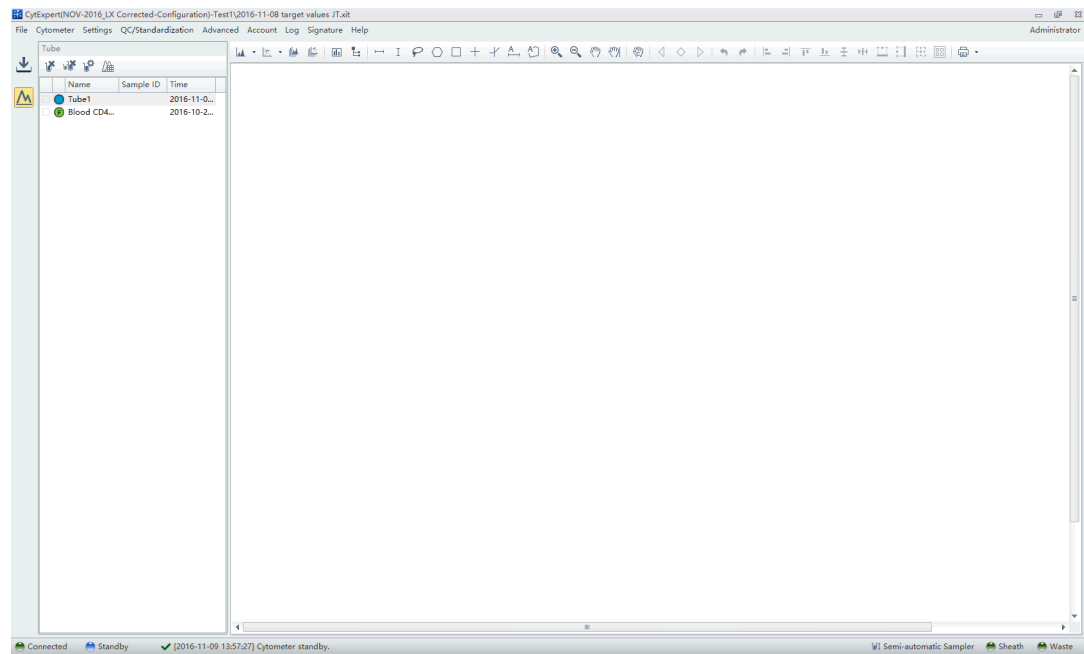
Imported data files appear in the Tube screen.



The **F** symbol in front of each data tube indicates that the data tube is an imported data file. Imported data files are copied and saved in the folder where the current experiment data files are saved.

Setting the Plots and Statistics

Opening the Analysis Screen

- 1 Select  on the left to enter the Analysis screen.



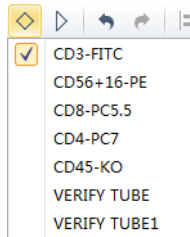
- 2 Copy plots obtained during data acquisition.
 - a. If you need original plots used during data acquisition, select  to access the Acquisition screen.
 - b. Select the appropriate plots.
 - c. Right-click the selected plots and select **Copy** from the drop-down menu or press Ctrl+C to copy.
 - d. Select  to return to the Analysis screen.
 - e. Select the required test tube from the tube list on the left side of the screen.
 - f. Right-click the plot area and select **Paste** from the drop-down menu or press Ctrl+V to paste the plot.


NOTE Pasted plots include all gates, but the gate names are reassigned.

-
- 3** New plots can be created according to need. After selecting the test tubes requiring analysis, use the plotting control buttons at the top of the screen to create a new plot.

NOTE Each graph in the **Analysis** screen may correspond to different data. Pay special attention to each plot's heading to avoid mistakes during analysis.

- 4** Use the sample selection controls in the graphics controls toolbar at the top of the page (see [Figure 2.1](#)) to change the data displayed in a plot.

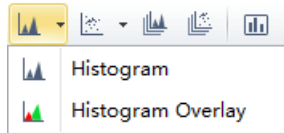


- a.** Select the plot requiring a change to the data displayed. By pressing and holding the Ctrl key while selecting plots, you can select several plots at one time.
- b.** Select one of the two triangular sample selection buttons (◀ or ▶) to choose between the previous sample and the next sample, or select  to specify which data to display.
-

Creating Histogram and Dot Plot Overlays

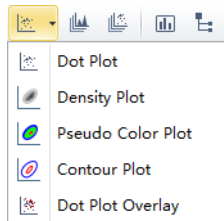
The CytExpert software supports histogram and dot plot data overlay functionality, allowing you to combine data from differing sources onto the same histogram or dot plot.

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




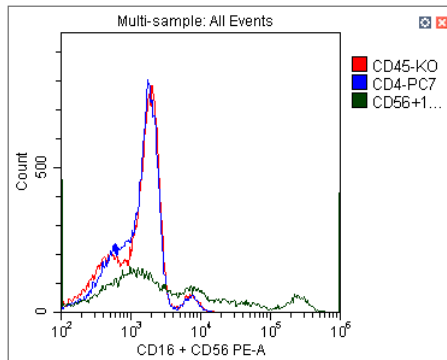
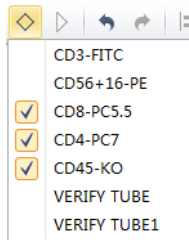
Or

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



IMPORTANT A maximum of 10 samples can be overlaid.

- 2 Select  to select samples for overlay display. Or, drag and drop samples from the tube list on the left into the histogram or dot plot overlay. The software automatically assigns different colors to different data.



To remove a sample, select  and uncheck the sample. Or, right-click the color legend and select **Remove [sample name]** or **Remove All Sample**. The corresponding data will no longer appear on the graph.

- 3 To change the color selections, right-click on the sample name in the legend located on the right side of the plot and select **Color** from the drop-down menu. A color pallet appears.

For configuring gates and generating statistics, refer to [CHAPTER 5, Data Acquisition and Sample Analysis](#).

Calculating Sample Volume and Concentration

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

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

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

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

- If using reference beads to calculate the concentration, select the *cells/μL(B)* checkbox and select the gated **Beads Population**. Enter the total number of reference beads as the **Beads Count**, as well as the sample volume in total. The software automatically calculates the original sample concentration based on the input values. (You can also enter the reference bead concentration directly in the beads count field and set the sample volume as 1.)

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

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

Data Review

Adjusting Compensation Settings

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

Header Statistics Population

Experiment Name

Tube Name

Sample ID

Record Time

Volume

Abort(%)

Expression

Name:

Equation:

Comment

Operator

Apply to:

Current Tube All Tubes Set As Default

[CytoFLEX LX Shown]

Statistics Setting

Header Statistics Population

Parent Population Events % Total % Parent Events/μL(V)

Events/μL(B)

Beads Population:

Beads Count: Sample Volume: μL

Parameter	Mean	Median	rCV	rSD	CV	SD
FSC-A						
SSC-A						
CD8 IR84...						
VIABILITY...						
HLA-DR V...						
CD45 V52...						
CD5 V610...						
CD34 V66...						
CD3 V763...						
CD20 NU...						
CD4 NU...						
CD38 NU...						

Area Height Area + Height

Preview

Apply to:

Current Tube All Tubes Set As Default

Tube Name: VC CD45RA FITC


Sample ID:

Volume(μL): 25.5

Population

All Events

Adjusting Compensation Settings

Data compensation can be carried out at any time. You can select the desired tube in the tube list on the left side of the screen and select  in the compensation controls, or select **Compensation Setup** in the Compensation menu. Refer to [Adjusting Compensation](#) in [CHAPTER 6, Compensation](#), for detailed instructions on adjusting compensation settings.

Exporting Results

Refer to [CHAPTER 5, Data Acquisition and Sample Analysis](#).

Overview

This chapter provides procedures for shutting down the CytoFLEX instrument.

Workflow:

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

This chapter contains information on:

- [Preparing the Cleaning Solution](#)
- [Shutting Down the Instrument](#)
- [Auto Shutdown \[CytoFLEX LX Only\]](#)

Preparing the Cleaning Solution

Required materials

Materials to prepare:

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

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

Shutting Down the Instrument

- 1 Run Daily Clean to clean the sample line. Refer to [Daily Clean](#) or [Daily Clean \[With Plate Loader\]](#) in [CHAPTER 10, Cleaning Procedures](#).
- 2 If necessary, empty all waste liquid from the waste container. Refer to [Emptying the 4 L Waste Container \[CytoFLEX\]](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

- 3** Remove the sample tube from the instrument and store according to your laboratory procedures.
- 4** Exit the software. The instrument automatically enters the standby state.
- 5** Turn the computer off.
- 6** Turn the Cytometer's main power switch off.
- 7** If there are any spills, clean the sample station. Refer to [Cleaning the Sample Station](#) in [CHAPTER 10, Cleaning Procedures](#).

Auto Shutdown [CytoFLEX LX Only]

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

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

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

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 can not be resolved, [contact us](#).

This chapter contains information on:

- [Precautions/Hazards](#)
- [Hazard Labels and Locations](#)
- [RoHS Notice](#)
- [Disposal Precaution](#)
- [Troubleshooting Table](#)
- [Backup and Restore](#)

Precautions/Hazards

Laser Related Hazards

Beckman Coulter design and manufacture of the instrument complies with the requirements governing the use and application of a laser specified in regulatory documents issued by the:

- U.S. Department of Health and Human Services
- Center for Devices and Radiological Health (CDRH)
- International Electrotechnical Commission (IEC)

In compliance with these regulatory documents, every measure has been taken to ensure the health and safety of users and laboratory personnel from the possible dangers of laser use.

Use the instrument according to the information in the manuals.

Use controls or adjustments or performance of procedures other than those specified herein might result in hazardous radiation exposure.

To ensure your safety, the Cytometer lasers are covered with protective shields. Do not remove these shields.

No user-servicable assemblies are accessible. Do not attempt to remove the laser or open it. The instrument has components that are dangerous to the operator. If any attempt has been made to defeat a safety feature, or if the instrument fails to perform as described in its manuals, disconnect the power and [contact us](#).

Laser Beam Hazards

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

- 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.



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



Risk of personal injury from radiation exposure. Never remove the shield surrounding a laser. Never remove covers.

CDRH-approved and IEC compliant labels are also placed near or on those covers that when removed might expose laser radiation. If necessary, a cover with a CDRH-approved or IEC compliant label must be removed by a qualified Beckman Coulter Representative only.

Refer to the following figures for the locations of the CDRH-approved and IEC compliant labels:

See [Figure 9.1](#) and [Figure 9.2](#) for the Laser Warning Label on the Cytometer optical bench.

See [Figure 9.3](#) and [Figure 9.4](#) for the Laser Warning Label on the optical bench (located Inside the Cytometer).

See [Figure 9.5](#) and [Figure 9.6](#) for the Laser Warning Labels on the Cytometer Back Cover.

The laser product is classified as CLASS 1 when all protective measures are in place. This product complies with 21 CFR Parts 1040.10 and 1040.11 as well as EN60825-1. See [Figure 9.1](#).

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

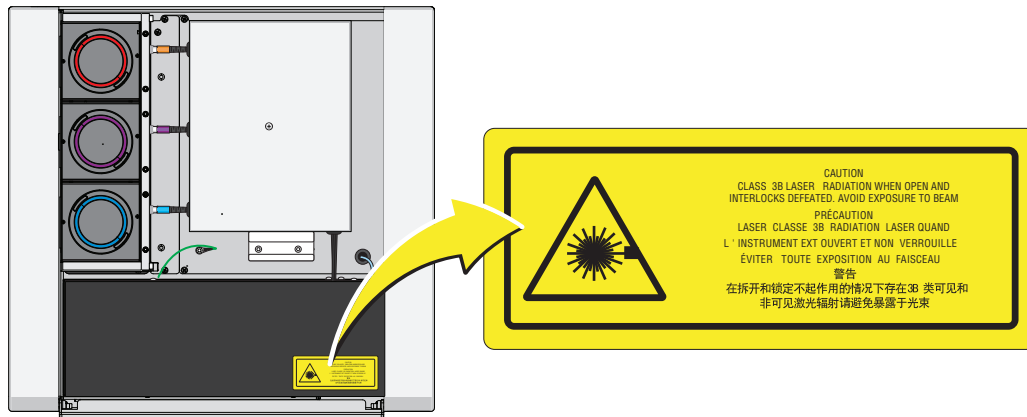


Figure 9.2 Laser Warning Label on the Laser Optical Bench [CytoFLEX LX]

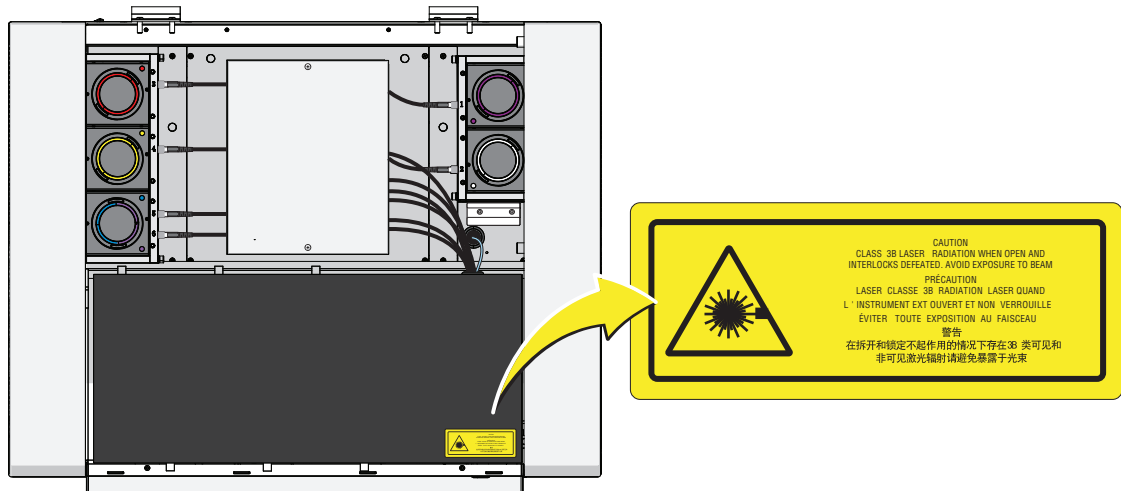


Figure 9.3 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX]

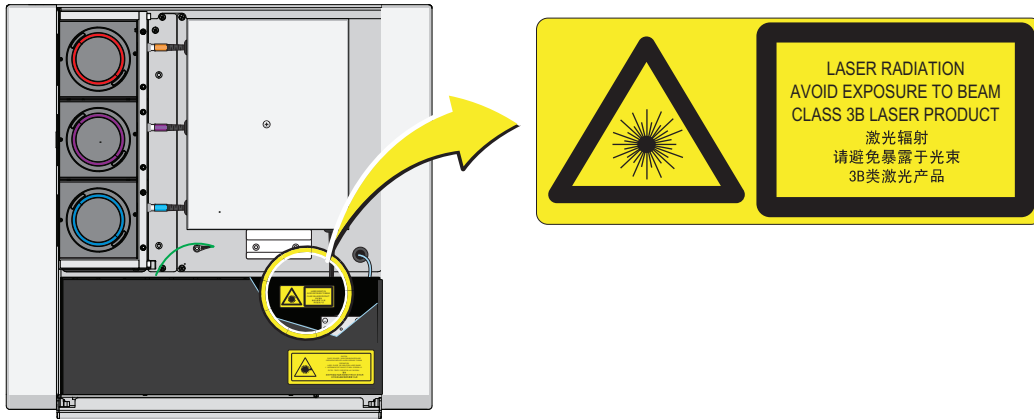


Figure 9.4 Laser Warning Label within the Optical Bench (Located Inside the Cytometer) [CytoFLEX LX]

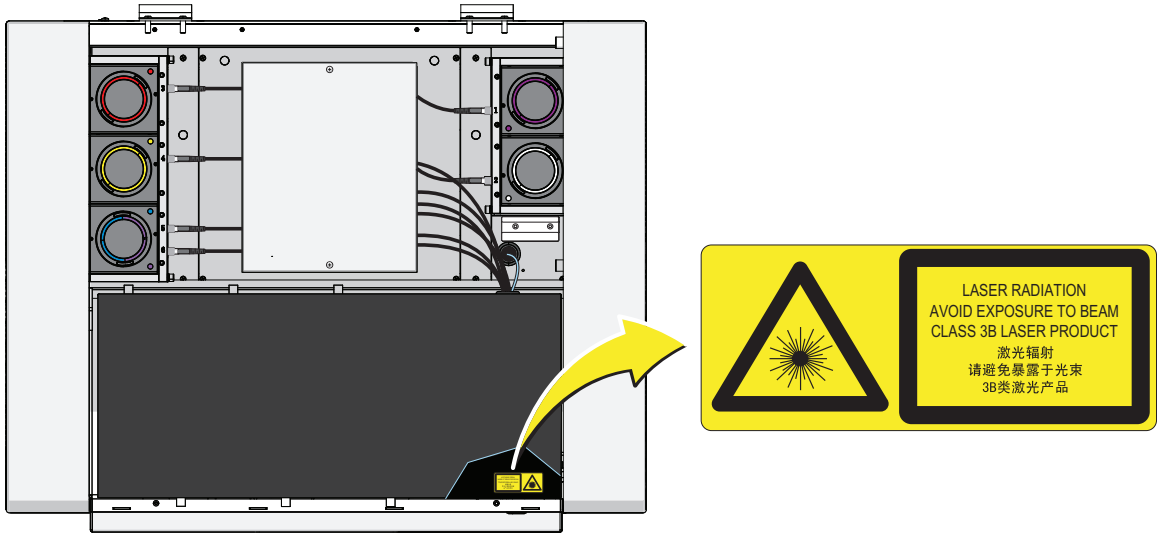


Figure 9.5 Laser Warning Labels on the Cytometer Back Cover [CytoFLEX]

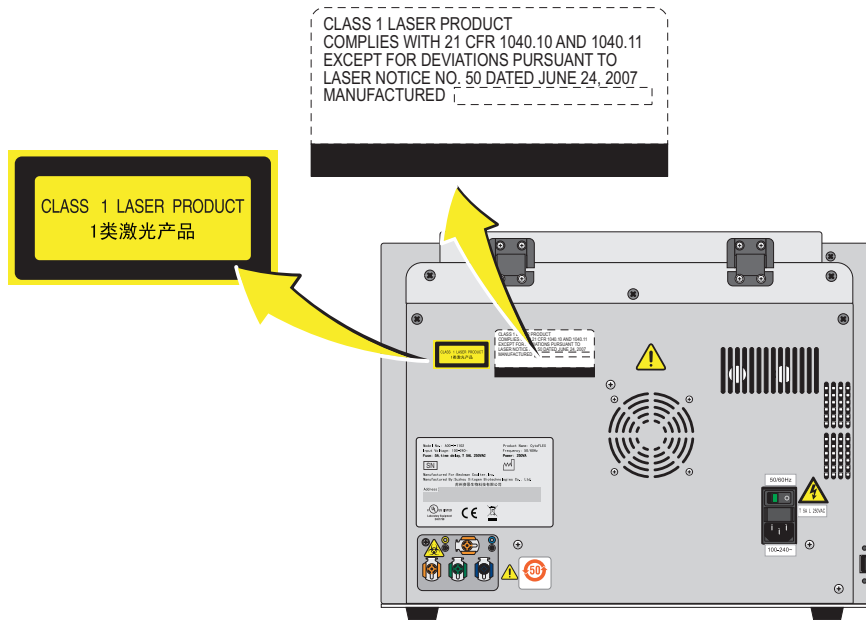
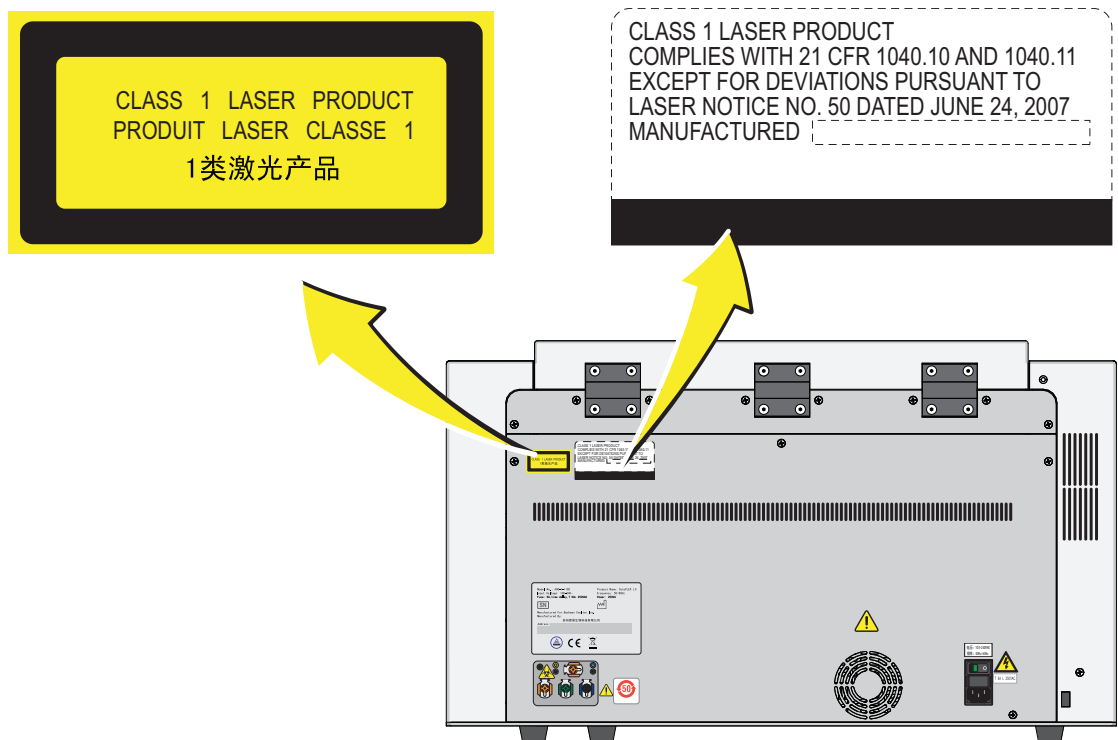


Figure 9.6 Laser Warning Labels on the Cytometer Back Cover [CytoFLEX LX]



Hazard Labels and Locations

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

NOTE If a label is missing or unclear, [contact us](#).

Biohazard Label and Location

Figure 9.7 Biohazard Label on the 4 L Fluid Containers

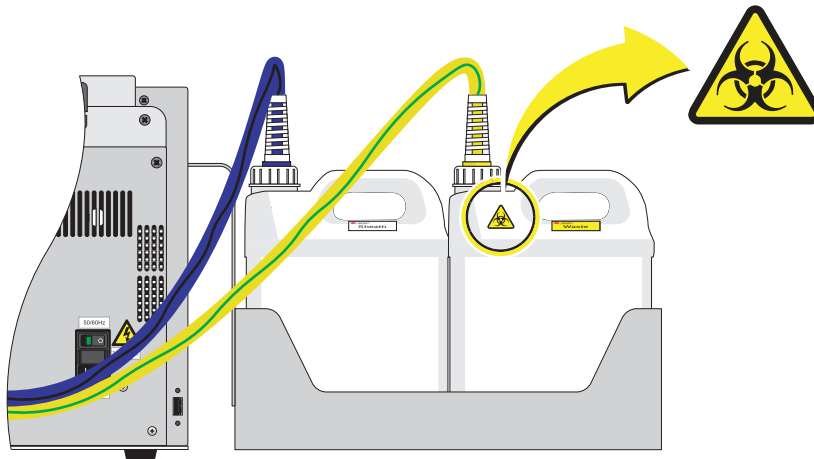


Figure 9.8 Biohazard Label on the 10 L Fluid Cubitainers

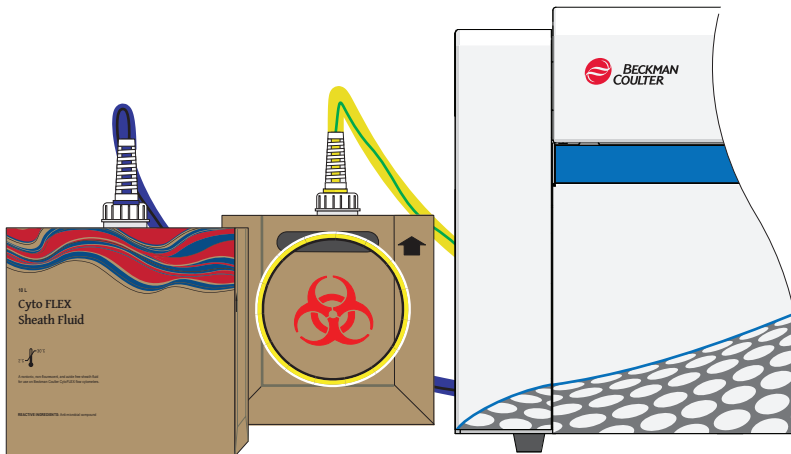
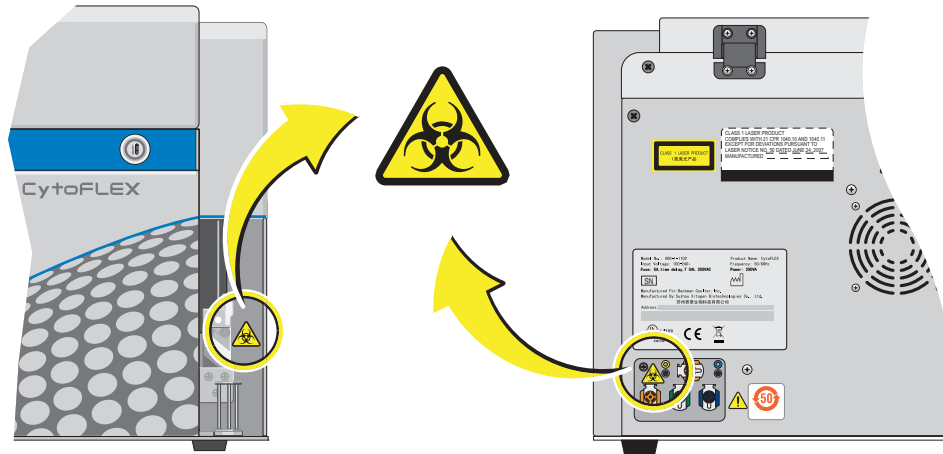
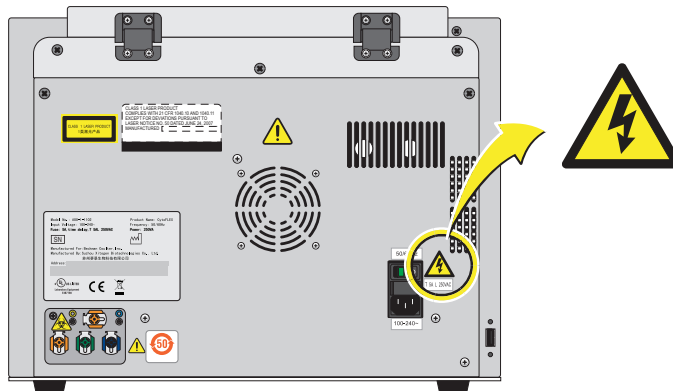


Figure 9.9 Biohazard Label Located in the Sample Station and on the Back of the Cytometer [CytoFLEX Shown]



Electrical Shock Hazard Label and Location

Figure 9.10 Electrical Shock Hazard Label by the Power Switch [CytoFLEX Shown]



Caution Labels and Location

Figure 9.11 Caution Labels [CytoFLEX Shown]

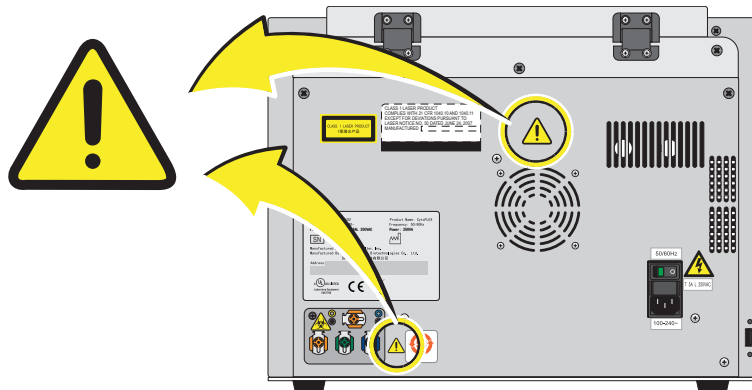
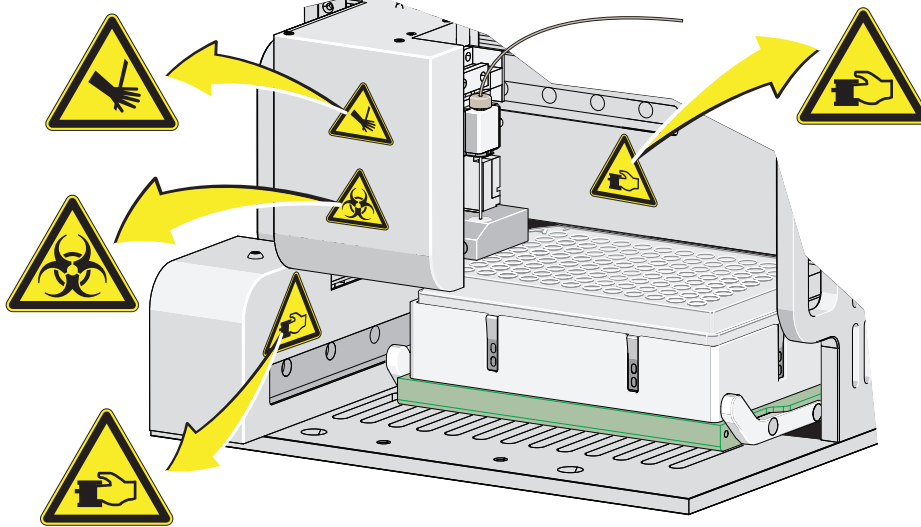


Plate Loader Hazard Labels and Location

Figure 9.12 Plate Loader Hazard Labels



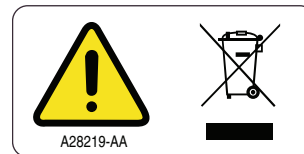
Disposal Of Electrical Instrumentation

It is very important that customers understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates:

- that the device was put on the European Market after August 13, 2005 and
- that the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take back program which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of device.



RoHS Notice

These labels and materials declaration table (the Table of Hazardous Substance's Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/T11364-2006 "Marking for Control of Pollution Caused by Electronic Information Products" requirements.

RoHS Caution Label

This label indicates that this electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.



RoHS Environmental Label

This label indicates that the electronic information product does not contain any toxic or hazardous substances. The center "e" indicates the product is environmentally safe and does not have an Environmentally Friendly Use Period (EFUP) date. Therefore, it can safely be used indefinitely. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.



Disposal Precaution



WARNING

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

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

Troubleshooting Table

Table 9.1 lists problems that you could encounter while running the CytoFLEX flow cytometer, the probable causes of each problem, and the corrective actions. These problems are listed alphabetically in the Index, under the primary entry “troubleshooting.”

Table 9.1 Troubleshooting

Problem	Probable Cause	Corrective Action
The Cytometer cannot be turned on.	<ul style="list-style-type: none"> The instrument is turned off in the Cytometer menu. [CytoFLEX LX] The power switch is in the off position and the Turn On selection will not function in the Cytometer menu. [CytoFLEX LX] The power cable is not securely connected. The fuse is blown. 	<ol style="list-style-type: none"> Ensure the power switch is in the on position on the back of the Cytometer. [CytoFLEX LX] Select Turn On in the Cytometer menu. [CytoFLEX LX] Ensure that the power cable is securely connected to the back of the Cytometer. Replace the fuse. Refer to CHAPTER 11, Replacing the Fuse in CHAPTER 11, Replacement/Adjustment Procedures. If the problem persists, contact us.
The Workstation cannot be turned on.	<ul style="list-style-type: none"> The power cable is not securely connected. The Workstation was restarted too fast. 	<ol style="list-style-type: none"> Ensure that the power cable is securely connected to the back of the Cytometer. Unplug the power cable. Wait 10 seconds, then plug the power cable back in. Then, restart the computer. If the problem persists, contact us.
The connection indicator light in the lower left corner of the software screen is red and displays <i>Disconnected and Error</i> .	<ul style="list-style-type: none"> Data connection error The Cytometer is not turned on. The Cytometer’s power cable is disconnected. 	<ol style="list-style-type: none"> Ensure that the USB data cable is securely connected to the back of the Cytometer and the back of the Workstation. Refer to Figure 1.17. Restart the software. Restart the Workstation. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. Turn on the Cytometer using the power switch on the back of the instrument. Verify that the power cable is securely connected to the back of the Cytometer. If the problem persists, contact us.

Table 9.1 Troubleshooting (*Continued*)


Problem	Probable Cause	Corrective Action
<p>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.</p>	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Ensure the sheath fluid/waste harnesses are secured to the correct container. 2. Ensure that the USB data cable is securely connected to the back of the Cytometer and the back of the Workstation. Refer to Figure 1.17. 3. Restart the Workstation. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. 4. Restart the software. <div style="border: 1px solid black; background-color: #ffcc00; padding: 5px; margin: 10px 0;">  WARNING </div> <p>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.</p> <ol style="list-style-type: none"> 5. Verify that the float of the sensor in the sheath fluid/waste container moves freely. 6. Replace the sheath fluid/waste harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. 7. If the problem persists, contact us.

Table 9.1 Troubleshooting (*Continued*)

Problem	Probable Cause	Corrective Action
<p>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.</p>	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Ensure the sheath fluid/waste harnesses are secured to the correct container. 2. Ensure that the USB data cable is securely connected to the back of the Cytometer and the back of the Workstation. Refer to Figure 1.17. 3. Restart the software. 4. Ensure the sheath fluid harness and/or the waste harness are properly connected. 5. Verify that the float of the sensor in the sheath fluid container and/or waste container moves freely. 6. Replace the sheath fluid harness and/or the waste harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. 7. If the problem persists, contact us.
<p>The sample tube holder cannot move up and down automatically.</p>	<p>The setting is incorrect.</p>	<ol style="list-style-type: none"> 1. Ensure that the sample injection mode in the software is in Semi-Automatic Injection mode. Refer to Selecting the Proper Sample Injection Mode in CHAPTER 3, Daily Startup. 2. If the problem persists, contact us.
<p>The sample flow rate is unstable.</p>	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. 2. Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures. 3. Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures. 4. Filter the sample using an appropriately sized mesh aperture filter. 5. Ensure that the sample tubing is properly connected. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. 6. Replace the sample probe and sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. 7. If the problem persists, contact us.

Table 9.1 Troubleshooting (*Continued*)

Problem	Probable Cause	Corrective Action
The sampling flow rate is too fast.	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Use the manual threshold setting to increase the threshold. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. 2. Calibrate the sample flow rate. Refer to Calibrating the Sample Flow Rate or Calibrating the Sample Flow Rate [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures. 3. Dilute the sample and adjust the concentration to approximately 10⁶/mL. 4. Filter the sample using an appropriately sized mesh aperture filter. 5. Restain the sample. 6. Replace the Sheath Fluid Filter. Refer to Replacing the Sheath Fluid Filter in CHAPTER 11, Replacement/Adjustment Procedures. 7. If the problem persists, contact us.
Laser power is low.	Communication error	<ol style="list-style-type: none"> 1. Reinitialize. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. 2. Restart the Cytometer. 3. If the problem persists, contact us.
Populations are drifting.	<ul style="list-style-type: none"> • Air bubbles are in the flow cell. • Air bubbles are in the system. • The sheath fluid harness float is restricted. 	<ol style="list-style-type: none"> 1. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. 2. Ensure that the sheath fluid harness and/or waste harness is not kinked. 3. Ensure that the sheath fluid harness and/or waste harness is securely connected. 4. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. 5. Verify that the float of the sensor in the sheath fluid container moves freely. 6. Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. 7. If the problem persists, contact us.

Table 9.1 Troubleshooting (*Continued*)

Problem	Probable Cause	Corrective Action
Population amplitude is decreasing and CV values are increasing.	<ul style="list-style-type: none"> • Air bubbles are in the flow cell. • The flow cell is dirty. • The sheath fluid harness float is restricted. 	<ol style="list-style-type: none"> 1. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. 2. Run the Deep Clean procedure. Refer to Deep Clean Procedure in CHAPTER 10, Cleaning Procedures. 3. Verify that the float of the sensor in the sheath fluid container moves freely. 4. Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. 5. If the problem persists, contact us.
The laser delay values are out of range.	<ul style="list-style-type: none"> • Air bubbles are in the flow cell. • Air bubbles are in the system. • The sheath fluid harness float is restricted. 	<ol style="list-style-type: none"> 1. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. 2. Ensure that the sheath fluid harness and/or waste harness is not kinked. 3. Ensure that the sheath fluid harness and/or waste harness is securely connected. 4. Verify that the float of the sensor in the sheath fluid container moves freely. 5. Replace the sheath fluid harness. Refer to Replacing the Sheath Fluid Harness and/or Waste Harness in CHAPTER 11, Replacement/Adjustment Procedures. 6. If the problem persists, contact us.

Table 9.1 Troubleshooting (*Continued*)

Problem	Probable Cause	Corrective Action
No data acquisition.	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Decrease the threshold setting. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. 2. Increase the gain setting. Refer to Adjusting the Gain in CHAPTER 5, Data Acquisition and Sample Analysis. 3. Ensure that the sheath fluid harness and/or waste harness is not kinked. 4. Ensure that the sheath fluid harness and/or waste harness is securely connected. 5. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. 6. Reinitialize. Refer to Initializing the Instrument in CHAPTER 3, Daily Startup. 7. Restart the Cytometer. 8. Verify that your sample does not have excessive debris. If it does: <ol style="list-style-type: none"> a. Filter the sample using an appropriately sized mesh aperture filter. b. Restain the sample. 9. Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures. 10. Replace the sample probe and the sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. 11. If the problem persists, contact us.
Data populations are normal on one laser, but too low on another laser.	The laser delay setting is incorrect.	<ol style="list-style-type: none"> 1. Ensure that the laser delay is set correctly. Refer to Setting Laser Delay in CHAPTER 11, Replacement/Adjustment Procedures. 2. If the problem persists, contact us

Table 9.1 Troubleshooting (*Continued*)


Problem	Probable Cause	Corrective Action
Data populations are not where they are expected.	<ul style="list-style-type: none"> • The detector configuration setting is incorrect. • The optical filter is not placed correctly. • QC was not completed. • Gain and threshold is not set correctly. 	<ol style="list-style-type: none"> 1. Ensure that the detector configuration is set correctly. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, <i>Data Acquisition and Sample Analysis</i>. 2. Ensure that the position of the optical filter in the WDM matches the detector configuration setting. Refer to Verifying, Selecting, Editing, and Creating Detector Configuration in CHAPTER 5, <i>Data Acquisition and Sample Analysis</i>. 3. Ensure that the optical filter is installed correctly. Refer to Replacing the Optical Filter in CHAPTER 11, <i>Replacement/Adjustment Procedures</i>. 4. Follow the QC procedure. Refer to CHAPTER 4, <i>Instrument Quality Control and Standardization</i>. 5. Review the gain and threshold settings. Refer to Adjusting the Gain and Adjusting the Threshold in CHAPTER 5, <i>Data Acquisition and Sample Analysis</i>. 6. Review the display ranges. Refer to Creating Plots and Gates in CHAPTER 5, <i>Data Acquisition and Sample Analysis</i>. 7. If the problem persists, contact us.
No changes occurred after manually adjusting compensation settings.	Compensation was applied to the wrong channel.	<p>Ensure that the adjustment is applied to the correct primary and secondary channels in the compensation matrix.</p> <p>Or</p> <p>Select  to modify compensation in the desired plot.</p>

Table 9.1 Troubleshooting (*Continued*)



Problem	Probable Cause	Corrective Action
<p>The calculation of the automatic compensation experiment is incorrect.</p>	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Ensure that the corresponding negative control tube and the individual positive tube acquired are from the same sample type. 2. Ensure that the single colors collected correspond to the correct tube name. 3. Ensure that the gate in the FSC/SSC plot encloses the correct sample population. 4. Ensure that the positive gate in each tube is correctly placed. 5. Modify the events to record to ensure that enough events are collected for the data population. 6. Select samples with a stronger positive signal as the positive control. <p>Or</p> <p>Use dedicated compensation beads such as VersaComp Antibody Capture Beads</p>
<p>The sample is flowing, but no signal appears in the plot.</p>	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Use either  or  to modify the display range. <p>Or</p> <p>Right-click the plot and select Property. The Plot Property window appears. Select Fit with sample.</p> <ol style="list-style-type: none"> 2. Ensure that the parent gate is gated correctly. 3. Change the display color. 4. Move the sample above the threshold using one of the following methods: <ul style="list-style-type: none"> • Decrease the threshold setting. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. • Increase the gain setting. Refer to Adjusting the Gain in CHAPTER 5, Data Acquisition and Sample Analysis.

Table 9.1 Troubleshooting (*Continued*)

Problem	Probable Cause	Corrective Action
The concentration calculation is incorrect.	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Ensure that the pipette used in sample processing is calibrated. 2. Verify that the concentration of the sample is between 2×10^4-10^7 events/mL. 3. Vortex the sample before loading and verify that the sample is evenly mixed before loading the sample. <ul style="list-style-type: none"> NOTE An excessively long sample loading time leads to sample settlement. 4. Ensure that the sample flow rate does not exceed 10,000 events/second. 5. Adjust the threshold to remove sample debris. 6. Ensure that the sample volume analyzed exceeds 10 μL. 7. Ensure that the following are correct: <ul style="list-style-type: none"> • Gain. Refer to Adjusting the Gain in CHAPTER 5, Data Acquisition and Sample Analysis. • Threshold. Refer to Adjusting the Threshold in CHAPTER 5, Data Acquisition and Sample Analysis. • Compensation settings. Refer to CHAPTER 6, Compensation 8. Ensure that the gating and population hierarchy are correct.
The sample probe is too low.	The sample probe is not securely connected.	<ol style="list-style-type: none"> 1. Ensure that the sample probe is securely connected to the sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. 2. Ensure the sample pump cover is securely fastened. 3. If the problem persists, contact us.
The wash station drips during backflush.	<ul style="list-style-type: none"> • The sample probe is not securely connected. • The wash station height adjustment is not correct. 	<ol style="list-style-type: none"> 1. Ensure that the sample probe is securely connected to the sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. 2. Ensure the sample pump cover is securely fastened. 3. If the problem persists, contact us.

Table 9.1 Troubleshooting (*Continued*)

Problem	Probable Cause	Corrective Action
The mixer is not functioning.	<ul style="list-style-type: none"> • Sample mixing is disabled in the software. • The mixer motor is defective. 	<ol style="list-style-type: none"> 1. Ensure sample mixing is enabled in the software. Refer to Changing Sample Mixing and Backflush Settings in CHAPTER 11, Replacement/Adjustment Procedures. 2. If the problem persists, contact us.
Instrument operations cannot be performed in the Acquisition screen.	<ul style="list-style-type: none"> • The instrument is in standby mode. • The software is frozen. • Data connection error. 	<ol style="list-style-type: none"> 1. Select Initialize. 2. Ensure that the power switch to the Cytometer is turned on. 3. Restart the software. 4. Restart the Workstation. 5. Ensure that the USB data cable is securely connected to the back of the Cytometer and the back of the Workstation. Refer to Figure 1.17. 6. If the problem persists, contact us.
Software installation fails.	Multiple issues.	Contact us .

Table 9.1 Troubleshooting (*Continued*)

Problem	Probable Cause	Corrective Action
<p>QC aborted due to low event rate.</p>	<ul style="list-style-type: none"> • The diluted CytoFLEX Daily QC Fluorosphere or CytoFLEX IR Daily QC Fluorosphere concentration is too low. • The sample probe is clogged. • The sample line is clogged. 	<ol style="list-style-type: none"> 1. Add 1 drop of CytoFLEX Daily QC Fluorospheres to the QC solution. Then, rerun QC. 2. Reload the target value file. Refer to Importing Lot-Specific Target Values in CHAPTER 4, Instrument Quality Control and Standardization. Then, rerun QC. 3. Prepare a new sample of the CytoFLEX Daily QC Fluorospheres. Then, rerun QC. 4. Clean the sample probe. Refer to Cleaning the Sample Probe in CHAPTER 10, Cleaning Procedures. Then, rerun QC. 5. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. Then, rerun QC. 6. Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures. Then, rerun QC. 7. Replace the sample probe and sample peristaltic pump tubing. Refer to Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing in CHAPTER 11, Replacement/Adjustment Procedures. Then, rerun QC. 8. If problem persists, contact us.

Table 9.1 Troubleshooting (*Continued*)

Problem	Probable Cause	Corrective Action
QC failed.	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Rerun QC. Refer to CHAPTER 4, Instrument Quality Control and Standardization. 2. Run Prime. Refer to Priming the Flow Cell in CHAPTER 11, Replacement/Adjustment Procedures. Then, rerun QC. 3. Prime the sheath fluid filter with sheath fluid as follows, then rerun QC. <ol style="list-style-type: none"> a. Remove the vent cap from the sheath fluid filter. b. Remove the rubber insert from the vent. c. Ensure that the instrument is in Standby. d. At the Workstation, select Cytometer > Prime. <p>IMPORTANT If the rubber insert and the vent cap are not reinstalled as soon as the sheath fluid approaches the vent port, the sheath fluid will overflow.</p> <ol style="list-style-type: none"> e. Wait until the sheath fluid approaches the vent port in the sheath fluid filter, then immediately reinstall the rubber insert and the vent cap to avoid overflow. 4. Run Daily Clean. Refer to Daily Clean in CHAPTER 10, Cleaning Procedures. Then, rerun QC. 5. Run Deep Clean. Refer to Deep Clean Procedure in CHAPTER 10, Cleaning Procedures. Then, rerun QC. 6. If the problem persists, contact us.

Table 9.2 Troubleshooting [With Plate Loader]

Problem	Probable Cause	Corrective Action
The sample probe comes in contact with the bottom of the well plate.	<ul style="list-style-type: none"> • Incorrect plate type selected. • The plate or plate holder is installed incorrectly. • The sample probe sampling position is not calibrated. 	<ol style="list-style-type: none"> 1. Verify that the correct plate type is selected in the Plate window. 2. Ensure the plate and the plate holder are installed correctly. Refer to Replacing the Plate Holder [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures. 3. Calibrate the sample probe sampling position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.
The dead volume of the plate wells are high.	<ul style="list-style-type: none"> • Incorrect plate type selected. • The sample probe sampling position is not calibrated. 	<ol style="list-style-type: none"> 1. Verify that the correct plate type is selected in the Plate window. 2. Calibrate the sample probe sampling position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.
Mixing does not sufficiently suspend the sample particles.	<ul style="list-style-type: none"> • Incorrect plate type selected. • Incorrect sample mixing setting. • The sample probe sampling position is not calibrated. 	<ol style="list-style-type: none"> 1. Verify that the correct plate type is selected in the Plate window. 2. Verify the sample mixing duration in the Plate window. 3. Calibrate the sample probe sampling position. Refer to Calibrating the Plate Position [With Plate Loader] in CHAPTER 11, Replacement/Adjustment Procedures.
Gain is over 3,000 and default gain settings are needed.	Corrupt/incorrect configuration file.	Reload the configuration file. Refer to Installing the Instrument Configuration File in APPENDIX A, Instrument Installation.
The configuration file does not match the instrument.	Corrupt/incorrect configuration file.	<ol style="list-style-type: none"> 1. Verify the correct configuration file is installed. 2. Reload the configuration file. Refer to Installing the Instrument Configuration File in APPENDIX A, Instrument Installation.
The Administrator forgot the Admin password.	The Admin password was forgotten.	<ol style="list-style-type: none"> 1. Select Forgot password. 2. Contact us
Plots are cut off when printing a PDF.	Plots need to be rearranged.	Rearrange the plots until the print preview screen shows all plots correctly.

Backup and Restore

IMPORTANT If you have the Electronic Record Management software option installed, ensure the following prior to attempting to restore data:

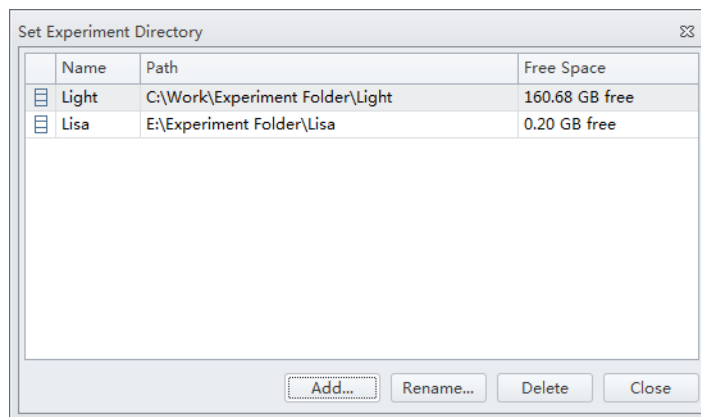
- The target system includes all of the disk volumes that each experiment directory is saved to.
- The user performing the restore has *Set Experiment Directory* permission in CytExpert.

NOTE These procedures are only available if you have either the User Management or Electronic Record Management software option installed.

Backup

1 If you have the 21CFR Tools software option installed:

- Launch CytExpert and select **Settings > Set Experiment Directory**. The Set Experiment Directory window appears.



- On the Set Experiment Directory, take note of the path to the backup location for each user, or for the user whose data you wish to back up.

Or

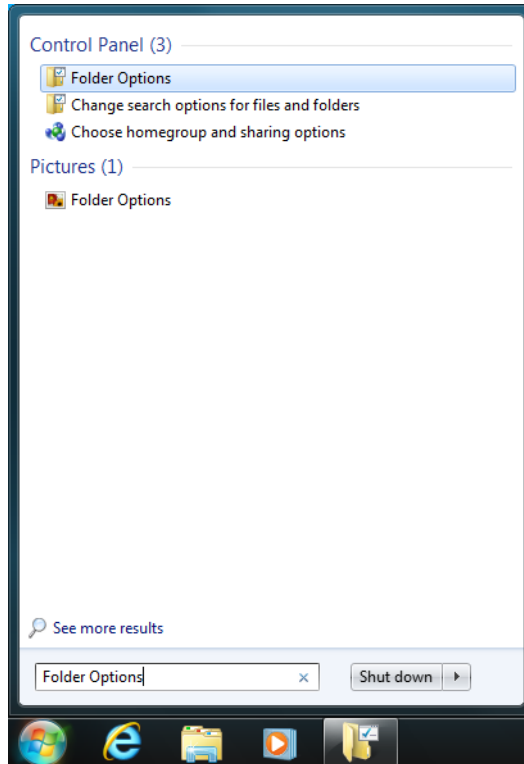
If you have User Management software option installed, skip to Step 2.

2 Exit CytExpert.

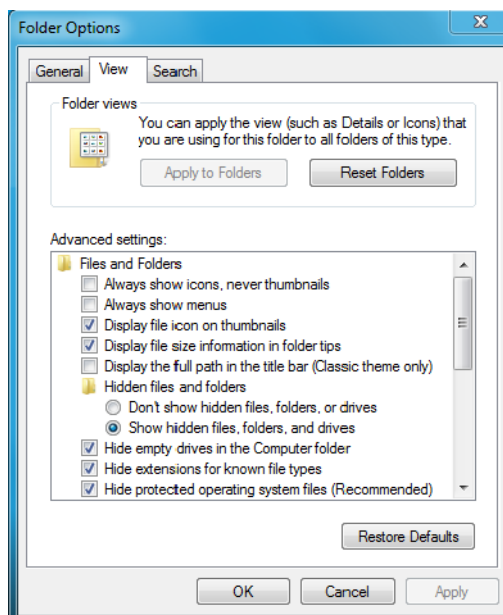
- 3 On the Workstation that is being backed up, go to C:\ProgramData\Beckman Coulter\CytExpert\Database\.

NOTE If the Program Data folder is not visible:

1. Select the Windows Start menu and search for *Folder Options*. The Folder Options window appears.



2. Select the **View** tab.



3. Select the *Show hidden files, folders, and drives* radio button.

4 Right-click on the file called *CytExpert.db* and select **Copy** from the pop-up menu.

5 Paste the file in your desired backup location.

If you have the 21CFR Tools software option installed, proceed to Step 6.

6 If you have the 21CFR Tools software option installed, go to the location of each experiment directory as noted in Step 1, and copy the folder to the backup location. Repeat this step for all the experiment directories that need to be backed up.

Restore

1 Exit CytExpert.

2 Go to your backup location.

IMPORTANT If the backup database file was renamed, rename it *CytExpert.db*.

3 Right-click on the file called *CytExpert.db* and select **Copy** from the drop down menu.

4 Go to C:\ProgramData\Beckman Coulter\CytExpert\Database\ on the system being restored and paste the CytExpert.db file.

If you have the 21CFR option installed, proceed to Step 5.

5 If you have the 21CFR Tools software option installed:

- a. Go to your backup location and copy the user directory's backup folder.
- b. Paste the folders in the path specified in the Set Experiment Directory window for each user.

Overview

This chapter describes how to carry out certain routine and nonscheduled cleaning procedures. Proper cleaning can help extend the service life of the instrument and ensure experimental accuracy. When conducting any cleaning, take all necessary biosafety precautions and use proper personal protective equipment.

This chapter contains information on:

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

Routine Cleaning

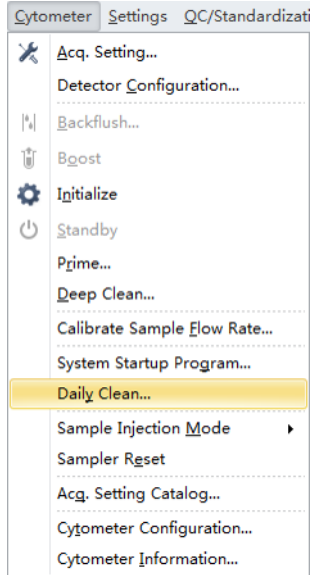
Daily Clean

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

After sampling an excessively large sample or a sample that can easily clog the sample probe, it is recommended to perform the Daily Clean procedure. Daily Clean can also be used to remove residual sample from previous tubes.

-
- 1 Open the CytExpert software and confirm that the instrument is connected and that it has already been initialized. Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#).

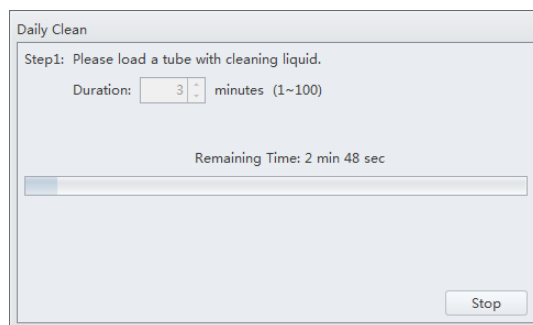
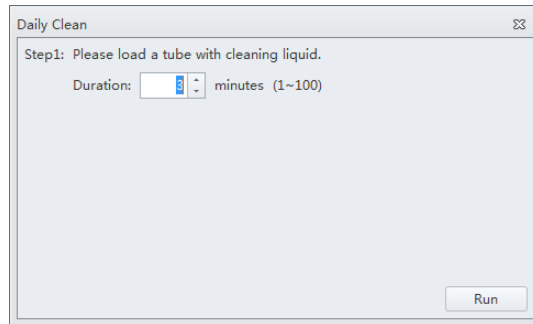
-
- 2 Select **Daily Clean** in the Cytometer menu.



-
- 3 Add 2 mL of FlowClean solution to an unused sample tube.
-
- 4 Add 3 mL of DI water to an unused sample tube.

- 5** Insert the sample tube with 2 mL of FlowClean solution into the sample holder and select **Run**.

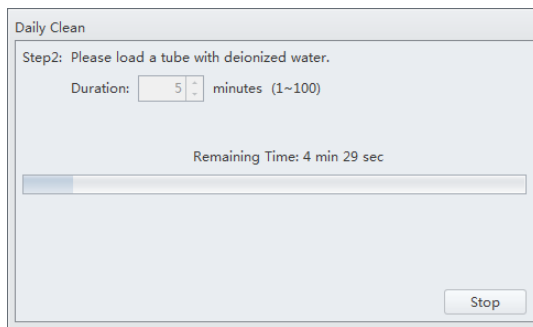
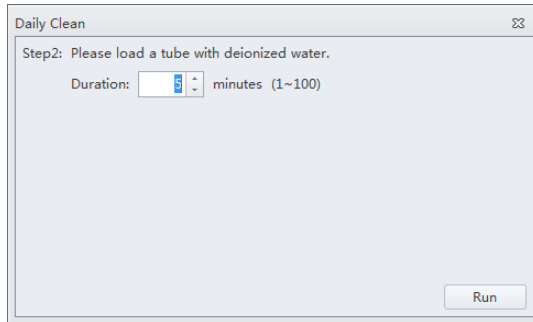
NOTE The default cleaning time is 3 minutes.



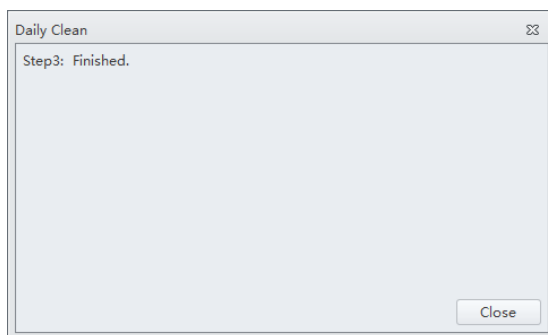
- 6** Remove the Flow Clean tube.

- 7 Insert the sample tube with 3 mL of DI water into the sample holder and select **Run** to perform the second step of the cleaning process.

NOTE The default cleaning time is 5 minutes.



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

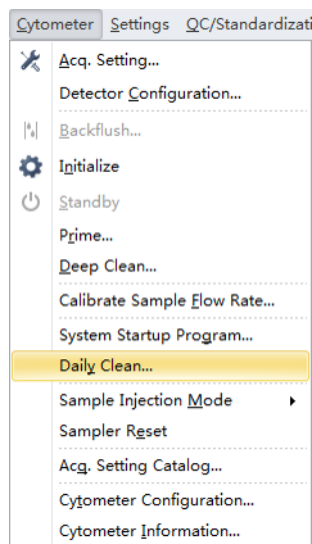


Daily Clean [With Plate Loader]

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

After sampling an excessively large sample or a sample that can easily clog the sample probe, it is recommended to perform the Daily Clean procedure. Daily Clean can also be used to remove residual sample from previous tubes.

- 1 Open the CytExpert software and confirm that the instrument is connected and that it has already been initialized. Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#).
- 2 Select **Daily Clean** in the Cytometer menu. The Daily Clean window appears. The plate loader automatically ejects the plate holder stage.



- 3 Follow the on screen software prompts and select the desired wells for cleaning agent and deionized water.

[CytoFLEX LX Shown]



IMPORTANT You must select at least one cleaning solution well and one water well.

- a. Select the desired wells for the cleaning agent and select **Set As Cleaning Agent Well**.
- b. Select the desired wells for the deionized water and select **Set As Deionized Water Well**.

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

- c. Select the *Turn off cytometer after daily clean* checkbox to automatically shutdown the cytometer after Daily Clean is finished. **[CytoFLEX LX Only]**

- 4 Select **Start** to start the cleaning procedure. The message *Please confirm that the correct plate is placed properly and press OK* appears. Select **OK**.

- 5 Select **Close**.

Cleaning the Sample Station



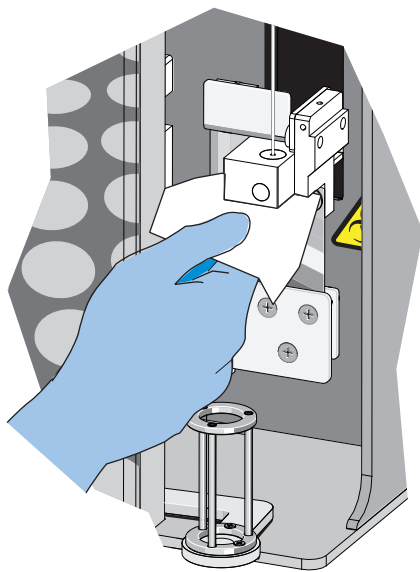
Carry out semi-automatic cleaning for the sample injection device once a week.

- 1 Ensure that the system has been shut down properly. Refer to [Shutting Down the Instrument in CHAPTER 8, Daily Shutdown](#).

WARNING

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

- 2 Use a piece of absorbent material with a 10% bleach solution (1 part bleach [5 to 6% sodium hypochlorite - available chlorine] with 9 parts DI water) to wipe off all surfaces in the sample station, while taking all necessary biological safety precautions.



- 3 Wipe off the bottom of the semi-automatic sample injection device.

Cleaning the Sample Probe



When problems such as blockage of the sample probe occur, it is required to replace or clean the sample probe.

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

WARNING

Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing might contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample probe and the sample peristaltic pump tubing in accordance with your local regulations and acceptable laboratory procedures.

- 2 Remove the sample probe. Refer to [Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).
- 3 Put the sample probe into a clean container and soak it in clean water. Use an ultrasonic cleaning device to clean for 2 minutes.
- 4 Reattach the sample probe to the sample peristaltic pump tubing and confirm that the bead on the sample probe touches the sleeve on the end of the sample peristaltic pump tubing.

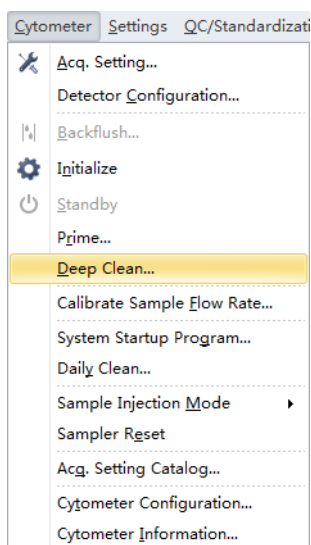
IMPORTANT To ensure that the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

- 5 Install the sample pump cover.
- 6 If ineffective, replace with a new sample probe. Refer to [Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

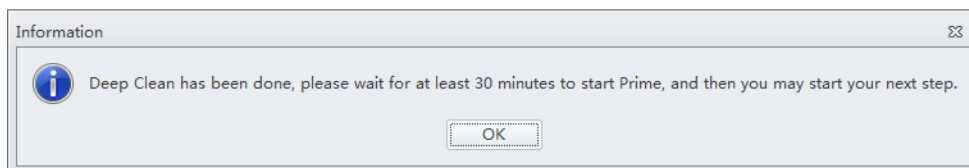
Deep Clean Procedure

Carry out a deep clean once a month to clean the instrument flow cell. If the unit will be shutdown and not used for more than 10 days, it is recommended to complete one deep clean before resuming use.

- 1 Place the instrument in standby state.
- 2 Remove the right-side cover. Refer to [Right-Side Cover Removal and Reinstallation](#) in CHAPTER 11, Replacement/Adjustment Procedures.
- 3 Confirm that the Deep Clean solution volume in the bottle located inside the Fluidics module is sufficient.
To prepare and add more Deep Clean solution, refer to [Adding the Deep Clean Solution](#) in CHAPTER 11, Replacement/Adjustment Procedures.
- 4 Select **Deep Clean** in the Cytometer menu. The software message *Are you sure to start deep clean?* appears. Select **Yes** to start the Deep Clean process in the instrument flow cell.

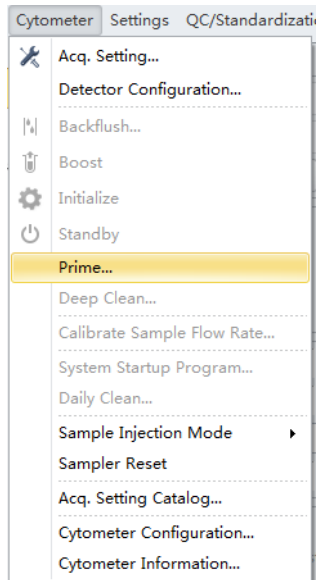


- 5 The status bar prompts that a deep clean is under way. Wait for the Deep Clean process to finish. The following software message appears:



Select **OK**.

- 6 Allow the cleaning solution to remain in the flow cell for approximately 30 minutes. If you are required to postpone the cleaning time, do not exceed 24 hours. During the Deep Clean cycle, the power supply of the unit can be turned off, but the instrument cannot be initialized.
- 7 Select **Prime** in the Cytometer menu. The software message *Are you sure to start Prime?* appears. Select Yes.



- 8 Run Daily Clean. Refer to [Daily Clean](#).
- 9 Perform initialization as required (see [Initializing the Instrument](#) in [CHAPTER 3, Daily Startup](#)) to carry out the next experiment or to turn off the instrument.
- 10 Reinstall the right-side cover. Refer to [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

Cleaning the 4 L Sheath Fluid Container

Clean the sheath fluid container once a month.

- 1 Confirm that the instrument is turned off or is in the standby state.
- 2 Remove the sheath fluid container from the Fluid Container holder.

-
- 3 Remove the sheath fluid harness from the sheath fluid container.

 - 4 Empty the residual sheath fluid from the sheath fluid container.

 - 5 Add about 50 to 100 mL of CytoFLEX Sheath Fluid to the sheath fluid container.

 - 6 Insert the sheath fluid harness back into the sheath fluid container and tightly close the sheath fluid container cap.

 - 7 Swirl the sheath fluid in the sheath fluid container, rinsing all surfaces.

 - 8 Empty the sheath fluid container.

 - 9 Refill the sheath fluid container. Refer to [Filling the 4 L Sheath Fluid Container \[CytoFLEX\]](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

Cleaning the 4 L Waste Container



Clean the waste container once a month.

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

 - 2 Remove the waste container from the Fluid Container holder.

WARNING

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

- 3 Remove the harness from the waste container.

 **WARNING**

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

- 4 Empty the waste container.

 **WARNING**

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

- 5 Add one liter of sodium hypochlorite solution with 0.5% active chlorine to the waste container.

- 6 Insert the waste harness back into the waste container and tightly close the waste container cap.

 **CAUTION**

Risk of damage to the sheath fluid harness and/or waste harness. Do not leave the sodium hypochlorite solution in the fluid containers longer than 10 minutes.

- 7 Let stand for 5 to 10 minutes.

 **WARNING**

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

- 8 Dispose of the sodium hypochlorite solution in accordance with your local regulations and acceptable laboratory procedures.

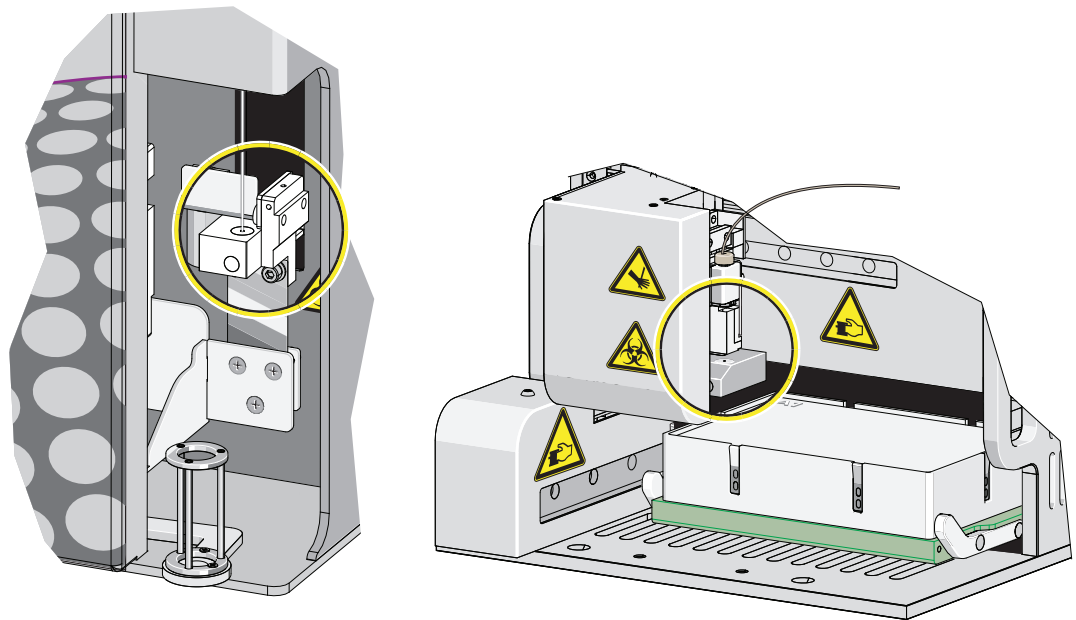
- 9 Use deionized water to rinse the waste container and the waste harness. Ensure that there is no sodium hypochlorite residue.

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

Nonscheduled Cleaning

Surface Cleaning and Disinfection

- 1 Wipe the CytoFLEX Series label of the instrument clean with water and wipe dry immediately.
- 2 If you have a plate loader installed on your instrument, remove the front cover. Refer to [Front Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).
- 3 Wipe the wash collar modules in the single tube station and the plate loader station with 100% isopropanol and wipe dry immediately.



- 4 If you have a plate loader installed on your instrument, reinstall the front cover. Refer to [Front Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

 **WARNING**

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

- 5 Prepare a cleaning solution of 1 part high-quality, fragrance-free bleach (5% or 6% solution of sodium hypochlorite - available chlorine) and 9 parts distilled water.

 **CAUTION**

Risk of instrument damage. The instrument label can peel or fade, and the wash collar can become brittle and crack over time if these surfaces are wiped clean using the bleach solution followed by the 70% ethanol. Do not clean the label or the wash collar with the bleach solution followed by the 70% ethanol. Only use the specified cleaning methods in Steps 1 and 3 to clean these surfaces.

 **CAUTION**

Risk of personal injury if electronic equipment is used near fumes or flammable gases. Ethanol is a flammability hazard. Avoid this risk by never using it in or near operating instruments.

- 6 Wipe down all exposed surfaces with the bleach solution and then 70% ethanol. Pay special attention to the Sampling area.
Be sure to avoid wiping the instrument label and wash collar module with the bleach solution and 70% ethanol.

Preparing the Instrument for Transport or Storage



When the instrument is to be transported or is not to be used for 30 days or more, complete the emptying processes to prevent instrument damage and to reduce the possibility of biological contamination. [Contact us](#) if you have any questions.

- 1 Run the Deep Clean procedure. Refer to [Deep Clean Procedure](#).
- 2 Run the Daily Clean procedure. Refer to [Daily Clean](#).

-
- 3 Clean the Sample Station. Refer to [Cleaning the Sample Station](#).

 - 4 Empty the sheath fluid container and the waste container (see [Emptying the 4 L Waste Container \[CytoFLEX\]](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)).

 - 5 Clean and disinfect all surfaces. Refer to [Surface Cleaning and Disinfection](#).

**WARNING**

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

- 6 Clean the sheath fluid container and the waste container. Refer to [Cleaning the 4 L Sheath Fluid Container](#).

- 7 Remove the right-side cover (see [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)).

- 8 Remove the Deep Clean solution bottle from the bracket, empty the Deep Clean solution bottle, and rinse with DI water. Then, attach the Deep Clean solution bottle to the bracket.

- 9 Remove the Plate Loader module if applicable (see [Plate Loader Module Removal and Reinstallation \[With Plate Loader\]](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)).

- 10 Power down and disconnect all the cables and sheath fluid and waste harnesses.

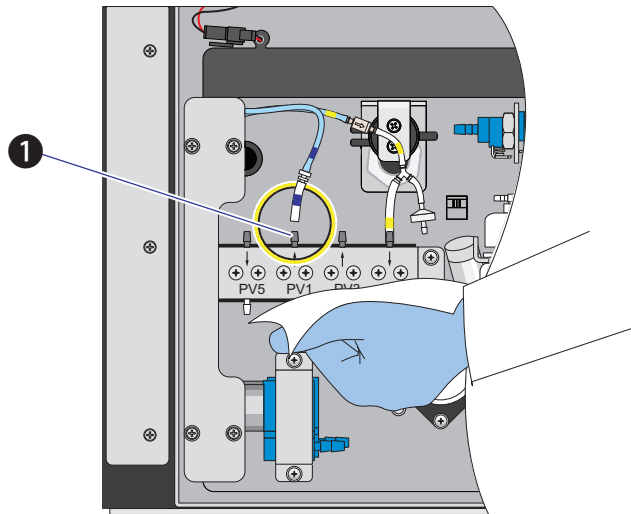
CAUTION

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

WARNING

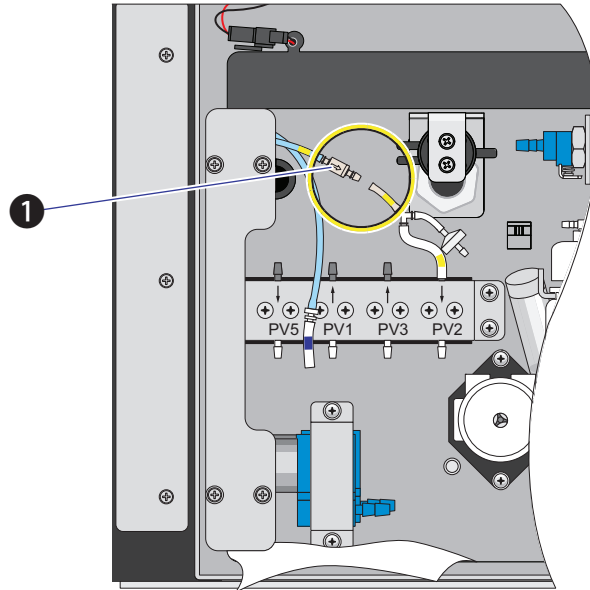
Risk of contamination from biohazardous material. Always wear PPE when performing this procedure as you can contact components with blood residue when handling the Fluidics module. Dispose of any absorbent materials used to collect or clean up leaks in accordance with the local regulations and acceptable laboratory procedures.

- 11 Disconnect the blue-labeled tubing from the pneumatic valve PV1 and hold the absorbent material under the disconnected tubing to collect any dripping liquid.



1. PV1

- 12** Disconnect the yellow-labeled tubing connected to the liquid damper to vent the flow cell, allowing it to drain.



1. Liquid damper

- 13** Verify that liquid has stopped dripping from the blue-labeled tubing.

NOTE The flow cell is empty when liquid stops dripping from the blue-labeled tubing.

- 14** Dispose of the absorbent material used to collect the liquid in accordance with the local regulations and acceptable laboratory procedures and cleanup any spills.

- 15** Reconnect the blue-labeled tubing to PV1.

- 16** Reconnect the yellow-labeled tubing to the liquid damper.

- 17** Reinstall the right-side cover (see [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)).

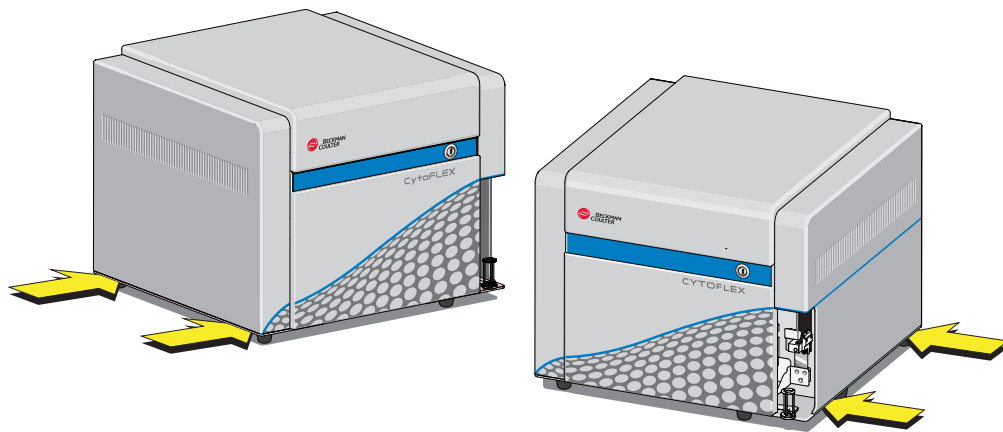
- 18** Ensure that the optical filters are seated properly.

- 19** Ensure that the top cover is tightly closed.

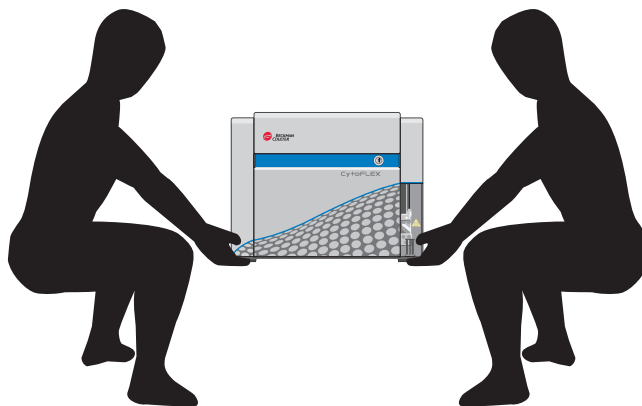
- 20 If the instrument is to be transported or stored, put the instrument and the Plate Loader module (if applicable) into the Beckman Coulter supplied packing and comply with the requirements in [Instrument Transportation and Storage](#) in [APPENDIX A, Instrument Installation](#), regarding correct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

Lifting and Carrying Instructions

- 1 Position a person on the left and right sides of the Cytometer.
- 2 Reach under the base of the Cytometer in the areas indicated by the arrows in the figure below.



- 3 Gently lift the Cytometer as shown in the figure below.





WARNING

Risk of personal injury. Use caution when lowering the Cytometer to avoid pinching fingers.

- 4 Lower the Cytometer to its designated location.

Replacement/Adjustment Procedures

Overview

This chapter describes how to carry out certain routine and nonscheduled maintenance procedures. Proper maintenance can help extend the service life of the instrument and ensure experimental accuracy. When conducting any maintenance work, take all necessary biosafety precautions.

IMPORTANT In addition to parts specifically discussed, for all replacement parts, use only parts provided by Beckman Coulter to ensure proper functioning of the instrument. Never disassemble any part of the instrument without prior authorization. Beckman Coulter assumes no responsibility for any instrument problems resulting from the use of any part not authorized by Beckman Coulter for use with the instrument.

This chapter contains information on:

- Routine Replacement/Adjustment
 - Front Cover Removal and Reinstallation
 - Right-Side Cover Removal and Reinstallation
 - Filling the 4 L Sheath Fluid Container [CytoFLEX]
 - Replacing the 10 L Sheath Fluid Container
 - Emptying the 4 L Waste Container [CytoFLEX]
 - Emptying the 10 L Waste Container
 - 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

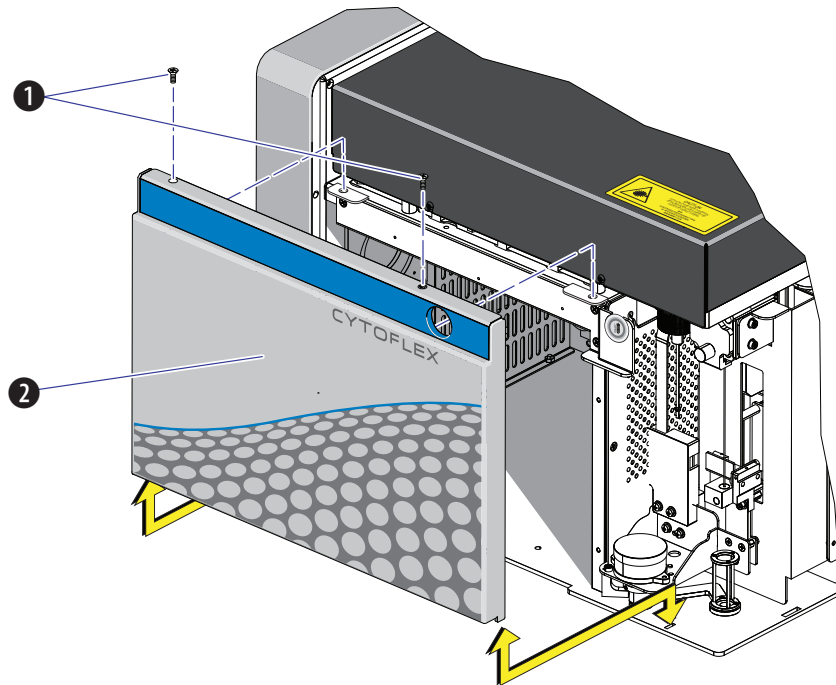
Removal



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.

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



1. Securing screws
2. Front cover

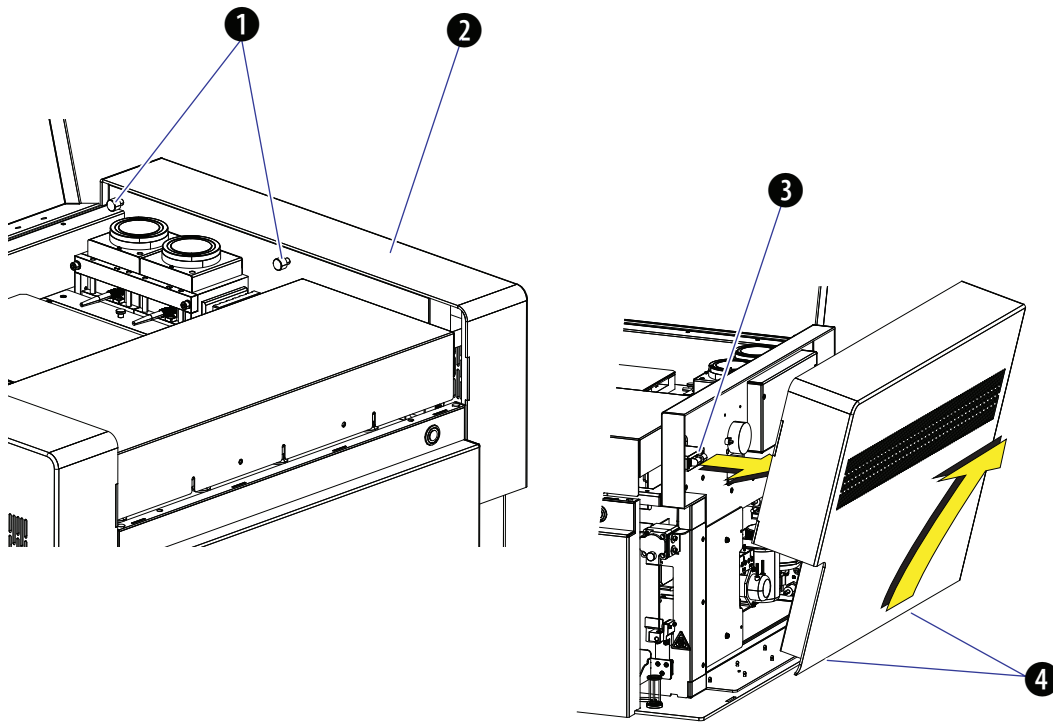
-
- 5** Lift the front cover up and out of the slots in the frame.

Reinstallation

- 1 Slide the tabs on the bottom of the front cover into the slots in the bottom of the frame.
 - 2 Push in the latches on the front cover to retract the pins, push the front cover into place, and release the latches to secure the cover.
 - 3 Close the top cover.
-

Right-Side Cover Removal and Reinstallation

IMPORTANT The CytoFLEX LX flow cytometer only requires you to unfasten the thumbscrews the first time. There is a retaining clip that is used to secure the right-side cover without the need for the thumbscrews, if desired. Once the thumbscrews are unfastened, the right-side cover can simply be pulled off of the retaining clip for removal and pushed onto the retaining clip for reinstallation. Ensure the top cover is open before removing the right-side cover from the retaining clip.

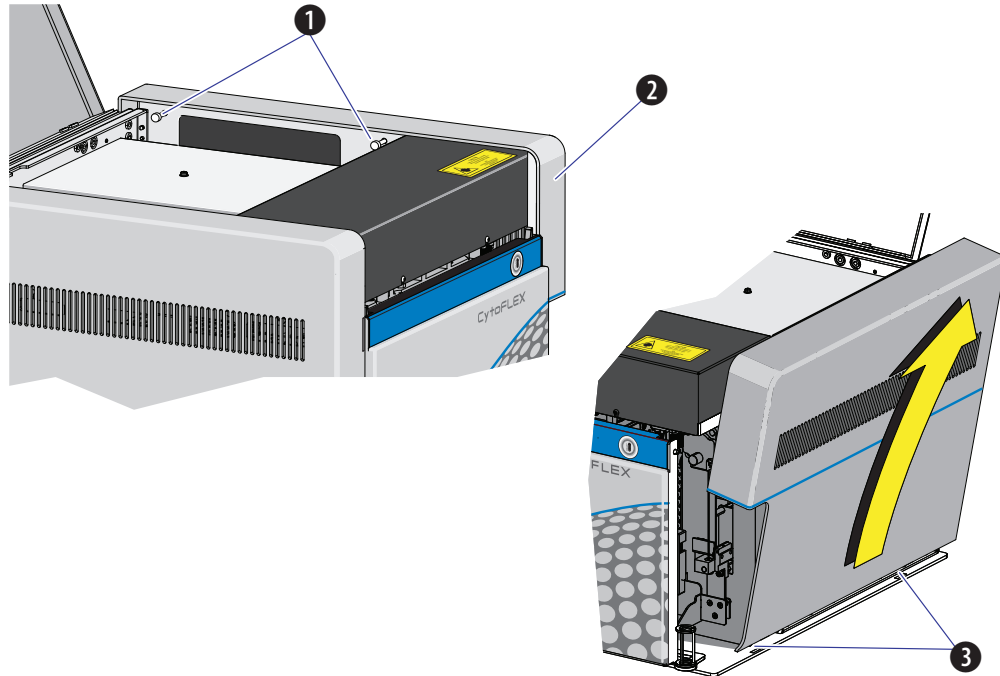


1. Thumbscrews
2. Right-side cover
3. Retaining clip
4. Tabs

Removal

- 1 Open the top cover.

-
- 2 Unfasten the two captive thumbscrews for the right-side cover.



- 1. Captive thumbscrews
- 2. Right-side cover
- 3. Tabs

-
- 3 Lift the right-side cover up and out of the slots in the frame.
-

Reinstallation

-
- 1 Slide the tabs on the bottom of the right-side cover into the slots in the bottom of the frame and push the cover into place.
-
- 2 Fasten the two captive thumbscrews to secure the right-side cover.
-
- 3 Close the top cover.
-

Filling the 4 L Sheath Fluid Container [CytoFLEX]

- 1 Confirm that the instrument is turned off or is in the standby state.
- 2 If necessary, remove any cardboard cutouts from the new CytoFLEX Sheath Fluid cubitainer. If you do not need a new cubitainer, skip to Step 7.
- 3 Locate the spigot inside the cardboard cutout.
- 4 Remove the cap and seal from the new sheath cubitainer. Be sure to completely remove the foil seal.
- 5 Screw on the spigot.
- 6 Unscrew the sheath fluid harness from the sheath fluid container and place in the fluid sensor holder cutout (refer to [Figure 1.7.](#)) to prevent contamination of the sheath fluid harness.

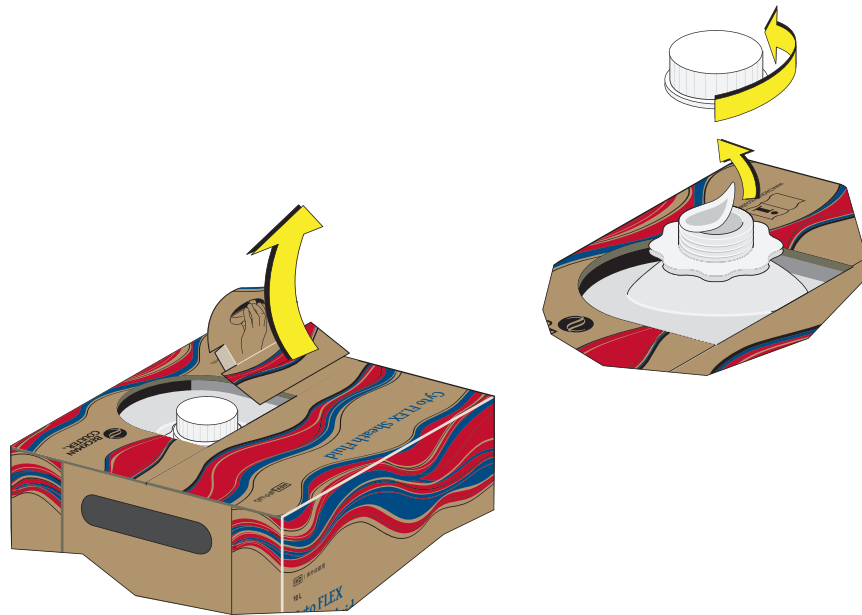
 **CAUTION**

Risk of instrument damage. Remove the sheath fluid container from the Fluid Container holder and fill away from the instrument to prevent spills that could damage the instrument circuitry.

- 7 Remove the sheath fluid container from the Fluid Container holder.
- 8 Hold the Sheath fluid container under the CytoFLEX Sheath Fluid cubitainer ensuring the cubitainer is resting on a stable surface.
- 9 Fill the sheath fluid container.
- 10 Reinstall the sheath fluid harness onto the sheath fluid container.
- 11 Place the sheath fluid container back into the Fluid Container holder.

Replacing the 10 L Sheath Fluid Cubitainer

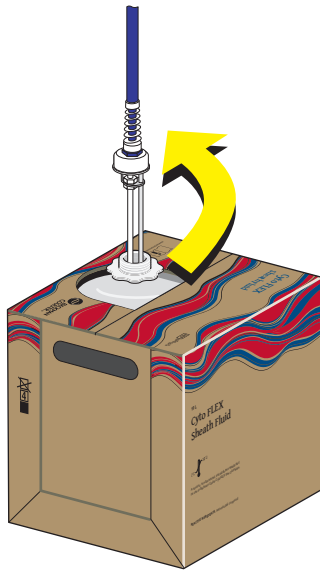
- 1 Confirm that the instrument is turned off or is in the standby state.
- 2 If necessary, remove any cardboard cutouts from the new CytoFLEX Sheath Fluid cubitainer. Remove the cap and seal from the new sheath fluid cubitainer. Be sure to completely remove the foil seal.



CAUTION

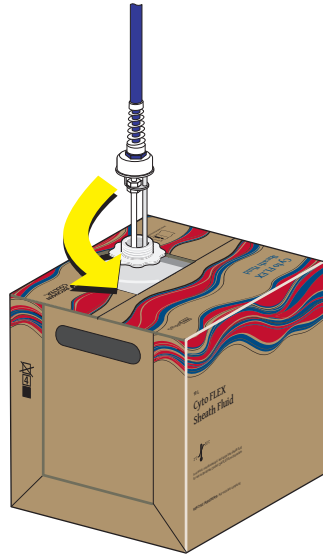
Misleading results could occur if you contaminate the sheath fluid. Be careful not to contaminate the sheath fluid. Do not let your fingers, paper towels, or other objects touch the pickup tube assembly.

- 3 Unscrew the plastic cap that secures the pickup tube assembly into the old sheath fluid container and lay it on a leakproof disposable container, such as a glove or beaker. Lift the pickup tube assembly straight up and out.



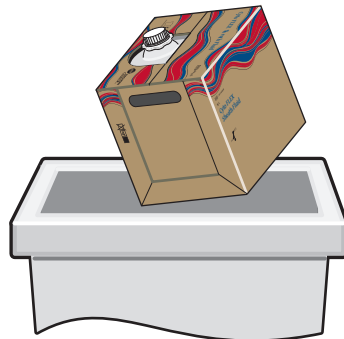
- 4 Inspect the pickup tube assembly and replace it if necessary.

- 5 Carefully insert the pickup tube assembly straight into the new sheath fluid cubitainer.
 Tighten the cap.



- 6 Place the 10 L sheath fluid cubitainer to the left of the instrument.

- 7 Put the cap from the new container onto the old container and dispose of the container properly.



Emptying the 4 L Waste Container [CytoFLEX]



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

2 Remove the waste harness (see [Figure 1.7](#)). The waste harness from the Cytometer is connected to a 4-L waste container.

3 Remove the waste container from the Fluid Container holder.

 **WARNING**

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

4 Empty the waste container. Dispose of the waste in accordance with your local regulations and acceptable laboratory procedures.

 **WARNING**

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

5 Add 400 mL of 5 to 6% bleach to the waste container.

6 Reinstall the waste harness in the waste container.

7 Put the waste container in the Fluid Container holder.

Emptying the 10 L Waste Cubitainer



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

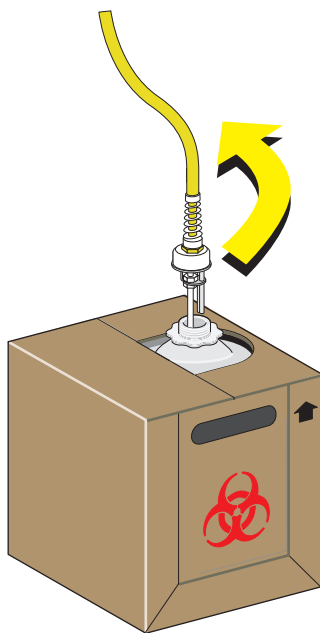
- 2 Lift the waste cubitainer and swirl it before removing the cap.



WARNING

Risk of biohazardous contamination if you have skin contact with the waste cubitainer, its contents, and its associated tubing. The waste cubitainer and 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 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.



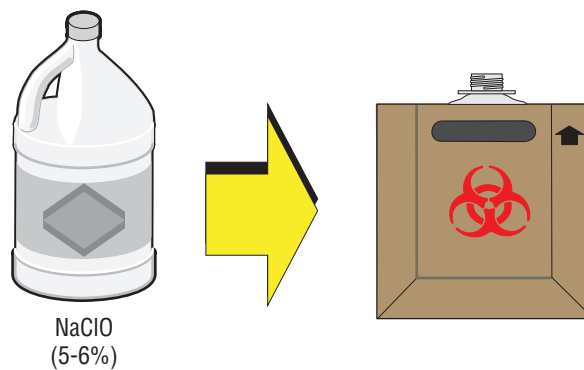
- 4 Empty the waste cubitainer according to your laboratory's procedures.

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.

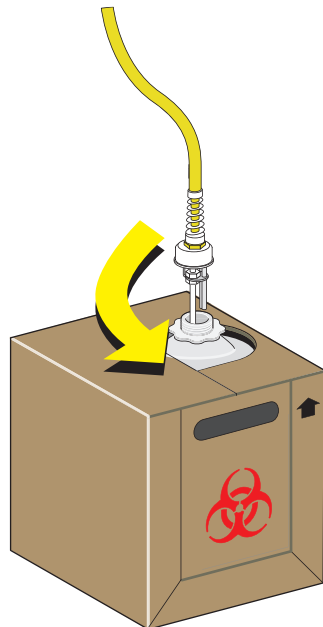
⚠ WARNING

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.

- 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.



- Replace the cap on the new waste cubitainer and securely tighten.






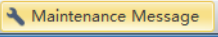

NOTE Properly dispose of the leakproof disposable container used in Step 3 after you screw the cap back on the waste container.

Managing the Maintenance Reminder


The maintenance reminder tracks the last maintenance date and initiates a reminder to complete maintenance for the following three items:

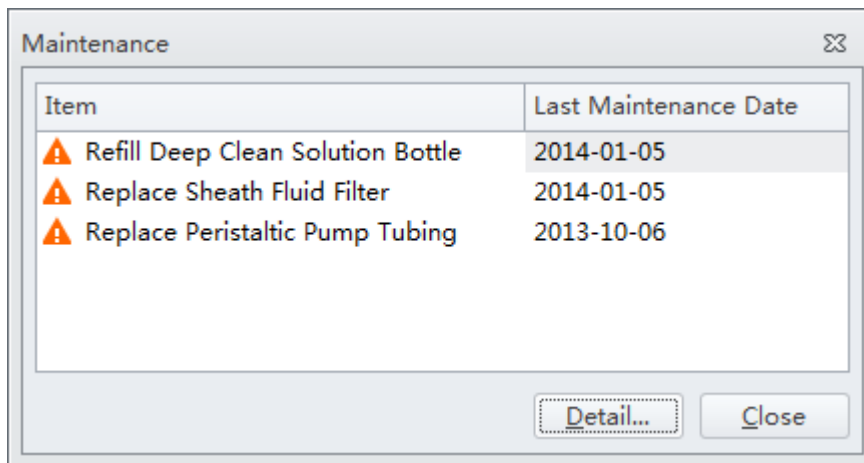
- Refill Deep Clean solution bottle (cleaning solution)
- Replace sheath fluid filter
- Replace sample peristaltic pump tubing

When reagents or parts have reached the designated use time limit in either days or number of uses, the Maintenance Message icon  Semi-automatic Sampler  Sheath  Waste appears in the right side of the status bar.

1 Select the Maintenance Message icon  from the status bar to access the Maintenance window. The expired item appears with a warning triangle  to the left of the item listed.

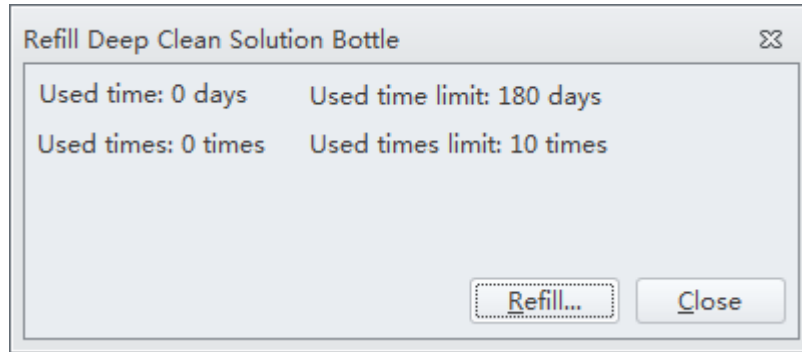
Or

Select **Maintenance** in the Advanced menu. The Maintenance window appears. The expired item is displayed with a warning triangle  to the left of the item listed.

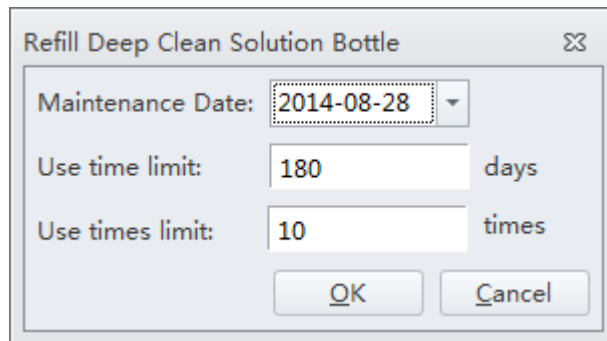


- 2 Select the desired item to manage, then choose one of the following:
- To manage refilling the Deep Clean solution bottle, go to Step 3.
 - To manage replacing the sheath filter, skip to Step 4.
 - To manage replacing the sample peristaltic pump tubing, skip to Step 5.

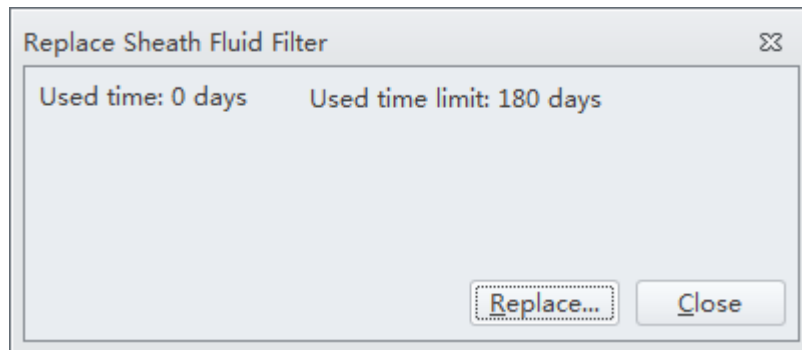
- 3 Select **Detail**. The Refill Deep Clean Solution Bottle window appears.



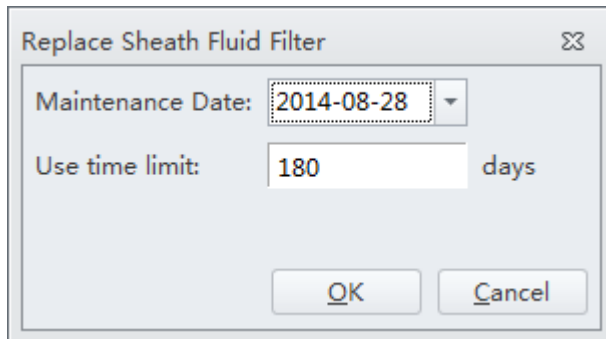
Select **Refill**. A pop-up window appears to reset the maintenance date as the current date that the maintenance was performed.



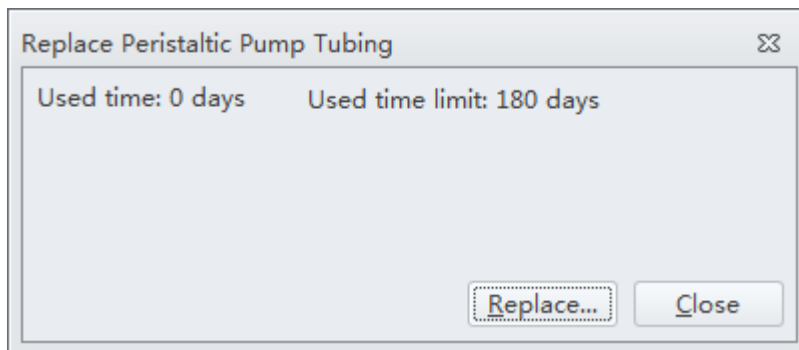
- 4 Select **Detail**. The Replace Sheath Fluid Filter window appears.



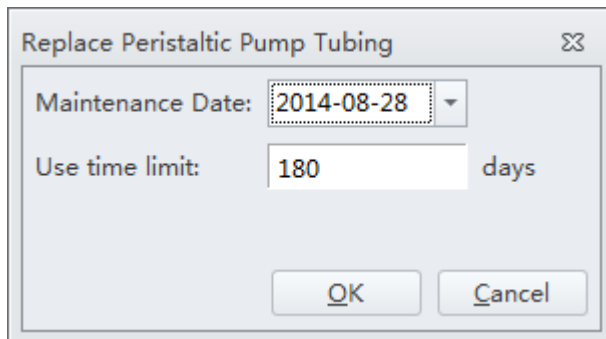
Select **Replace**. A pop-up window appears to reset the maintenance date as the current date that the maintenance was performed.



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



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



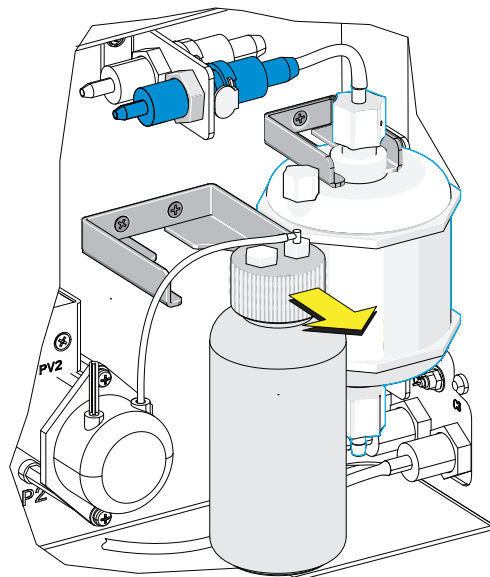
Adding the Deep Clean Solution

Check occasionally whether the Deep Clean solution in the Deep Clean solution bottle is sufficient. Replace the Deep Clean solution when the maintenance reminder prompts you to do so.

⚠ WARNING

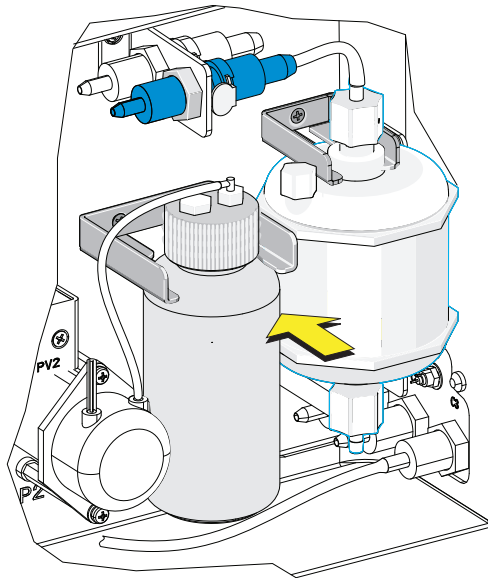
Risk of chemical injury from Contrad® 70 reagent. To avoid contact with the Contrad® 70 reagent, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

- 1 Make 60 mL of Deep Clean solution by mixing 30 mL of Contrad® 70 and 30 mL DI water in the Deep Clean solution bottle and swirl the solution gently to create the Deep Clean solution.
- 2 Verify that the Cytometer is in standby state or is turned off.
- 3 Remove the right-side cover of the instrument. Refer to [Right-Side Cover Removal and Reinstallation](#).
- 4 Remove the Deep Clean solution bottle and open the cap.



- 5 Add 60 mL Deep Clean solution to the bottle.

- 6 Tighten the cap, and attach the Deep Clean solution bottle to the bracket.



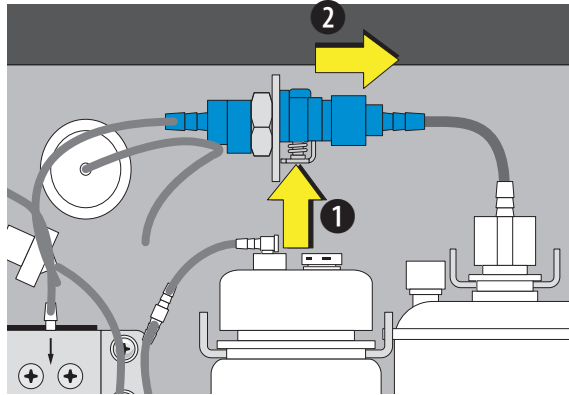
- 7 Reinstall the right-side cover (see [Right-Side Cover Removal and Reinstallation](#)), and fasten the thumbscrews.
- 8 Reset the maintenance reminder tracker. Refer to [Managing the Maintenance Reminder](#).

Replacing the Sheath Fluid Filter

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

- 1 Select **Standby** on the left of the screen to place the instrument in standby state, or shut off the Cytometer's power.
- 2 Remove the right-side cover. Refer to [Right-Side Cover Removal and Reinstallation](#).

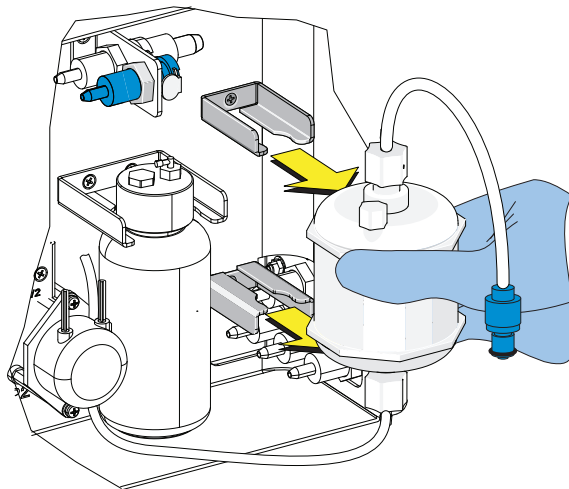
- 3 Press the spring piece on the quick connector and the Cytometer on the upper side of the filter, and disconnect the quick connector.



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

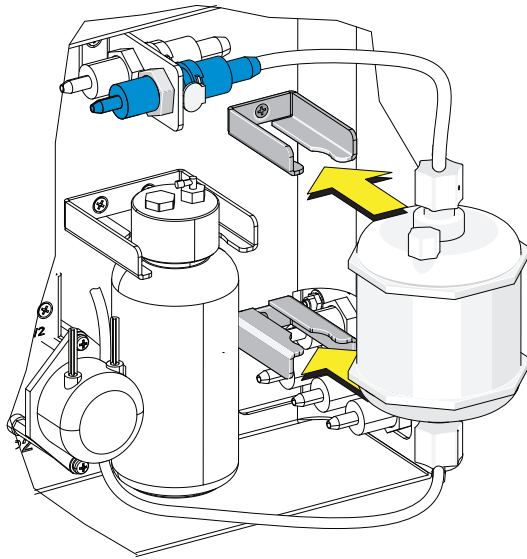
NOTE The spring is on the opposite side.

- 5 Remove the sheath fluid filter from the bracket.

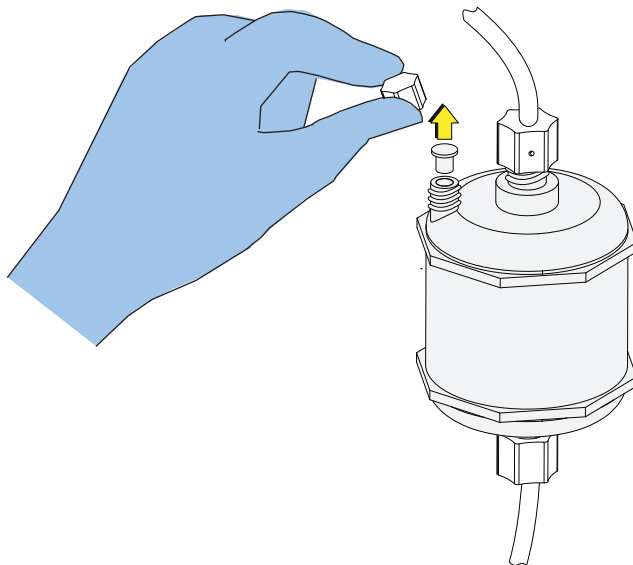


- 6 Connect the new, unused filter using the quick connector springs.

-
- 7 Attach the filter to the filter bracket.



-
- 8 Remove and set aside the vent cap and the rubber insert.



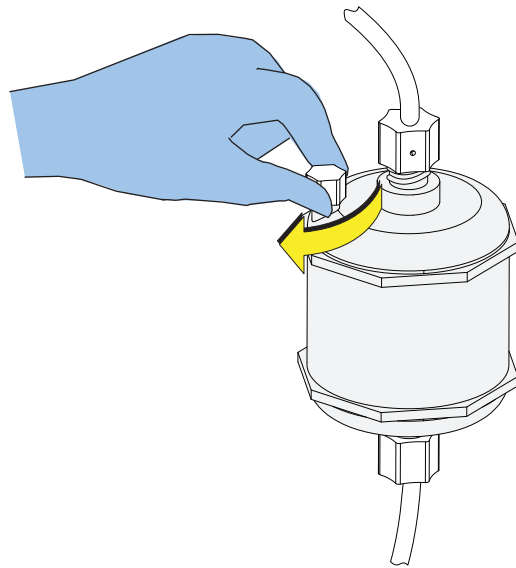
-
- 9 Turn on the Cytometer and open the software.

-
- 10 Select **Prime** in the Cytometer menu.

CAUTION

Risk of instrument damage. If the vent cap is not sealed tightly, unstable flow rate and leakage of the sheath fluid can occur.

- 11 Observe the liquid level in the filter during the prime cycle. When the liquid level reaches the upper section of the filter, reinstall the rubber insert, and lock the vent cap to prevent air leakage.



- 12 Reinstall the right-side cover (see [Right-Side Cover Removal and Reinstallation](#)) and lock the screw.
- 13 If the problem persists, [contact us](#).
- 14 Run the system startup program. Refer to [Running the System Startup Program with the Single Tube Loader](#) in [CHAPTER 3, Daily Startup](#).
- 15 Reset the maintenance reminder tracker. Refer to [Managing the Maintenance Reminder](#).

Replacing the Sample Probe and/or the Sample Peristaltic Pump Tubing



WARNING

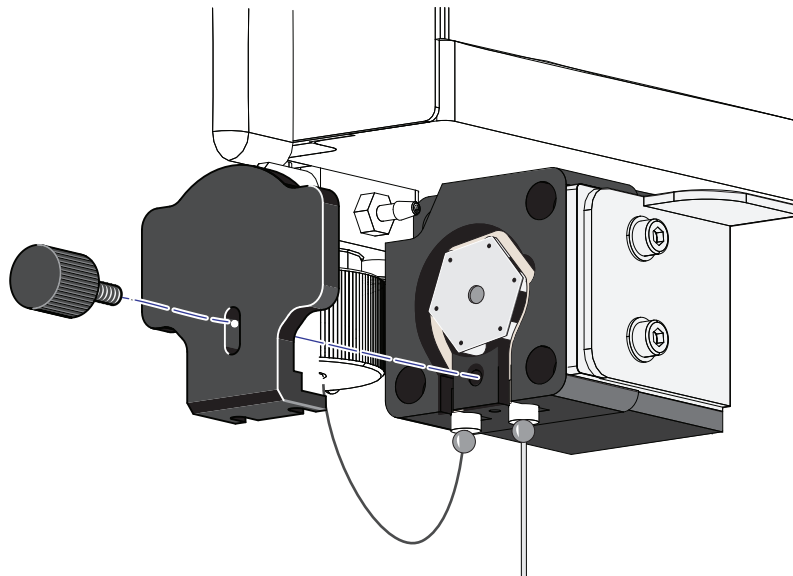
Risk of biohazardous contamination if you have skin contact with the sample probe or the sample peristaltic pump tubing. The sample probe and the sample peristaltic pump tubing could contain residual biological material and must be handled with care. Clean up spills immediately. Dispose of the sample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

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

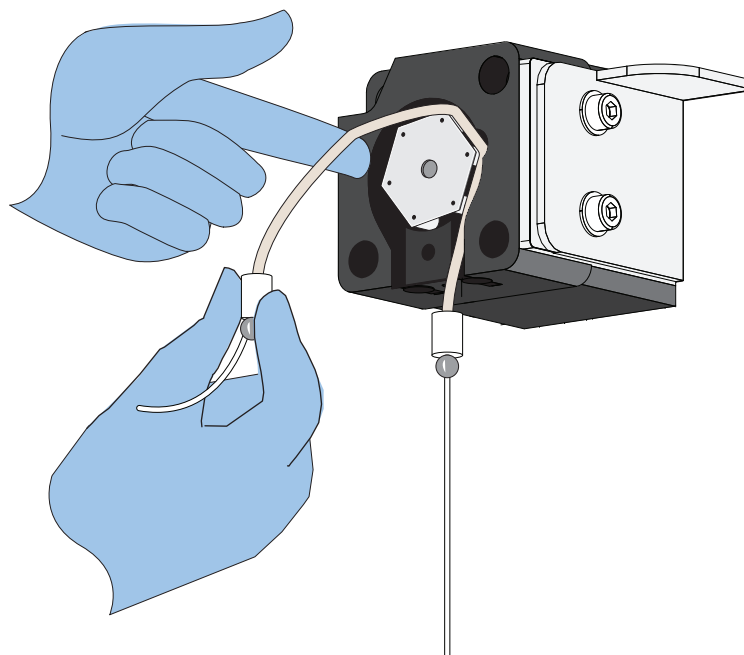
It is recommended to replace the tubing of the sample peristaltic pump every six months, as tubing used for an excessively long time can cause degradation of the stability of the sample flow and increase of the CV detected.

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

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



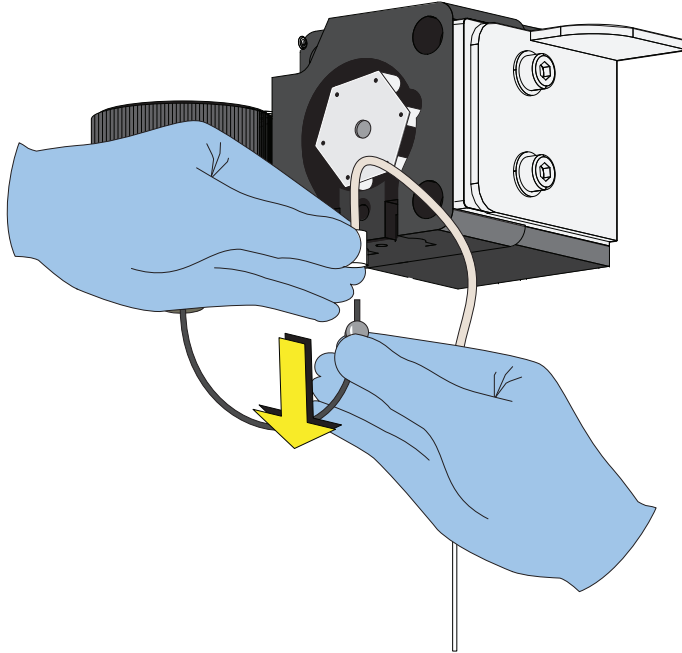
-
- 5 Take out the sample peristaltic pump tubing.



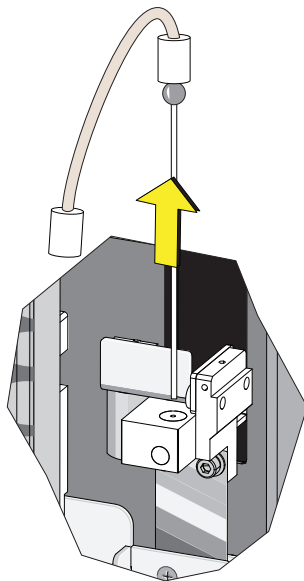
CAUTION

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

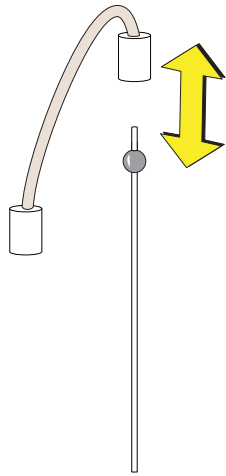
- 6 Remove the sample PEEK tubing from the sample peristaltic pump tubing.



- 7 Lift the sample probe out of the wash station.

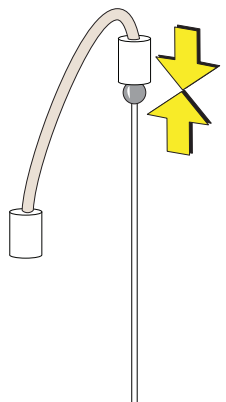


-
- 8** Remove the sample peristaltic pump tubing from the sample probe.

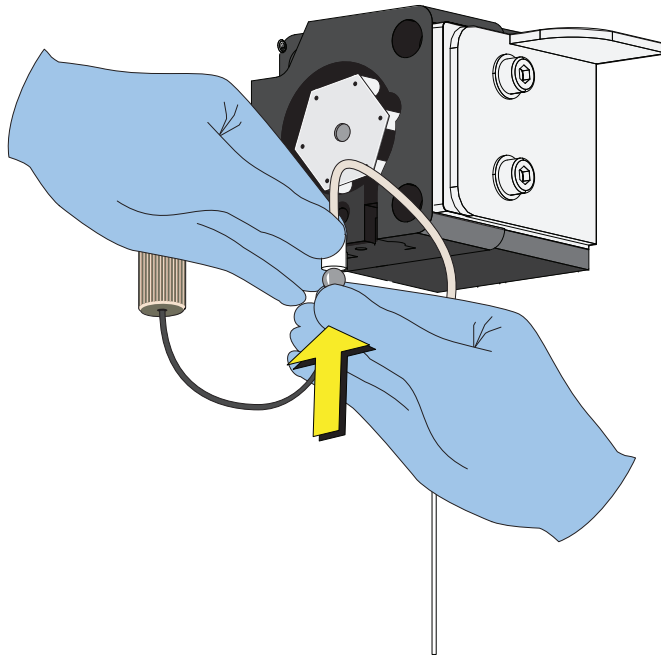


-
- 9** Dispose of the old sample probe and/or sample peristaltic pump tubing in accordance with your local regulations and acceptable laboratory procedures.

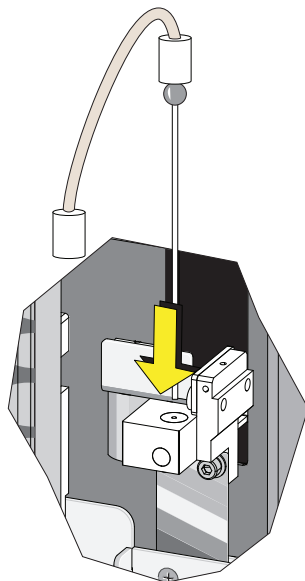
-
- 10** Connect the sample peristaltic pump tubing to the sample probe.



11 Connect the sample PEEK tubing to the sample peristaltic pump tubing.

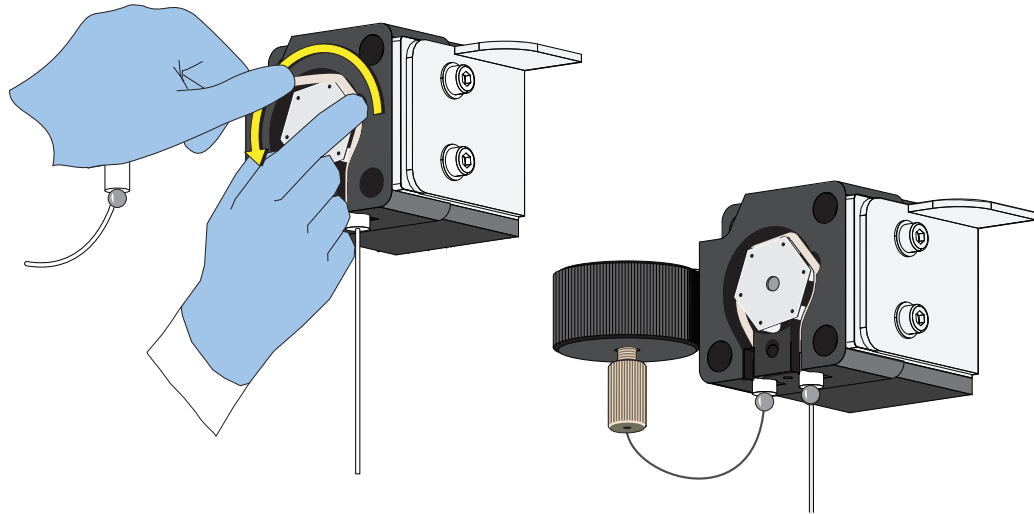


12 Slide the sample probe into the wash station.

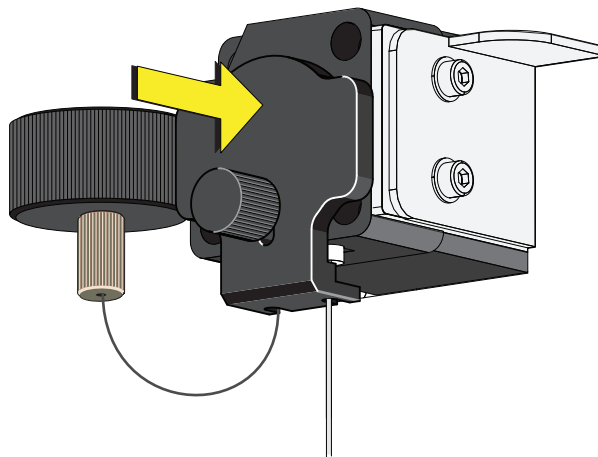


-
- 13** Install the sample peristaltic pump tubing, taking care not to use any sharp tools, ensuring that the tube is fully inserted into the groove.

NOTE You can use the sample pump cover thumbscrew to install the sample peristaltic pump tubing.



-
- 14** Install the sample pump cover.



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

-
- 15** Reinstall the right-side cover (see [Right-Side Cover Removal and Reinstallation](#)), and lock with the screw.
-

Replacing the Sample Probe Assembly [With Plate Loader]

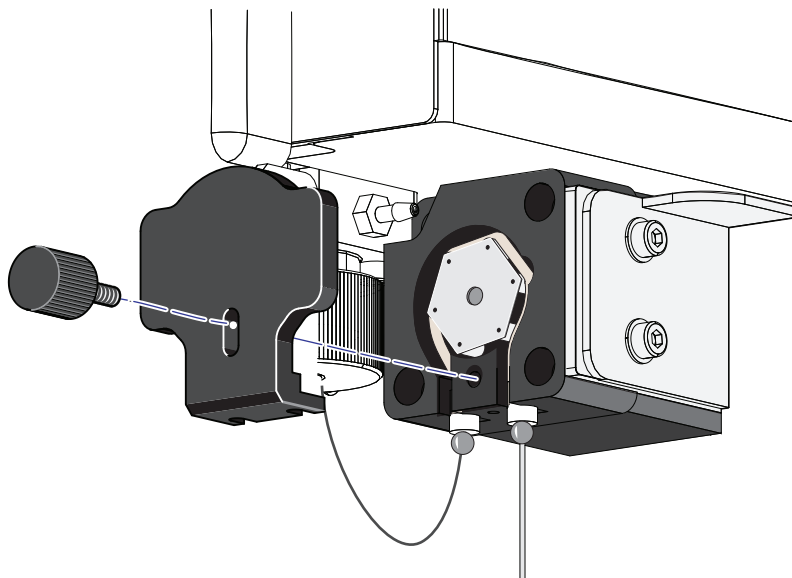


IMPORTANT If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, [contact us](#) to replace the sample probe assembly.

WARNING

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

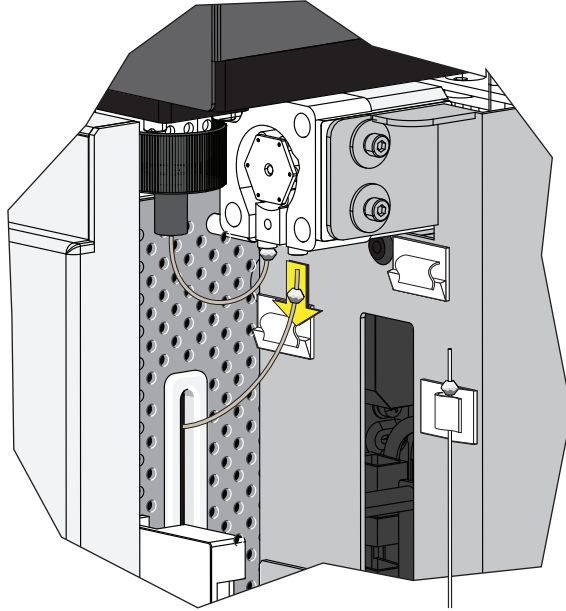
- 1 Turn the Cytometer's main power switch off.
- 2 Remove the right-side cover. Refer to [Right-Side Cover Removal and Reinstallation](#) in CHAPTER 11, Replacement/Adjustment Procedures.
- 3 Remove the front cover. Refer to [Front Cover Removal and Reinstallation](#) in CHAPTER 11, Replacement/Adjustment Procedures.
- 4 Remove the sample pump cover thumbscrew and the sample pump cover.



CAUTION

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

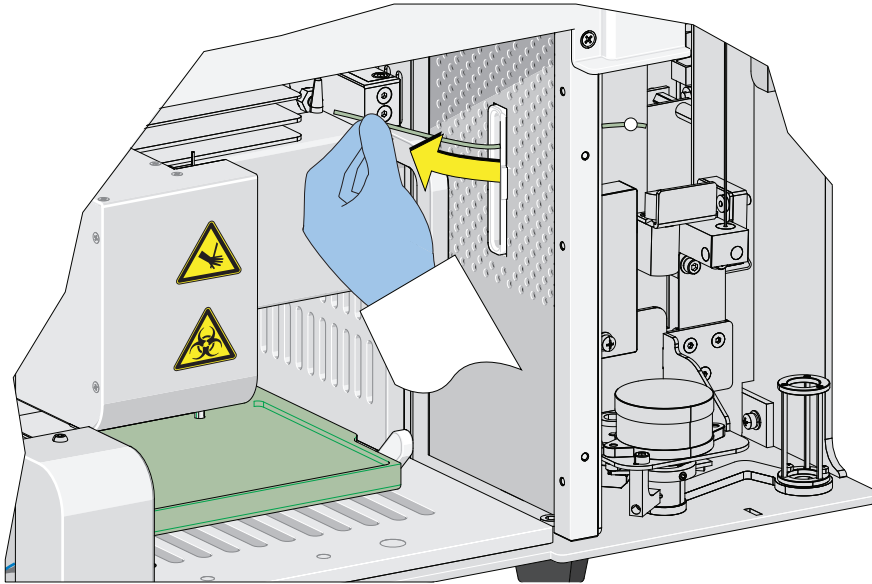
- 5 Remove the plate loader PEEK tubing from the sample peristaltic pump tubing.



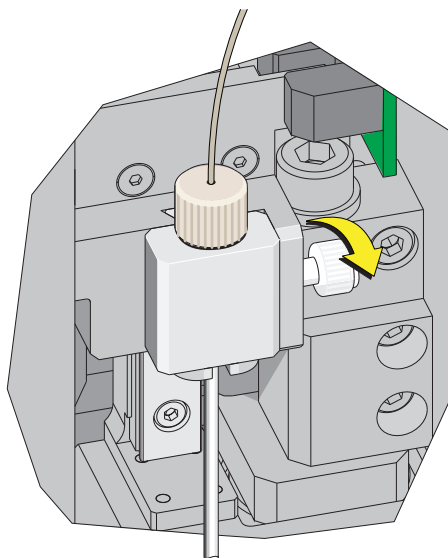
⚠ CAUTION

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

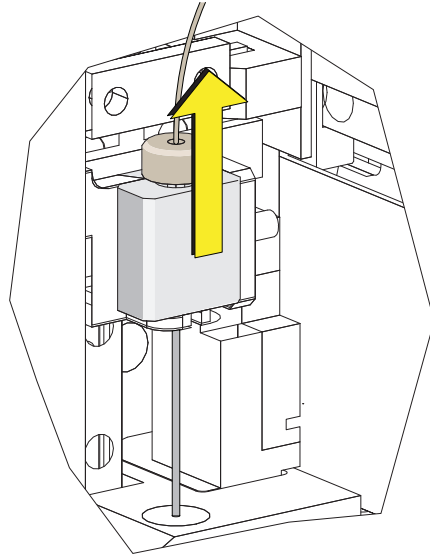
- 6 Pull the plate loader PEEK tubing through the slot so the tubing sits inside the instrument.



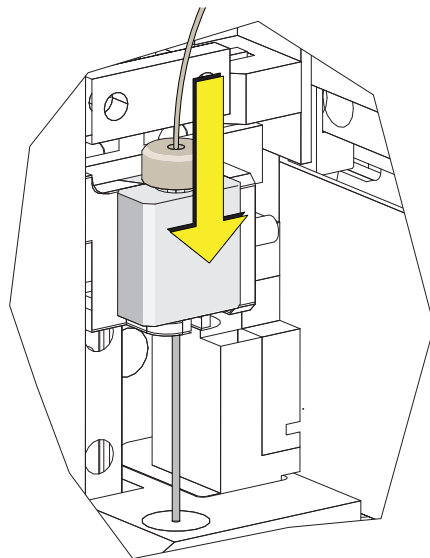
- 7 Loosen the white plastic thumbscrew behind the plate loader sample probe.



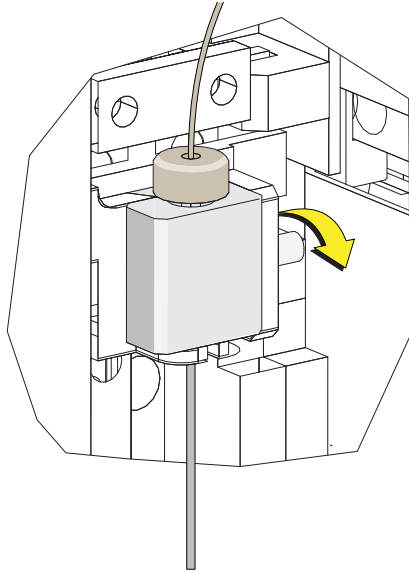
- 8 Lift the plate loader sample probe assembly straight up to remove it from the probe holder.



- 9 Align the tongue on the plate loader sample probe assembly with the groove in the probe holder and slide the probe down until it is flush with the probe holder.



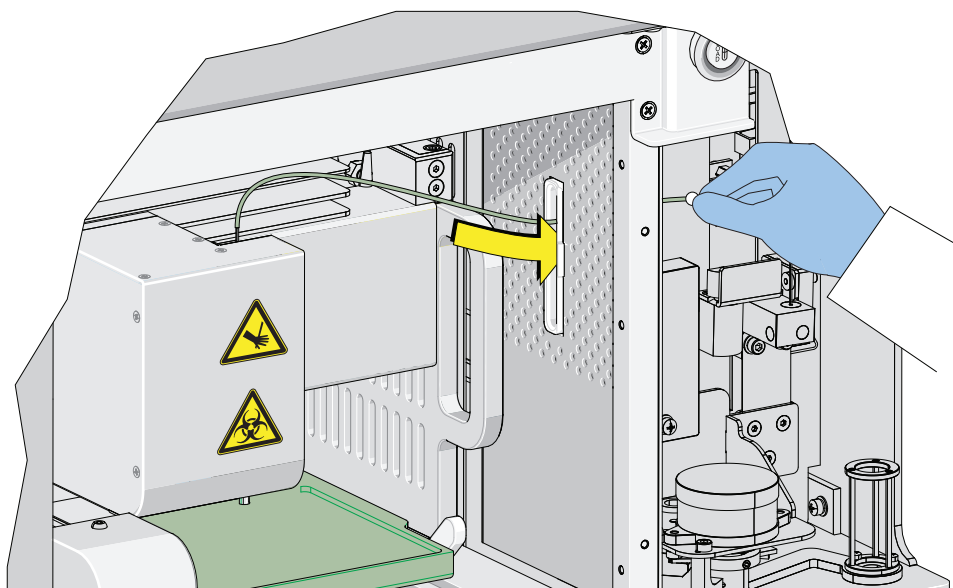
- 10 Tighten the white plastic thumbscrew.



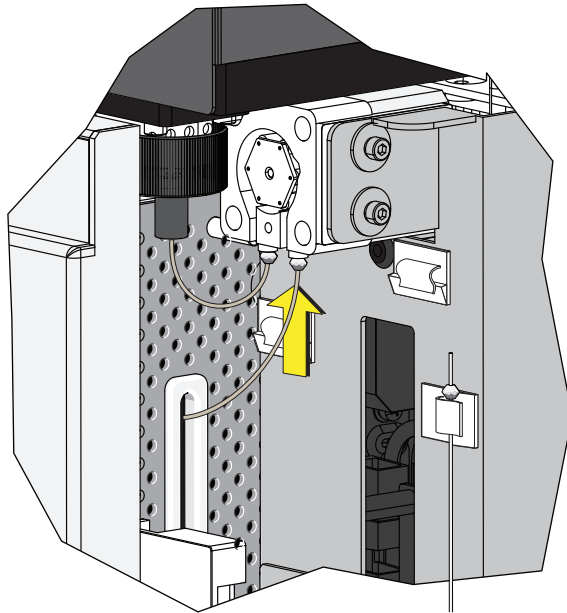
CAUTION

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

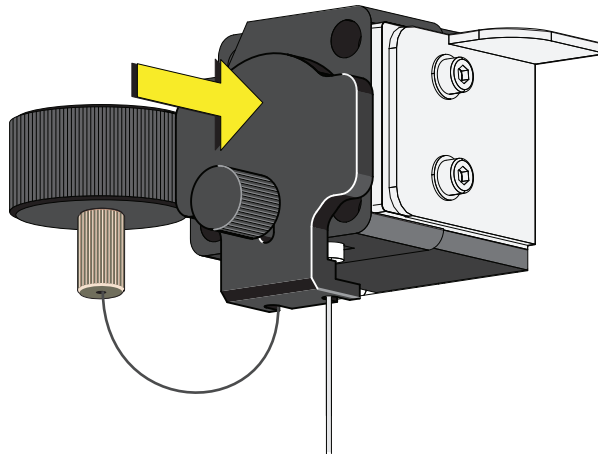
- 11 Slide the new plate loader PEEK tubing through the slot into the single tube sample station area.



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



13 Install the sample pump cover.



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

14 Reinstall the right-side cover (refer to [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)), and lock with the screw.

15 Close the top cover.

-
- 16 Turn the Cytometer's main power switch on.
-

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



WARNING

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

NOTE If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, refer to [Switching the Sample Probe from the Single Tube Sample Station to the Plate Loader \[With Sample Injection Mode Control Knob\]](#) in [APPENDIX C, Sample Injection Mode Control Kit](#) for instructions on switching between the single tube mode and the plate loader mode.

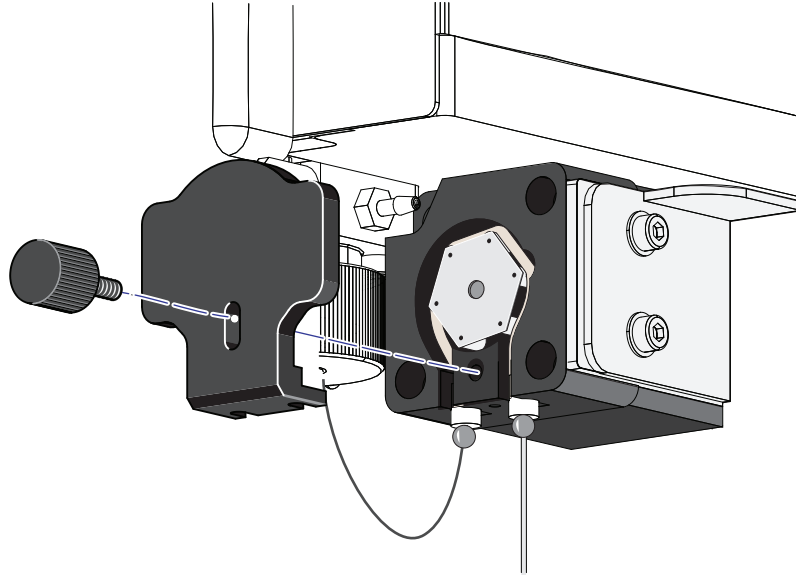
-
- 1 Switch to the Plate Loader sample injection mode. Refer to [Selecting the Plate Loader Sample Injection Mode \[With Plate Loader\]](#) in [CHAPTER 3, Daily Startup](#).

 - 2 Lift the top cover.

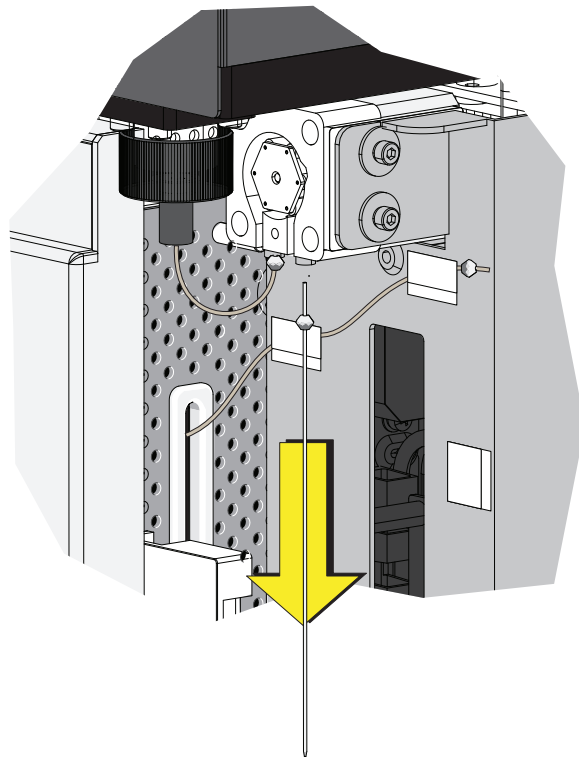
 - 3 Remove the right-side cover. Refer to [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

 - 4 Remove the front cover. Refer to [Front Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

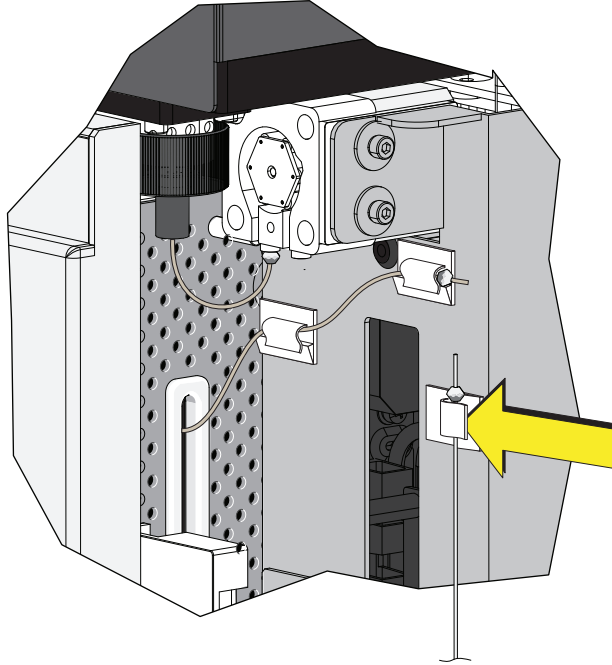
- 5 Remove the sample pump cover thumbscrew and the sample pump cover.



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



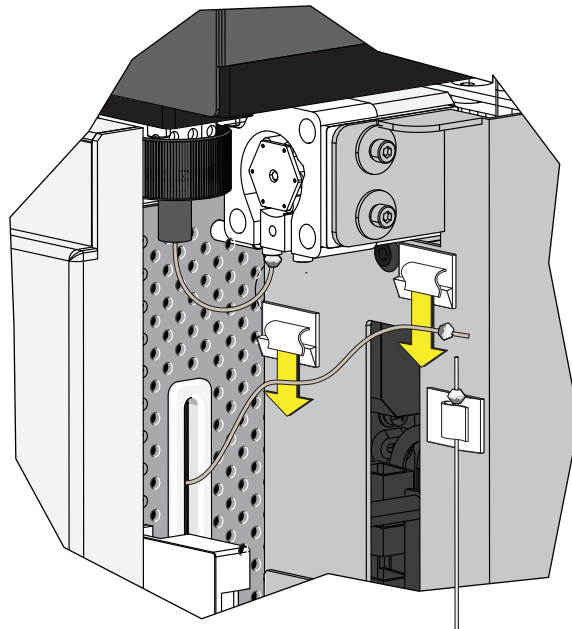
- 7 Place the sample probe in the white clip located on the right side of the sample station.



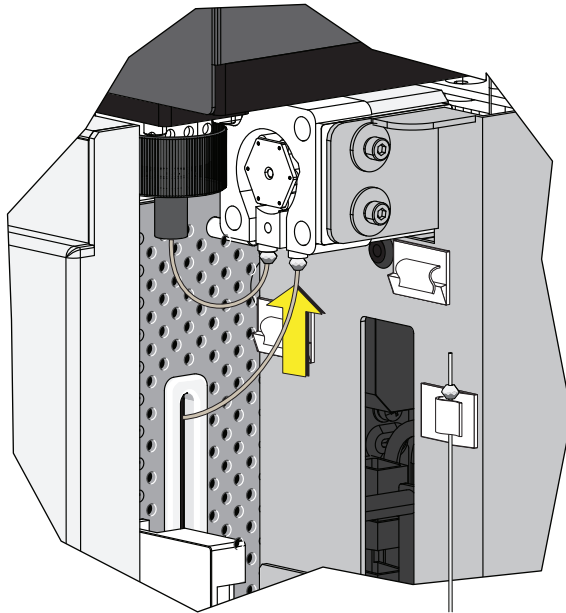
CAUTION

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

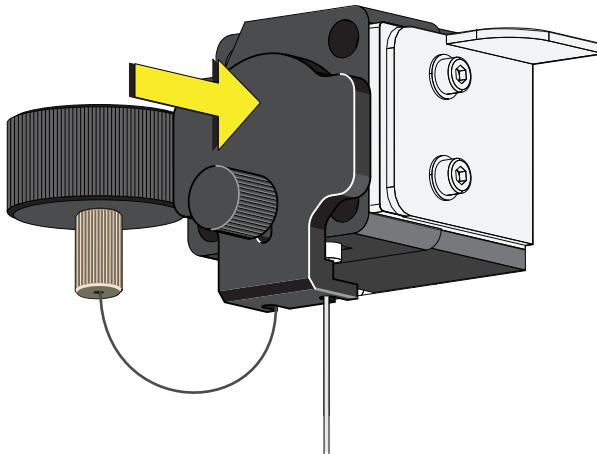
- 8 Remove the plate loader PEEK tubing from the two white clips located at the top of the sample station.



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



-
- 10 Reinstall the sample pump cover.



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

-
- 11 Reinstall the right-side cover (refer to [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)), and lock with the screw.

-
- 12 Close the top cover.

-
- 13 Turn the Cytometer's main power switch on.
-

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



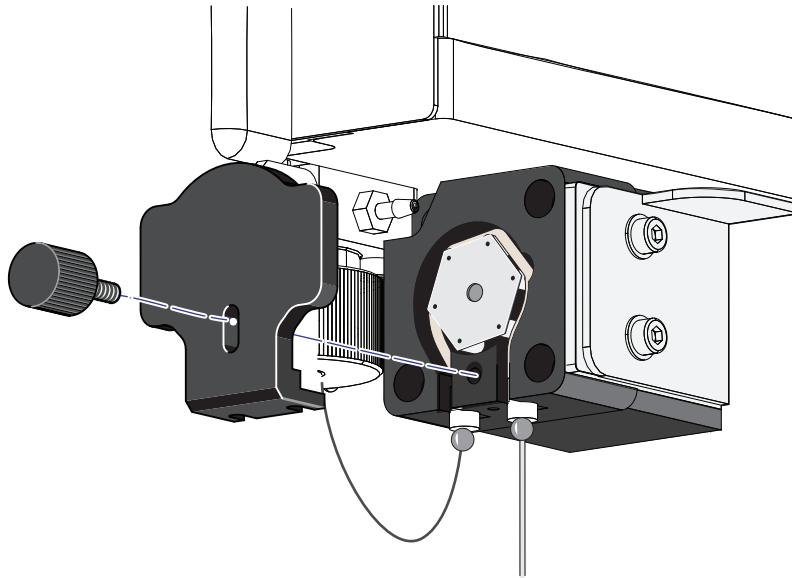
WARNING

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

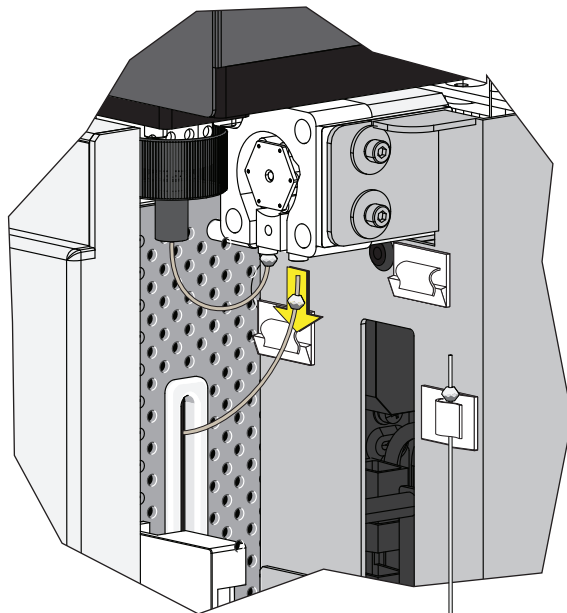
NOTE If you have the Sample Injection Mode Control Kit installed on your CytoFLEX Series instrument, refer to [Switching the Sample Probe from the Plate Loader to the Single Tube Sample Station \[With Sample Injection Mode Control Knob\]](#) in [APPENDIX C, Sample Injection Mode Control Kit](#) for instructions on switching between the single tube mode and the plate loader mode.

- 1 Switch to either the Semi-Automatic or manual sample injection mode. Refer to [Selecting the Proper Sample Injection Mode](#) in [CHAPTER 3, Daily Startup](#).
- 2 Lift the top cover.
- 3 Remove the right-side cover. Refer to [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).
- 4 Remove the front cover. Refer to [Front Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

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



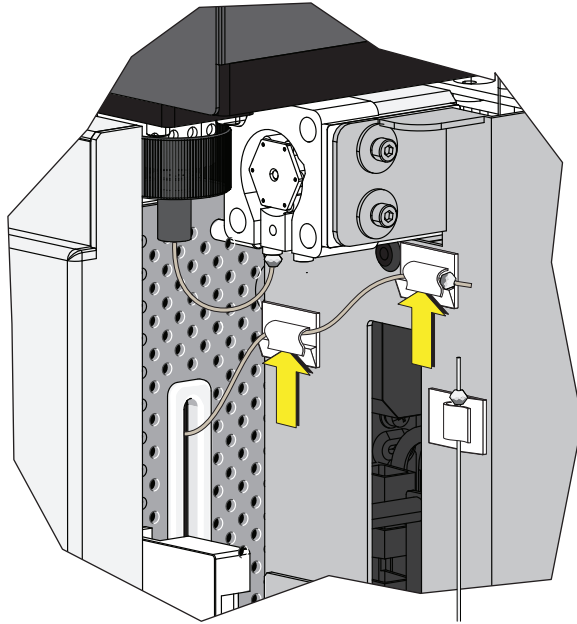
-
- 6 Remove the plate loader PEEK tubing from the sample peristaltic pump tubing.



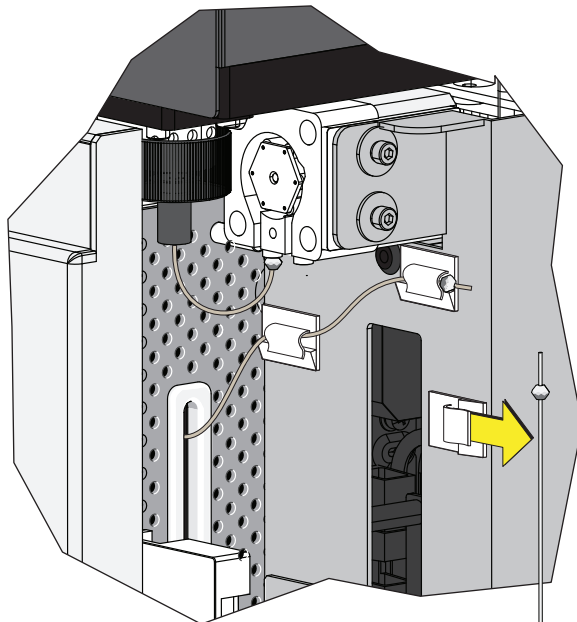
CAUTION

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

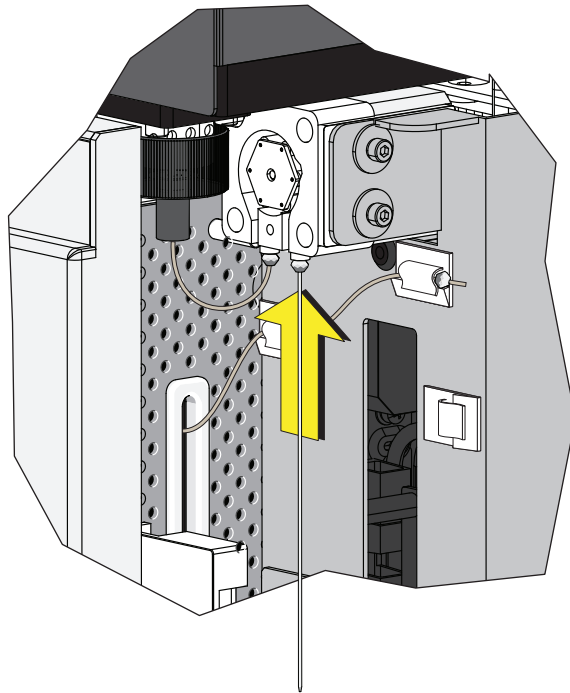
- 7 Place the plate loader PEEK tubing in the two white clips located at the top of the sample station.



- 8 Remove the sample probe from the white clip located on the right side of the sample station.



- 9 Connect the sample probe to the sample peristaltic pump tubing.



- 10 Run [Daily Clean](#) in [CHAPTER 10, Cleaning Procedures](#).

Inspecting the Liquid Flow Path for Leaks

⚠ WARNING

The liquid flow tubing can aged and cracked or the connector can be loosened. Liquid leakage can lead to biological harm. To reduce occurrence of such problems, carry out liquid flow tubing inspection every six months and ensure that the Fluidics module functions without any leaks. If any leaks are found when using the Cytometer, stop the experiment immediately and look for the source of the leak.

- 1 Remove the right-side cover of the instrument. Refer to [Right-Side Cover Removal and Reinstallation](#).
- 2 Perform instrument initialization to enable the sheath fluid to flow. Refer to [Initializing the Instrument](#) in [CHAPTER 3, Daily Startup](#).

-
- 3 Check the connectors and tubes in the Fluidics module, and check whether any liquid leaks out.

 - 4 Check the sheath fluid, backflush, and waste liquid connector on the back of the Cytometer, and check whether any liquid leaks out.

 - 5 Place the instrument in standby state, complete the priming procedure, and check whether the Fluidics module has any liquid leakage.

 - 6 If any liquid leaks out and the point of liquid leakage is from the filter, try to tighten the filter connector and check again. If the problem persists, replace the sheath fluid filter.

 - 7 If any liquid leaks out from any other connector or tube, stop running the instrument and [contact us](#).
-

Priming the Flow Cell

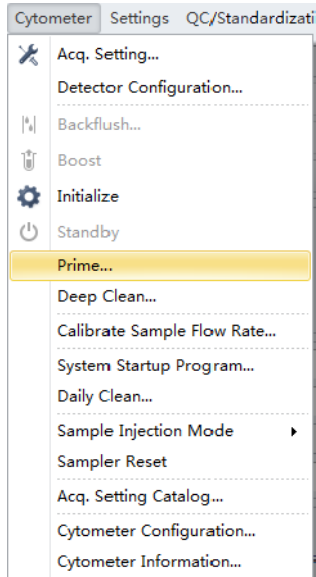
Priming of the flow cell is required when:

- The instrument is not used for a long period of time.
- The sheath fluid is refilled.
- The instrument is being used for the first time.
- The signal of the instrument is poor or the signal drifts.
- The sheath filter is replaced.

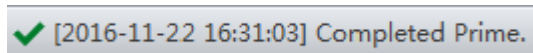
-
- 1 Ensure that the instrument is in standby state.

NOTE If the instrument is not already in the standby state, select **Standby** from the Cytometer Menu or select **Standby** in the Data Acquisition Control screen.

- 2 Select **Prime** from the Cytometer Menu to prime the flow cell. Wait for the beep and for the Instructions window to close.



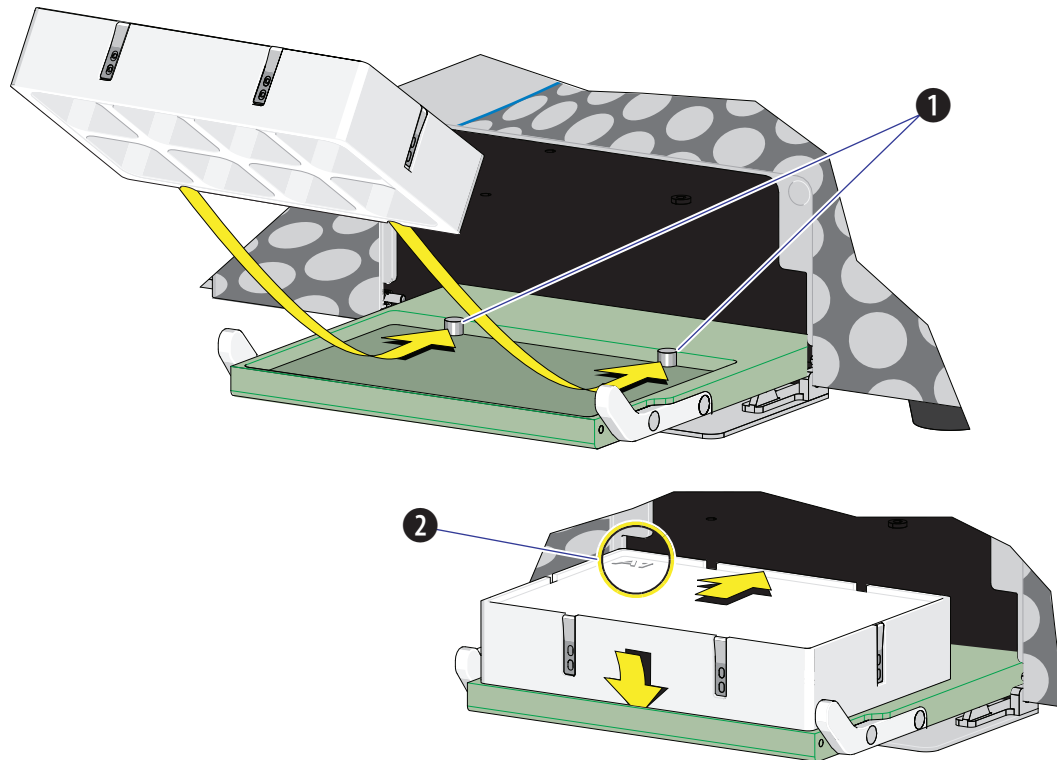
Otherwise, look for the status bar to display that priming has completed.



- 3 Run Daily Clean to clean the sample line. Refer to [Daily Clean](#) in [CHAPTER 10, Cleaning Procedures](#)

Replacing the Plate Holder [With Plate Loader]

Figure 11.1 Plate Holder Stage



1. Pins
2. Position A1

CAUTION

The plate holder must be secured tightly to the plate holder stage with position A1 located in the top, left corner of the plate holder stage (refer to [Figure 11.1](#)) to prevent instrument damage.

Slide the notches on the bottom of the plate holder (refer to [Figure 1.15](#)) over the pins (refer to [Figure 11.1](#)).

Plate Loader Module Removal and Reinstallation [With Plate Loader]



WARNING

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

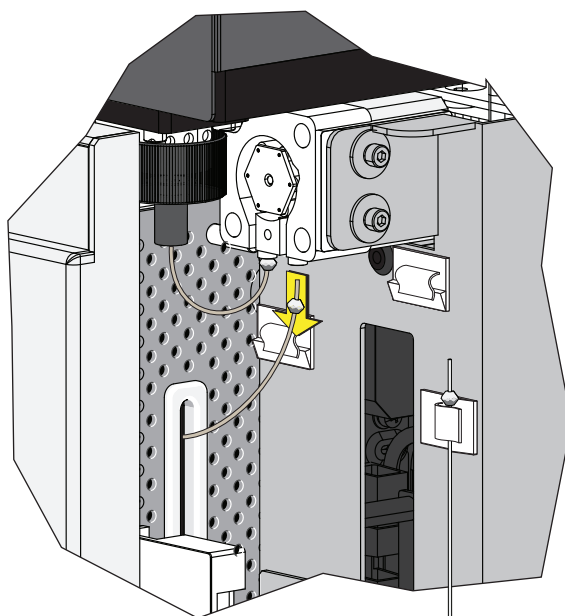
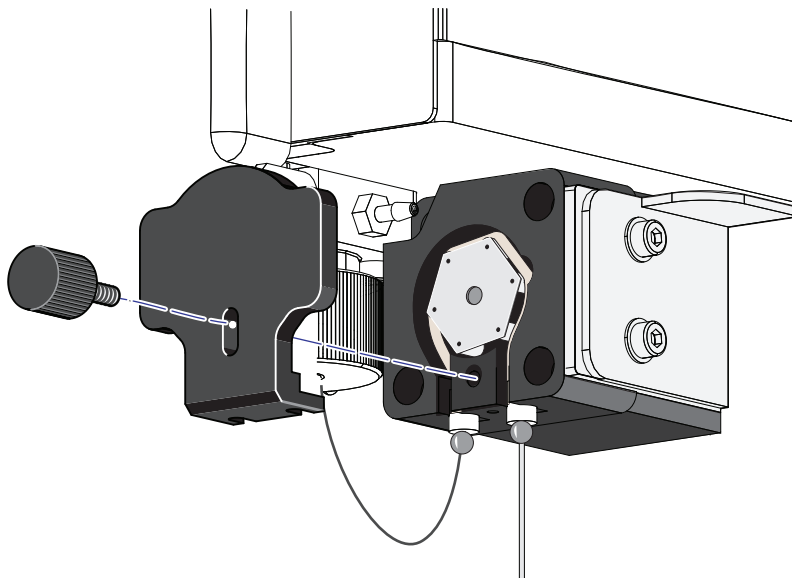
Removal

-
- 1** Power down the instrument and disconnect the power cable from the wall.

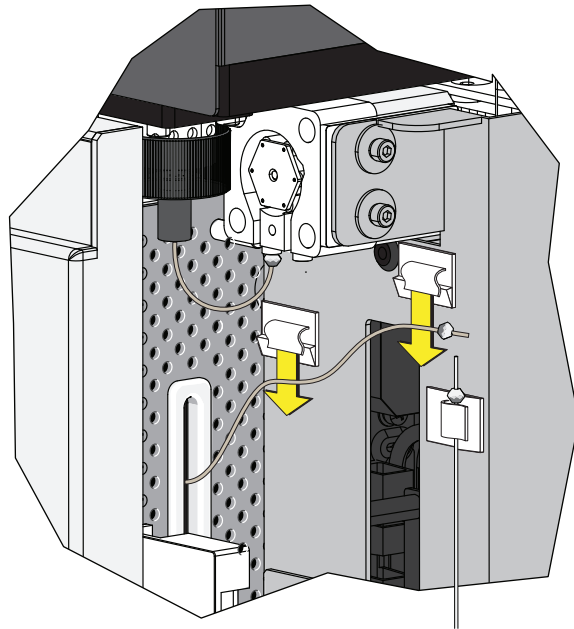
 - 2** Lift the top cover and remove the front cover. Refer to [Front Cover Removal and Reinstallation](#).

 - 3** Place absorbent material on the stage in the Plate Loader module.

- 4** In the Sample Station, disconnect that end of the plate loader PEEK tubing.
- If the plate loader PEEK tubing is attached to the sample peristaltic pump tubing, remove the sample pump cover and disconnect the plate loader PEEK tubing.



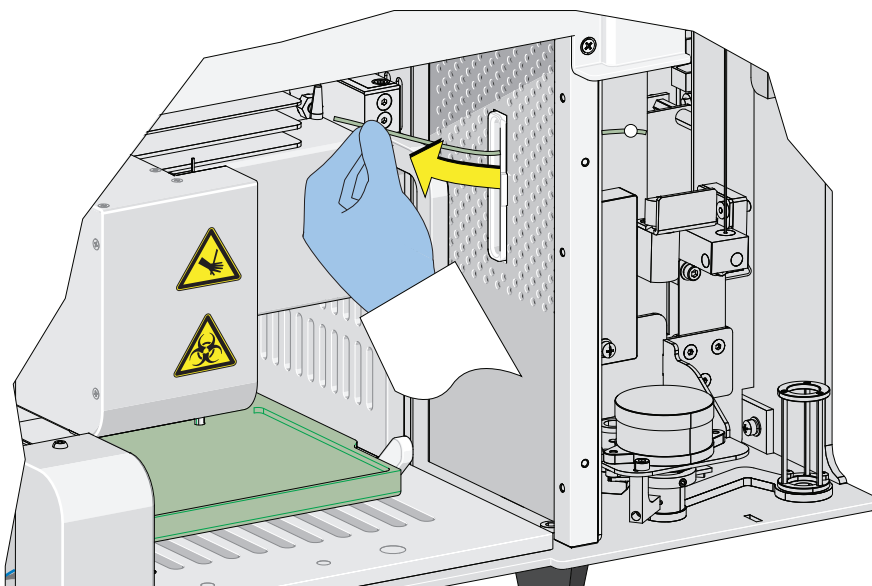
- If the plate loader PEEK tubing is in the plate loader PEEK tubing clips, remove the tubing from the clips.



CAUTION

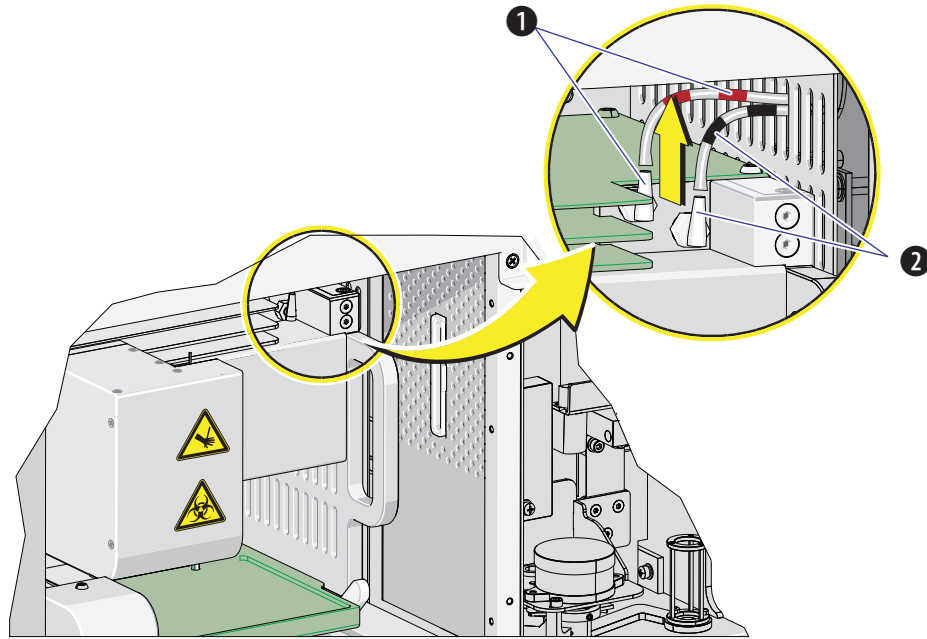
The PEEK tubing can be deformed which could affect sample flow. When routing the Plate Loader PEEK tubing to and from the Sampling Station, be careful not to pinch, crimp, stretch, or break the tubing.

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



- 6 Remove the red and black marked tubes that are attached to the left and right connectors in the Plate Loader module, respectively, as shown in [Figure 11.2](#).

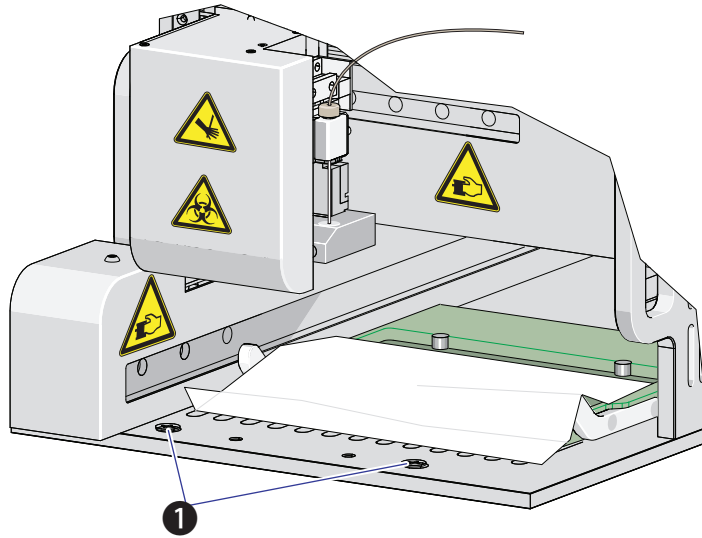
Figure 11.2 Removing the Tubings from the Fluidics Module to the Plate Loader



1. Red
2. Black

- 7 Remove the two countersunk M4 x 10 Phillips-head screws in the Plate Loader module. Refer to [Figure 11.3](#).

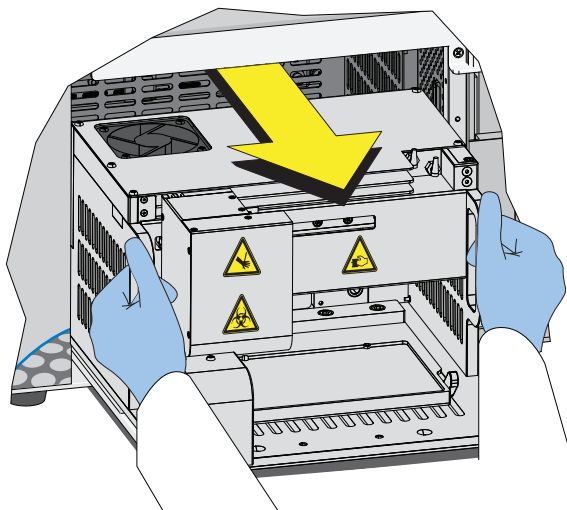
Figure 11.3 Plate Loader Module Securing Screws



1. Securing screws

- 8 Slide the Plate Loader module out of the Cytometer. Refer to [Figure 11.4](#).

Figure 11.4 Removing the Plate Loader from the Cytometer



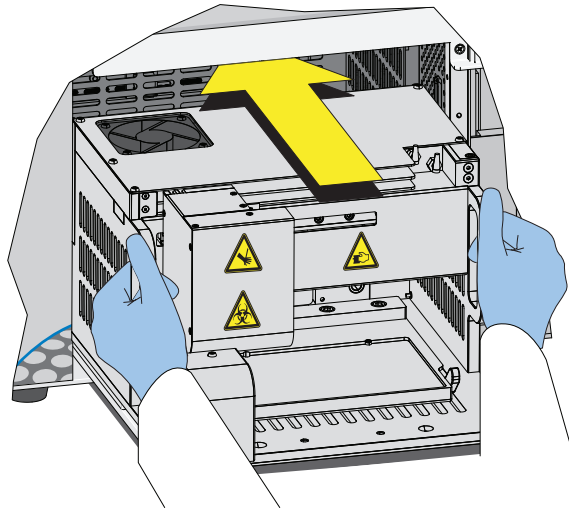
Installation

- 1 Remove the front cover. Refer to [Front Cover Removal and Reinstallation](#).

2 Remove the right-side cover. Refer to [Right-Side Cover Removal and Reinstallation](#).

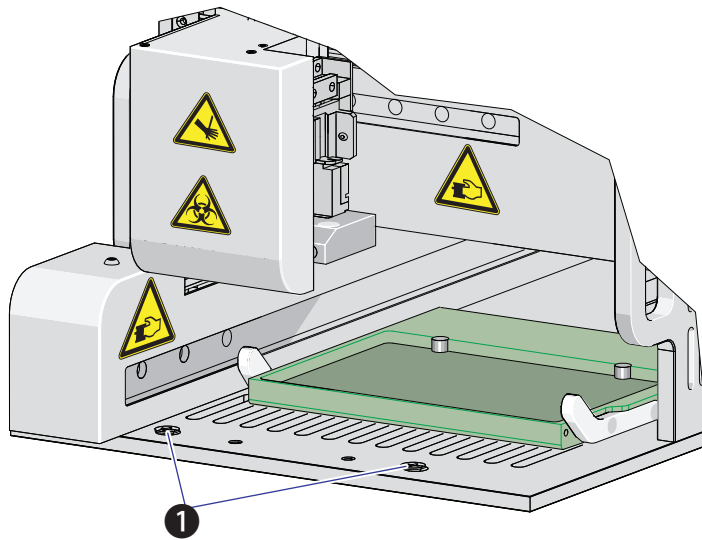
3 Slide the Plate Loader module in to the Cytometer. Refer to [Figure 11.5](#).

Figure 11.5 Installing the Plate Loader in to the Cytometer



4 Install the two countersunk M4 x 10 Phillips-head screws in the Plate Loader module. Refer to [Figure 11.6](#).

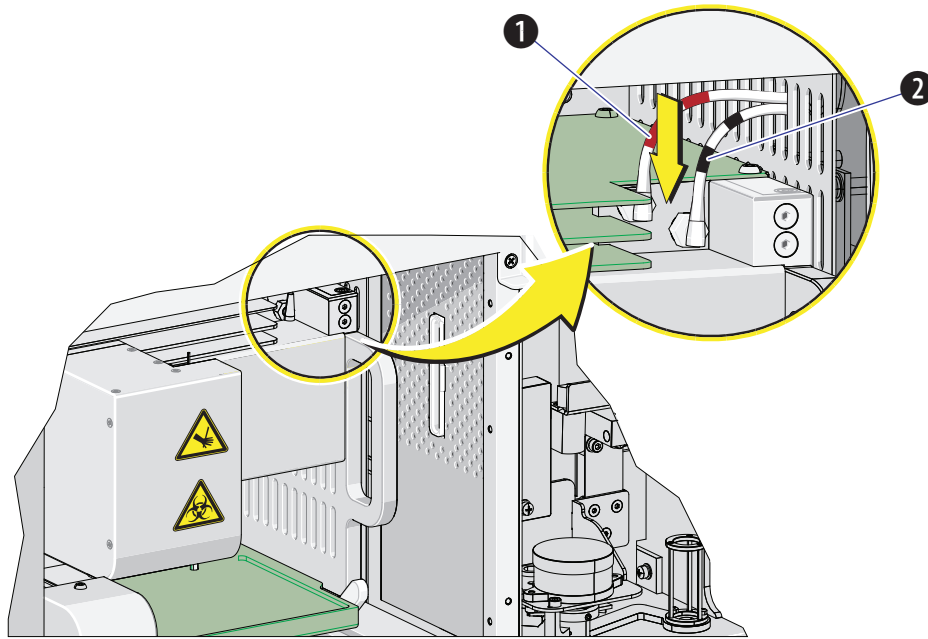
Figure 11.6 Plate Loader Module Securing Screws



1. Securing screws

- 5 Install the red and black marked tubings that are attached to the left and right connectors in the Plate Loader module, respectively, as shown in [Figure 11.7](#).

Figure 11.7 Connecting the Tubings from the Fluidics Module to the Plate Loader

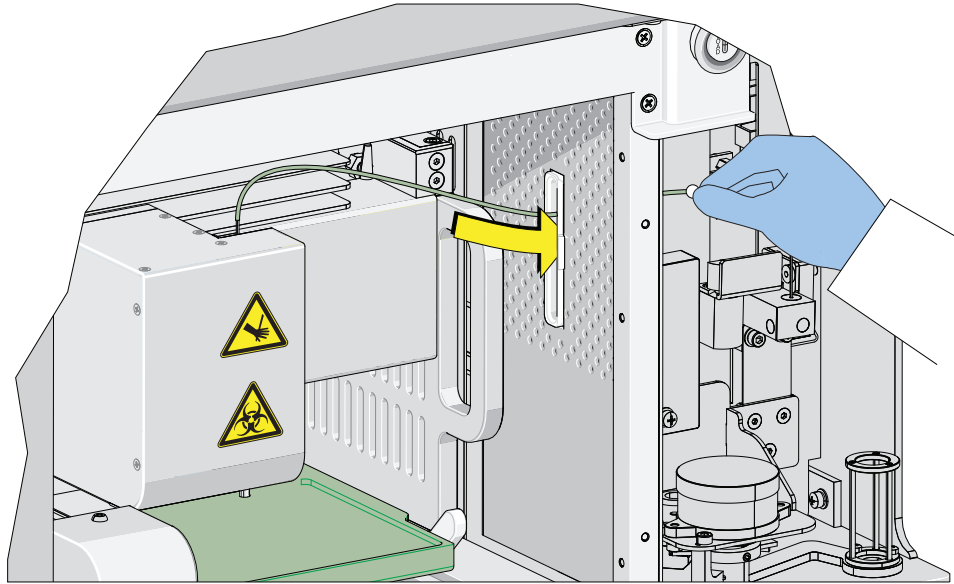


1. Red
2. Black

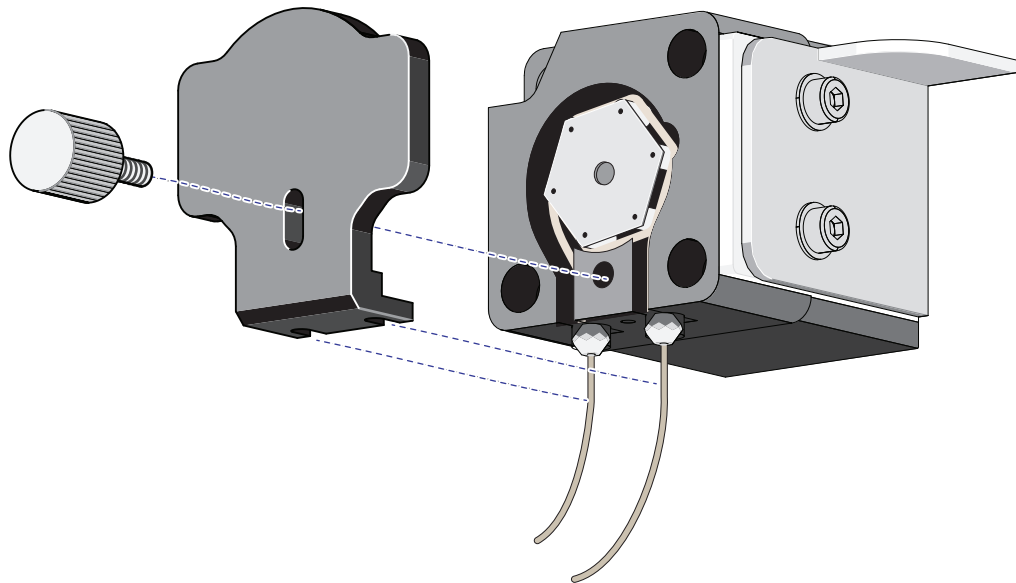
⚠ CAUTION

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

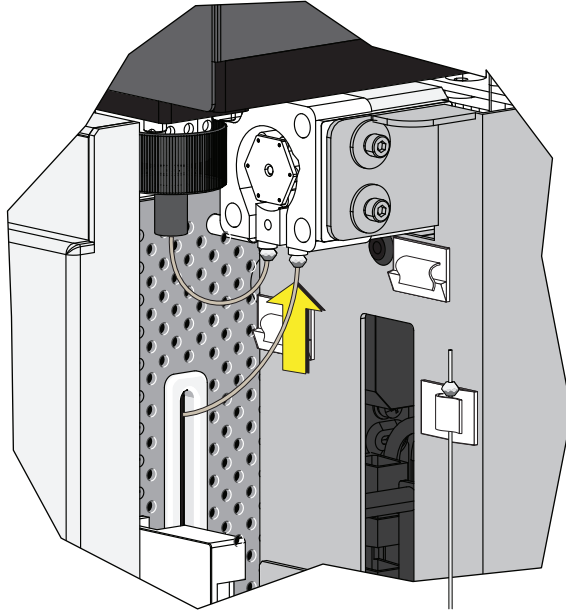
- 6 Slide the new plate loader PEEK tubing through the slot into the single tube sample station area.



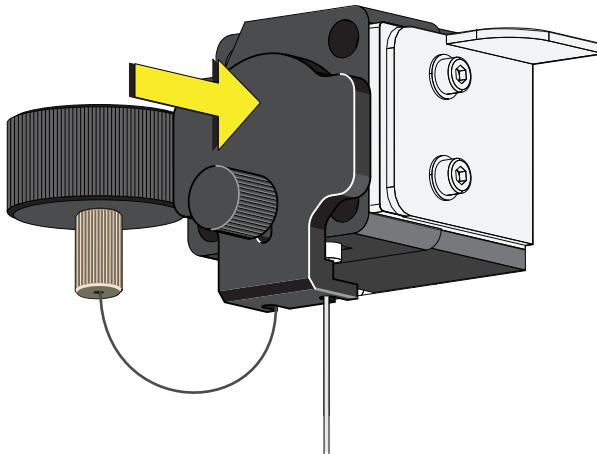
- 7 Remove the sample pump cover thumbscrew and the sample pump cover.



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



-
- 9 Reinstall the sample pump cover.



NOTE To ensure the proper placement of the sample probe, push the sample pump cover up while installing the thumbscrew.

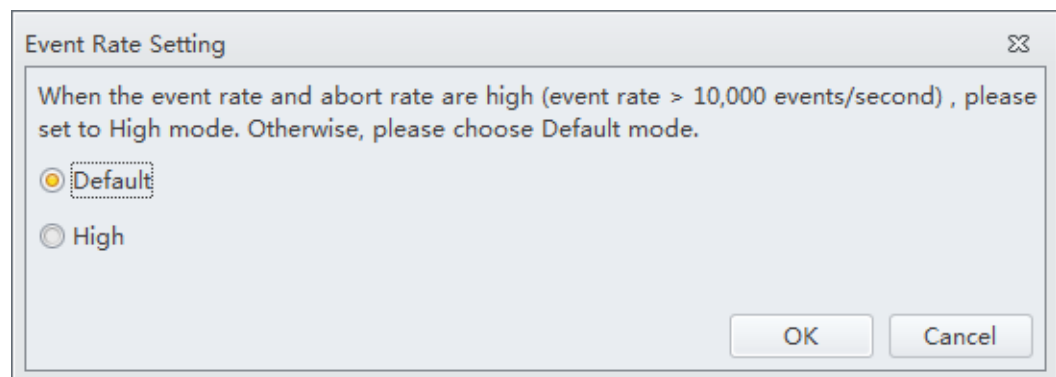
-
- 10 Replace the right-side cover (refer to [Right-Side Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#)), and lock with the screw.
-
- 11 Replace the front cover. Refer to [Front Cover Removal and Reinstallation](#).

- 12 Close the top cover.
- 13 Turn the Cytometer's main power switch on.
- 14 Verify that the system is operating correctly by running QC in the Plate Loader mode. Refer to [Preparing the QC Sample \[With Plate Loader\]](#) and [Collecting QC Data \[With Plate Loader\]](#) in CHAPTER 4, Instrument Quality Control and Standardization.

Changing the Event Rate Setting

The Event Rate Setting adjusts the collection setting around signal measurement so that the system is able to optimize the acquisition of events ensuring optimal sensitivity and abort rates when acquiring at higher event rates.

- 1 Select **Event Rate Setting** in the Advanced menu. The Event Rate Setting window appears.



- 2 Select **High** if the event rate is >10,000 events/second.
Or
Select **Default** if the event rate is <10,000 events/second.
- 3 Select **OK**.

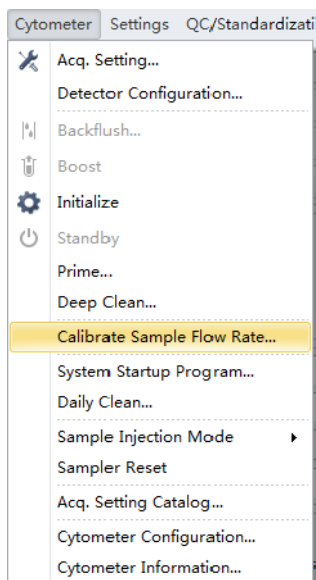
Nonscheduled Replacement/Adjustment

Calibrating the Sample Flow Rate

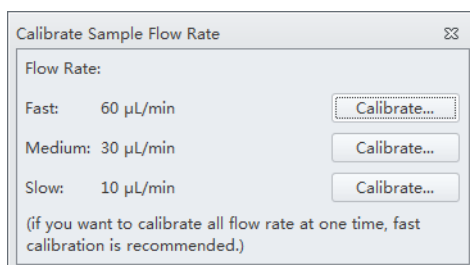
Calibrate the sample flow rate:

- After replacing the sample peristaltic pump tubing.
- If a precise volumetric measurement is required.
The accuracy of concentration calculations can be affected by the sample flow rate.

- 1 Verify that the instrument is in the initialized state.
- 2 Select **Calibrate Sample Flow Rate** in the Cytometer menu.



- 3 Select the flow rate to be calibrated. You can carry out calibration only for one speed and apply it to the setting of all the speeds. It is recommended to use a fast speed calibration.



-
- 4 Use a calibrated electric balance to measure the weight of one sample tube containing at least 1 mL clean deionized water. Record the data and enter it into the software.

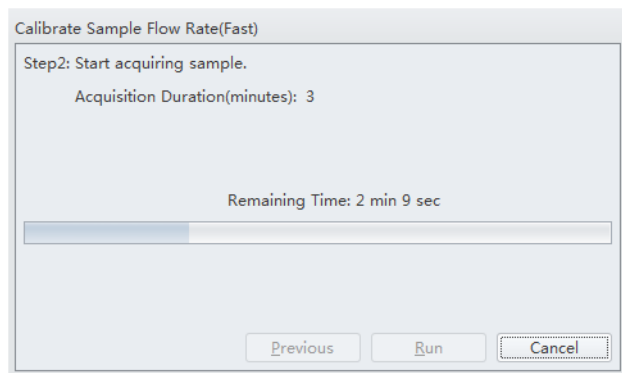
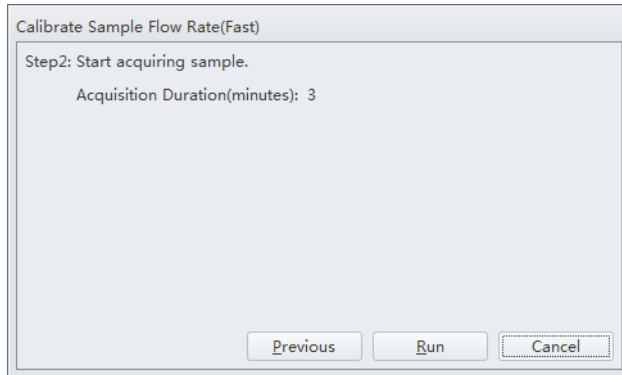
The screenshot shows a dialog box titled "Calibrate Sample Flow Rate(Fast)". Inside, it says "Step1: Please prepare at least 1mL of deionized water, and enter the weight" followed by an empty text input field and "g.". Below that, it says "Acquisition Duration(minutes):" followed by a spin box containing the number "3". At the bottom right, there are two buttons: "Next" and "Cancel".

-
- 5 Select **Next** and put the sample tube in the sample loading position (see [Figure 1.12](#)).

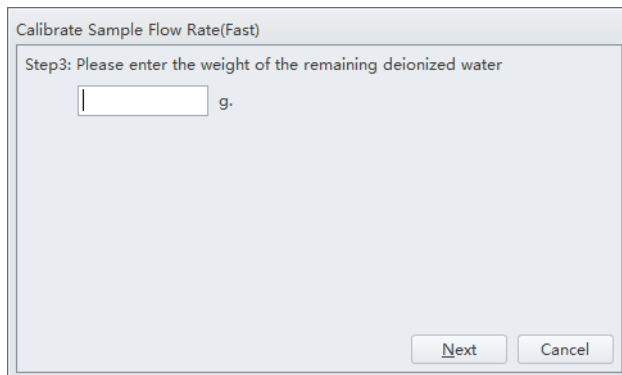
-
- 6 Select **Initialize** to start the sample run.

The screenshot shows the same dialog box titled "Calibrate Sample Flow Rate(Fast)". It now says "Step2: Start acquiring sample." and "Acquisition Duration(minutes): 3". At the bottom, there are three buttons: "Previous", "Initialize", and "Cancel".

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

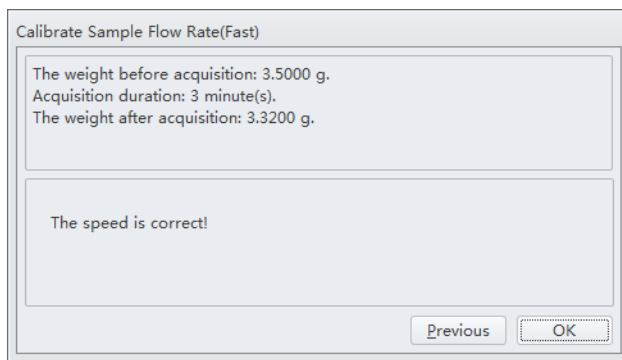


8 Wait for the sample run to finish, remove the sample tube, and use the electronic balance to measure the weight and record the value.

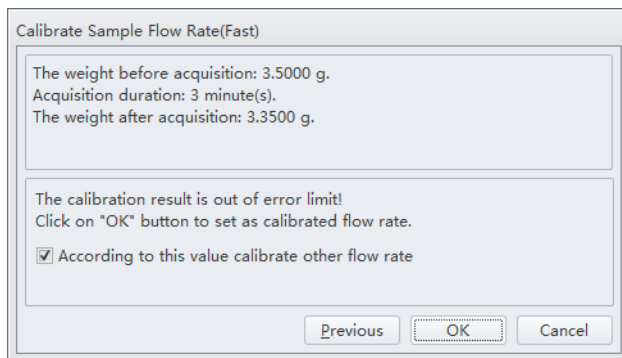


- 9 Select **Next** to determine whether the current flow rate is required to be calibrated. According to need, select whether any other flow rate is required to be calibrated.

If no calibration is required, the current setting is kept.



If deviation occurs, the setting is automatically corrected.



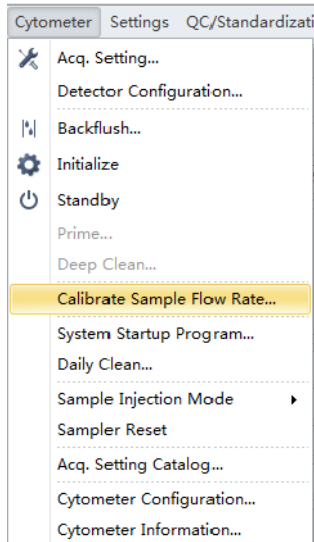
Calibrating the Sample Flow Rate [With Plate Loader]

Calibrate the sample flow rate:

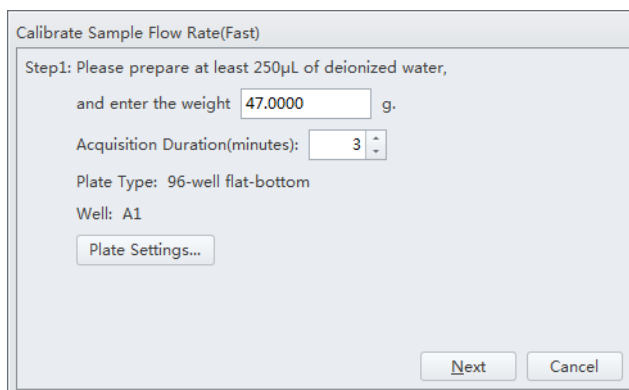
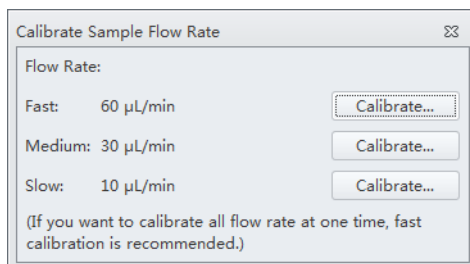
- After replacing the sample peristaltic pump tubing.
- If a precise volumetric measurement is required.
 The accuracy of concentration calculations can be affected by the sample flow rate.

- 1 Verify that the instrument is in the initialized state.

- 2 Select **Calibrate Sample Flow Rate** in the Cytometer menu. The plate loader automatically ejects the plate holder stage.



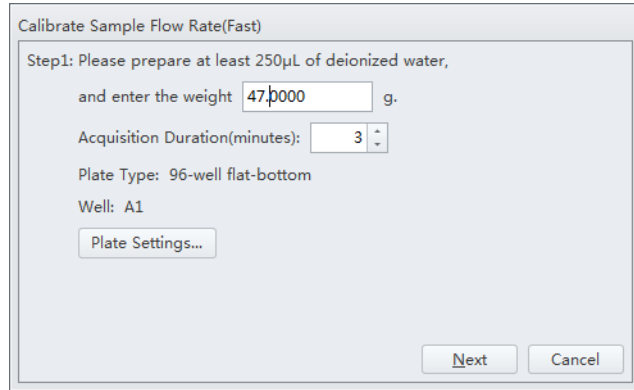
- 3 Select the sampling speed that needs to be calibrated and select **Calibrate**. The Calibrate Sample Flow Rate window appears.



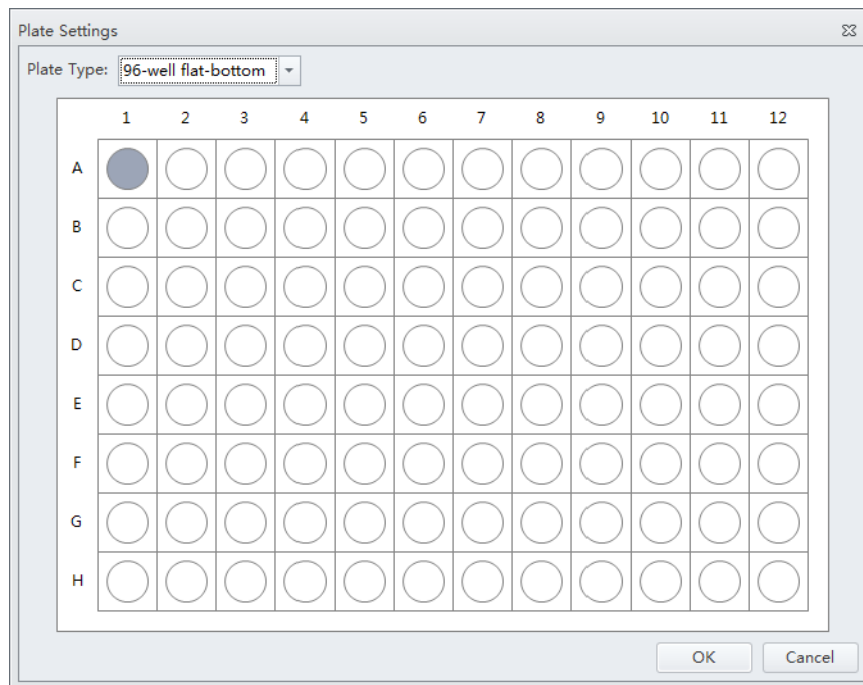
- 4 Follow the onscreen software prompts, then weigh the sample plate.

5 Enter the weight of the sample plate and set the acquisition time.

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

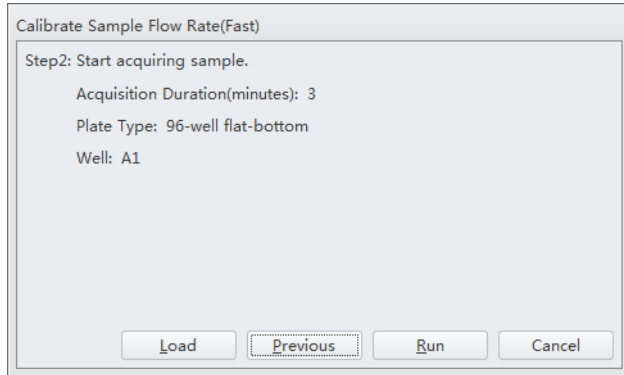


6 Select **Plate Settings** to set the sample well and plate type.



7 Select **OK** to save the settings.

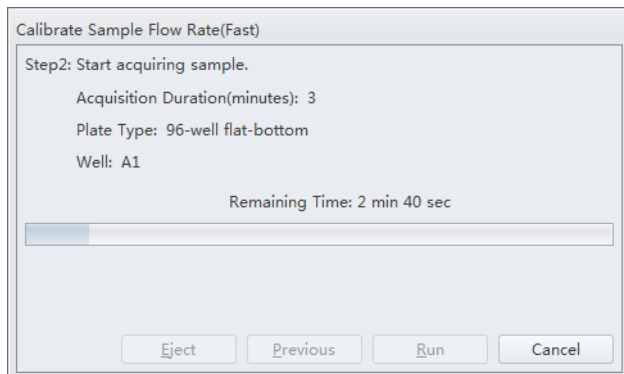
- 8 Select **Next** to proceed to the next step. The plate loader automatically ejects the plate holder stage.



- 9 Place the sample plate onto the plate loader.

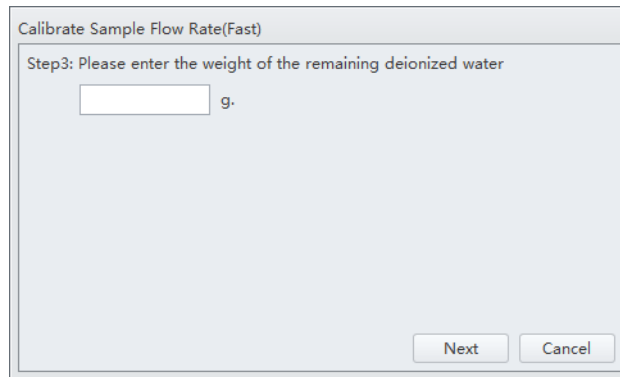
- 10 Verify the settings and select **Load** to load the plate.

- 11 Select **Run**. The message *Please confirm that the correct plate is placed properly and press OK* appears. Select **OK**. The system begins acquiring the sample.

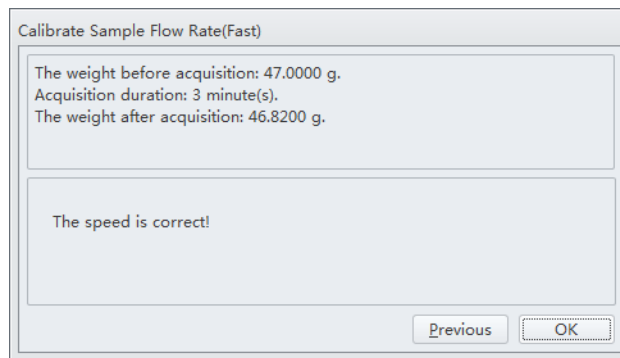


- 12 The plate loader automatically ejects the plate holder stage after the sample is acquired. Weigh the plate.

13 Enter the remaining weight value and select **Next** to confirm the calibration.

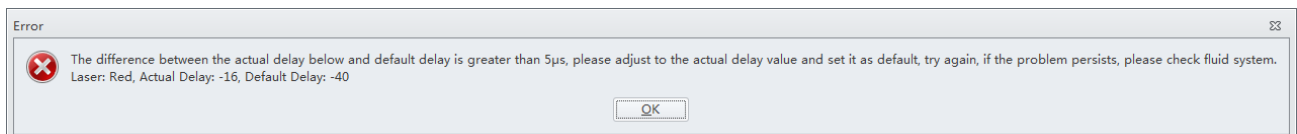


14 Select **OK**.



Setting Laser Delay

Laser delay is preset for QC. Only change the laser delay if the software prompts you that there is a difference in the actual delay and the default delay.



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

Detector	Current(μ s)	Calc(μ s)	Set Calc to Current
Red	-45.12	-	Set
Blue	0.00	-	Set
Violet	38.08	-	Set

Buttons: Set All, Set As Default, Default, Close

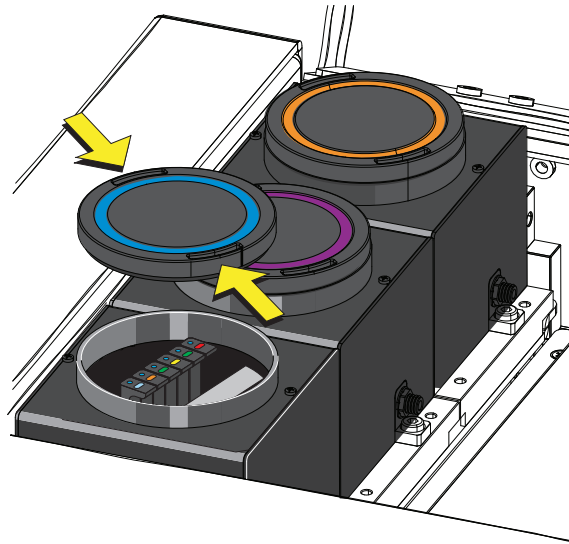
- 2 Set the current to the actual delay for the specified detector stated in the error message received.
- 3 Select **Set as Default**.
- 4 Select **Close**.

Replacing the Optical Filter

When the optical filter is damaged or it is required to use an optical filter with a non-standard wavelength, you must replace the optical filter yourself. For the specific part number of the optical filter, consult your Beckman Coulter Representative or your local dealer.

- 1 Confirm that the instrument is in the standby state or that the instrument is turned off.
- 2 Confirm the laser corresponds to the channel in which the optical filter is to be replaced.
- 3 Open the top cover of the instrument.

- 4 Press the spring piece of the WDM cap corresponding to the laser, and open the WDM cap.



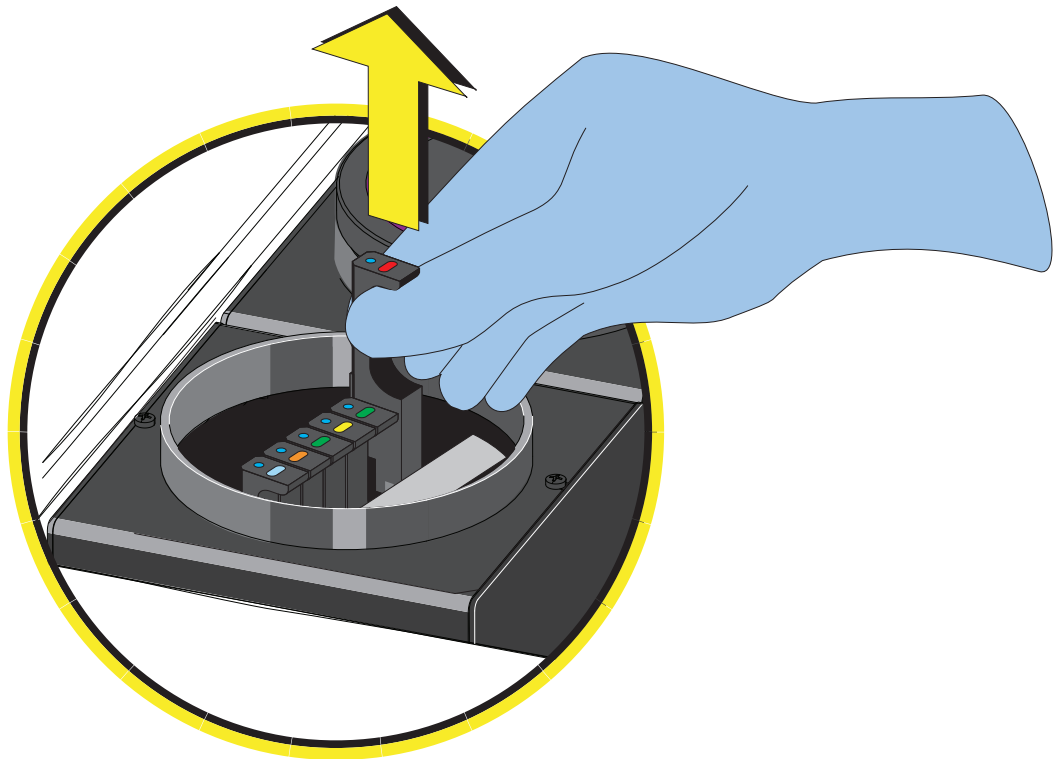
⚠ CAUTION

Risk of damage to the optical filter. Do not touch the optical filter glass piece. Touching the optical filter glass piece can obscure and/or scratch the optical filter glass piece.

⚠ CAUTION

Risk of damage to the optical filter. Pull the filter straight up when removing it from the WDM. Removing the filter at an angle could chip the edge of the filter glass.

- 5 Use vertical force to remove the optical filter to be replaced, and note the color identification and wavelength identification on the optical filter bracket.



⚠ CAUTION

Risk of damage to the optical filter. Push the filter straight down when inserting it into the WDM. Inserting the filter at an angle could chip the edge of the filter glass.

- 6 Insert the optical filter to be installed vertically into the corresponding position, taking care to align the wavelength identification with the left, and that the bracket is inserted into the bottom.

- 7 Close the WDM cap and the instrument top cover.
- 8 Turn on the Cytometer and open the software.
- 9 Select **Detector Configuration** in the Cytometer menu and create a new instrument configuration based on the settings of the new optical filter. Refer to [Verifying, Selecting, Editing, and Creating Detector Configuration](#) in CHAPTER 5, *Data Acquisition and Sample Analysis*.
 Set this new configuration as the current configuration.

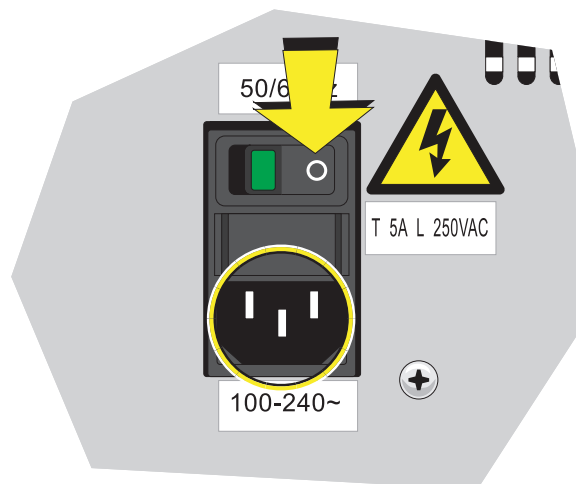
Replacing the Fuse

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

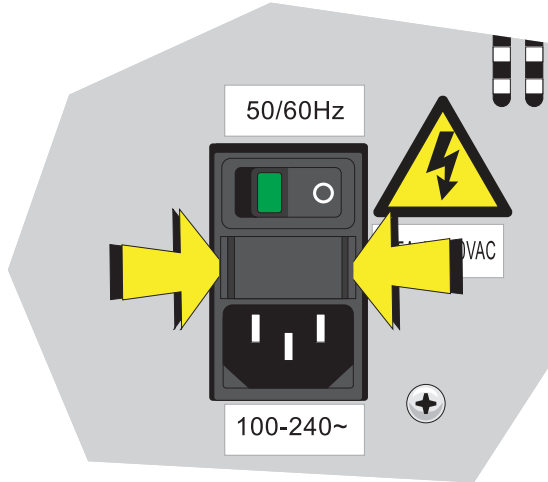


Risk of personal injury. A shock hazard exists if the power cable is connected. Turn off the Cytometer and disconnect the primary power cable before performing these procedures.

- 1 Turn off the Cytometer, and disconnect the power cable.



- 2 Press both sides of the fuse holder of the instrument inwards using a flat head screwdriver, and pull out the fuse holder.



IMPORTANT Select well-performing products that comply with the specifications required, to ensure that the instrument can function normally and safely.

- 3 Check whether the fuse installed is blown, and replace the blown fuse with a new one.
The specifications of the fuse required are: T 5 AL 250 VAC, delay blow fuse, 5A, 250 VAC, 5 x 20 mm. Beckman Coulter recommends using SCHURTER 0034.3124.



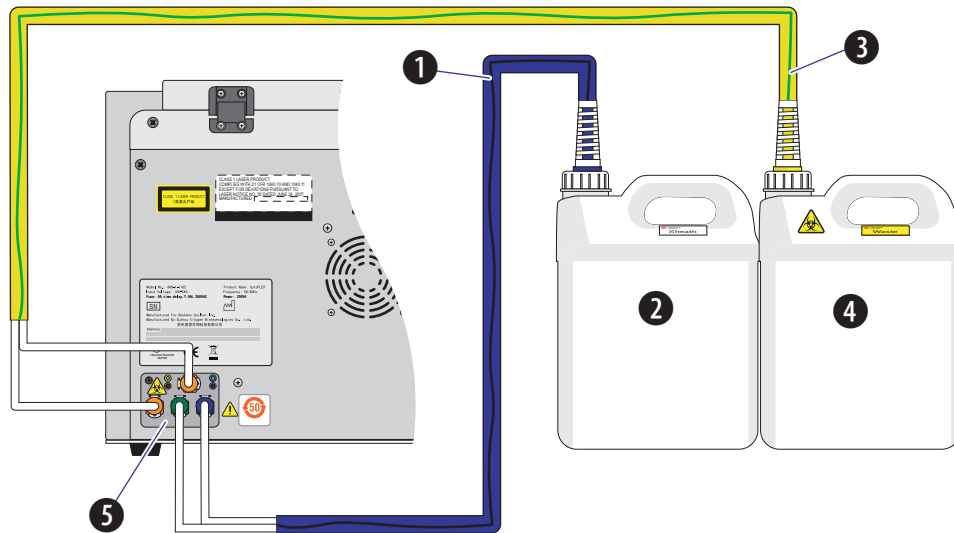
- 4 Insert the fuse holder.

-
- 5 Reconnect the power cable.
-

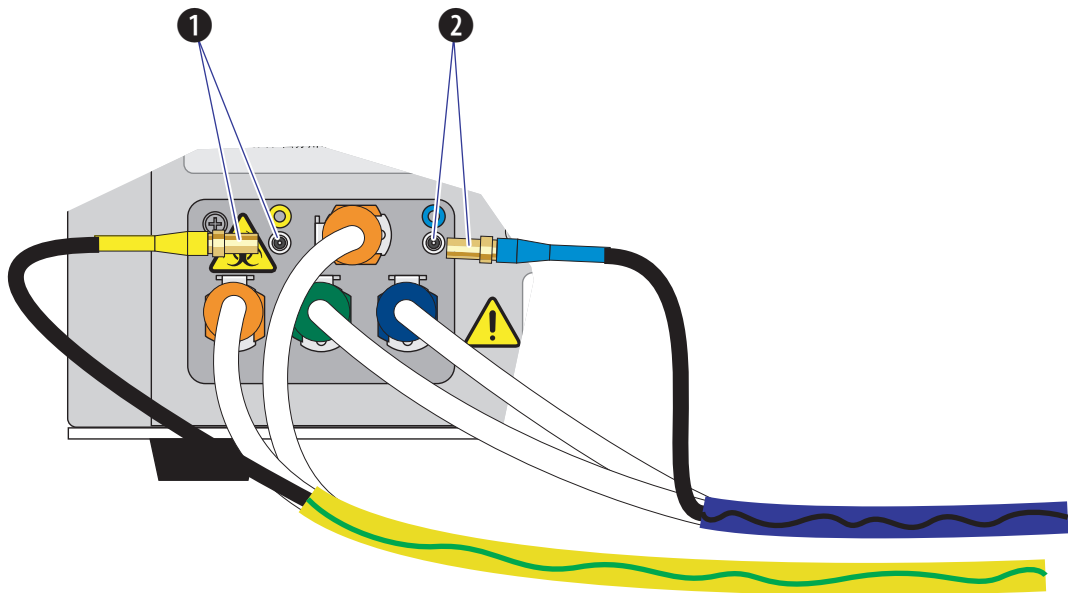
Replacing the Sheath Fluid Harness and/or Waste Harness

Replace the sheath fluid harness and/or the waste harness if you have a faulty sheath fluid sensor and/or waste sensor.

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



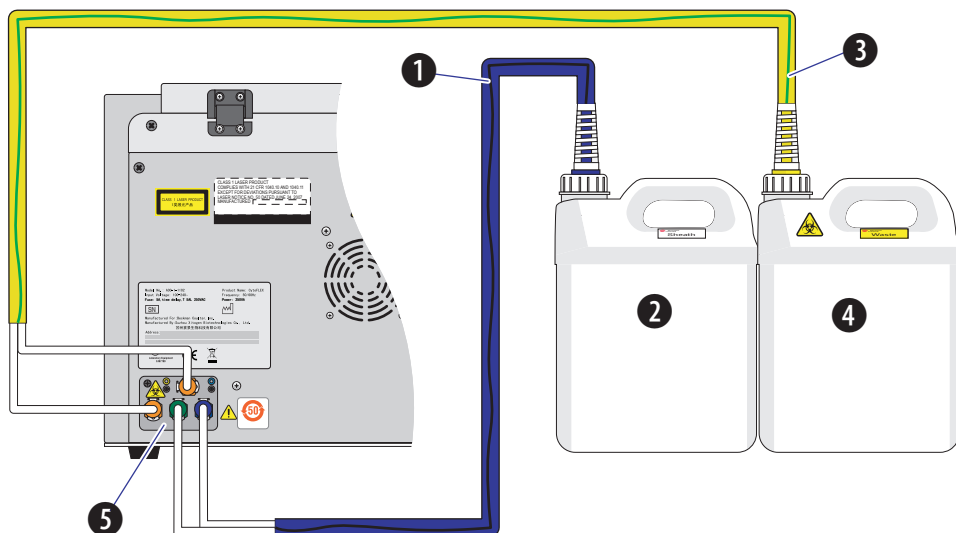
Disconnect the waste (1) and/or sheath (2) level sensors.



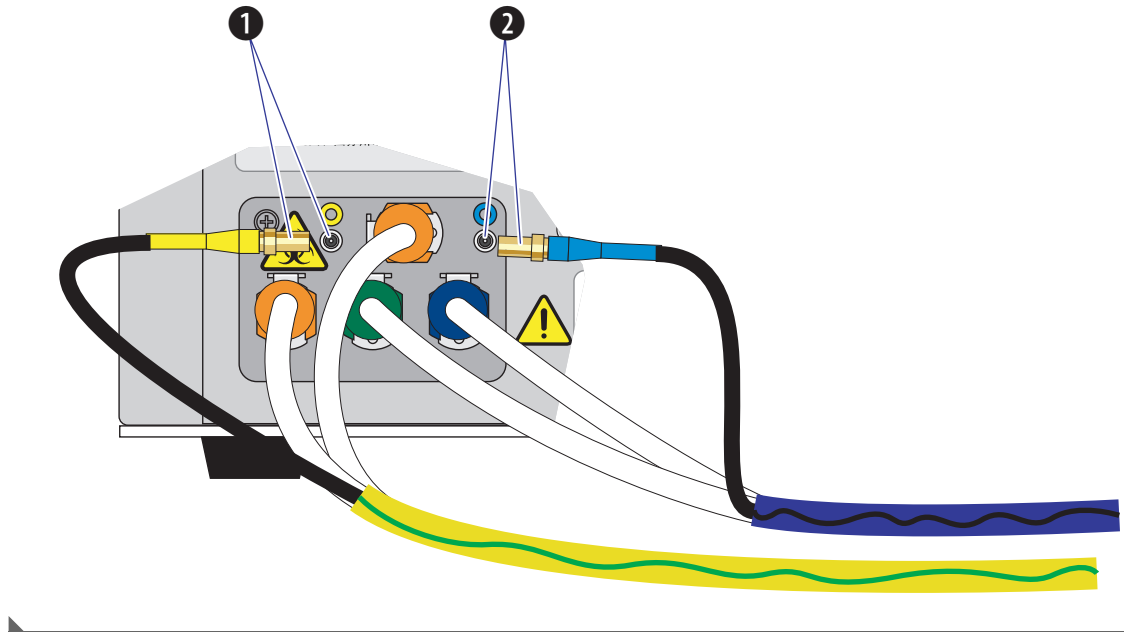
4 Dispose of the sheath fluid harness and/or the waste harness according to your laboratory procedures.

5 Insert the new sheath and/or waste pickup tubing into the appropriate container.

6 Connect the blue harness (1) from the sheath fluid container (2) and/or the yellow harness (3) from the waste container (4) to the fluid connector panel (5) on the back right corner of the instrument according to the color code.



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



Changing Sample Mixing and Backflush Settings



! WARNING

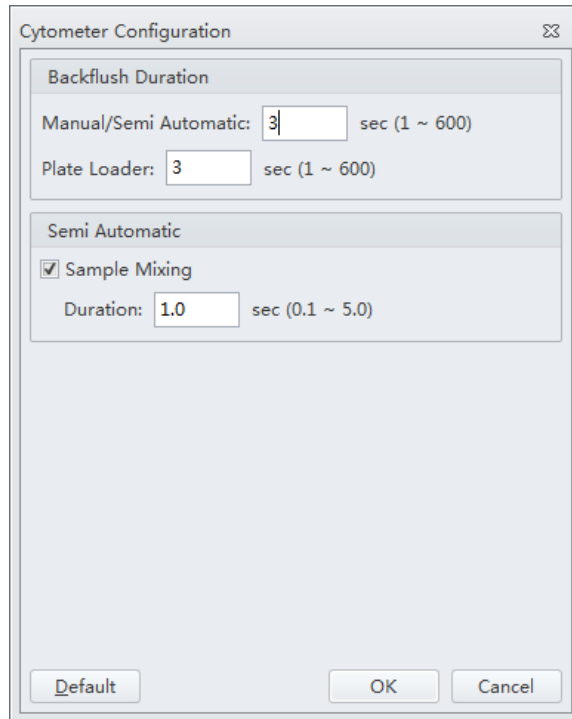
Risk of biohazardous contamination. Enabling sample mixing for 1.5-mL and 2-mL sample tubes in the semi-automatic sample injection mode can result in sample splashing. Exceeding 300- μ L sample volume when using 1.5-mL and/or 2-mL sample tubes can also result in sample splashing. Disable sample mixing in the semi-automatic sample injection mode when using 1.5-mL and 2-mL sample tubes and do not exceed 300- μ L sample volume.

The sample mixer can be enabled or disabled if necessary. The sample mixing duration can also be increased or decreased if necessary.

Whenever a sample is likely to leave residue or cause contamination, the backflush time can be increased to reduce cross contamination.

- 1 Open the CytExpert software and confirm that the instrument is connected. Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#).

- 2 Select **Cytometer Configuration** in the Cytometer menu. The Cytometer Configuration window appears.



- 3 Select the Sample Mixing checkbox to enable sample mixing.
Or
Deselect the Sample Mixing checkbox to disable sample mixing.

- 4 Change the sample mixing duration to the desired time.

NOTE The default setting is 1 second. Select **Default** to set the Cytometer configuration settings back to the factory default settings.

- 5 Change the backflush duration to the desired time for either the Manual/Semi-Automatic sample injection mode or the Plate Loader sample injection mode depending on the current sample injection mode selected.

NOTE The default setting is 3 seconds without the Sample Injection Mode Control Kit installed or 4 seconds [**CytoFLEX and CytoFLEX S**] or 6 seconds [**CytoFLEX LX**] with the Sample Injection Mode Control Kit installed. Select **Default** to set the Cytometer configuration settings back to the factory default settings.

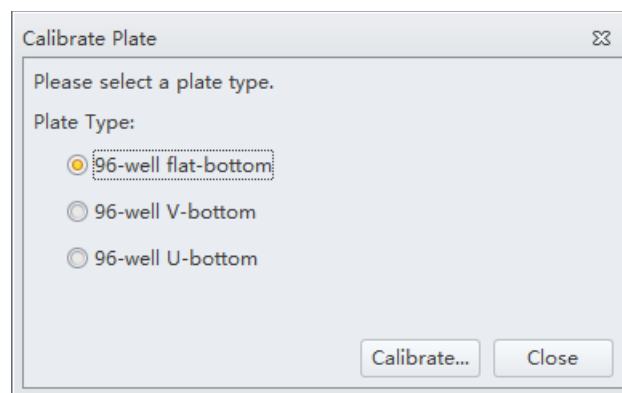
-
- 6 Select **OK**.
-

Calibrating the Plate Position [With Plate Loader]

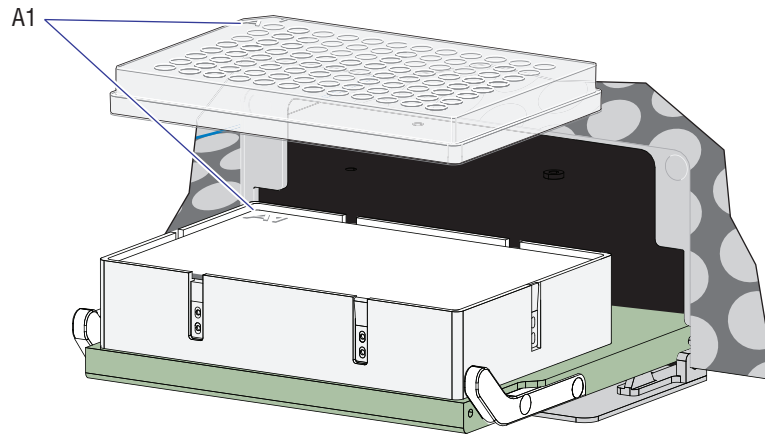
Use the following procedure to calibrate the plate position and the sample probe position:

- Upon installation
- After a new plate type is defined for first use
- After changing plate manufacturers of the same previously calibrated plate type
- When optimizing plate performance

-
- 1 Remove the front cover. Refer to [Front Cover Removal and Reinstallation](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).
 - 2 Select **Calibrate Plate** in the Advanced menu. The plate loader automatically ejects the plate holder stage and the Calibrate Plate window appears.

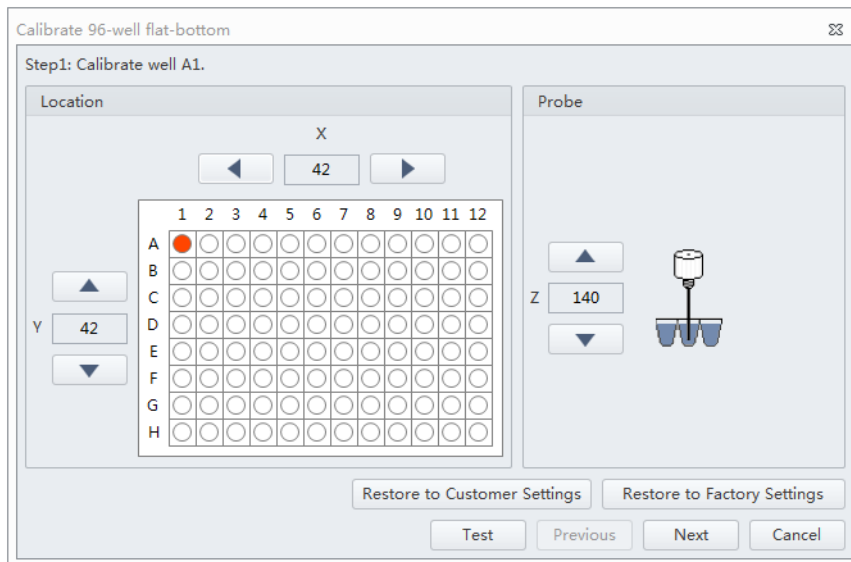


- 3 Select the plate type and place the plate on the plate holder.



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

- 4 Select **Calibrate**. The message *Are you sure the plate is loaded?* appears. Select **OK**. The plate loader loads the plate holder stage and the sample probe moves to the sample aspiration position of well A1.



CAUTION

Risk of instrument damage. Do not crash the probe into the bottom of the plate as this will cause irreparable damage to the probe. Move the probe one step at a time when lowering the probe on the Z-axis toward the bottom of the plate. You will hear a click when the probe makes contact with the bottom of the well, this is the correct position for the Z-axis of the probe. Do not move the probe any lower after you hear the click.

- 5 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.

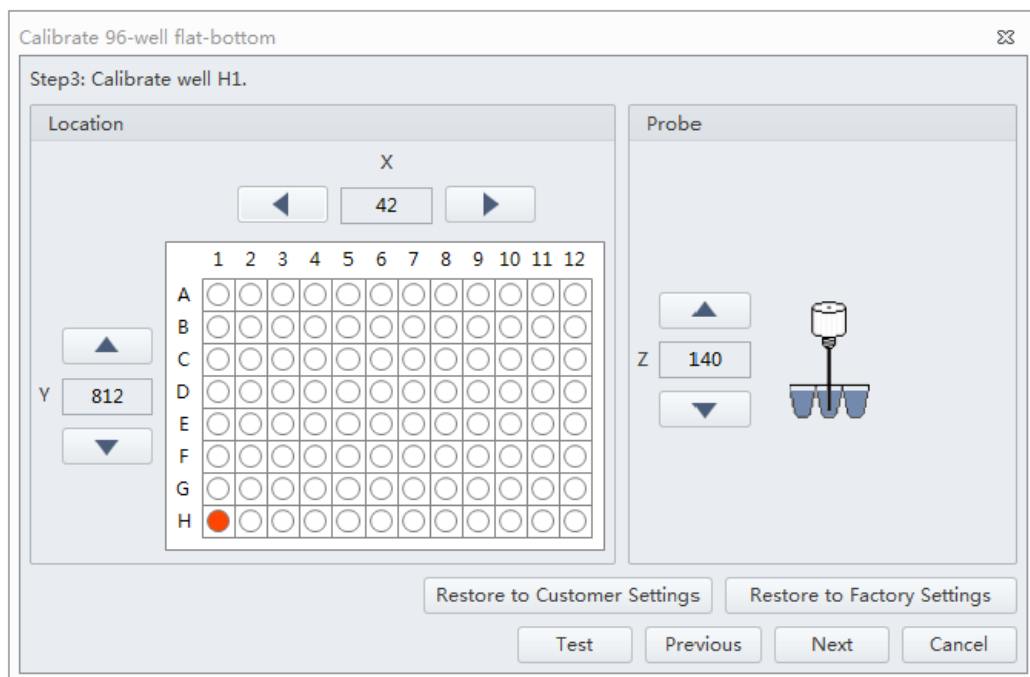
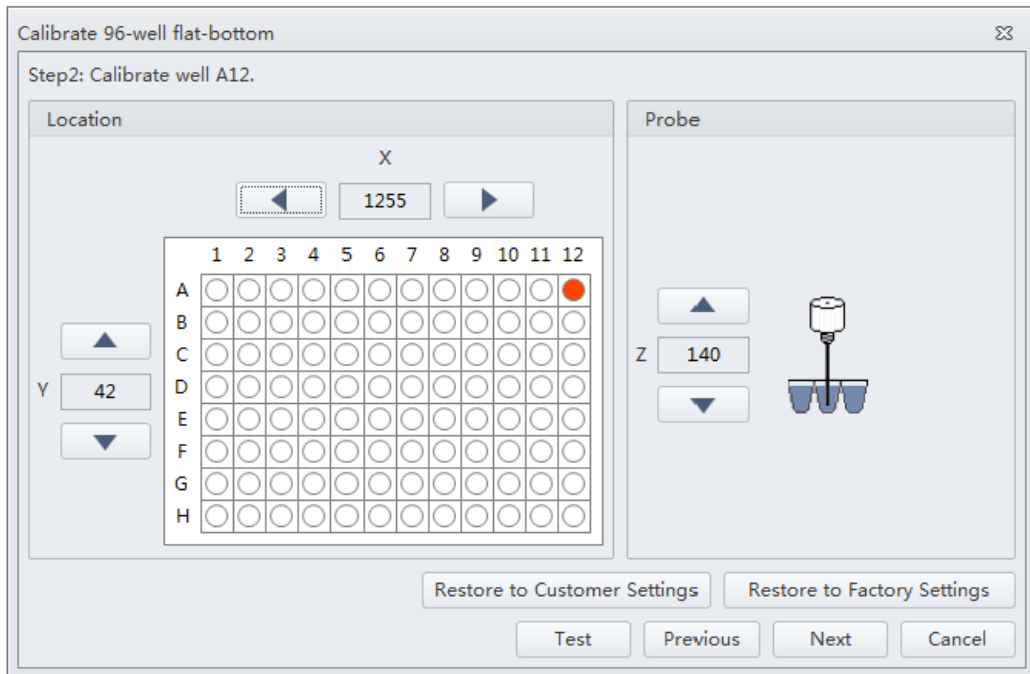
NOTE The sample probe should just make contact with the bottom of the well.

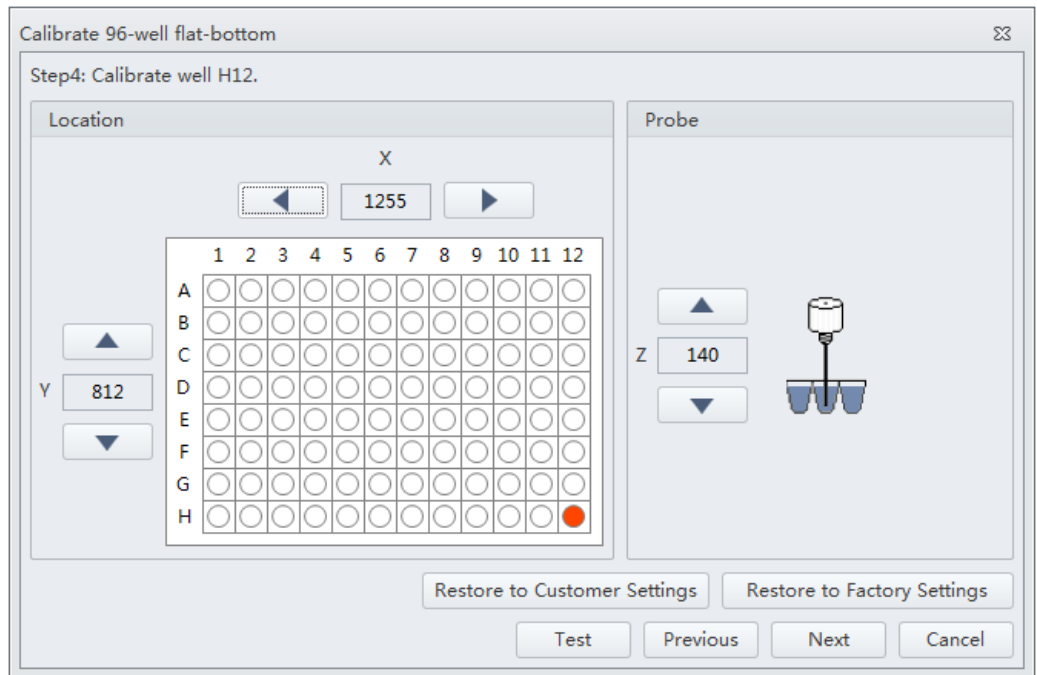
NOTE The X-axis arrows moves the sample probe well position left and right. The Y-axis arrows moves the sample probe well position forward and back. The Z-axis arrows moves the sample probe up and down.

- 6 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 if necessary.

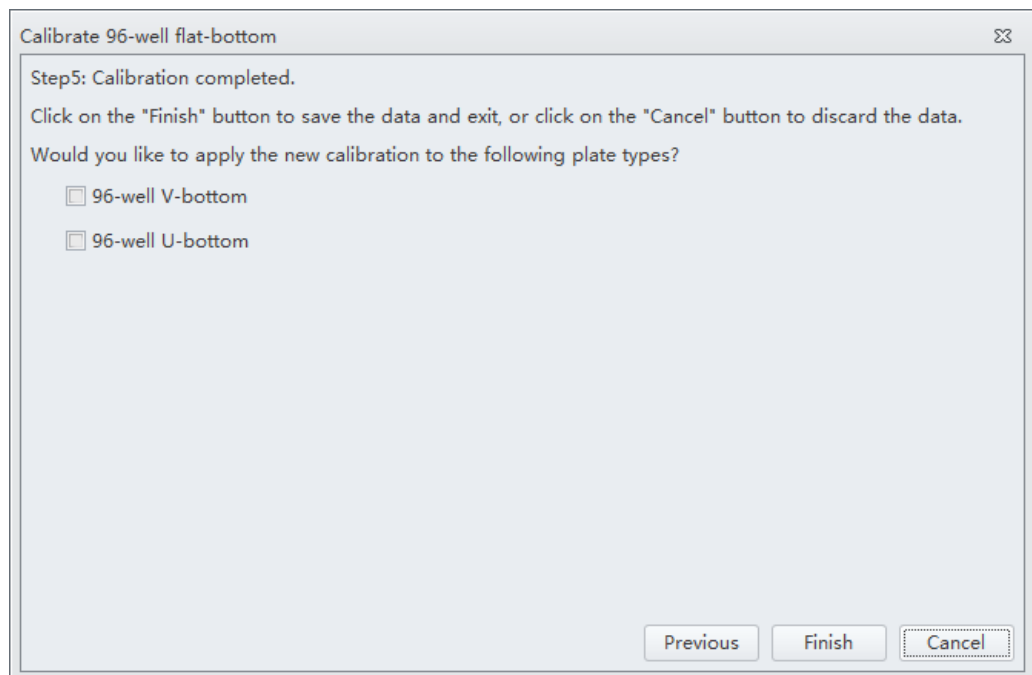
7 Select **Next** to move to the next well.

8 Repeat Steps 5-7 for wells A12, H1, and H12.





- 9 Select additional plate types to be calibrated on the Calibration completed window, if necessary. Place the corresponding plate on the plate holder and repeat Steps 5-8.



- 10 Select **Finish** to save the settings.

11 Select **Close** to quit the program.

12 Reinstall the front cover. Refer to [Front Cover Removal and Reinstallation](#) in **CHAPTER 11, Replacement/Adjustment Procedures**.

Overview

[CytoFLEX]: Your instrument may have been shipped directly to your laboratory, in which case you will need to set up and connect the Cytometer and the Workstation. Refer to this chapter for the instrument installation procedure.

Validate the environment	→	Open the package	→	Install the instrument	→	Prepare to boot
--------------------------	---	------------------	---	------------------------	---	-----------------

[CytoFLEX LX]: The CytoFLEX LX is installed by your Beckman Coulter Service Representative. Do not open the box or crate. Wait for a qualified Beckman Coulter Service Representative.

This chapter contains information on:

- [Instrument Transportation and Storage](#)
- [Installation Environment Validation](#)
- [Unpacking the Instrument and Inspecting the Materials for Defects or Omissions \[CytoFLEX\]](#)
- [CytExpert Software Installation Options](#)
- [Installing the Software \[CytoFLEX\]](#)
- [Upgrading the CytExpert Software](#)
- [Reinstalling the CytExpert Software](#)

Instrument Transportation and Storage

Refer to [Preparing the Instrument for Transport or Storage](#) in [CHAPTER 10, Cleaning Procedures](#), prior to transportation or storage.

Attention to the following items is required when transporting or storing the instrument:

- Take caution to protect the instrument from exposure to rain or sunlight.
- Always place the instrument on a flat, stable surface, and take note of the symbol for *this side up*.
- Temperature range: see [Temperature and Humidity](#).
- To prevent extrusion, the load on top cannot exceed 100 kg.
- CytoFLEX Cytometer net weight 23 kg, gross weight 27 kg; transport the instrument using only appropriate equipment so as to guard against personal injury.
- CytoFLEX LX Cytometer net weight 23 kg, gross weight 27 kg; transport the instrument using only appropriate equipment so as to guard against personal injury.

Installation Environment Validation

IMPORTANT This instrument is intended for indoor use only.

Verify whether the installation environment satisfies the following requirements:

Worktable



Risk of instrument damage. Place the instrument on a level surface. Failing to do so places the system in danger of toppling and can result in damage. Take all necessary precautions throughout the process of storing or transporting the instrument.

- The tabletop must be smooth and level.
- Minimum tabletop load bearing capacity [CytoFLEX]: 50 kg
- Minimum tabletop load bearing capacity [CytoFLEX LX]: 100 kg.
- The tabletop must not vibrate or shake.
- Minimum tabletop dimensions [CytoFLEX]: 120 cm x 80 cm; minimum vertical space above tabletop: 80 cm
- Minimum tabletop dimensions [CytoFLEX LX]: 200 cm x 80 cm; minimum vertical space above tabletop: 100 cm
- Position the instrument in such a manner that it will facilitate disconnection of the power cable at the instrument end.

Ventilation and Cleaning

IMPORTANT If necessary, use ventilation equipment, but airflow must not be allowed to blow directly on the system, as it can affect the reliability of the data.

- Ensure that the working environment is well ventilated for proper heat dissipation.
- Maintain a clearance of at least 20 cm from the back of the instrument for heat dissipation.
- Keep the environment as dust free as possible.
- Avoid direct exposure to sunlight.
- Avoid placing near heat sources or exposing to drafts.
- Avoid corrosives or flammable gases.

Power Source



DANGER

Risk of electric shock and/or instrument damage. Ensure that the power source is properly grounded. Improper grounding can cause electric shock and damage the system. Verify that the output voltage of the power outlet conforms to the system requirements and that a 5 A, time delay, T 5 AL 250 VAC fuse is installed. To prevent personal injury, Beckman Coulter recommends using a power source designed to protect against electrical shock.



CAUTION

Possible instrument damage could occur if you use an extension cord or a power strip to connect the Cytometer. Always plug the Cytometer into a dedicated outlet with an isolated ground.

The power source requirements are as follows:

- This instrument has been tested to and meets all applicable requirements for CE Marking.
- This instrument complies with the emission and immunity requirements described in IEC 61326-1 and 61326-2-6 series.
- 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 $\leq \pm 2^\circ\text{C}$ per hour.
- **CytoFLEX LX:** Ambient temperature: 15-30°C with fluctuations of no more than $\leq \pm 2^\circ\text{C}$ per hour.
- Relative humidity: 15% RH-80% RH, non-condensing.

Waste Disposal



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

The waste line from the Cytometer is connected to a waste container/cubitainer. Dispose of the system's waste in accordance with your local regulations and acceptable laboratory procedures.

The waste line supplied with the instrument can be connected to an open drain. If you use an open drain, mechanically secure the waste tube into the drain so the tube cannot accidentally come out of the drain. This prevents spillage.

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

Take care to store the instrument in a suitable environment where it can remain in the proper position.

Check that the following components on the packing list are present:

- Cytometer
- Cables
- Computer

- Mouse
- Keyboard
- Monitor
- Fluid Container holder [CytoFLEX]
- Sheath fluid container
- Waste container
- Sheath fluid tubing
- Waste tubing
- USB configuration key
- Software USB

Installing the Instrument and Connecting the Equipment [CytoFLEX]

IMPORTANT Use the appropriate power cable plug for your geographic region.



Risk of erroneous results. Place the Fluid Containers and the instrument on the same, level surface. An excessive difference in height can alter the flow velocity.



Possible instrument damage could occur if you use an extension cord or a power strip to connect the Cytometer. Always plug the Cytometer into a dedicated outlet with an isolated ground.

-
- 1 Remove the Cytometer, the Fluid Containers, the accompanying holder, the computer, the monitor, and the keyboard and mouse from the each respective box placing them flat on the instrument worktable.

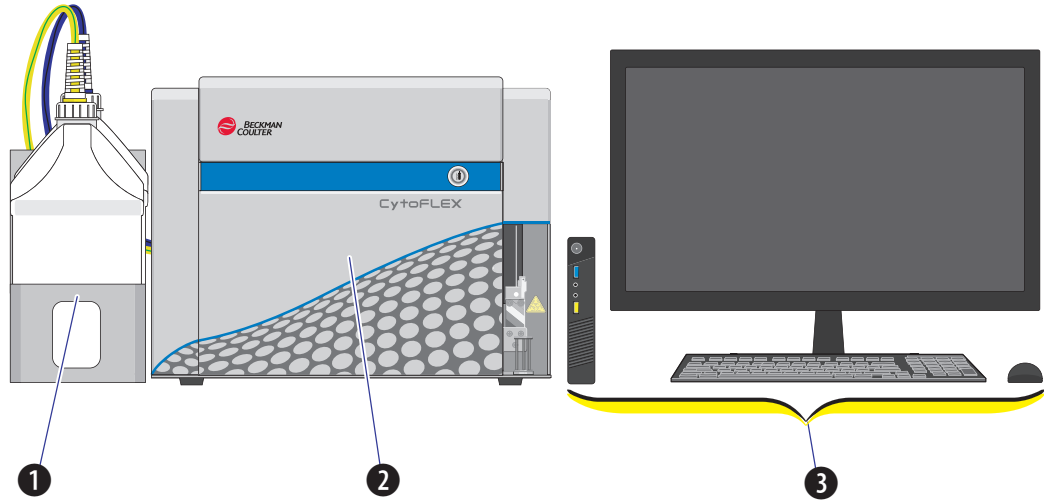
NOTE The Fluid Container holder must be on the same plane as the Cytometer.

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

-
- 2 Ensure a minimum clearance of 20 cm on both sides and to the back of the Cytometer to maintain enough room to access the on/off controls for the Cytometer devices.

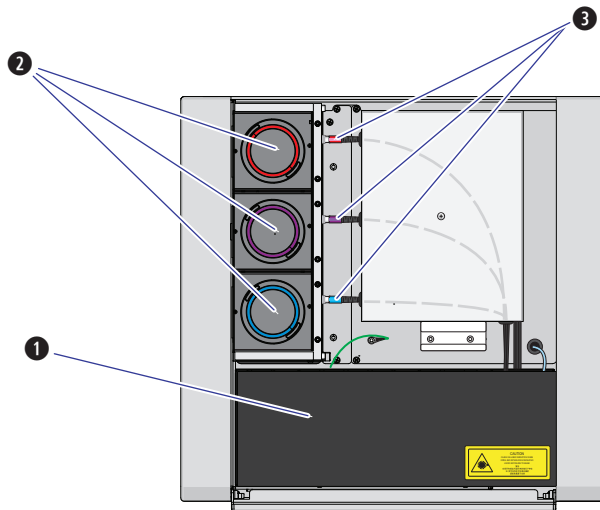
- 3** After removing the monitor and attaching the base, place them on the worktable with the computer.

[CytoFLEX Without Plate Loader Shown]

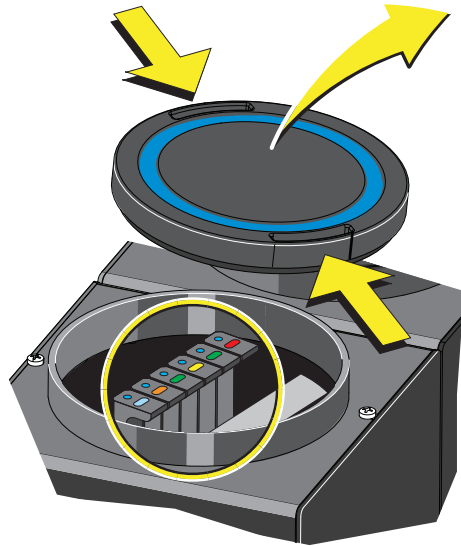


- 1. Fluid Containers.** Place on the left side of the Cytometer.
- 2. Cytometer.** Place between the Fluid Containers and the Workstation.
- 3. Workstation.** Place on the right side of the Cytometer.

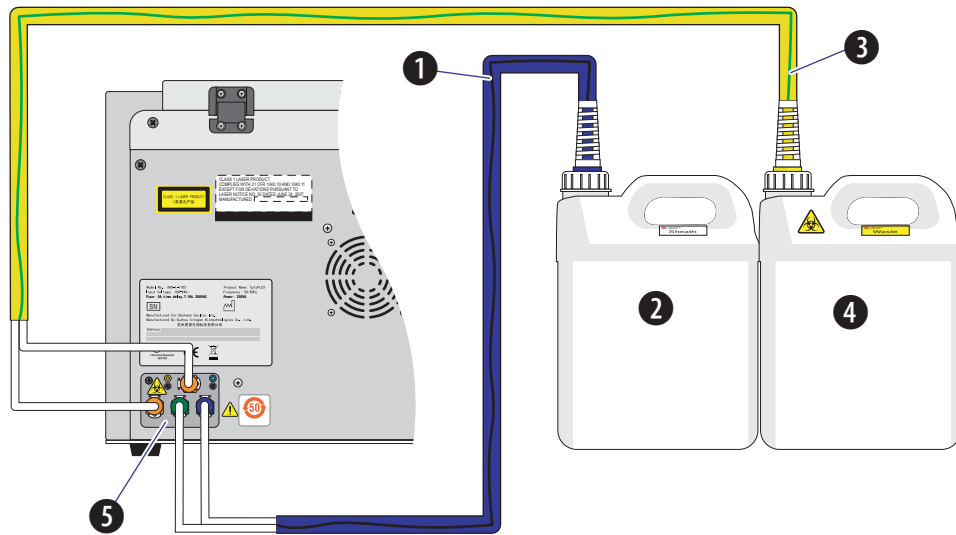
- 4** Open the top cover of the Cytometer. Check inside to verify that the optical bench cover (1) is tightly closed and that the optical fibers (3) and WDMs (2) are securely connected.



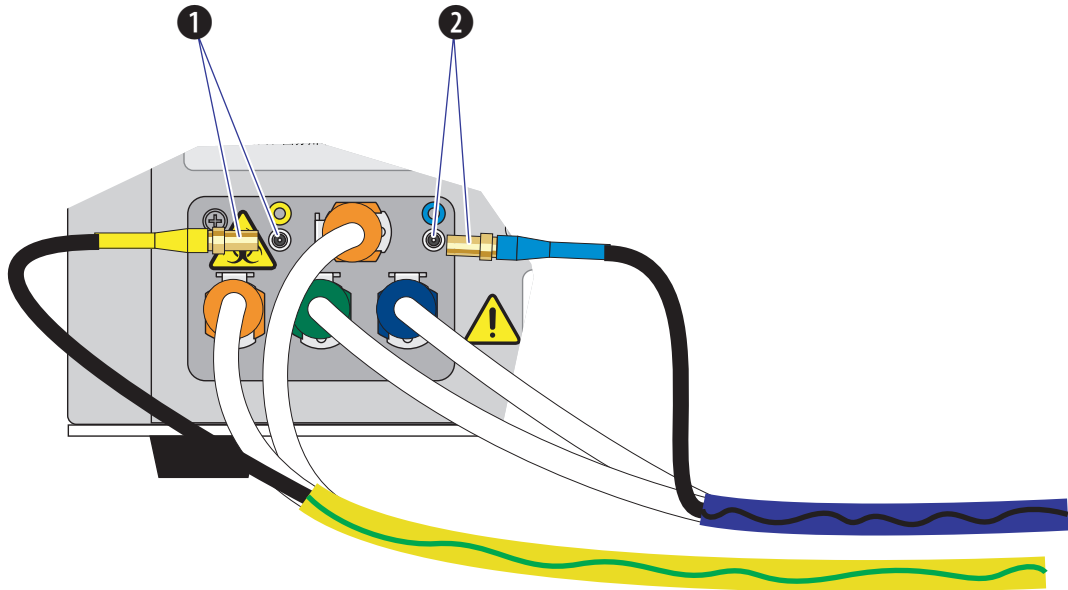
- Remove any shipping tape and open the cap of each WDM. Verify that the light filters inside are in place.



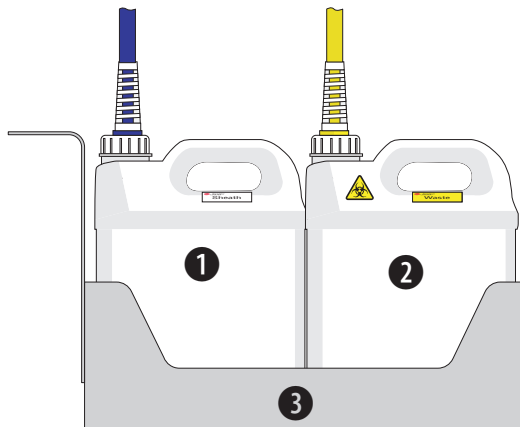
- Insert sheath and waste pickup tubing into the appropriate container.
- Connect the blue harness (1) from the sheath fluid container (2) and the yellow harness (3) from the waste container (4) to the fluid connector panel (5) on the back right corner of the Cytometer according to the color code.



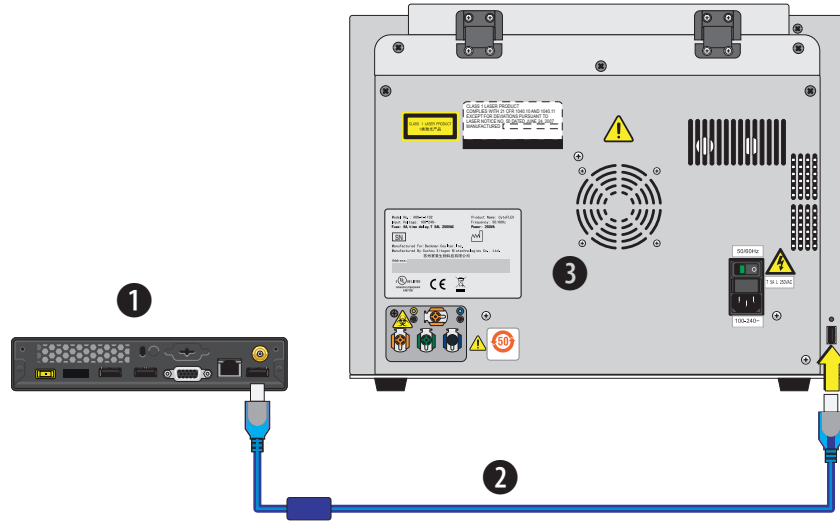
-
- 8** Connect the waste (1) and sheath (2) level sensors.



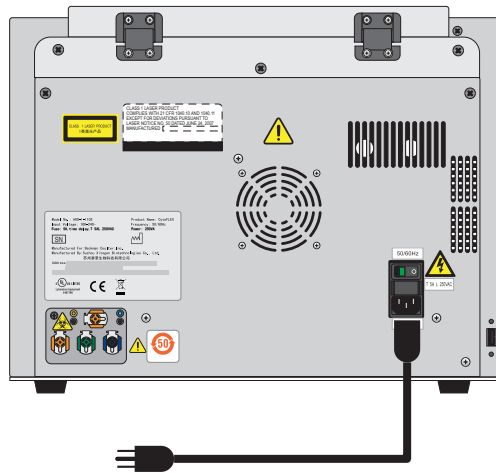
-
- 9** Place the sheath fluid container (1) and the waste container (2) in the Fluid Container holder (3).



- 10** Set up the supplied computer (1) and connect the USB cable (2) from the back of the Cytometer (3) to a USB port on the back of the computer.



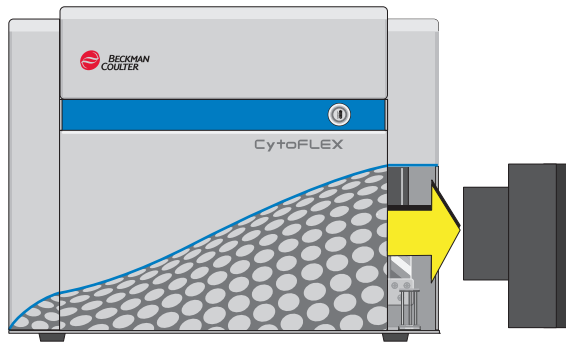
- 11** Plug the Cytometer power cable in to the back of the Cytometer.



- 12** Connect the computer keyboard, mouse, and monitor.

-
- 13** Remove the foam support holding the sample loader in the front-right corner of the instrument.

[CytoFLEX Without Plate Loader Shown]



-
- 14** Add Deep Clean solution to the Deep Clean solution bottle. Refer to [Adding the Deep Clean Solution](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

-
- 15** Clean the sheath fluid container. Refer to [Cleaning the 4 L Sheath Fluid Container](#) in [CHAPTER 10, Cleaning Procedures](#).

-
- 16** Fill the Sheath fluid container. Refer to [Filling the 4 L Sheath Fluid Container \[CytoFLEX\]](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

⚠ WARNING

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

-
- 17** Add 400 mL of 5 to 6% bleach to the waste container.

-
- 18** Install the CytExpert software. Refer to [Installing the Software \[CytoFLEX\]](#).

-
- 19** Turn on the instrument. Refer to [Turning On the Instrument](#) in [CHAPTER 3, Daily Startup](#).

-
- 20** Open the CytExpert software. Refer to [Logging Into the Software](#) in [CHAPTER 3, Daily Startup](#).

-
- 21** Run the System Startup Program. Refer to [Running the System Startup Program with the Single Tube Loader](#) in [CHAPTER 3, Daily Startup](#).

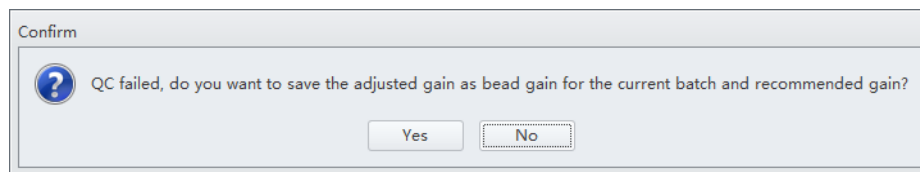
22 Prime the instrument three times.

23 Prepare a QC sample. Refer to [Preparing the QC Sample](#) in [CHAPTER 4, Instrument Quality Control and Standardization](#).

24 Import the lot-specific target values files. Refer to [Importing Lot-Specific Target Values](#) in [CHAPTER 4, Instrument Quality Control and Standardization](#).

25 Perform a QC to establish the target gain values for your instrument. Refer to [Collecting QC Data](#) in [CHAPTER 4, Instrument Quality Control and Standardization](#).

26 If QC fails, the following software message appears. Select **Yes**.



NOTE Target gain values must be established upon installation. QC could fail up to three times upon installation until target gain values are established.

27 Repeat Steps [23-26](#) until the target gain values are established and QC has passed.

NOTE If QC fails more than three times, [contact us](#).

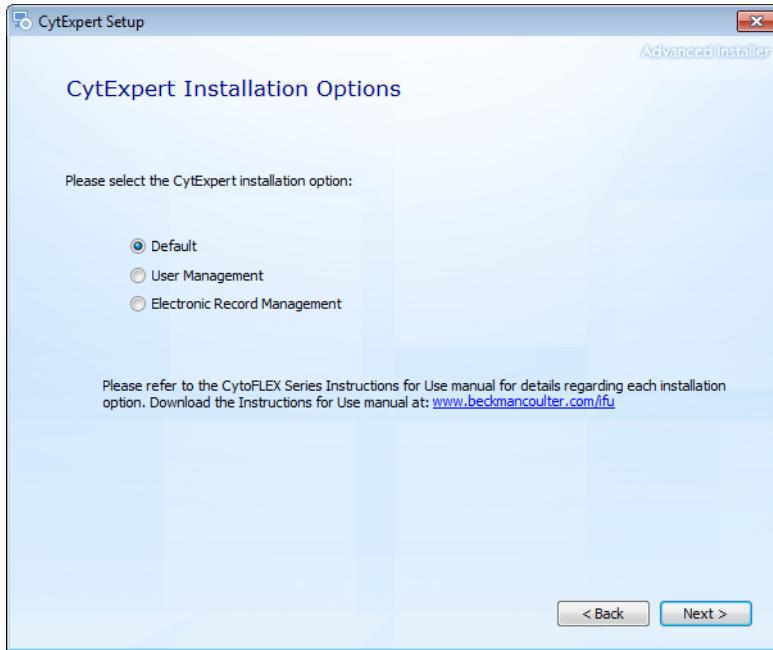
28 Within five business days, activate your Warranty by [contacting us](#) and providing them with your latest QC run results.

Installing the Instrument and Connecting the Equipment [CytoFLEX LX]

The CytoFLEX LX is installed by Beckman Coulter service.

CytExpert Software Installation Options

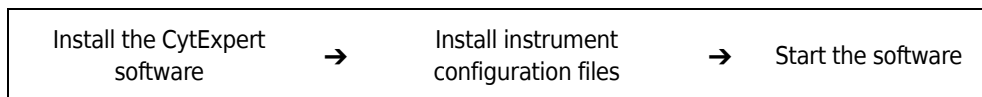
CytExpert software version 2.0 and higher has three installation options upon install.



- **CytExpert Default software option.** User Login is not required to run the system.
- **CytExpert User Management software option.** User Login is required to run the system. Contains features and functionality that facilitates user and role management.
- **CytExpert Electronic Record Management software option.** User Login is required to run the system. Contains features and functionality that facilitates compliance with 21 CFR Part 11 guidelines for Electronic Records and Signatures.

Installing the Software [CytoFLEX]

The installation process workflow is as follows:



The CytExpert software can be installed on any computer that meets the minimum specifications (see [Instrument Specifications](#) in [CHAPTER 1, System Overview](#)) for analysis-only use.

Required Materials

The following materials are required to install the CytExpert software:

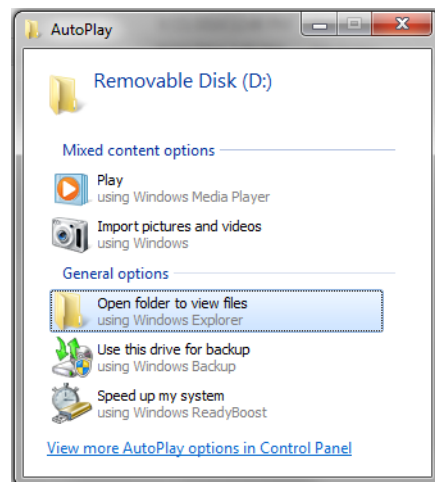
- CytoFLEX Series flow cytometer.
- CytoFLEX Series Workstation.
- CytExpert software installation USB.
- Authorized Beckman Coulter USB configuration key.

Installing the CytExpert Software

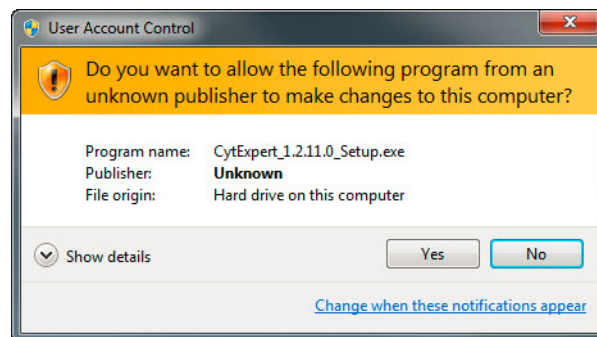
IMPORTANT Follow this procedure when installing the CytExpert software for the first time.

- 1 Insert the software USB into the computer.

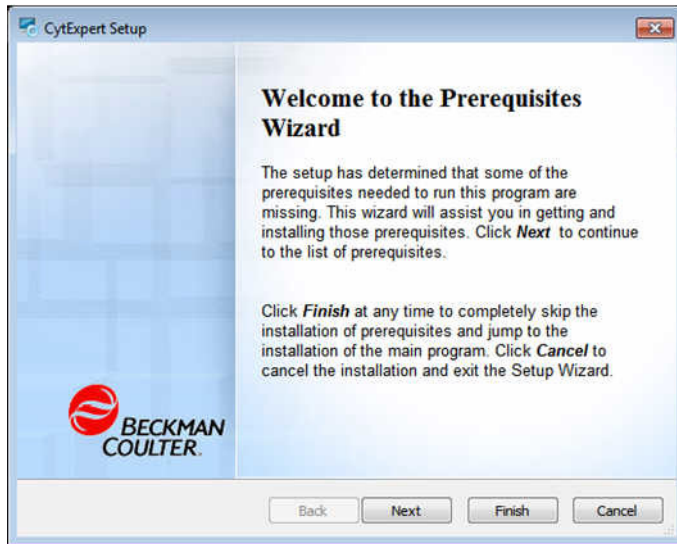
NOTE If the Autoplay window appears, select Open folder to view files.



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

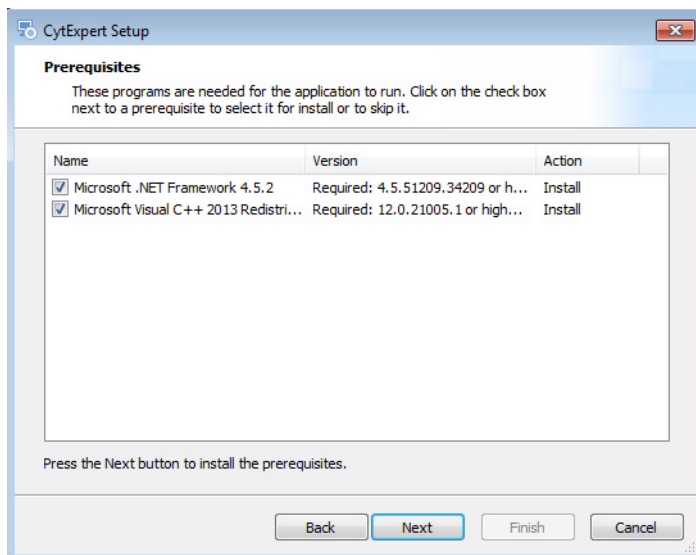


- 3 Select **Yes**. The CytExpert Setup Welcome window appears.

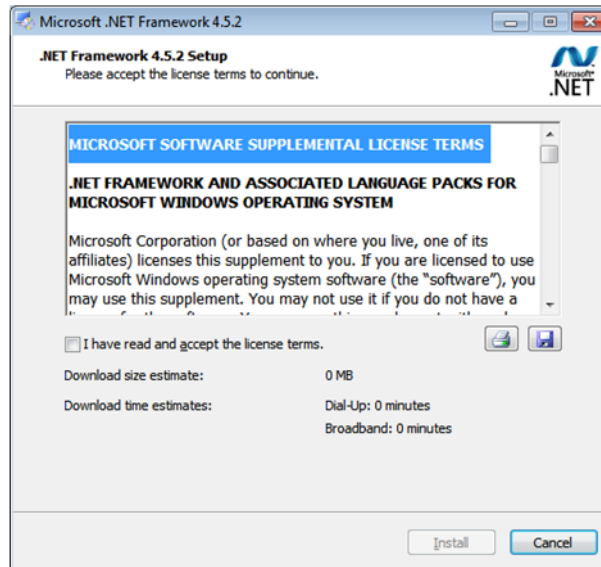


- 4 Select **Next**.

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

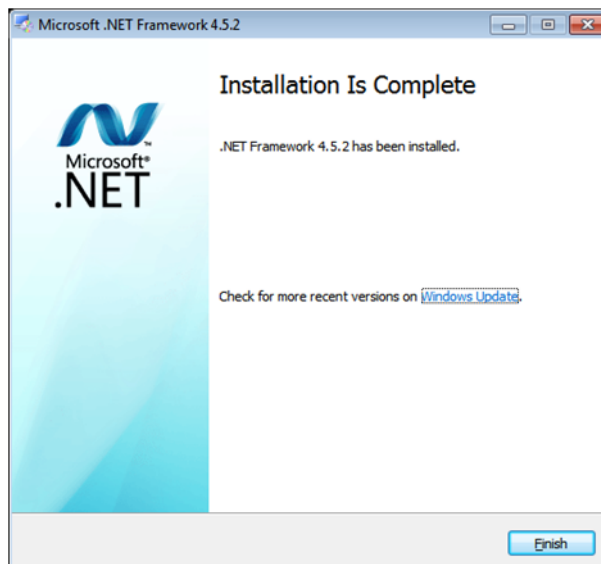


- 6 Select **Next**. The Microsoft .NET Framework window appears.

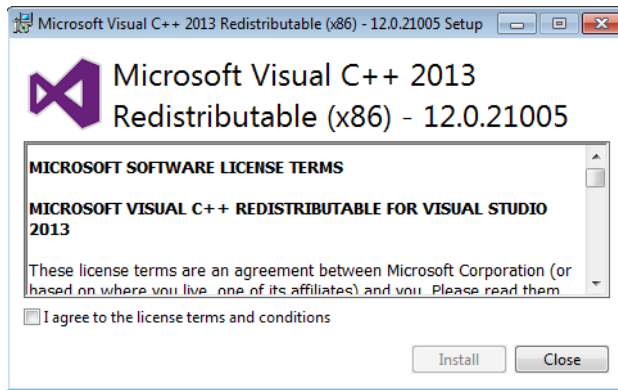


- 7 Select the *I have read and accept the license terms* checkbox.

- 8 Select **Install**. The Installation is Complete window appears when installation has finished.

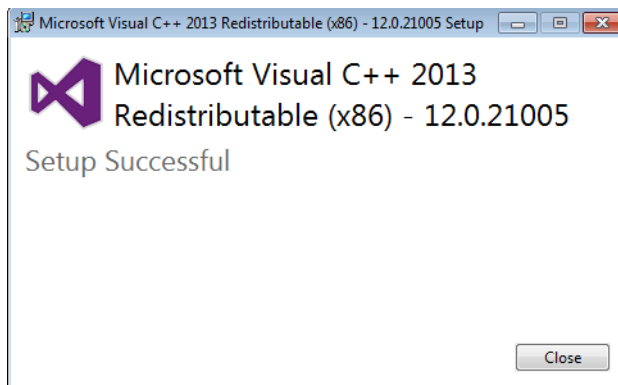


-
- 9 Select **Finish**. The Microsoft Visual C++ 2010 Redistributable Setup window appears.

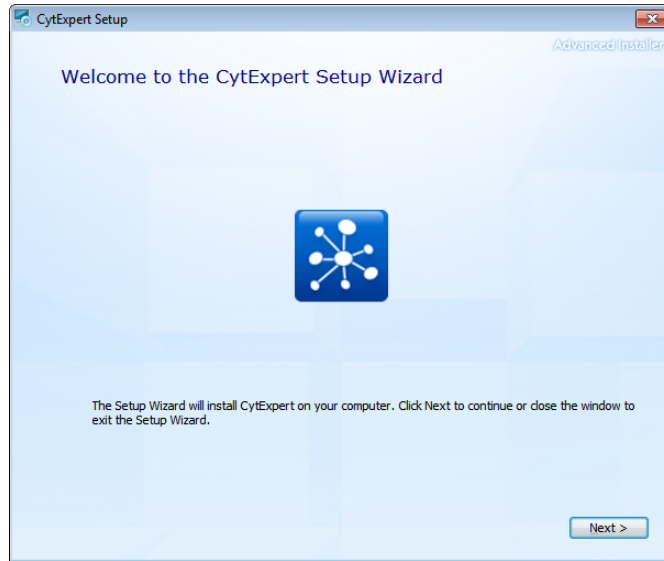


-
- 10 Select the *I have read and accept the license terms* checkbox.

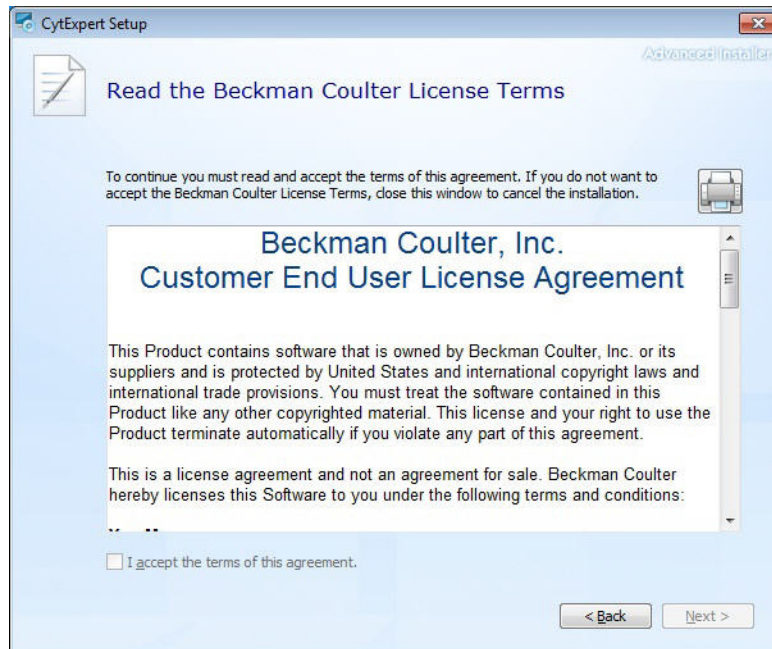
-
- 11 Select **Install**. The Setup Successful window appears when installation has finished.



12 Select **Close**. The Welcome to the CytExpert Setup Wizard window appears.



13 Select **Next**. The Beckman Coulter License Terms window appears.

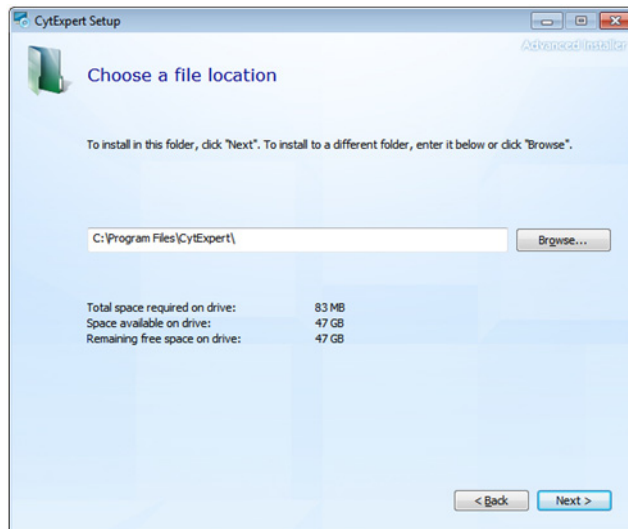


14 Read the Beckman Coulter Customer End User License Agreement.

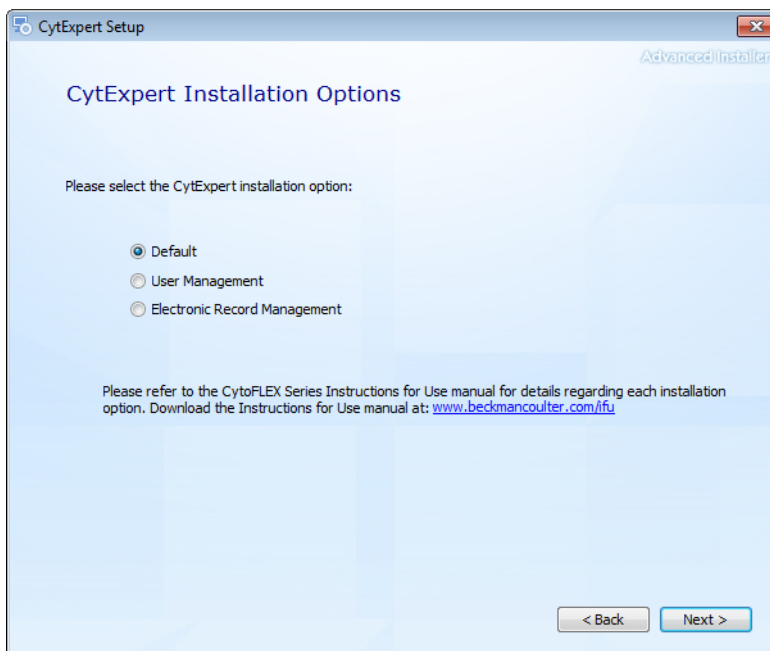
15 Select the *I accept the terms of this agreement* checkbox.

NOTE The checkbox is not selectable until you scroll all the way to the end of the agreement.

16 Select **Next**. The Choose a file location window appears.

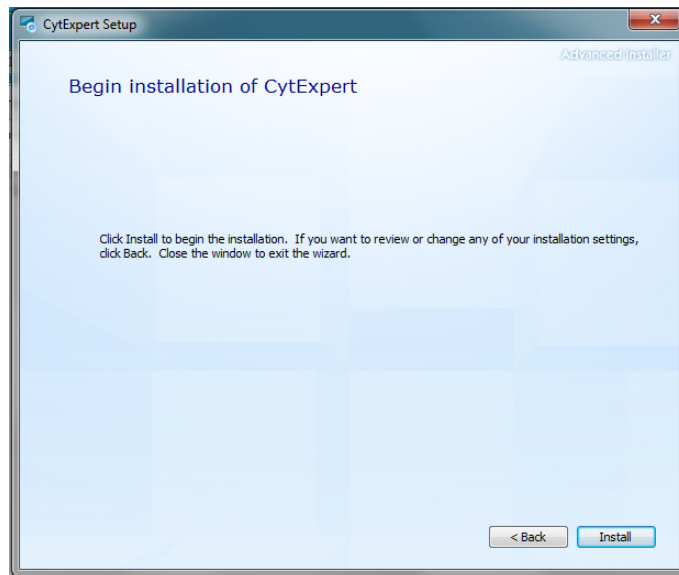


17 Select **Next**. The Installation Options window appears.

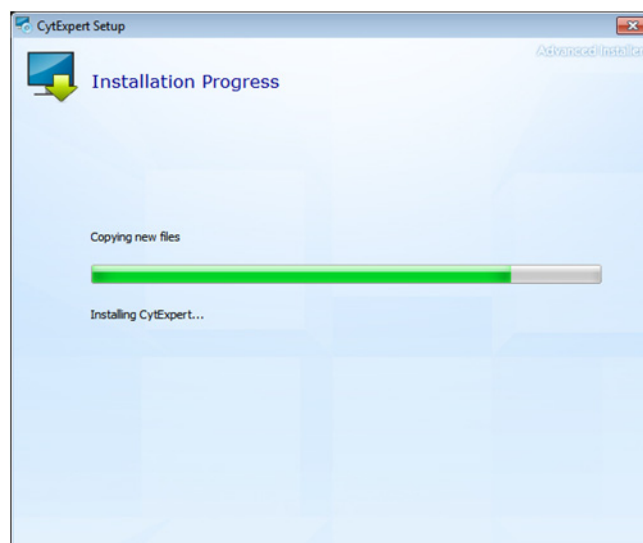


18 Select the desired installation option. Refer to [CytExpert Software Installation Options](#).

19 Select **Next**. The Begin installation of CytExpert window appears.

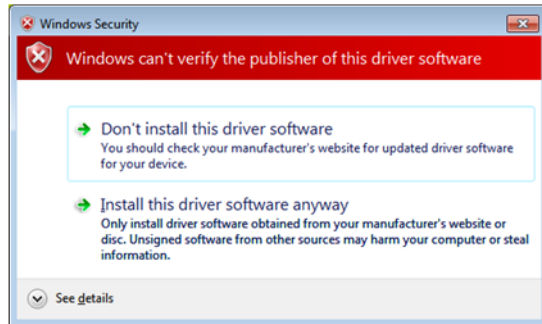


20 Select **Install** to begin installing the software. The Installation Progress window appears.

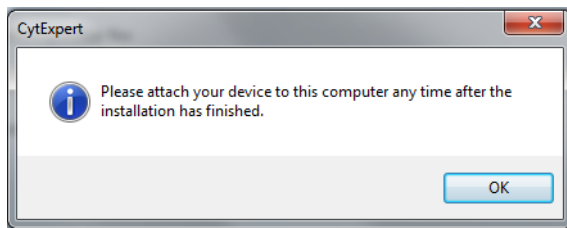


NOTE The software will install into the default file path provided unless otherwise specified.

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

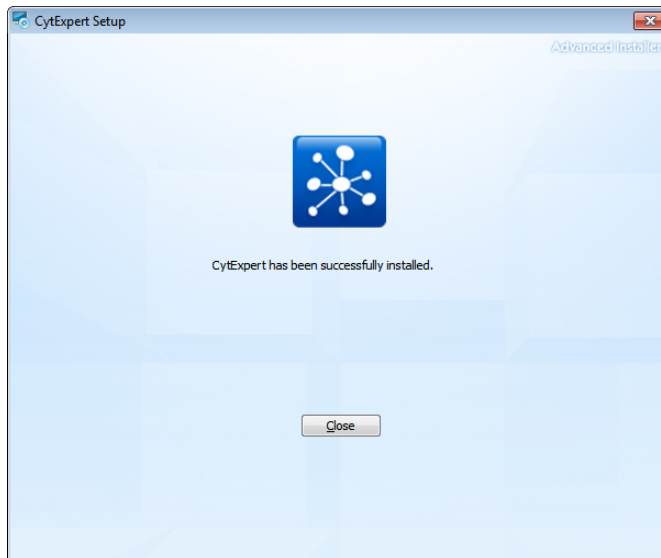


- 22 The following software prompt appears. Select **OK**.



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

- 23 Wait for the software to finish installing. The install complete window appears.



24 Select **Close** to finish the CytExpert software installation.

25 Install the instrument configuration file. Refer to [Installing the Instrument Configuration File](#).

Installing the Instrument Configuration File

CAUTION

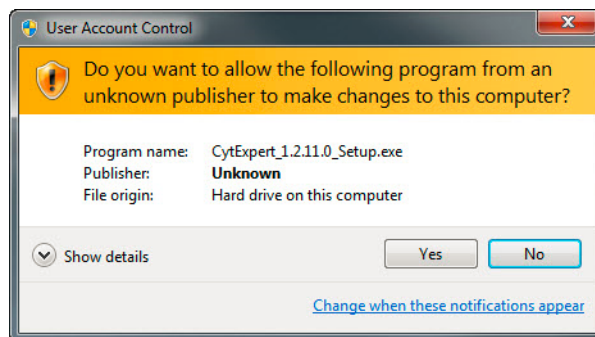
Risk of erroneous results or instrument damage. Only install the configuration file that matches your instrument. Installing an incorrect configuration file could cause erroneous results or instrument damage.

Use this procedure to install the configuration settings for the instrument. If the CytExpert software will not be connected to a Cytometer, this step can be skipped.

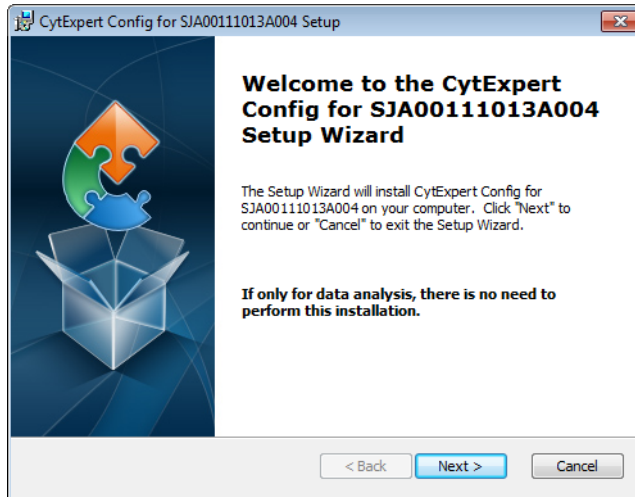
IMPORTANT You must install the CytExpert software before installing the instrument configuration file. Refer to [Installing the Software \[CytoFLEX\]](#).

1 Select and run **CytExpert_2.0_Config_Setup_XXXX.exe**. The User Account Control window appears.

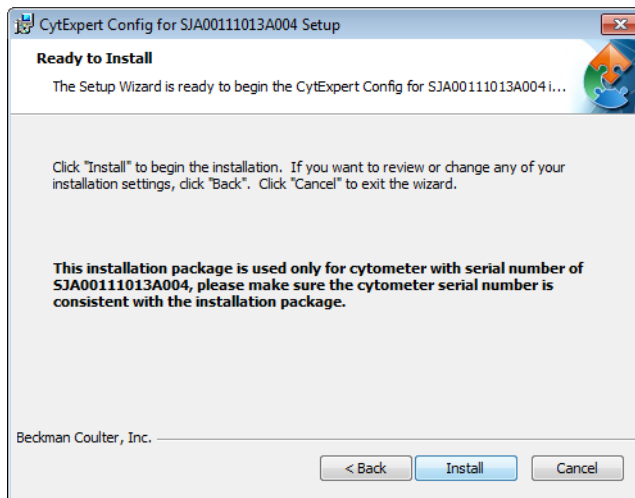
NOTE XXXX refers to the serial number of the instrument.



- 2 Select **Yes**. The CytExpert Config Welcome window appears.

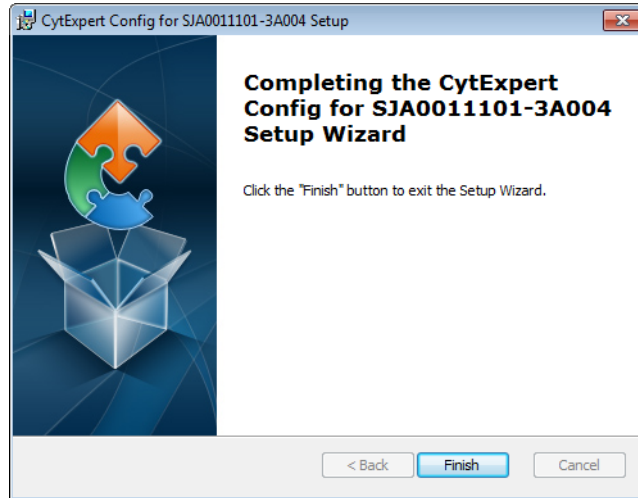


- 3 Select **Next**. The CytExpert Config Ready to Install window appears.



- 4 Verify that the serial name displayed at the top of the window is correct.

- 5 Select **Install**. When the installation has finished, the Completing the CytExpert Config Setup Wizard window appears.

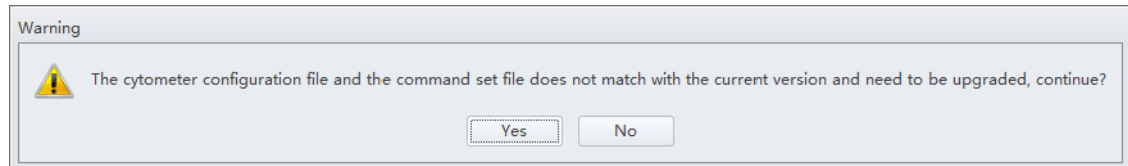


- 6 Select **Finish**.

- 7 If you get the following message, select **OK**. When you launch CytExpert, the system will automatically upgrade your configuration file. Proceed to Step 8.



- 8 Launch CytExpert. A System Warning message appears. Select **Yes** to update the 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 3, Daily Startup](#), for detailed instructions on opening the software and confirming the connection status.

NOTE If the software shows *Connected*, data collection and analysis can be completed.

Upgrading the CytExpert Software

Use this procedure to upgrade to software version 2.0 or higher from any software version prior to software version 2.0.

If you only need to upgrade to the CytExpert Default software option, you should follow the procedure below.

If you need to install either the CytExpert User Management software option or the CytExpert Electronic Record Management software option, you should first follow the procedure below then follow the reinstallation procedure. Refer to [Reinstalling the CytExpert Software](#).

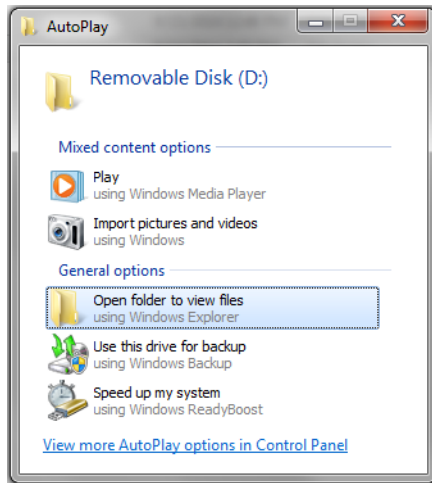
Refer to [CytExpert Software Installation Options](#) for the differences between each software option available.

CAUTION

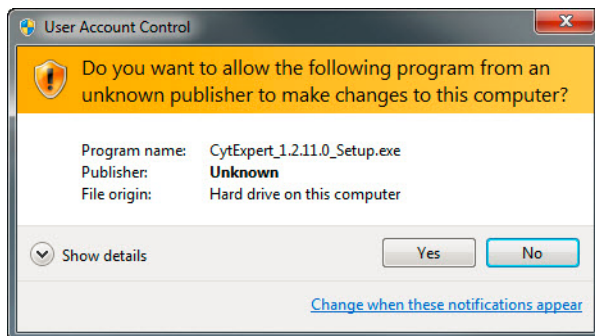
Risk of data loss. Reinstalling the CytExpert Software could overwrite your database. Ensure you backup your database prior to software reinstallation.

- 1 Insert the software USB into the computer.

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



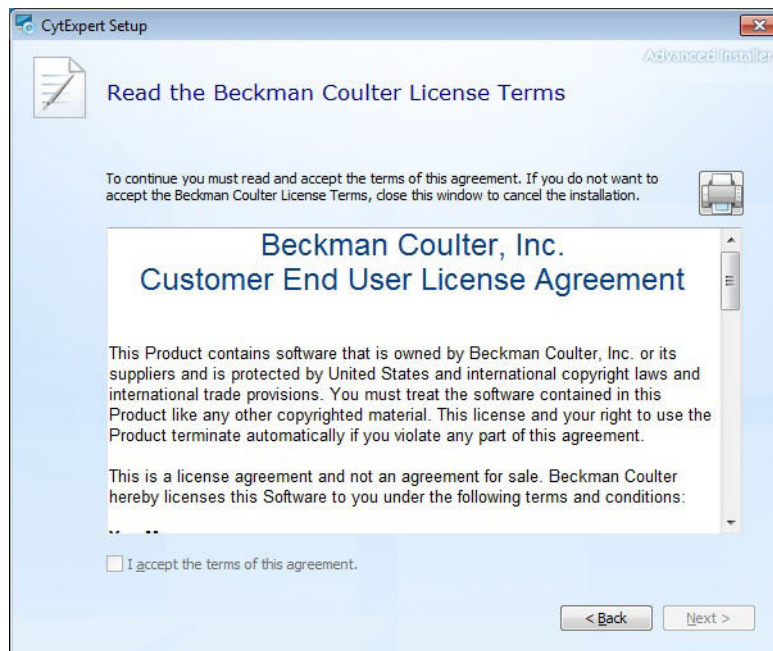
- 2 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.



- 3 Select **Yes**. The Welcome to CytExpert Setup Wizard window appears.



- 4 Select **Next**. The Beckman Coulter License Terms window appears.

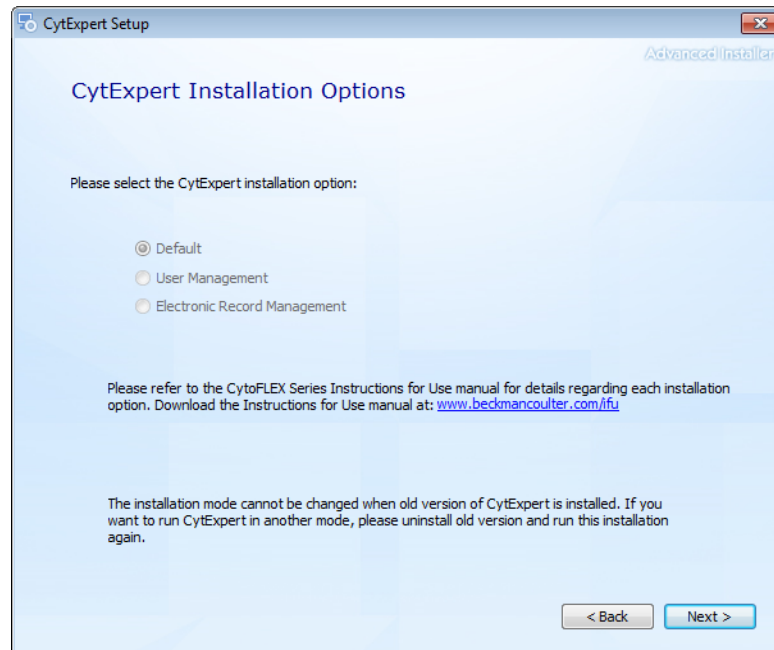


- 5 Read the Beckman Coulter Customer End User License Agreement.

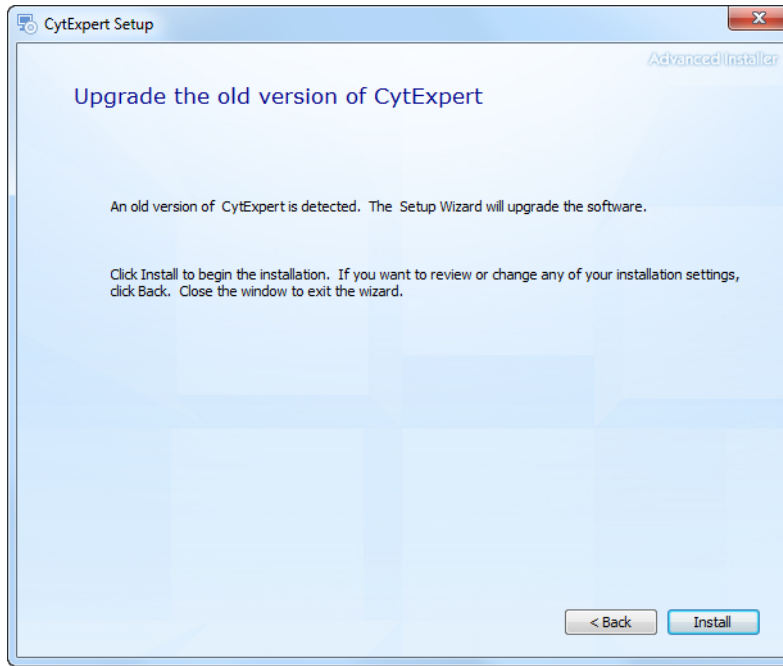
6 Select the *I accept the terms of this agreement* checkbox.

NOTE The checkbox is not selectable until you scroll all the way to the end of the agreement.

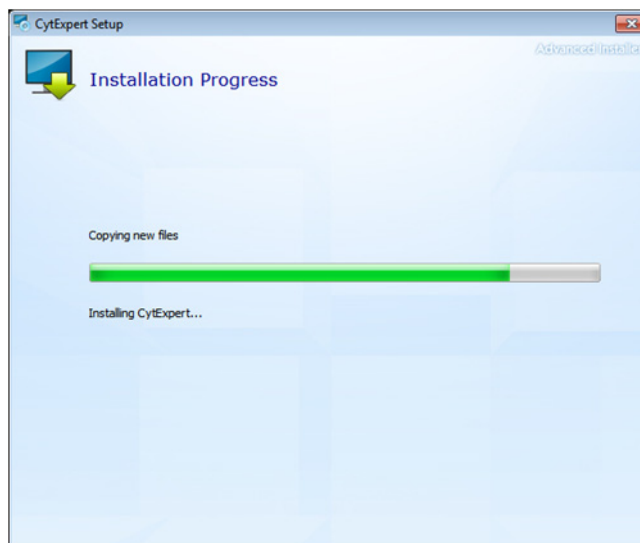
7 Select **Next**. The Installation Options window appears.



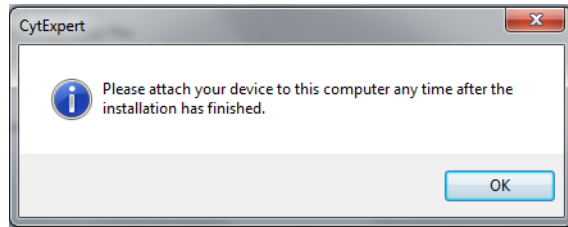
- 8 Select **Next**. If you are upgrading your software from a previous version and the previous version is not uninstalled first, the following window appears.



- 9 Select **Install** to begin installing the software. The Installation Progress window appears.

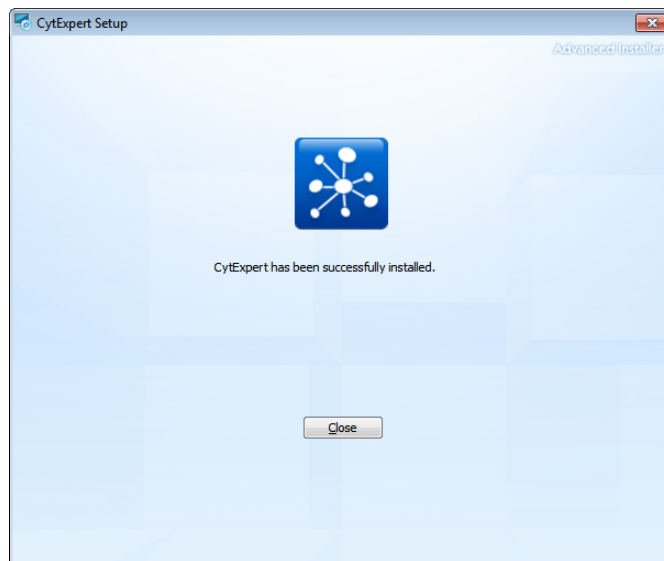


10 If the following software prompt appears, select **OK**.



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

11 Wait for the software to finish installing. The install complete window appears.



12 Select **Close** to finish the CytExpert software installation.

Reinstalling the CytExpert Software

Use this procedure to:

- Change the software option installed in all software versions 2.0 and higher. Refer to [CytExpert Software Installation Options](#) for the differences between each software option available.

NOTE If you are upgrading to software version 2.0 or higher from any software version prior to software version 2.0, refer to [Upgrading the CytExpert Software](#).

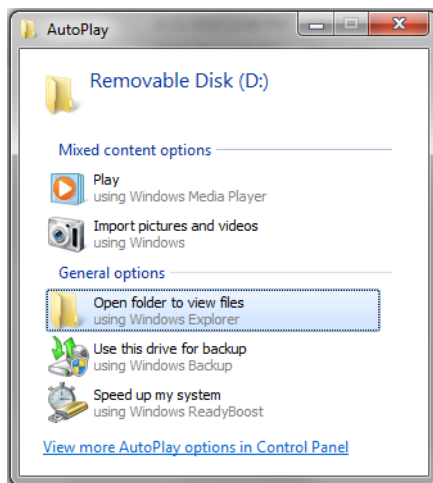
- Reinstall the same version of software.
- Upgrade your software to a version newer than software version 2.0.

⚠ CAUTION

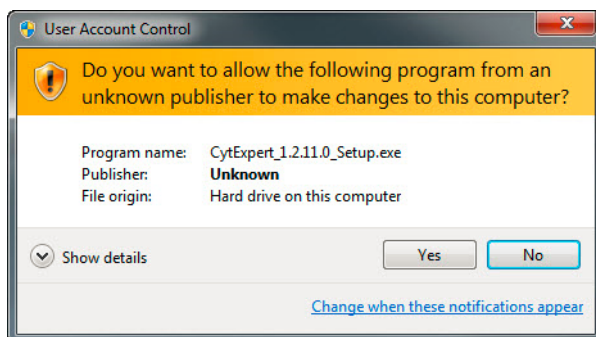
Risk of data loss. Reinstalling the CytExpert Software could overwrite your database. Ensure you backup your database prior to software reinstallation.

- 1 Backup your CytExpert data if you previously had a software option other than the Default software option installed. Refer to [Backup and Restore](#) in [CHAPTER 9, Troubleshooting](#).
- 2 Insert the software USB into the computer.

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



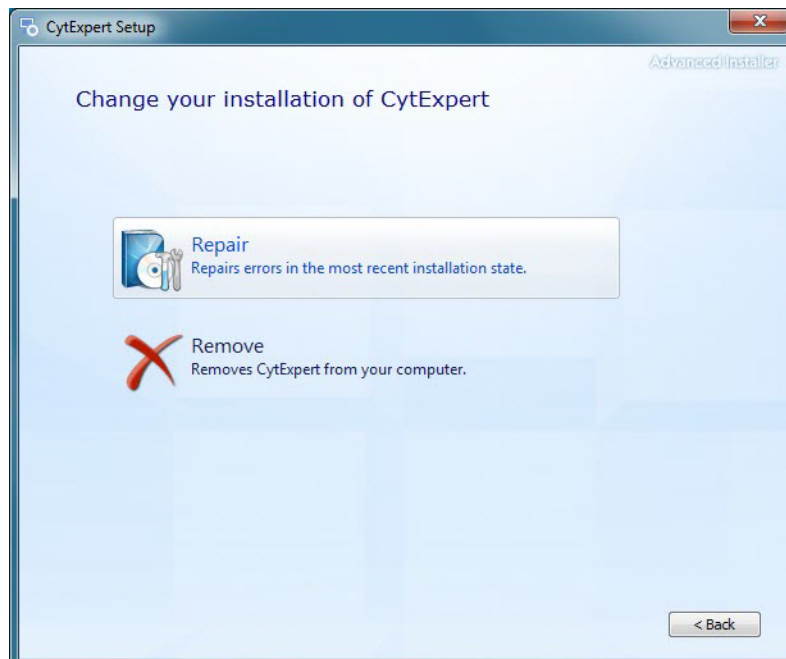
- 3 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.



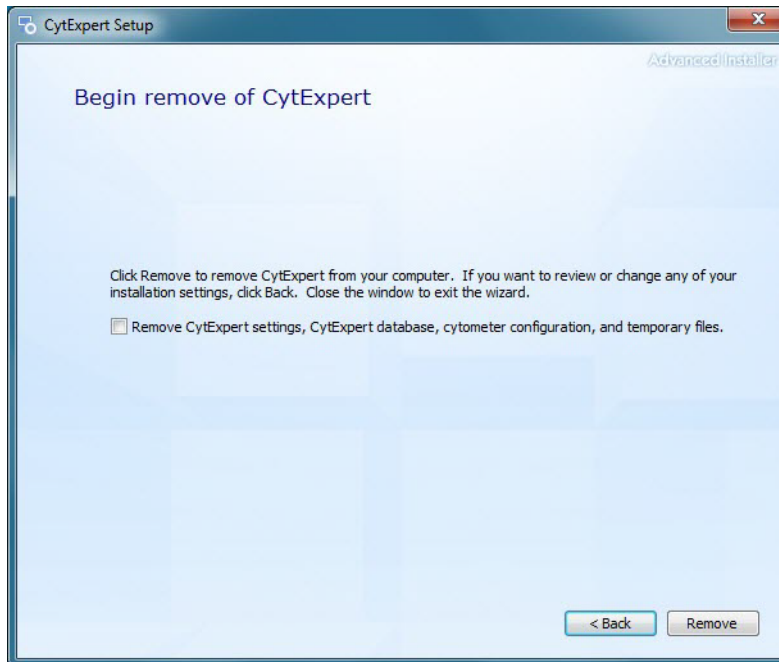
- 4 Select **Yes**. The Welcome to CytExpert Setup Wizard window appears.



- 5 Select **Next**. The Change your installation of CytExpert screen appears.



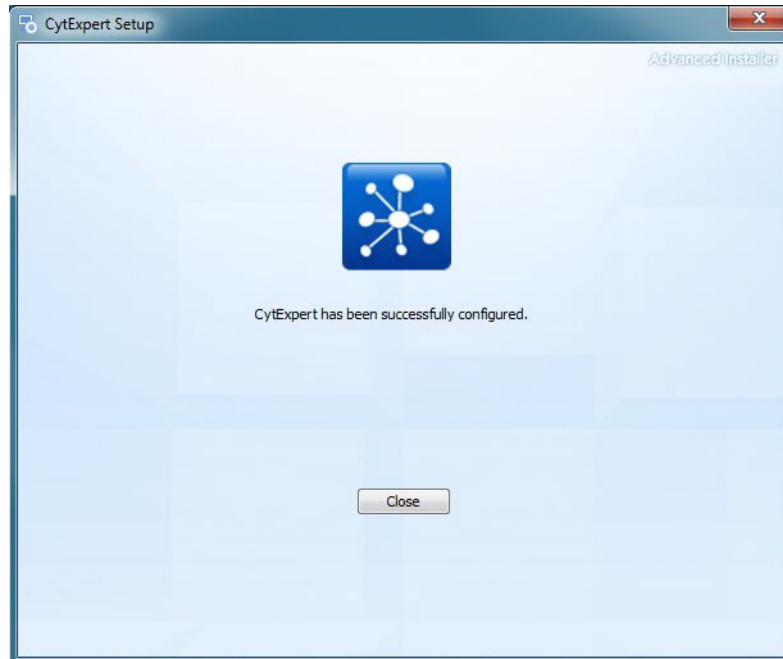
- 6 Select **Remove**. The Begin Remove of CytExpert screen appears.



IMPORTANT If the *Remove CytExpert settings, CytExpert database, cytometer configuration, and temporary files* checkbox is checked, your settings, database, configuration files, and temporary files will be overwritten and you will need to reinstall your cytometer configuration and restore any databases you might have.

- 7 Ensure the *Remove CytExpert settings, CytExpert database, cytometer configuration, and temporary files* checkbox is unchecked.

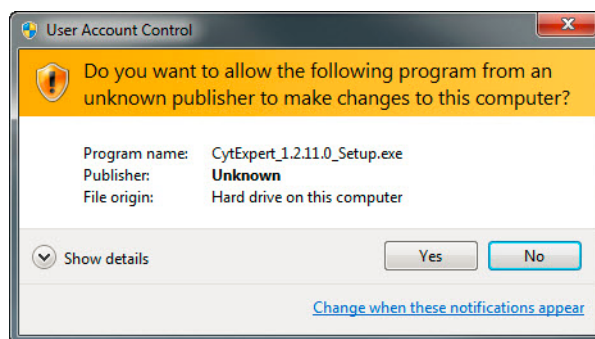
- 8 Select **Remove**. When software removal is complete, the software displays the message *CytExpert has been successfully configured*.



- 9 Select **Close**.

- 10 Navigate back to the software USB folder.

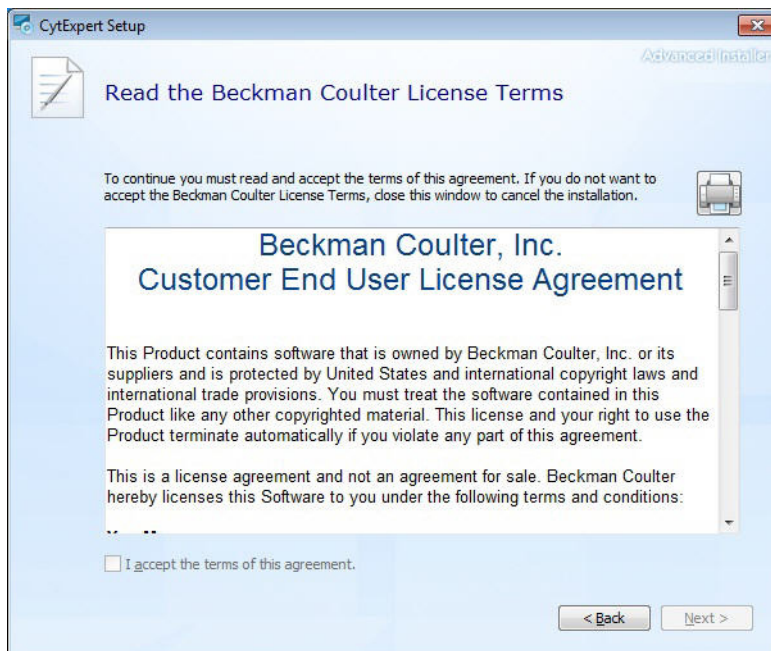
- 11 Select **CytExpert_X.X_Setup.exe**. The User Account Control window appears.



12 Select **Yes**. The Welcome to CytExpert Setup Wizard window appears.



13 Select **Next**. The Beckman Coulter License Terms window appears.

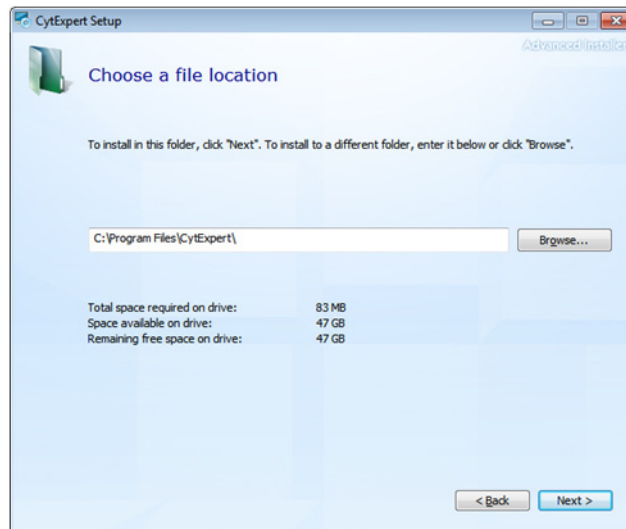


14 Read the Beckman Coulter Customer End User License Agreement.

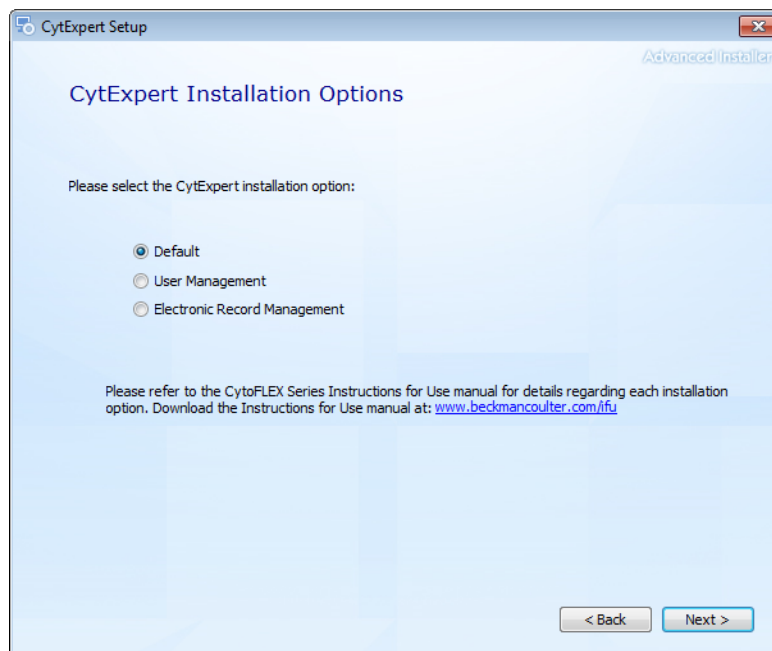
15 Select the *I accept the terms of this agreement* checkbox.

NOTE The checkbox is not selectable until you scroll all the way to the end of the agreement.

16 Select **Next**. The Choose a file location window appears.

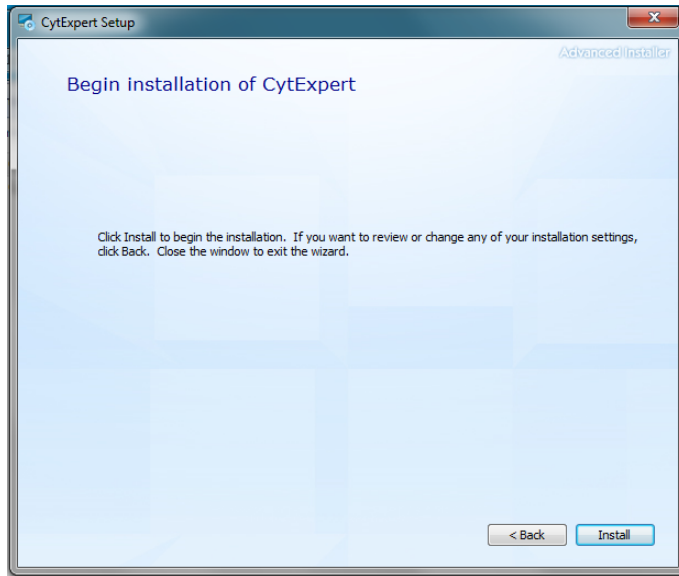


17 Select **Next**. The Installation Options window appears.

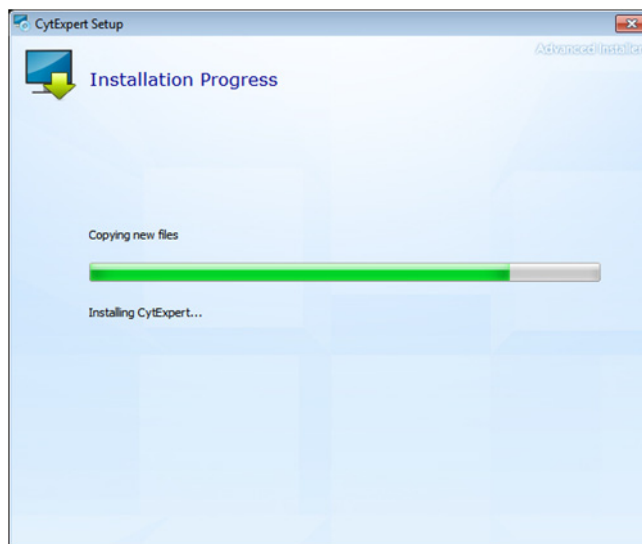


18 Select the desired installation option. Refer to [CytExpert Software Installation Options](#).

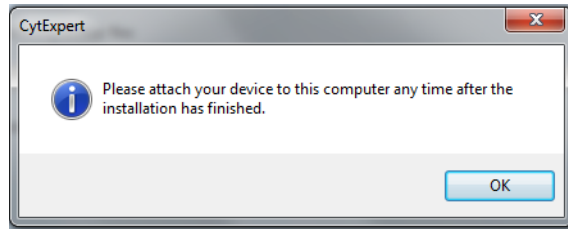
19 Select **Next**. The Begin installation of CytExpert window appears.



20 Select **Install** to begin installing the software. The Installation Progress window appears.

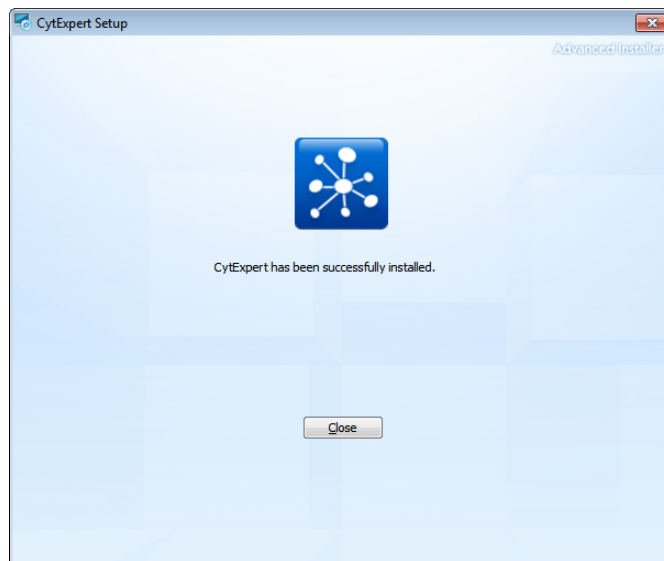


21 If the following software prompt appears, select **OK**.



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

22 Wait for the software to finish installing. The install complete window appears.



23 Select **Close** to finish the CytExpert software installation.

CytExpert Electronic Record Management

Overview

IMPORTANT You must have the CytExpert Electronic Record Management software option installed to use the features listed below. Refer to [CytExpert Software Installation Options](#) in [APPENDIX A, Instrument Installation](#).

Beckman Coulter's CytoFLEX with CytExpert software version 2.0 and higher contains features and functionality that facilitates compliance with 21 CFR Part 11 guidelines for Electronic Records and Signatures. This electronic record management includes controls for user identification, permissions, electronic signatures, data integrity, operation and experiment logs and audit trails. CytExpert software version 2.0 and higher contains a database that uses checksum matching to prevent tampering of the records and files that are indexed in the Closed File System.

This chapter contains information on:

- [Software Menu](#)
- [Experiment Management](#)
- [Log](#)
- [Electronic Signature](#)
- [User Management](#)

Software Menu

The CytExpert Electronic Record Management software option includes additional software menu items that are not necessarily available in the CytExpert Default software option or the CytExpert User Management software option. Refer to [Figure 2.3](#) and [Figure 2.4](#) for comprehensive software menu trees and details on which menu item applies to each software option.

Experiment Management



Risk of file corruption. Do not add, delete, or modify data from the Windows Explorer directory. Manage all data changes using Experiment Explorer to ensure file indexing remains intact.

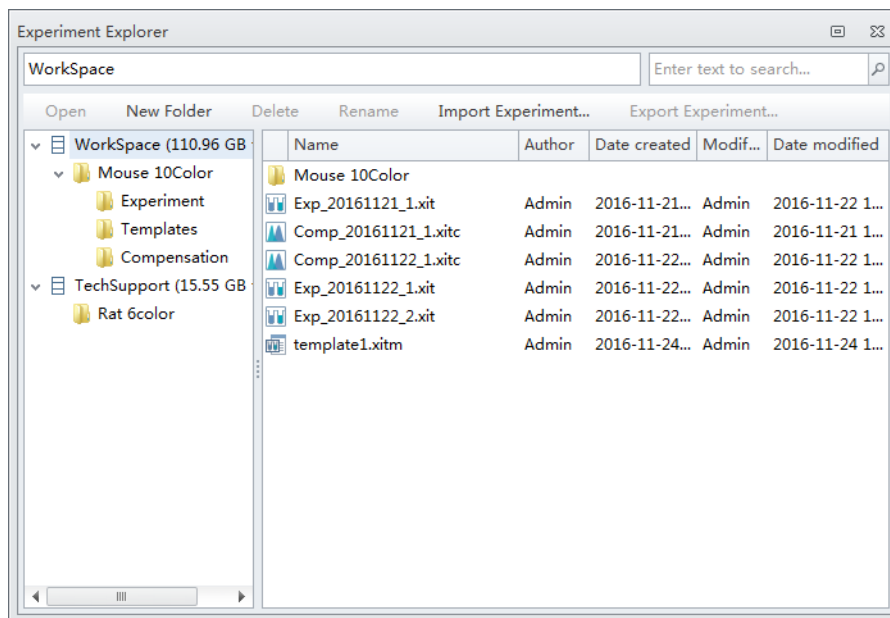
Closed File System

The closed file system provides audit trail capability for CytExpert experiment files. The closed file system provides a secure layer between the actual Windows Explorer files and the CytExpert users to retain file integrity.

Three file types are managed by the closed file system:

- Experiment files
- Compensation experiment files
- Experiment template files

The Experiment Explorer dialog can be accessed by selecting **File > Experiment Explorer**. The Experiment Explorer dialog functions the same as Windows file explorer.



Other mode experiment, compensation experiment, and experiment template files can be imported into the closed file system. These closed file system experiments can also be exported. Refer to [Importing an Experiment/Template](#) and [Exporting an Experiment/Template](#).

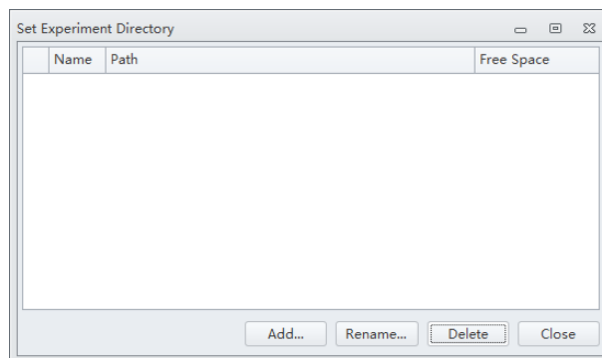
Experiment Directory Management

When launching CytExpert for the first time after installing the software, the Administrator must setup at least one experiment directory for experiment files.

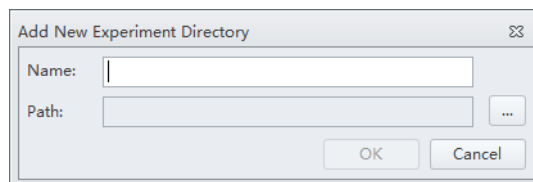
NOTE Only one experiment directory can be created per disk.


Setting Up the Experiment Directory

- 1 Select **Settings > Set Experiment Directory**. The Set Experiment Directory window appears.

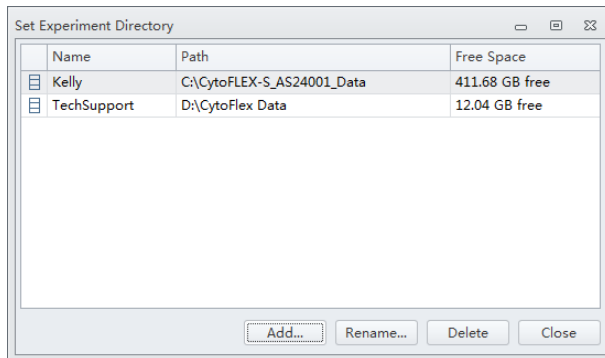


- 2 Select **Add**. The Add New Experiment Directory window appears.



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

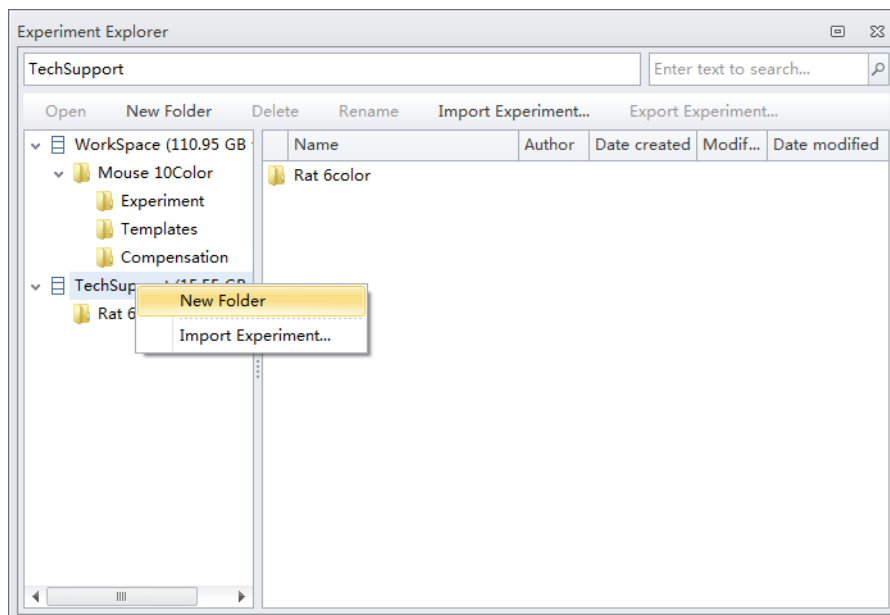
5 Select **OK**. The specified folder appears in the Set Experiment Directory window.



NOTE Select **Rename** to rename the experiment directory. Select **Delete** to delete an experiment directory.

Folder Hierarchy Management

Select **File > Experiment Explorer** to view Experiment Explorer. Select **New Folder** from the right-click drop down menu or from the Experiment Explorer toolbar to create a new subfolder.

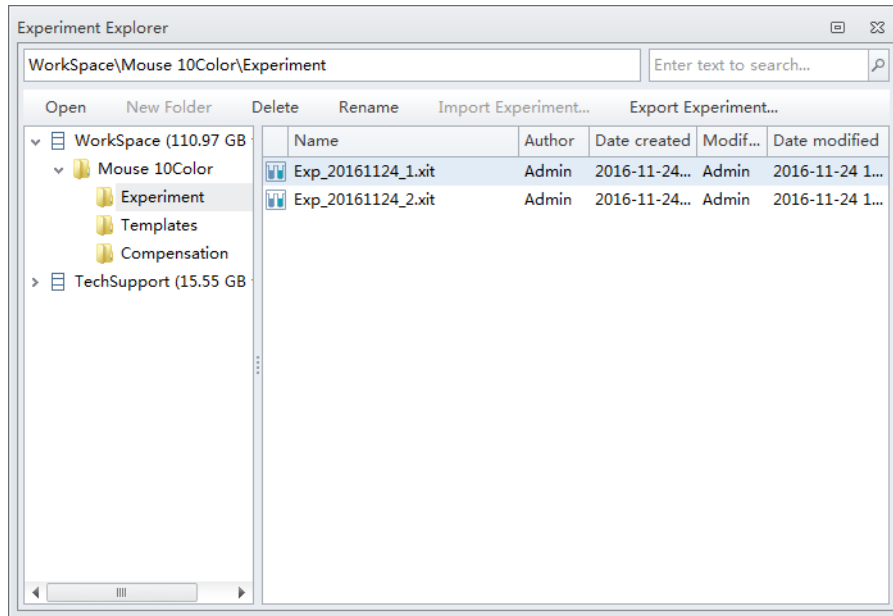


Select **Rename** from the right-click drop down menu or from the Experiment Explorer toolbar to rename a subfolder.

Select **Delete** from the right-click drop down menu or from the Experiment Explorer toolbar to delete a subfolder.

Experiment Related Operations

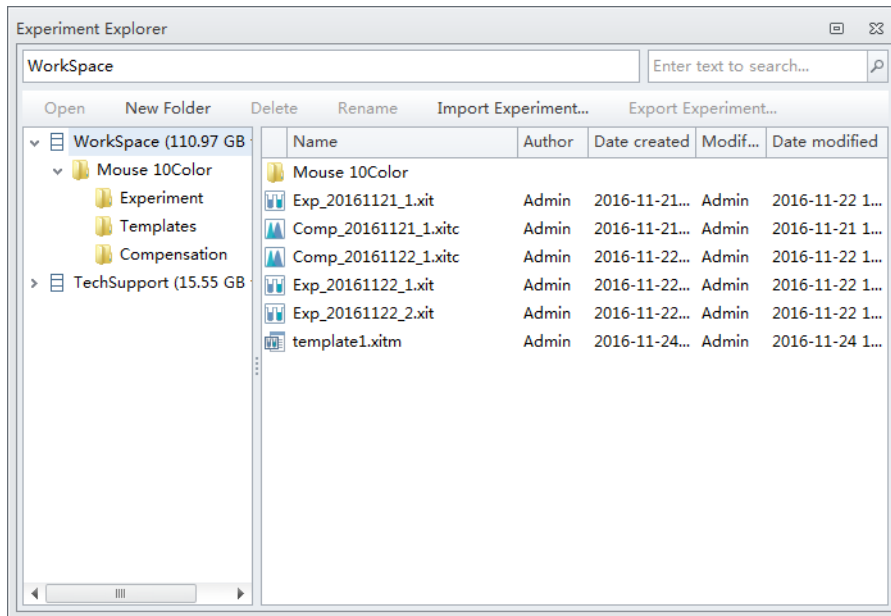
The Experiment Explorer dialog appears in place of the Windows File Explorer in the following operations: New/Open Experiment, New/Open Compensation Experiment, Save As, Save Experiment As Template, Recent Template, and New Experiment from Template.



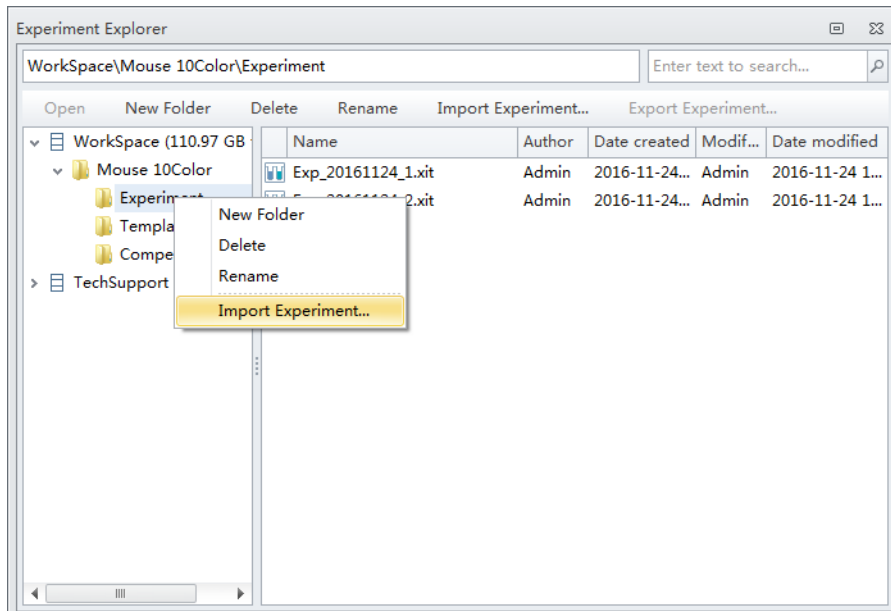
Importing an Experiment/Template

Use the following procedure to import experiment (.xit), compensation (.xipc), or experiment from template (.xitm) files into the system.

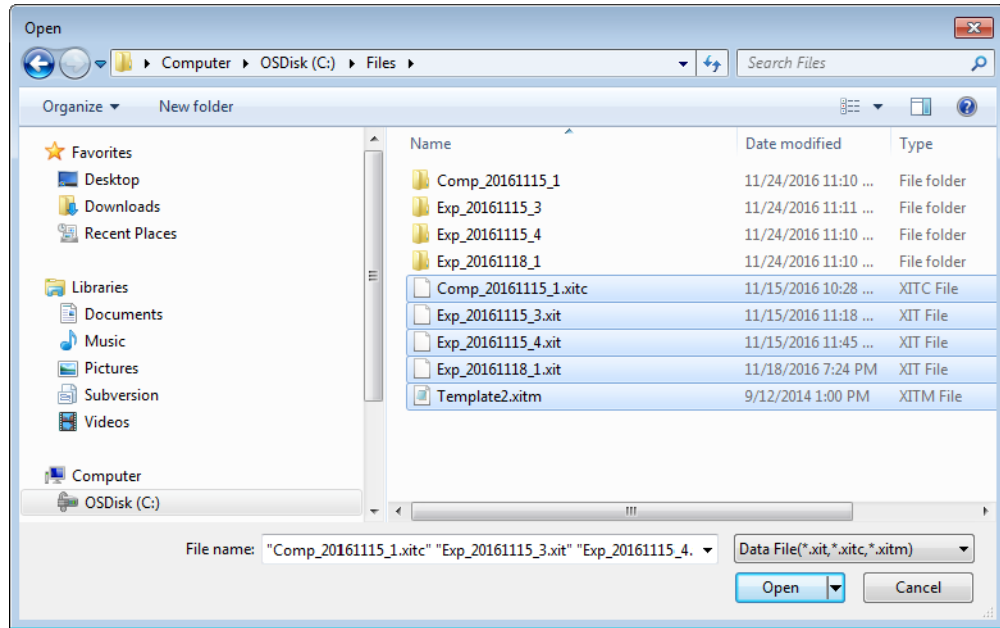
- 1 Select **File > Experiment Explorer**. The Experiment Explorer window appears.



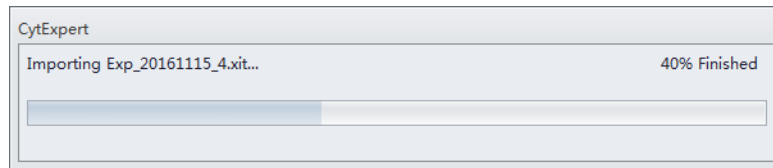
- 2 Select **Import Experiment** from the right-click drop down menu or from the Experiment Explorer toolbar.



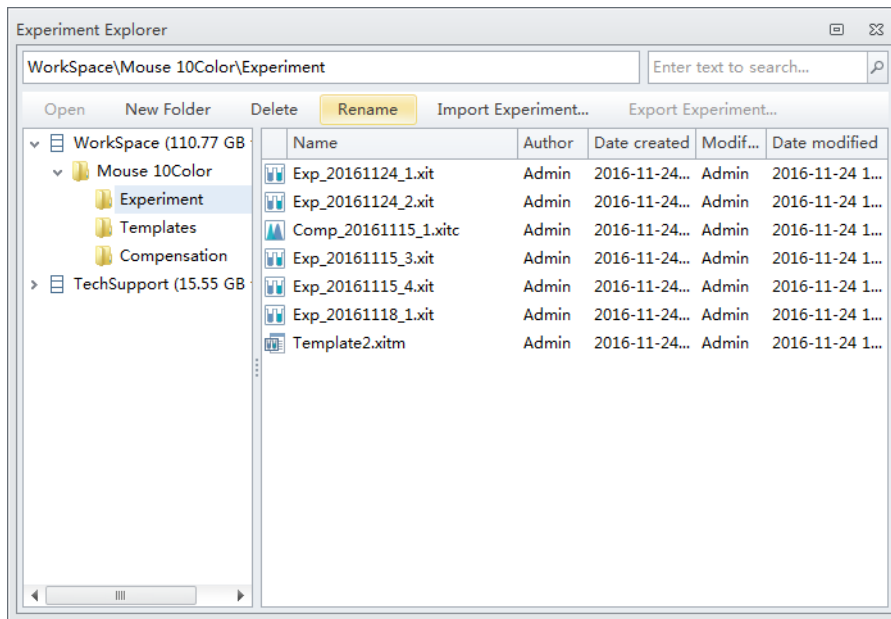
3 Browse to the desired file path to import and select **Open**.



A progress bar appears when importing files.



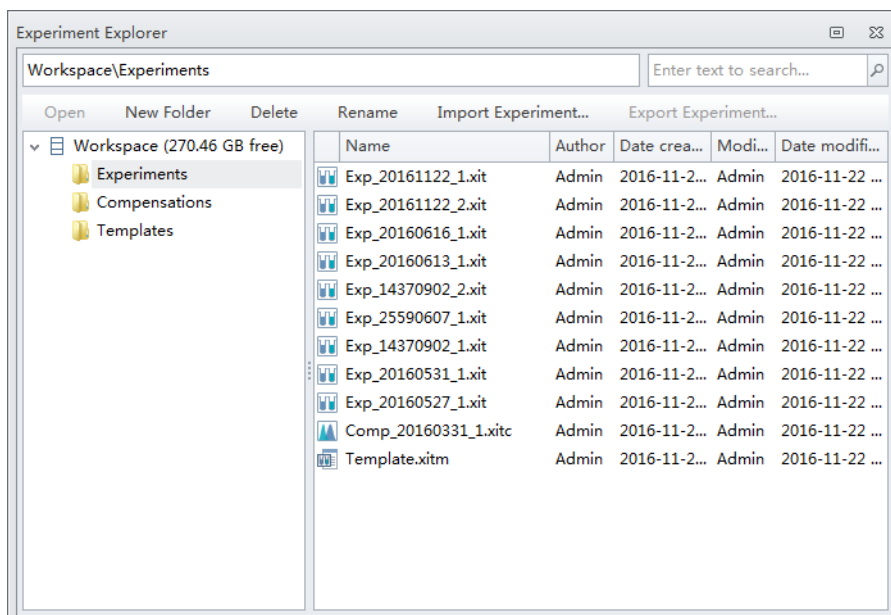
Once the import is complete, the imported files display in the Experiment Explorer.



NOTE Users can import .xit, .xitc, and .xitm files individually or multiple files at a time.

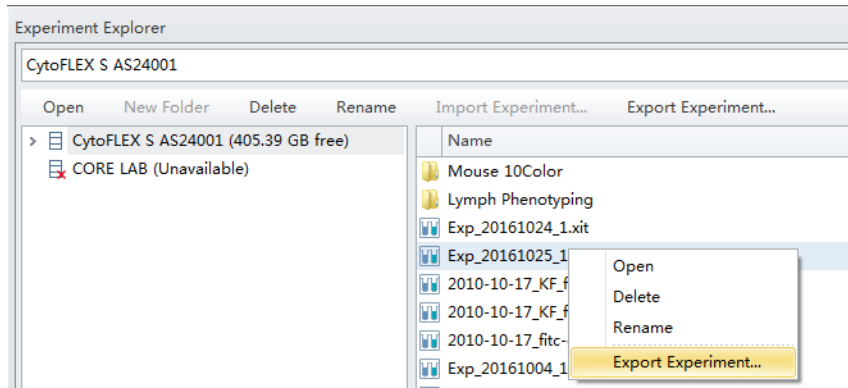
Exporting an Experiment/Template

- 1 Select **File > Experiment Explorer**. The Experiment Explorer window appears.

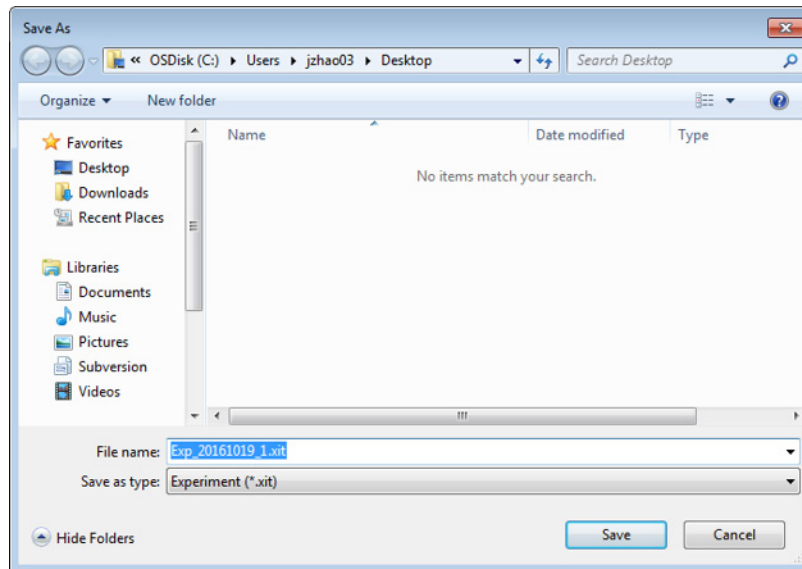


2 To export a single experiment:

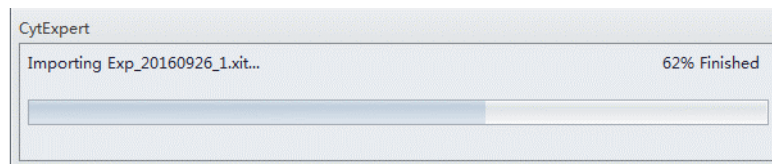
- a. Select **Export Experiment** from the right-click drop down menu or from the Experiment Explorer toolbar.**



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



A progress bar appears when exporting files.

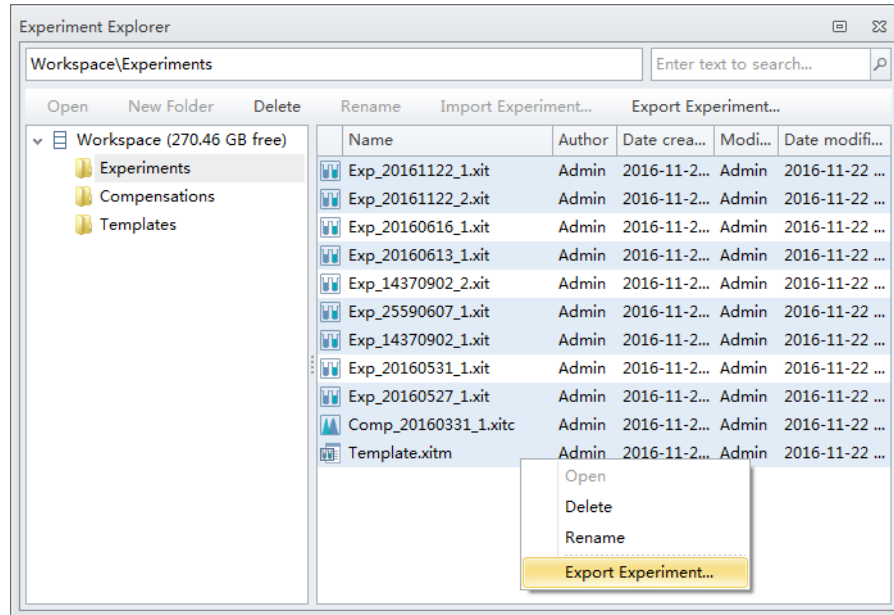


Once the export is complete, the exported file displays in the target folder.

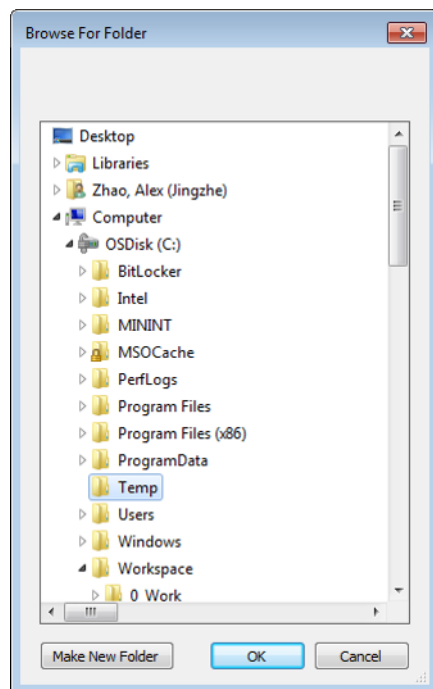
OR

To export multiple experiments:

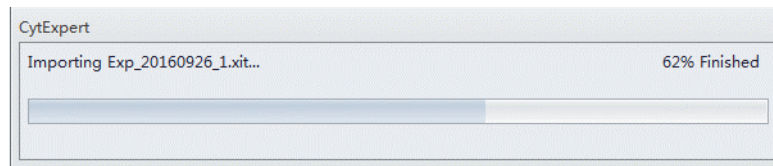
- a. Select all of the experiments to be exported then select **Export Experiment** from the right-click drop down menu or from the Experiment Explorer toolbar.



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



A progress bar appears when exporting files.



Once the export is complete, the exported file displays in the target folder.

Log

The CytExpert Electronic Record Management software option includes three logs:

Experiment Operation Log — The experiment operation log lists the audit trail records related to the experiment operations based on query criteria.

System Operation Log — The system operation log lists the log records associated with the system configuration changes based on query criteria.

User Management Operation Log — The user management operation log lists all of the log records related to the user management operations based on query criteria.

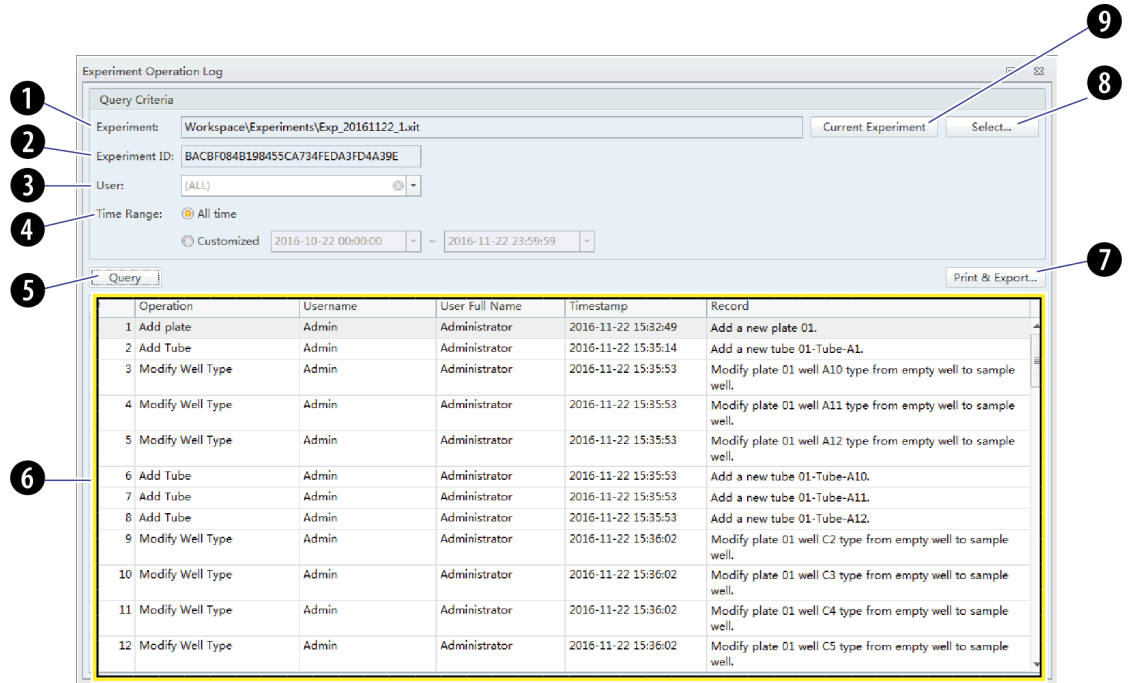
NOTE All three logs support print and export to PDF file functions.

Experiment Operation Log

The Experiment Operation Log generates an experiment audit trail record when performing experiment operations.

Select **Log > Experiment Operation Log** to open the Experiment Operation Log window. Refer to [Figure B.1](#).

Figure B.1 Experiment Operation Log Window



1. **Experiment:** Used to specify the experiment file criteria.

NOTE If an experiment is open, the current experiment displays in the Experiment section.

2. **Experiment ID:** Used to specify the experiment ID query criteria.

3. **User:** Used to specify the user query criteria.

NOTE The default selection all users.

4. **Time Range:** Used to specify the operation time range query criteria.

5. **Query** : Runs the query on the specified query criteria.

6. **Query display:** Displays the queried results.

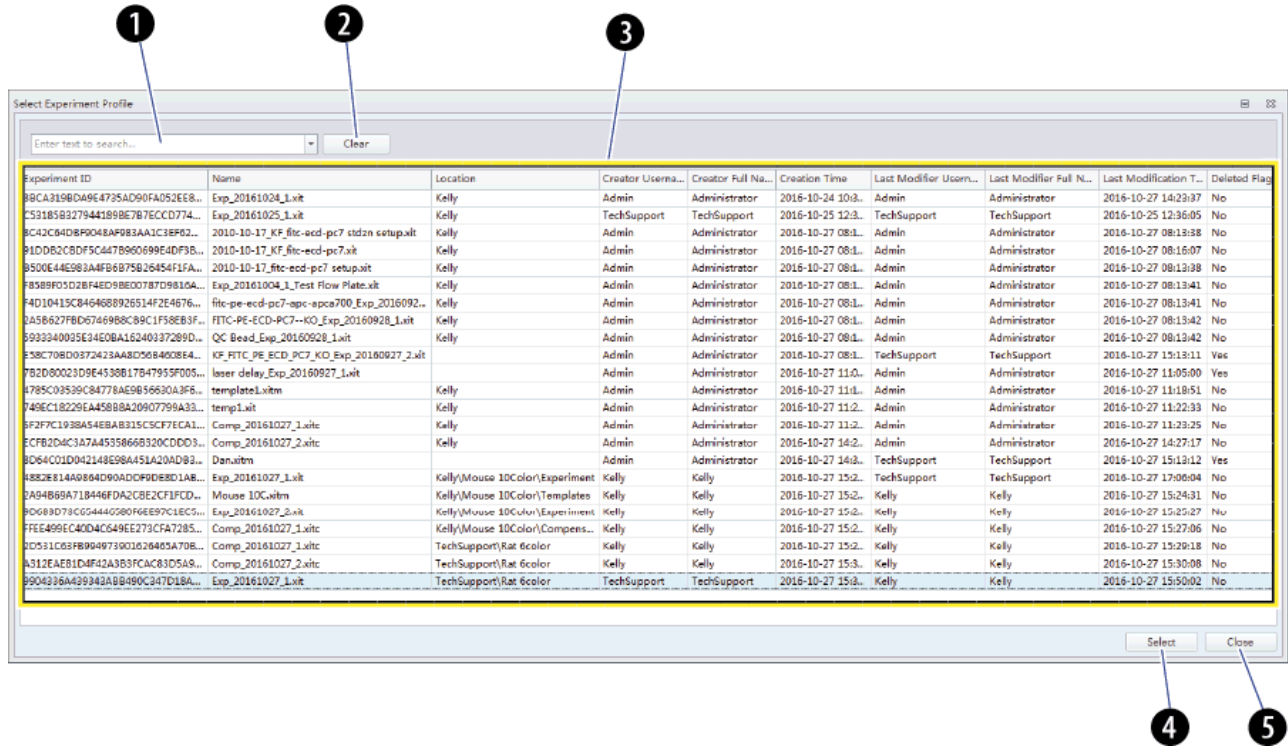
7. **Print & Export...** : Displays the print and export preview dialog. Refer to [Figure B.3](#).

8. **Select...** : Selects an experiment from the Select Experiment Profile window. Refer to [Figure B.2](#).

9. **Current Experiment** : Selects the experiment that is currently open.

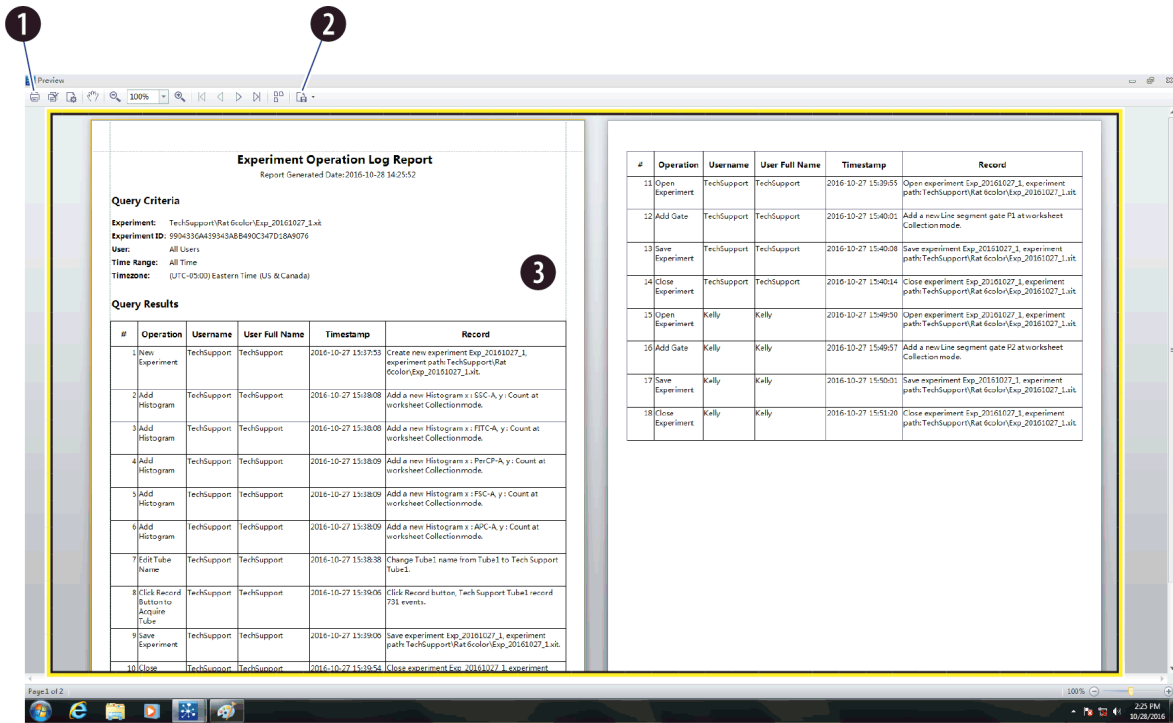
NOTE An experiment must be open for the button to be selectable.

Figure B.2 Select Experiment Profile Window



1. **Keyword search:** Used to search for keywords in the experiment display list.
2. **Clear:** Clears the keyword search.
3. **Experiment display list:** Displays the experiments in a list.
4. **Select:** Selects an experiment.
5. **Close:** Closes the Select Experiment Profile window.

Figure B.3 Print and Export Preview Window



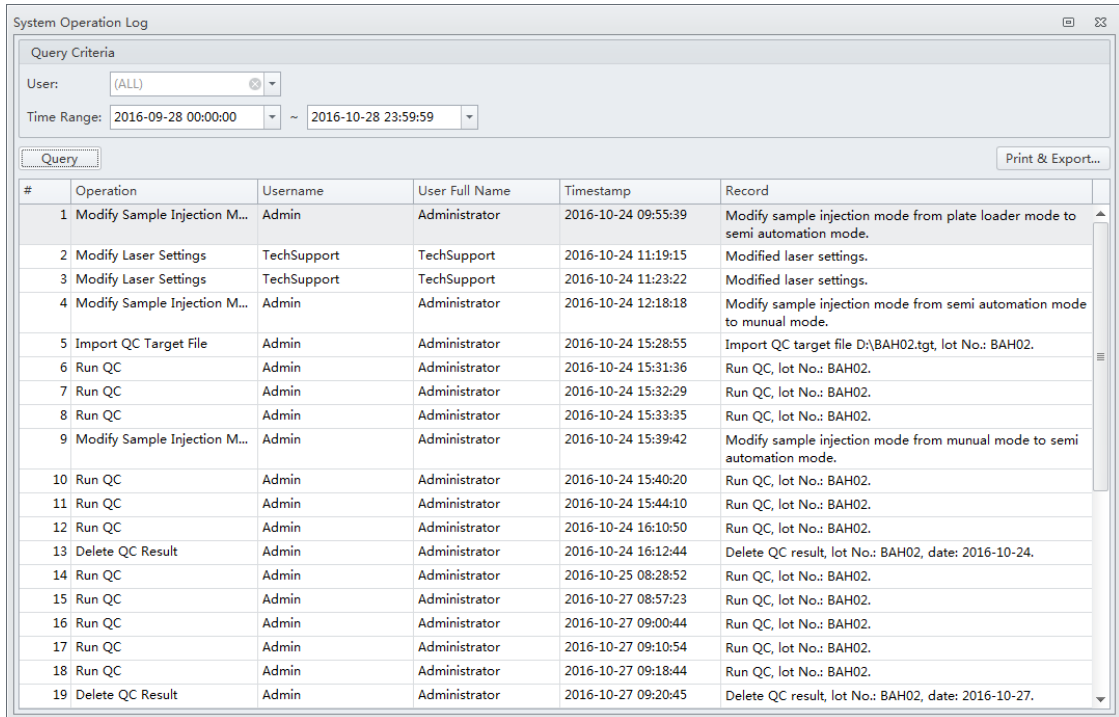
1. **Print:** Prints the report.
2. **Export to PDF:** Exports the report as a PDF.
3. **Report View:** Displays the detailed report view.

System Operation Log

The System Operation Log generates system log records for settings, configuration, maintenance, and QC.

Select **Log > System Operation Log** to open the System Operation Log window. Refer to [Figure B.4](#).

Figure B.4 System Operation Log Window



The screenshot shows the 'System Operation Log' window. At the top, there is a 'Query Criteria' section with a 'User:' dropdown set to '(ALL)' and a 'Time Range:' dropdown set to '2016-09-28 00:00:00' to '2016-10-28 23:59:59'. Below this is a 'Query' button and a 'Print & Export...' button. The main area is a table with the following data:

#	Operation	Username	User Full Name	Timestamp	Record
1	Modify Sample Injection M...	Admin	Administrator	2016-10-24 09:55:39	Modify sample injection mode from plate loader mode to semi automation mode.
2	Modify Laser Settings	TechSupport	TechSupport	2016-10-24 11:19:15	Modified laser settings.
3	Modify Laser Settings	TechSupport	TechSupport	2016-10-24 11:23:22	Modified laser settings.
4	Modify Sample Injection M...	Admin	Administrator	2016-10-24 12:18:18	Modify sample injection mode from semi automation mode to manual 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 manual mode to semi automation mode.
10	Run QC	Admin	Administrator	2016-10-24 15:40:20	Run QC, lot No.: BAH02.
11	Run QC	Admin	Administrator	2016-10-24 15:44:10	Run QC, lot No.: BAH02.
12	Run QC	Admin	Administrator	2016-10-24 16:10:50	Run QC, lot No.: BAH02.
13	Delete QC Result	Admin	Administrator	2016-10-24 16:12:44	Delete QC result, lot No.: BAH02, date: 2016-10-24.
14	Run QC	Admin	Administrator	2016-10-25 08:28:52	Run QC, lot No.: BAH02.
15	Run QC	Admin	Administrator	2016-10-27 08:57:23	Run QC, lot No.: BAH02.
16	Run QC	Admin	Administrator	2016-10-27 09:00:44	Run QC, lot No.: BAH02.
17	Run QC	Admin	Administrator	2016-10-27 09:10:54	Run QC, lot No.: BAH02.
18	Run QC	Admin	Administrator	2016-10-27 09:18:44	Run QC, lot No.: BAH02.
19	Delete QC Result	Admin	Administrator	2016-10-27 09:20:45	Delete QC result, lot No.: BAH02, date: 2016-10-27.

NOTE The System Operation Log uses the same query criteria and functions as the Experiment Operation Log. Refer to [Figure B.1](#).

User Management Operation Log

The User Management Operation Log generates audit log records for user management, role management, account policies, password changes, and login/logout records.

Select **Log > User Management Operation Log** to open the User Management Operation Log window. Refer to [Figure B.5](#).

Figure B.5 User Management Operation Log Window

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

NOTE The User Management Operation Log uses the same query criteria and functions as the Experiment Operation Log. Refer to [Figure B.1](#).

Electronic Signature

Signing an Experiment

- 1 Select **Signature > Sign** to sign an experiment. The Sign window appears.

Sign

Username: Admin

Password:

Comment:

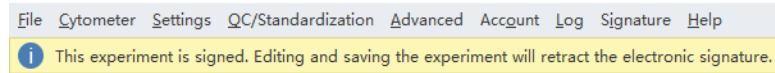
OK Cancel

- 2 If necessary, enter comments related to the experiment.

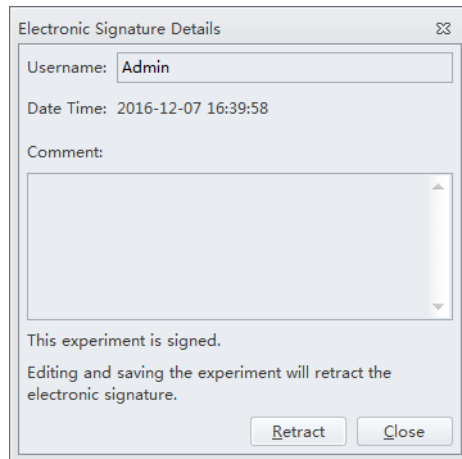
3 Enter your password.

4 Select **OK**.

The signature status displays in the top left corner of the main UI.



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



Retracting an Experiment Signature

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



2 Select **Retract**. The following message appears: *Are you sure you want to remove the electronic signature?*

3 Select **Yes** to confirm the retraction of your signature or **No** to cancel.

The following message appears after editing and saving a signed experiment: *Saving the experiment will retract the electronic signature of this experiment, continue?* Select **Yes** to remove the signature or **No** to cancel.

The following message appears before starting data acquisition: *Acquiring data will retract the electronic signature of this experiment, continue?* Select **Yes** to remove the signature or **No** to cancel.

User Management

User Administration

Logging In and Out of the Software

Refer to [Logging Into the Software](#) and [Logging Out of the Software](#) in CHAPTER 3, Daily Startup.

Role Management

Refer to [Role Management](#) in CHAPTER 2, Using the CytExpert Software.

User Management

Refer to [User Management](#) in CHAPTER 2, Using the CytExpert Software.

Account Policies

Refer to [Account Policies](#) in CHAPTER 2, Using the CytExpert Software.

Sample Injection Mode Control Kit

Overview

The Sample Injection Mode Control Kit is a mechanical knob installed by your service engineer that enables users to switch between the Plate Loader sample injection mode and the Semi-Automatic or manual sample injection mode. The switch eliminates the need to manually re-route the tubing.

This chapter contains information on:

- [Performance Characteristics \[With the Sample Injection Mode Control Knob\]](#)
- [Sample Injection Mode Control Kit Components](#)
- [Switching the Sample Probe from the Single Tube Sample Station to the Plate Loader \[With Sample Injection Mode Control Knob\]](#)
- [Switching the Sample Probe from the Plate Loader to the Single Tube Sample Station \[With Sample Injection Mode Control Knob\]](#)

Performance Characteristics [With the Sample Injection Mode Control Knob]

Performance [CytoFLEX With Plate Loader]	
Throughput [With Plate Loader] ^a	10 second acquisition without mixing or backflush: <35 min.
	10 second acquisition with 3 second mixing and 4 second backflush: < 50 min.

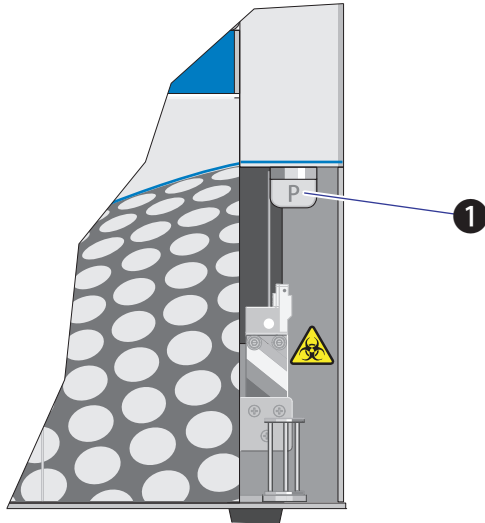
a. This performance characteristic is different if you do not have the Sample Injection Mode Control Kit installed on your CytoFLEX flow cytometer. Refer to [Performance Characteristics](#) in [CHAPTER 1, System Overview](#).

Performance [CytoFLEX LX With Plate Loader]	
Throughput [With Plate Loader] ^a	10 second acquisition without mixing or backflush: <38 min.
	10 second acquisition with 3 second mixing and 6 second backflush: < 56 min.

a. This performance characteristic is different if you do not have the Sample Injection Mode Control Kit installed on your CytoFLEX LX flow cytometer. Refer to [Performance Characteristics](#) in [CHAPTER 1, System Overview](#).

Sample Injection Mode Control Kit Components

Figure C.1 Sample Injection Mode Control Knob



1. Switch module knob

NOTE The **P** indicates the instrument is set to Plate Loader sample injection mode. The **T** indicates the instrument is set to the Semi-Automatic or manual sample injection mode.

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



WARNING

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

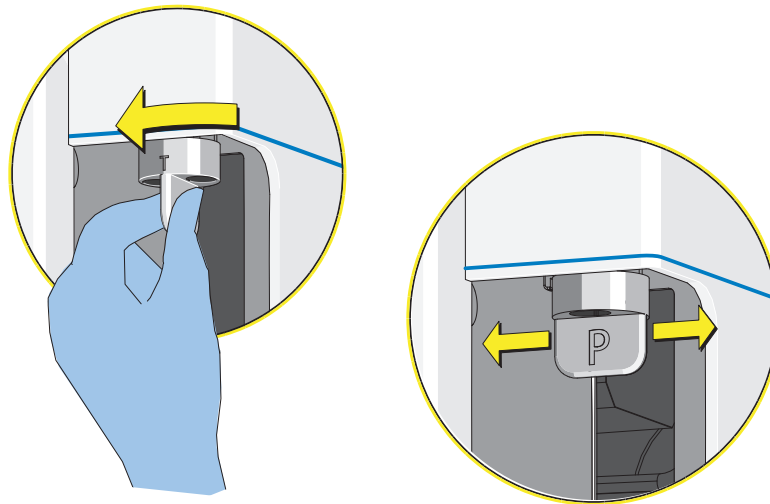
Use universal precautions when working with pathogenic materials. Means must be available to decontaminate the instrument and to dispose of biohazardous waste.

CAUTION

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

NOTE If you do not have the Sample Injection Mode Control Kit installed, refer to [Changing the Sample Probe from the Single Tube Sample Station to the Plate Loader \[CytoFLEX With Plate Loader\]](#) in CHAPTER 11, Replacement/Adjustment Procedures.

- 1 Switch to the Plate Loader sample injection mode. Refer to [Selecting the Plate Loader Sample Injection Mode \[With Plate Loader\]](#) in CHAPTER 3, Daily Startup.
- 2 Turn the switch module knob to the right (clockwise) until the flat side of the knob is parallel to the front panel and the P is facing you. Ensure that the knob is turned as far as it will go.



The system is now ready for use in the Plate Loader sample injection mode.

NOTE If you do not see any events upon running an acquisition:

1. Select to stop the acquisition.
2. Ensure that the switch module knob is turned as far as it will go.
3. Rerun the acquisition.
4. If the problem persists, [contact us](#).

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



WARNING

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

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

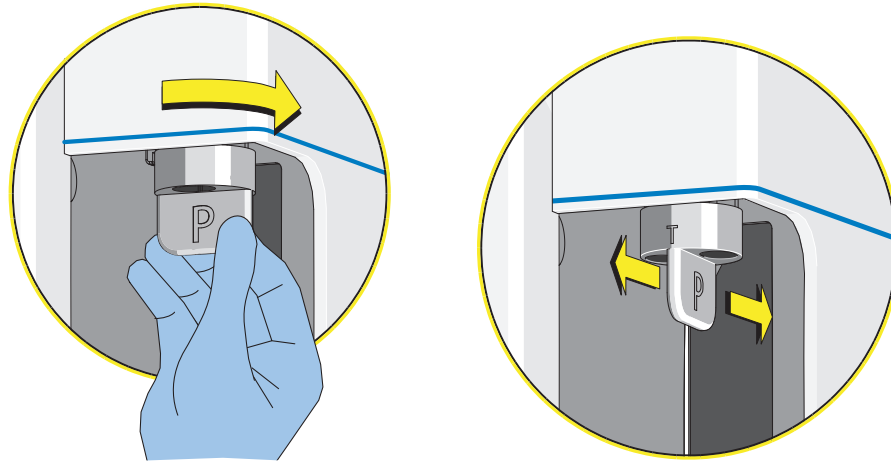
CAUTION

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

NOTE If you do not have the Sample Injection Mode Control Kit installed, refer to [Changing the Sample Probe from the Plate Loader to the Single Tube Sample Station \[CytoFLEX With Plate Loader\]](#) in [CHAPTER 11, Replacement/Adjustment Procedures](#).

- 1 Switch to either the Semi-Automatic or manual sample injection mode. Refer to [Selecting the Proper Sample Injection Mode](#) in [CHAPTER 3, Daily Startup](#).

- 2 Turn the switch module knob to the left (counterclockwise) until the flat side of knob is perpendicular to the front panel and the T is facing you. Ensure that the knob is turned as far as it will go.



The system is now ready for use in either the Semi-Automatic or manual sample injection mode.

NOTE If you do not see any events upon running an acquisition:

1. Select 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](#).

Sample Injection Mode Control Kit

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

Table of Hazardous Substances

Table of Hazardous Substances

The Hazardous Substances Names and Concentration are shown in [Table D.1](#) and [Table D.2](#).

Table D.1 有毒有害物质名称及含量的标识格式 Table of Hazardous Substances Name and Concentration [CytoFLEX]

电子电气产品号码 EEP Part Number: A00-1-1102	产品名称 Product Name: CytoFLEX 产品型号 Product Model Number: A00-1-1102					
部件名称 Component Name	有毒有害物质或元素 Hazardous Substances Name					
	铅 (Pb)	汞 (Hg)	镉 (Cd)	六价铬 (Cr ⁶⁺)	多溴联苯 (PBB)	多溴二苯醚 (PBDE)
印刷电路板组件 Circuit Boards	X	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	X	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

This table is prepared in accordance with the provisions of SJ/T 11364

0: 表示该有毒有害物质在该部件所有均质材料中的含量均在GB/T 26572标准规定的限量要求以下

x: 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出GB/T 26572标准规定的限量要求

(企业可在此处, 根据实际情况对上表中打“x”的技术原因进行进一步说明)

0: Indicates that the toxic or hazardous substances contained in all of the homogenous materials for this part is below the limit requirements in GB/T 26572.

X: Indicates that the toxic or hazardous substance contained in at least one of the homogenous materials used for this part in above the limit requirement in GB/T 26572.

Table D.2 有毒有害物质名称及含量的标识格式 Table of Hazardous Substances Name and Concentration [CytoFLEX LX]

电子电气产品号码 EEP Part Number: B90883	产品名称 Product Name: CytoFLEX LX 产品型号 Product Model Number: B90883					
部件名称 Component Name	有毒有害物质或元素 Hazardous Substances Name					
	铅 (Pb)	汞 (Hg)	镉 (Cd)	六价铬 (Cr ⁶⁺)	多溴联苯 (PBB)	多溴二苯醚 (PBDE)
印刷电路板组件 Circuit Boards	X	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	X	0	0	0	0	0
发动机/泵/阀门/ Motors/Pumps/Valves	0	0	0	0	0	0
电线 Cables	X	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	X	0	0	0	0	0
包装材料 Packing Material	0	0	0	0	0	0

This table is prepared in accordance with the provisions of SJ/T 11364

0: 表示该有毒有害物质在该部件所有均质材料中的含量均在GB/T 26572标准规定的限量要求以下

x: 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出GB/T 26572标准规定的限量要求

(企业可在此处, 根据实际情况对上表中打“x”的技术原因进行进一步说明)

0: Indicates that the toxic or hazardous substances contained in all of the homogenous materials for this part is below the limit requirements in GB/T 26572.

X: Indicates that the toxic or hazardous substance contained in at least one of the homogenous materials used for this part in above the limit requirement in GB/T 26572.

Table of Hazardous Substances
Table of Hazardous Substances

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

KO — Krome Orange	rCV — robust coefficient of variation
LED — light emitting diode	RH — relative humidity
L — liter	RoHS — Restriction of Hazardous Substances Directive
LJ — Levey-Jennings	RPTM — real-time messaging protocol
LWH — length, width, height	S/N — serial number
m — meter	SNR — signal to noise ratio
MB — megabyte	SSC — side scatter
MFI — median fluorescence intensity	USB — universal serial bus
MHz — megahertz	USPTO — United States Patent and Trademark Office
min — minute	UV — ultraviolet
mL — milliliter	V — volts
mm — millimeter	VA — volt-ampere
mW — milliwatt	VAC — voltage alternating current
NA — numerical aperture	VSSC — violet side scatter
NaClO — sodium hypochlorite solution	WDM — wavelength division multiplexer
NaN₃ — sodium azide	W — watts
nm — nanometer	
Nt Wt — net weight	
PB — Pacific Blue™ dye	
PC5 — Phycoerythrin-Cy™5 tandem dye	
PC5.5 — Phycoerythrin-Cy™5.5 tandem dye	
PC7 — Phycoerythrin-Cy™7 tandem dye	
PE — Phycoerythrin	
PEEK — polyether ether ketone	
PerCP — Peridinin-Chlorophyll	
PI — Propidium Iodide	
PN — part number	
QC — quality control	
RAM — random access memory	

Символи

- ,
- define, [Abbreviations-1](#)
- "
- 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](#)

Числові дані

- 10 L sheath fluid cubitainer
 - replacing, [11-7](#)
- 10 L waste cubitainer (CytoFLEX LX)
 - emptying, [11-10](#)
- 4 L sheath fluid container
 - to clean, [10-10](#)
- 4 L sheath fluid container (cleaning)
 - routine maintenance, [10-10](#)
- 4 L sheath fluid container (CytoFLEX)
 - filling, [11-6](#)
- 4 L waste container
 - to clean, [10-11](#)
- 4 L waste container (cleaning)
 - routine maintenance, [10-11](#)
- 4 L waste container (CytoFLEX)
 - emptying, [11-9](#)

A

- A
 - define, [Abbreviations-1](#)
- AC
 - define, [Abbreviations-1](#)
- account
 - unlock, [2-26](#)
- account menu, [2-21](#)
- account policies, [2-30](#)
- acoustic noise level, [1-25](#)
- 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, [5-36](#)
- add
 - vertex to auto polygon gate, [5-50](#)
- adding
 - Deep Clean solution, [11-17](#)
 - standardization item, [4-24](#)
- adding channels
 - for compensation, [6-21](#)
- adjust compensation
 - manually, [6-16](#)
- adjusting
 - autogate movement, [5-51](#)
 - compensation, [6-16](#)
 - compensation settings, [7-8](#)
 - gain, [5-55](#)
 - threshold, [5-57](#)
- adjusting auto gates, [5-48](#)
- adjustment
 - nonscheduled, [11-56](#)
 - routine, [11-2](#)
- advanced menu, [2-20](#)
- alarm
 - Fluidics module, [1-12](#)
 - mute, [1-12](#)

- alarm does not sound when the waste container
 - is full or the sheath fluid container is low
 - and the software status display is red, 9-11
 - analysis
 - screen, 7-1
 - analysis screen, 2-9
 - to open, 7-3
 - analyzing
 - data, 5-64
 - APC
 - define, [Abbreviations-1](#)
 - APC-A7500
 - define, [Abbreviations-1](#)
 - APC-Cy7
 - define, [Abbreviations-1](#)
 - API
 - define, [Abbreviations-1](#)
 - applying
 - standardization in QC, 4-26
 - auto gates
 - adjusting, 5-48
 - creating, 5-48
 - auto polygon gate
 - add vertex, 5-50
 - auto recalculate
 - turn off, 5-50
 - turn on, 5-50
 - auto shutdown, 5-6, 8-2, 10-6
 - auto startup, 8-2
 - autogate
 - adjusting movement, 5-51
 - extent, 5-52
 - movement, 5-51
 - autosampling system
 - Deep Clean solution peristaltic pump, 1-12
- B**
- backflush settings
 - to change, 11-71
 - backup
 - database, 9-23
 - manual injection mode, 3-9
 - band-pass filter, 1-7, [Abbreviations-1](#)
 - band-pass filters, 1-6
 - BCI
 - define, [Abbreviations-1](#)
 - BMP
 - define, [Abbreviations-1](#)
- bottle
 - Deep Clean solution, 1-12
 - BP
 - define, [Abbreviations-1](#)
- C**
- calculating
 - compensation values, 6-8, 6-13
 - concentration, 7-7
 - sample volume, 7-7
 - calculation of the automatic compensation
 - experiment is incorrect, 9-17
 - calculations
 - compensation, 6-1
 - calibrating
 - plate position [with plate loader], 11-73
 - sample flow rate, 11-56
 - sample flow rate with plate loader, 11-59
 - caution label
 - RoHS, 9-9
 - CDRH
 - define, [Abbreviations-1](#)
 - CFSE
 - define, [Abbreviations-1](#)
 - change
 - password, 2-26
 - changing
 - mixer and backflush settings, 11-71
 - tube name, 5-36
 - changing sample probe
 - from plate loader to single tube sample station, 11-39
 - from single tube sample station to plate loader, 11-34
 - channel
 - to set, 5-36
 - channels
 - adding for compensation, 6-21
 - fluorescence, 5-26, 5-27
 - to set, 5-36
 - characteristics
 - performance, 1-28
 - check
 - waste and reagent levels (10 L fluid cubitainers), 3-4
 - waste and reagent levels (4 L fluid containers), 3-2
 - cleaning
 - 4 L sheath fluid container, 10-10

- 4 L waste container, [10-11](#)
 - nonscheduled, [10-13](#)
 - routine, [10-1](#)
 - sample probe, [10-8](#)
 - sample station, [10-7](#)
 - surfaces, [10-13](#)
- cleaning and ventilation
 - installation environment validation, [A-2](#)
- cleaning solution
 - to prepare, [8-1](#)
- cleaning the 4 L sheath fluid container
 - routine maintenance, [10-10](#)
- cleaning the 4 L waste container
 - routine maintenance, [10-11](#)
- cleaning the sample station
 - routine maintenance, [10-7](#)
- closed file system
 - electronic record management, [B-2](#)
- cm
 - define, [Abbreviations-1](#)
- collecting
 - data, [5-32](#)
 - QC data, [4-10](#)
 - QC data with plate loader, [4-12](#)
- collection
 - acquisition screen, [2-5](#)
- collection conditions
 - to set, [5-58](#)
- compensation
 - adding channels, [6-21](#)
 - calculations, [6-1](#)
 - to adjust, [6-16](#)
 - to manually adjust, [6-16](#)
- compensation controls
 - compensation experiment screen, [2-11](#)
- compensation experiment
 - to create, [6-2](#)
- compensation experiment screen, [2-10](#)
 - compensation controls, [2-11](#)
- compensation experiment with plate loader
 - creating, [6-10](#)
- compensation library
 - to manage, [6-20](#)
- compensation matrix
 - to create from previously acquired data, [6-14](#)
 - to generate, [6-4](#), [6-13](#)
- compensation sample
 - prepare, [6-4](#), [6-13](#)
- compensation settings
 - adjust, [7-8](#)
 - exporting, [6-19](#)
 - importing and exporting, [5-75](#)
 - compensation settings from compensation library
 - to import, [6-17](#)
 - compensation settings from compensation matrix files
 - to import, [6-16](#)
 - compensation values
 - to calculate, [6-8](#), [6-13](#)
- components
 - Cytometer, [1-2](#)
 - Fluid Containers/Cubitainers, [1-2](#)
 - fluidics system, [1-10](#)
 - instrument, [1-1](#)
 - main, [1-2](#)
 - optical, [1-4](#)
 - plate holder, [1-18](#)
 - plate loader, [1-16](#)
 - sample injection mode control kit, [C-2](#)
 - Workstation, [1-2](#)
- concentration
 - calculation, [7-7](#)
 - sample volume, [7-7](#)
- concentration calculation is incorrect, [9-18](#)
- concluding
 - experiment, [5-78](#)
- configuration
 - system, [1-20](#)
- configuring
 - acquisition settings, [5-36](#)
- confirming results, [4-16](#)
- connection indicator light in the lower left corner of the software screen is red and displays Disconnected and Error, [9-10](#)
- conventions used, [-xxix](#)
- copying
 - experiments, [7-1](#)
 - previously acquired experiment, [7-1](#)
- 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, [6-2](#)
 - compensation experiment with plate loader, [6-10](#)

- compensation matrix from previously
 - acquired data, [6-14](#)
 - detector configuration, [5-25](#)
 - dot plot overlays, [7-5](#)
 - heat map (plate loader), [5-15](#)
 - histogram overlays, [7-5](#)
 - Levey-Jennings charts, [4-19](#)
 - new experiment, [5-1](#)
 - new experiment with plate loader, [5-3](#)
 - plots and gates, [5-39](#)
 - user roles, [2-28](#)
 - users, [2-24](#)
 - creating auto gates, [5-48](#)
 - CSV
 - define, [Abbreviations-1](#)
 - custom statistics
 - setting, [5-62](#)
 - customer-replaceable parts
 - ordering, [D-1](#)
 - customized parameters
 - setting, [5-61](#)
 - CV
 - define, [Abbreviations-1](#)
 - CytExpert
 - installation options, [A-12](#)
 - CytExpert API test client
 - setup, [2-37](#)
 - CytExpert software
 - reinstall, [A-29](#)
 - upgrade, [A-24](#)
 - instrument, [1-25](#)
 - dimensions, [1-25](#)
 - instrument, [1-25](#)
 - dimensions, [1-25](#)
 - Cytometer, [1-2](#), [1-3](#), [A-6](#)
 - Cytometer cannot be turned on, [9-10](#)
 - cytometer menu, [2-17](#)
- D**
- daily shutdown
 - routine maintenance, [10-1](#)
 - daily shutdown with plate loader
 - routine maintenance, [10-5](#)
 - daily start up
 - routine maintenance, [10-1](#)
 - daily start up with plate loader
 - routine maintenance, [10-5](#)
 - data
 - analyzing, [5-64](#)
 - collecting, [5-32](#)
 - exporting, [5-64](#)
 - sampling, [5-32](#)
 - to import, [7-1](#)
 - data populations are normal on one laser, but
 - too low on another laser, [9-15](#)
 - data populations are not where they are
 - expected, [9-16](#)
 - data sheets, material safety
 - how to order, [1-24](#)
 - database
 - backup, [9-23](#)
 - restore, [9-23](#)
 - Deep Clean
 - procedure, [10-8](#)
 - routine maintenance, [10-8](#)
 - Deep Clean solution
 - to add, [11-17](#)
 - to prepare, [11-17](#)
 - Deep Clean solution bottle
 - fluidics module, [1-12](#)
 - Deep Clean solution peristaltic pump
 - autosampling system, [1-12](#)
 - default
 - detector configuration, [5-26](#)
 - default password, [2-23](#), [A-24](#)
 - default username
 - username
 - default, [A-24](#)
 - define
 - %, [Abbreviations-1](#)
 - quality control (QC), [4-1](#)
 - symbols, [-xi](#)
 - defining the negative population, [6-4](#)
 - delete
 - experiment directory (electronic record
 - management), [B-4](#)
 - heat map parameter, [5-17](#)
 - deleting
 - heat map (plate loader), [5-23](#)
 - standardization items, [4-26](#)
 - user roles, [2-29](#)
 - users, [2-25](#)
 - detector (WDM)
 - optical components, [1-4](#)
 - detector configuration
 - to create, [5-25](#)

- to edit, [5-25](#)
 - to select, [5-25](#)
 - to verify, [5-25](#)
 - detector configuration default, [5-26](#)
 - detector unit
 - wavelength division multiplexor (WDM), [1-5](#)
 - disabling lasers, [5-54](#)
 - disinfection
 - surfaces, [10-13](#)
 - disposal
 - precaution, [9-9](#)
 - waste, [A-4](#)
 - DNA
 - define, [Abbreviations-1](#)
 - dot plot overlays
 - to create, [7-5](#)
 - dual-parameter plots, [2-32](#)
- E**
- ECD
 - define, [Abbreviations-1](#)
 - editing
 - detector configuration, [5-25](#)
 - standardization item parameters, [4-25](#)
 - EFUP
 - define, [Abbreviations-1](#)
 - electronic devices
 - fuse, [1-21](#), [1-23](#)
 - load button, [1-21](#), [1-23](#)
 - power switch, [1-21](#), [1-23](#)
 - electronic record management
 - closed file system, [B-2](#)
 - electronic signature, [B-16](#)
 - experiment directory management, [B-3](#)
 - experiment management, [B-2](#)
 - experiment operation log, [B-11](#)
 - experiment related operations, [B-5](#)
 - exporting an experiment/template, [B-8](#)
 - folder hierarchy management, [B-4](#)
 - importing an experiment/template, [B-6](#)
 - log, [B-11](#)
 - software menu, [B-1](#)
 - system operation log, [B-14](#)
 - user management, [B-18](#)
 - user management operation log, [B-15](#)
 - electronic signature
 - electronic record management, [B-16](#)
 - retract, [B-17](#)
 - EMF
 - define, [Abbreviations-1](#)
 - emptying
 - 10 L waste cubitainer (CytoFLEX LX), [11-10](#)
 - 4 L waste container (CytoFLEX), [11-9](#)
 - EMR
 - define, [Abbreviations-1](#)
 - enabling lasers, [5-54](#)
 - 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](#)
 - environmental label
 - RoHS, [9-9](#)
 - exit the software
 - start page operations, [2-2](#)
 - experiment
 - concluding, [5-78](#)
 - sign, [B-16](#)
 - experiment (new)
 - creating, [5-1](#)
 - 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-11](#)
 - experiment related operations
 - electronic record management, [B-5](#)
 - experiment saving, [5-78](#)
 - experiment selection
 - start page, [3-24](#)
 - experiment settings, [2-34](#)
 - experiments
 - to copy, [7-1](#)
 - exporting
 - compensation settings, [5-75](#), [6-19](#)
 - data, [5-64](#)
 - FCS files, [5-70](#)
 - heat map (plate loader), [5-23](#)
 - instrument settings, [5-73](#), [5-74](#)
 - plots of multiple tubes as picture files, [5-73](#)
 - results, [7-9](#)

- statistics table of multiple tubes as a picture file, [5-73](#)
 - user logs, [2-31](#)
- exporting and experiment/template
 - electronic record management, [B-8](#)
- extent
 - autogate, [5-52](#)
- F**
- FAPD
 - define, [Abbreviations-1](#)
- FCS
 - define, [Abbreviations-1](#)
- FCS files
 - exporting, [5-70](#)
- files menu, [2-16](#)
- filling
 - 4 L sheath fluid container (CytoFLEX), [11-6](#)
- filling sheath fluid container, [3-2](#)
- filter
 - optical, [1-5](#)
 - sheath fluid, [1-12](#)
- FITC
 - define, [Abbreviations-1](#)
- flow cell
 - to prime, [11-43](#)
- flow cell waste out
 - fluidic connection, [1-13](#), [3-3](#), [3-5](#)
- Fluid Container holder, [1-10](#)
- Fluid Containers, [1-2](#), [1-3](#), [A-6](#)
- Fluid Containers/Cubitainers
 - component, [1-2](#)
 - fluidics system, [1-11](#)
 - fluidics system component, [1-10](#)
- fluid sensor holder cutout, [1-10](#)
- fluid status information displays red for Sheath and/or Waste even though the sheath fluid container is full and the waste container is empty, [9-12](#)
- fluidic connections
 - flow cell waste out, [1-13](#), [3-3](#), [3-5](#)
 - sheath fluid in, [1-13](#), [3-3](#), [3-5](#)
 - sheath fluid level sensor connector, [1-13](#), [3-3](#), [3-5](#)
 - sheath return, [1-13](#), [3-3](#), [3-5](#)
 - waste level sensor connector, [1-13](#), [3-3](#), [3-5](#)
 - waste out, [1-13](#), [3-3](#), [3-5](#)
- Fluidics module
 - alarm, [1-12](#)
- fluidics module
 - Deep Clean solution bottle, [1-12](#)
 - fluidics system, [1-12](#)
 - fluidics system component, [1-10](#)
 - sheath fluid filters, [1-12](#)
- fluidics system
 - components, [1-10](#)
 - Fluid Containers/Cubitainers, [1-11](#)
 - fluidics module, [1-12](#)
- fluidics system components
 - Fluid Containers/Cubitainers, [1-10](#)
 - fluidics module, [1-10](#)
- fluorescence channels, [5-26](#), [5-27](#)
- folder hierarchy management
 - electronic record management, [B-4](#)
- four-quadrant gates
 - for dual-parameter plots, [2-33](#)
- front cover
 - removal, [11-2](#)
- FSC
 - define, [Abbreviations-1](#)
- functions
 - software, [2-1](#)
- fuse
 - electronic devices, [1-21](#), [1-23](#)
 - to replace, [11-67](#)
- G**
- gain
 - to adjust, [5-55](#)
- gate settings, [2-36](#)
- gates
 - to create, [5-39](#)
- gates for dual-parameter plots
 - four-quadrant gates, [2-33](#)
 - lasso gates, [2-33](#)
 - polygon gates, [2-33](#)
 - rectangle gates, [2-33](#)
- gates for single-parameter plots
 - line-segment gates, [2-33](#)
 - vertical gates, [2-33](#)
- GB
 - define, [Abbreviations-1](#)
- generating
 - compensation matrix, [6-4](#), [6-13](#)
- Ghz
 - define, [Abbreviations-1](#)
- Gr. Wt.
 - define, [Abbreviations-1](#)

graphic and gating styles, [2-32](#)
 graphics
 printing, [5-75](#)

H

H
 define, [Abbreviations-1](#)
 hazard
 laser, [9-1](#)
 hazards
 laser beam, [9-2](#)
 radiation, [9-2](#)
 hazards/precautions, [9-1](#)
 heat map (plate loader)
 creating, [5-15](#)
 deleting, [5-23](#)
 exporting, [5-23](#)
 modifying, [5-22](#)
 refreshing, [5-21](#)
 heat map parameter
 deleting, [5-17](#)
 help menu, [2-22](#)
 histogram overlays
 to create, [7-5](#)
 humidity and temperature
 installation environment validation, [A-4](#)
 Hz
 define, [Abbreviations-1](#)

I

IEC
 define, [Abbreviations-1](#)
 importing
 compensation settings, [5-75](#)
 compensation settings from compensation
 library, [6-17](#)
 compensation settings from compensation
 matrix files, [6-16](#)
 data, [7-1](#)
 instrument settings, [5-73](#), [5-74](#)
 lot-specific target values, [4-6](#)
 previously acquired data, [7-1](#)
 standardization item, [4-26](#)
 importing an experiment/template
 electronic record management, [B-6](#)
 information
 ordering, [1-24](#)
 initializing the instrument, [3-25](#)

inspecting materials
 unpacking, [A-4](#)
 inspection
 liquid flow tubing, [11-42](#)
 pre-boot, [3-1](#)
 inspection when unpacking, [A-4](#)
 installation
 environment validation, [A-2](#)
 instrument, [A-1](#)
 software, [A-12](#)
 installation category, [1-25](#)
 installation options
 CytExpert, [A-12](#)
 installing
 instrument configuration file, [A-21](#)
 Plate Loader module, [11-46](#)
 software, [A-13](#)
 installing the instrument and connecting the
 equipment
 unpacking inspection, [A-5](#), [A-11](#)
 instrument
 components, [1-1](#)
 initializing, [3-25](#)
 inspecting materials, [A-4](#)
 installation, [A-1](#)
 maintenance, [11-1](#)
 parameters, [1-26](#)
 shut off, [8-1](#)
 transportation and storage, [A-1](#)
 unpacking, [A-4](#)
 instrument characteristics, [1-25](#)
 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-21](#)
 instrument dimensions, [1-25](#)
 instrument operations cannot be performed in
 the Acquisition screen, [9-19](#)
 instrument settings
 exporting, [5-74](#)
 importing, [5-74](#)
 importing and exporting, [5-73](#)
 instrument specifications, [1-25](#)
 instrument transport or storage
 to prepare, [10-14](#)
 IR

- define, [Abbreviations-1](#)
 - items
 - adding for standardization, [4-24](#)
 - deleting for standardization, [4-26](#)
 - editing for standardization, [4-25](#)
 - importing for standardization, [4-26](#)
- K**
- kg
 - define, [Abbreviations-1](#)
 - KO
 - define, [Abbreviations-2](#)
- L**
- L
 - define, [Abbreviations-2](#)
 - label
 - RoHS caution, [9-9](#)
 - RoHS environmental, [9-9](#)
 - labels
 - disposal of electrical instrumentation,
 - warning, [9-8](#)
 - RoHS caution, [9-9](#)
 - RoHS environmental, [9-9](#)
 - to set, [5-36](#)
 - language settings, [2-37](#)
 - laser
 - hazards, [9-1](#)
 - settings, [5-53](#)
 - standardizing target values, [4-23](#)
 - target power settings (CytoFLEX LX only), [5-54](#)
 - laser beam
 - hazards, [9-2](#)
 - laser delay
 - to set, [11-63](#)
 - laser delay values are out of range, [9-14](#)
 - laser lines, [1-26](#)
 - laser power is low, [9-13](#)
 - laser target power settings (CytoFLEX LX only), [5-54](#)
 - laser wavelength, [1-26](#)
 - lasers
 - disable, [5-54](#)
 - enable, [5-54](#)
 - lasso gates
 - for dual-parameter plots, [2-33](#)
 - launching the software, [2-1](#)
- LED
 - define, [Abbreviations-2](#)
- Levey-Jennings charts
 - to create, [4-19](#)
- line segment gates
 - for single-parameter plots, [2-33](#)
- liquid flow tubing
 - inspection, [11-42](#)
- liquid flow tubing inspection
 - routine maintenance, [11-42](#)
- LJ
 - define, [Abbreviations-2](#)
- load button
 - electronic devices, [1-21](#), [1-23](#)
- log
 - electronic record management, [B-11](#)
 - user management operation, [2-31](#)
- log in
 - software, [3-6](#)
- log menu, [2-21](#)
- logs
 - exporting, [2-31](#)
 - viewing, [2-31](#)
- lot-specific target values
 - to import, [4-6](#)
- LWH
 - define, [Abbreviations-2](#)
- M**
- m
 - define, [Abbreviations-2](#)
 - main components, [1-2](#)
 - maintenance
 - instrument, [11-1](#)
 - See also* routine maintenance; nonscheduled maintenance
 - maintenance reminder
 - to manage, [11-14](#)
 - managing
 - compensation library, [6-20](#)
 - maintenance reminder, [11-14](#)
 - manual
 - adjust compensation, [6-16](#)
 - manual injection mode
 - running small sample volumes, [3-9](#)
 - material safety data sheets (MSDS/SDS)
 - how to order, [1-24](#)
 - MB
 - define, [Abbreviations-2](#)

- menu
 - account, 2-21
 - acquisition and analysis screen, 2-15
 - advanced, 2-20
 - cytometer, 2-17
 - files, 2-16
 - help, 2-22
 - log, 2-21
 - 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, 9-19
- mixer settings
 - to change, 11-71
- mL
 - define, [Abbreviations-2](#)
- mm
 - define, [Abbreviations-2](#)
- modifying
 - heat map (plate loader), 5-22
 - user roles, 2-29
 - users, 2-25
 - well settings with plate loader, 5-11
- modifying well settings, 5-11
 - applying existing well settings to additional wells, 5-12
 - moving well location [with plate loader], 5-13
- movement
 - adjusting for autogates, 5-51
 - autogate, 5-51
- moving
 - well location [with plate loader], 5-13
- MSDS (material safety data sheets)
 - how to order, 1-24
- multiple tube plots
 - export as picture files, 5-73
- multiple tube statistics table
 - export as picture files, 5-73
- mute
 - alarm, 1-12
 - mute alerter icon, 1-12
- mW
 - define, [Abbreviations-2](#)
- N**
- 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, 6-4
- new experiment
 - creating, 5-1
- new experiment with plate loader
 - creating, 5-3
- nm
 - define, [Abbreviations-2](#)
- no changes occurred after manually adjusting
 - compensation settings, 9-16
- no data acquisition, 9-15
- nonscheduled cleaning, 10-13
- nonscheduled maintenance
 - adding the Deep Clean solution, 11-17
 - calibrating the sample flow rate, 11-56
 - calibrating the sample flow rate with plate loader, 11-59
 - cleaning the sample probe, 10-8
 - preparing the instrument for storage or transport, 10-14
 - replacing the fuse, 11-67
 - replacing the optical filter, 11-64
- nonscheduled replacement/adjustment, 11-56
- Nt. Wt.
 - define, [Abbreviations-2](#)
- O**
- open a compensation experiment
 - start page operations, 2-2
- open an experiment
 - start page operations, 2-2
- open software, 3-6
- opening

- analysis screen, 7-3
 - 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, 1-9
 - optical filter, 1-5
 - to replace, 11-64
 - ordering
 - customer-replaceable parts, D-1
 - ordering information, 1-24
 - overlay dot plots, 2-32
 - overlay histograms, 2-32
- P**
- page setup settings, 2-36
 - parameter (heat map)
 - delete, 5-17
 - parameters
 - editing standardization items, 4-25
 - instrument, 1-26
 - password
 - change, 2-26
 - default, 2-23, A-24
 - reset, 2-26
 - 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, 1-28
 - sample injection mode control knob, C-1
 - performing
 - daily quality control, 4-1
 - PI
 - define, [Abbreviations-2](#)
 - picture files
 - to export, 5-73
 - plate holder
 - components, 1-18
 - plate holder with plate loader
 - replacing, 11-45
 - plate loader
 - components, 1-16
 - Plate Loader module
 - installing, 11-46
 - removing, 11-46
 - plate loader PEEK tubing
 - replacing, 11-28
 - plate loader PEEK tubing (replacing)
 - routine maintenance, 11-28
 - plate position [with plate loader]
 - calibrating, 11-73
 - plot area
 - acquisition screen, 2-8
 - plot display conditions
 - to set, 5-60
 - plot settings, 2-35
 - plots
 - dual-parameter, 2-32
 - overlay dot plots, 2-32
 - overlay histograms, 2-32
 - single-parameter, 2-32
 - to create, 5-39
 - to set, 7-3
 - PN
 - define, [Abbreviations-2](#)
 - policies
 - account, 2-30
 - pollution degree, 1-25
 - polygon gates
 - for dual-parameter plots, 2-33
 - population amplitude is decreasing and CV
 - values are increasing, 9-14
 - populations are drifting, 9-13
 - position
 - sample tube holder, 1-15
 - power source
 - installation environment validation, A-3
 - power source inspection
 - pre-boot, 3-5
 - power switch
 - electronic devices, 1-21, 1-23
 - pre-boot inspection, 3-1
 - check waste and reagent levels (10 L fluid

- cubitainers), [3-4](#)
 - check waste and reagent levels (4 L fluid containers), [3-2](#)
 - power source inspection, [3-5](#)
 - workstation connections inspection, [3-5](#)
- precaution
 - disposal, [9-9](#)
- precautions
 - safety, [-x](#)
- precautions/hazards, [9-1](#)
- preparation process
 - QC sample (CytoFLEX Daily QC Fluorospheres), [4-3](#)
 - QC sample (CytoFLEX Daily QC Fluorospheres) with plate loader, [4-4](#)
 - QC sample (CytoFLEX IR Daily QC Fluorospheres), [4-3](#)
 - QC sample (CytoFLEX IR Daily QC Fluorospheres) with plate loader, [4-5](#)
- preparing
 - compensation sample, [6-4](#), [6-13](#)
 - Deep Clean solution, [11-17](#)
 - instrument for storage or transport, [10-14](#)
 - QC sample, [4-3](#)
 - QC sample with plate loader, [4-4](#)
 - the cleaning solution, [8-1](#)
- preparing QC sample
 - required materials, [4-3](#)
- preparing QC sample with plate loader
 - required materials, [4-4](#)
- previously acquired data
 - to import, [7-1](#)
- previously acquired experiment
 - to copy, [7-1](#)
- priming
 - flow cell, [11-43](#)
- printing
 - graphics, [5-75](#)
- procedure
 - Deep Clean, [10-8](#)
- product description, [1-1](#)

Q

- QC
 - applying standardization, [4-26](#)
 - define, [Abbreviations-2](#)
 - laser target values, [4-23](#)
 - result manager, [4-21](#)
- QC aborted due to low event rate, [9-20](#)

- QC data
 - collecting, [4-10](#)
- QC data with plate loader
 - collecting, [4-12](#)
- QC experiment screen, [2-12](#), [2-13](#)
- QC failed, [9-21](#)
- QC menu tree
 - software, [2-15](#)
- QC report screen, [2-12](#)
- QC sample
 - preparation, [4-3](#)
- QC sample (CytoFLEX Daily QC Fluorospheres)
 - preparation process, [4-3](#)
- QC sample (CytoFLEX Daily QC Fluorospheres) with plate loader
 - preparation process, [4-4](#)
- QC sample (CytoFLEX IR Daily QC Fluorospheres)
 - preparation process, [4-3](#)
- QC sample (CytoFLEX IR Daily QC Fluorospheres) with plate loader
 - preparation process, [4-5](#)
- QC sample with plate loader
 - preparation, [4-4](#)
- QC screen
 - navigation, [2-13](#)
- QC software menu tree, [2-15](#)
- QC/Standardization menu, [2-20](#)
- quality control
 - perform daily, [4-1](#)
- quality control (QC)
 - define, [4-1](#)

R

- radiation
 - hazards, [9-2](#)
- RAM
 - define, [Abbreviations-2](#)
- rCV
 - define, [Abbreviations-2](#)
- reagent levels (10 L fluid cubitainers)
 - check, [3-4](#)
- reagent levels (4 L fluid containers)
 - check, [3-2](#)
- rectangle gates
 - for dual-parameter plots, [2-33](#)
- refreshing
 - heat map (plate loader), [5-21](#)
- reinstall

- CytExpert software, [A-29](#)
 - reinstallation procedures
 - right-side cover, [11-5](#)
 - removal procedures
 - front cover, [11-2](#)
 - right-side cover, [11-4](#)
 - removing
 - Plate Loader module, [11-46](#)
 - rename
 - experiment directory (electronic record management), [B-4](#)
 - replacement
 - nonscheduled, [11-56](#)
 - routine, [11-2](#)
 - replacing
 - 10 L sheath fluid cubitainer, [11-7](#)
 - fuse, [11-67](#)
 - optical filter, [11-64](#)
 - plate holder with plate loader, [11-45](#)
 - plate loader PEEK tubing, [11-28](#)
 - sample peristaltic pump tubing for sample loading, [11-22](#)
 - sheath fluid filter, [11-18](#)
 - sheath fluid harness and/or waste harness, [11-69](#)
 - replacing sheath fluid cubitainer, [3-4](#)
 - replacing the peristaltic pump for sample loading
 - routine maintenance, [11-22](#)
 - replacing the sample probe for plate loader
 - routine maintenance, [11-28](#)
 - replacing the sheath fluid filter
 - routine maintenance, [11-18](#)
 - required materials
 - QC sample preparation, [4-3](#)
 - QC sample preparation with plate loader, [4-4](#)
 - software installation, [A-13](#)
 - reset
 - password, [2-26](#)
 - restore
 - database, [9-23](#)
 - result manager
 - QC, [4-21](#)
 - results
 - confirming, [4-16](#)
 - to export, [7-9](#)
 - retract
 - electronic signature, [B-17](#)
 - RH
 - define, [Abbreviations-2](#)
 - right-side cover
 - reinstallation, [11-5](#)
 - removal, [11-4](#)
 - RoHS
 - caution label, [9-9](#)
 - define, [Abbreviations-2](#)
 - environmental label, [9-9](#)
 - role management, [2-26](#)
 - roles
 - creating, [2-28](#)
 - deleting, [2-29](#)
 - modifying, [2-29](#)
 - routine cleaning, [10-1](#)
 - routine maintenance
 - cleaning the 4 L sheath fluid container, [10-10](#)
 - cleaning the 4 L waste container, [10-11](#)
 - cleaning the sample station, [10-7](#)
 - daily startup and shutdown, [10-1](#)
 - daily startup and shutdown with plate loader, [10-5](#)
 - Deep Clean, [10-8](#)
 - liquid flow tubing inspection, [11-42](#)
 - replacing the plate loader PEEK tubing, [11-28](#)
 - replacing the sample peristaltic pump tubing for sample loading, [11-22](#)
 - replacing the sheath fluid filter, [11-18](#)
 - routine replacement/adjustment, [11-2](#)
 - RPTM
 - define, [Abbreviations-2](#)
 - running
 - single positive control samples, [6-6](#)
 - system startup program, [3-14](#)
 - system startup program with plate loader, [3-17](#)
 - running small sample volumes
 - manual injection mode, [3-9](#)
- ## S
- S/N
 - define, [Abbreviations-2](#)
 - safety
 - precautions, [-x](#)
 - sample
 - checks before running, [5-24](#)
 - sample acquisition position
 - sample tube holder position, [1-15](#)
 - sample flow rate

- calibrating, [11-56](#)
- sample flow rate is unstable, [9-12](#)
- sample flow rate with plate loader
 - calibrating, [11-59](#)
- sample injection control knob
 - performance characteristics, [C-1](#)
- sample injection mode
 - selecting, [3-9](#)
- sample injection mode control kit
 - components, [C-2](#)
- sample is flowing, but no signal appears in the plot, [9-17](#)
- sample loading
 - backup, [3-9](#)
- sample loading position
 - sample tube holder position, [1-15](#)
- sample peristaltic pump tubing
 - to replace, [11-22](#)
- sample peristaltic pump tubing for sample loading
 - to replace, [11-22](#)
- sample peristaltic pump tubing for sample loading (replacing)
 - routine maintenance, [11-22](#)
- sample probe, [1-14](#)
 - sample station, [1-14](#)
 - to clean, [10-8](#)
- sample probe change
 - from plate loader to single tube sample station, [11-39](#)
 - from single tube sample station to plate loader, [11-34](#)
- sample probe for plate loader
 - replacing, [11-28](#)
- sample probe is too low, [9-18](#)
- sample probe switch
 - from plate loader to single tube sample station, [C-4](#)
 - from single tube sample station to plate loader, [C-2](#)
- sample station, [1-14](#)
 - sample probe, [1-14](#)
 - sample tube holder, [1-14](#)
 - to clean, [10-7](#)
 - washer station and mixer, [1-14](#)
- sample station (cleaning)
 - routine maintenance, [10-7](#)
- sample tube holder
 - sample station, [1-14](#)
- sample tube holder cannot move up and down
 - automatically, [9-12](#)
- sample tube holder position
 - sample acquisition position, [1-15](#)
 - sample loading position, [1-15](#)
 - standby position, [1-15](#)
- sample wells with plate loader
 - setting, [5-7](#)
- sampling
 - data, [5-32](#)
- sampling flow rate is too fast, [9-13](#)
- saving
 - experiment, [5-78](#)
- screen
 - acquisition, [2-3](#)
 - analysis, [2-9](#), [7-1](#), [7-3](#)
 - compensation experiment, [2-10](#)
 - QC experiment, [2-12](#), [2-13](#)
 - QC report, [2-12](#)
 - software, [2-1](#)
- SDS (safety data sheets)
 - how to order, [1-24](#)
- selecting
 - detector configuration, [5-25](#)
 - proper sample injection mode, [3-9](#)
- setting
 - channel, [5-36](#)
 - channels, [5-36](#)
 - collection conditions, [5-58](#)
 - custom statistics, [5-62](#)
 - customized parameters, [5-61](#)
 - labels, [5-36](#)
 - laser delay, [11-63](#)
 - laser target power settings (CytoFLEX LX only), [5-54](#)
 - plot display conditions, [5-60](#)
 - sample wells with sample wells, [5-7](#)
 - the plots and statistics, [7-3](#)
- setting up
 - violet side scatter channel (VSSC), [5-29](#)
- settings
 - acquisition, [5-36](#)
 - experiment, [2-34](#)
 - gate, [2-36](#)
 - language, [2-37](#)
 - laser, [5-53](#)
 - page setup, [2-36](#)
 - plot, [2-35](#)
 - software, [2-34](#)
 - tube, [2-35](#)
- settings menu, [2-19](#)

- setup
 - CytExpert API test client, [2-37](#)
 - experiment directory (electronic record management), [B-3](#)
- sheath fluid container, [1-10](#)
 - to fill, [3-2](#)
- sheath fluid container 4 L (CytoFLEX)
 - filling, [11-6](#)
- sheath fluid container/cubitainer, [1-11](#)
- sheath fluid cubitainer
 - to replace, [3-4](#)
- sheath fluid cubitainer 10 L
 - replacing, [11-7](#)
- sheath fluid filter
 - to replace, [11-18](#)
- sheath fluid filter (replacing)
 - routine maintenance, [11-18](#)
- sheath fluid filters
 - fluidics module, [1-12](#)
- sheath fluid harness and sensor, [1-10](#), [1-11](#)
- sheath fluid in
 - fluidic connection, [1-13](#), [3-3](#), [3-5](#)
- sheath fluid level sensor connector
 - fluidic connection, [1-13](#), [3-3](#), [3-5](#)
- sheath return
 - fluidic connection, [1-13](#), [3-3](#), [3-5](#)
- shut off
 - the instrument, [8-1](#)
- shutdown
 - automatic, [5-6](#), [8-2](#), [10-6](#)
- shutdown daily
 - routine maintenance, [10-1](#)
- shutdown daily with plate loader
 - routine maintenance, [10-5](#)
- sign
 - experiment, [B-16](#)
- signature menu, [2-22](#)
- single positive control samples
 - to run, [6-6](#)
- single-parameter plots, [2-32](#)
- SNR
 - define, [Abbreviations-2](#)
- software
 - functions, [2-1](#)
 - launching, [2-1](#)
 - log in, [3-6](#)
 - main screen, [2-1](#)
 - open, [3-6](#)
 - to install, [A-13](#)
 - to start, [A-24](#)
- software installation, [A-12](#)
 - installing CytExpert software, [A-13](#)
 - installing instrument configuration file, [A-21](#)
 - required materials, [A-13](#)
 - starting the software, [A-24](#)
- software installation fails, [9-19](#)
- 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, [2-34](#)
- specifications
 - characteristics, [1-25](#)
 - instrument, [1-25](#)
- SSC
 - define, [Abbreviations-2](#)
- standardization, [4-22](#)
 - applying in QC, [4-26](#)
 - laser target values, [4-23](#)
- standardization item
 - adding, [4-24](#)
 - deleting, [4-26](#)
 - importing, [4-26](#)
- standardization item parameters
 - editing, [4-25](#)
- standby position
 - sample tube holder position, [1-15](#)
- start page
 - main software screen, [2-2](#)
 - selecting experiments, [3-24](#)
- 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, [8-2](#)

- startup daily
 - routine maintenance, 10-1
 - startup daily with plate loader
 - routine maintenance, 10-5
 - station
 - sample, 1-14
 - statistics
 - to set, 7-3
 - status bar
 - acquisition screen, 2-9
 - storing the instrument, A-1
 - surface
 - cleaning and disinfection, 10-13
 - switching sample probe
 - from plate loader to single tube sample station, C-4
 - from single tube sample station to plate loader, C-2
 - symbols
 - define, -xi
 - system configuration, 1-20
 - system operation log
 - electronic record management, B-14
 - system startup program
 - to run, 3-14
 - system startup program with plate loader running, 3-17
- T**
- table
 - troubleshooting, 9-10
 - target power settings (CytoFLEX LX only)
 - laser, 5-54
 - target values
 - laser standardization, 4-23
 - temperature and humidity
 - installation environment validation, A-4
 - test tubes
 - acquisition screen, 2-7
 - threshold
 - to adjust, 5-57
 - transporting the instrument, A-1
 - troubleshooting, 9-1
 - alarm does not sound when the waste container is full or the sheath fluid container is low and the software status display is red, 9-11
 - calculation of the automatic compensation experiment is incorrect, 9-17
 - concentration calculation is incorrect, 9-18
 - connection indicator light in the lower left corner of the software screen is red and displays Disconnected and Error, 9-10
 - Cytometer cannot be turned on, 9-10
 - data populations are normal on one laser, but too low on another laser, 9-15
 - data populations are not where they are expected, 9-16
 - fluid status information displays red for Sheath and/or Waste even though the sheath fluid container is full and the waste container is empty, 9-12
 - instrument operations cannot be performed in the Acquisition screen, 9-19
 - laser delay values are out of range, 9-14
 - laser power is low, 9-13
 - mixer is not functioning, 9-19
 - no changes occurred after manually adjusting compensation settings, 9-16
 - no data acquisition, 9-15
 - population amplitude is decreasing and CV values are increasing, 9-14
 - populations are drifting, 9-13
 - QC aborted due to low event rate, 9-20
 - QC failed, 9-21
 - sample flow rate is unstable, 9-12
 - sample is flowing, but no signal appears in the plot, 9-17
 - sample probe is too low, 9-18
 - sample tube holder cannot move up and down automatically, 9-12
 - sampling flow rate is too fast, 9-13
 - software installation fails, 9-19
 - table, 9-10
 - wash station drips during backflush, 9-18
 - Workstation cannot be turned on, 9-10
 - tube
 - settings, 2-35
 - tube name
 - to change, 5-36
 - turn off
 - auto recalculate, 5-50
 - turn on
 - auto recalculate, 5-50
 - turning the power on, 3-6
- U**
- unlock

- user account, 2-26
 - 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, 6-4
 - upgrade
 - CytExpert software, A-24
 - USB
 - define, [Abbreviations-2](#)
 - user account
 - unlock, 2-26
 - user management, 2-22
 - electronic record management, B-18
 - user management operation
 - log, 2-31
 - user management operation log
 - electronic record management, B-15
 - user roles
 - creating, 2-28
 - deleting, 2-29
 - modifying, 2-29
 - users
 - creating, 2-24
 - deleting, 2-25
 - modifying, 2-25
 - using unstained samples, 6-4
 - USPTO
 - define, [Abbreviations-2](#)
 - 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, 5-25
 - vertex
 - add to auto polygon gate, 5-50
 - vertical gates
 - for single-parameter plots, 2-33
- viewing
 - user logs, 2-31
- violet side scatter channel (VSSC)
 - to set up, 5-29
- VSSC
 - define, [Abbreviations-2](#)
- W**
- W
 - define, [Abbreviations-2](#)
- wash station and mixer
 - sample station, 1-14
 - wash station drips during backflush, 9-18
 - waste container, 1-10
 - waste container 4 L (CytoFLEX)
 - emptying, 11-9
 - waste container/cubitainer, 1-11
 - waste cubitainer 10 L (CytoFLEX LX)
 - emptying, 11-10
 - waste disposal, A-4
 - waste fluid harness and sensor, 1-10, 1-11
 - waste harness
 - to replace, 11-69
 - waste level sensor connector
 - fluidic connection, 1-13, 3-3, 3-5
 - waste levels (10 L fluid cubitainers)
 - check, 3-4
 - waste levels (4 L fluid containers)
 - check, 3-2
 - waste out
 - fluidic connection, 1-13, 3-3, 3-5
 - wavelength
 - laser, 1-26
 - wavelength division multiplexer (WDM), 1-5
 - WDM
 - define, [Abbreviations-2](#)
 - well settings with plate loader
 - modifying, 5-11
 - copying
 - wells, 5-12
 - cutting
 - wells, 5-12
 - modifying well settings
 - copying wells, 5-12
 - cutting wells, 5-12
 - pasting wells, 5-12
 - pasting
 - wells, 5-12
 - running

- samples, [5-13](#)
- sample, [5-13](#)
- sample injection mode
 - selecting, [3-12](#)
- selecting
 - proper sample injection mode, [3-12](#)
- Workstation, [1-2](#), [1-3](#), [A-6](#)
 - component, [1-2](#)
- Workstation cannot be turned on, [9-10](#)
- workstation connections inspection
 - pre-boot, [3-5](#)
- worktable
 - installation environment validation, [A-2](#)

Beckman Coulter, Inc.

Customer End User License Agreement

This Product contains software that is owned by Beckman Coulter, Inc. or its suppliers and is protected by United States and international copyright laws and international trade provisions. You must treat the software contained in this Product like any other copyrighted material. This license and your right to use the Product terminate automatically if you violate any part of this agreement.

This is a license agreement and not an agreement for sale. Beckman Coulter hereby licenses this Software to you under the following terms and conditions:

You May:

1. Use this software in the computer supplied to you by Beckman Coulter;
2. Maintain one copy of this software for backup purposes (the backup copy shall be supplied by Beckman Coulter);
3. After written notification to Beckman Coulter, transfer the entire Product to another person or entity, provided you retain no copies of the Product software and the transferee agrees to the terms of this license agreement.

You May Not:

1. Use, copy or transfer copies of this Software except as provided in this license agreement;
2. Alter, merge, modify or adapt this Software in any way including disassembling or decompiling;
3. Loan, rent, lease, or sublicense this Software or any copy.

Limited Warranty

Beckman Coulter warrants that the software will substantially conform to the published specifications for the Product in which it is contained, provided that it is used on the computer hardware and in the operating system environment for which it was designed. Should the media on which your software arrives prove defective, Beckman Coulter will replace said media free of charge within 90 days of delivery of the Product. This is your sole remedy for any breach of warranty for this software.

Except as specifically noted above, Beckman Coulter makes no warranty or representation, either expressed or implied, with respect to this software or its documentation including quality, performance, merchantability, or fitness for a particular purpose.

No Liability for Consequential Damages

In no event shall Beckman Coulter or its suppliers be liable for any damages whatsoever (including, without limitation, damages for loss of profits, business interruption, loss of information, or other pecuniary loss) arising out of the use of or inability to use the Beckman Coulter Product software. Because some states do not allow the exclusion or limitation of liability for consequential damages, the above limitation might not apply to you.

General

This agreement constitutes the entire agreement between you and Beckman Coulter and supersedes any prior agreement concerning this Product software. It shall not be modified except by written agreement dated subsequent to the date of this agreement signed by an authorized Beckman Coulter representative. Beckman Coulter is not bound by any provision of any purchase order, receipt, acceptance, confirmation, correspondence, or otherwise, unless Beckman Coulter specifically agrees to the provision in writing. This agreement is governed by the laws of the State of California.

Related Documents

Operating Instructions

PN B49006

- Introduction
- System Overview
- Using the CytExpert Software
- Daily Startup
- Instrument Quality Control and Standardization
- Data Acquisition and Sample Analysis
- Compensation
- Data Review
- Daily Shutdown
- Troubleshooting
- Cleaning Procedures
- Replacement/Adjustment Procedures
- Instrument Installation
- CytExpert Electronic Record Management
- Sample Injection Mode Control Kit
- Table of Hazardous Substances
- Abbreviations

CytoFLEX Flow Cytometer Quick Start Guide

PN B49008

CytoFLEX Setup Guide

PN B53767

www.beckmancoulter.com

