BD LSRFortessa™ X-30
BD LSRFortessa™ X-50
BD LSRFortessa™ X-30 Dual
BD FACSymphony™ A3
BD FACSymphony™ A5
BD FACSymphony™ A3 Dual
Flow Cytometers User's Guide

For Research Use Only

bdbiosciences.com
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Class 1 Laser Product

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

FCC information

WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

NOTICE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense. Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits. This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations. Cet appareil numérique de la classe A respecte toutes les exigences du Réglement sur le matériel brouilleur du Canada.

Compliance information

NOTICE: This laboratory equipment has been tested and found to comply with the EMC and the Low Voltage Directives. This includes FCC, Part 15 compliance for a Class A Digital Device.

CAUTION: Any unauthorized modifications to this laboratory equipment may affect the Regulatory Compliance items stated above.

History

Revision	Date	Change made
23-17876-00	8/2017	Initial release

Contents

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About this guide

This chapter covers the following topics:

What this guide covers

This guide describes the procedures necessary to operate and maintain the following Special Order Research Product (SORP) flow cytometers:

- BD LSRFortessa™ X-30
- BD LSRFortessa™ X-50
- BD LSRFortessa™ X-30 Dual
- BD FACSymphony™ A3
- BD FACSymphony™ A5
- BD FACSymphony™ A3 Dual

BD Biosciences changed the names of the BD LSRFortessa X-30, X-50, and X-30 Dual flow cytometer models to BD FACSymphony A3, A5, and A3 Dual, respectively.

Cytometer old name	Cytometer new name
BD LSRFortessa X-30	BD FACSymphony A3
BD LSRFortessa X-50	BD FACSymphony A5
BD LSRFortessa X-30 Dual	BD FACSymphony A3 Dual

Because many cytometer functions are controlled by BD FACSDiva™ software, this guide also contains information about software features required for basic cytometer setup and operation.

This guide assumes you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, see the documentation provided with your computer.

Conventions

Introduction

The following table lists the safety symbols used in this guide to alert you to potential hazards.

Safety symbols

Symbol	Meaning
	Caution alert Identifies a hazard or unsafe practice that could result in data loss, material damage, minor injury, severe injury, or death
	Biological hazard
	Electrical hazard
	Laser hazard

About the documentation

Introduction

This topic describes the documentation available with the BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual flow cytometers.

The following list includes the available documentation for the system.

- *BD FACSDiva Software Reference Manual:* Includes instructions or descriptions for installation and setup, workspace components, acquisition controls, analysis tools,

and data management. Access this manual from the BD FACSDiva Software Help menu (Help > Documentation > Reference Manual), or by double-clicking the shortcut on the desktop.

- *BD Cytometer Setup and Tracking Application Guide:* Describes how to use the BD™ Cytometer Setup and Tracking (CS&T) features in BD FACSDiva software.
- The *BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual Flow Cytometers Site Preparation Guide:* Contains specifications for:
  - Cytometer weight and size
  - Temperature and other environmental requirements
  - Electrical requirements
- The *BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual Flow Cytometers Safety and Limitations:* Provides descriptions of safety and warning labels, general system hazards, specific risks, and laser, electrical, and biological hazards.
- *BD High Throughput Sampler User's Guide:* Describes how to set up and operate the BD™ High Throughput Sampler (HTS) option. It also contains a description of BD FACSDiva software features specific to the HTS.
- *BD FACSFlow™ Supply System User's Guide:* Describes the optional automated sheath and waste fluid control system.

Publication formats

This guide is provided in PDF format to provide an eco-friendly option.

Instrument technical support

Introduction

This topic describes how to get technical assistance.

Contacting technical support

If technical assistance is required, contact your local BD Biosciences customer support representative or supplier. Visit our website bdbiosciences.com for up-to-date contact information.

When contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number
- Version of BD FACSDiva software you are using
- Any error messages
- Details of recent system performance

More information

- What this guide covers (page 10)

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Introduction

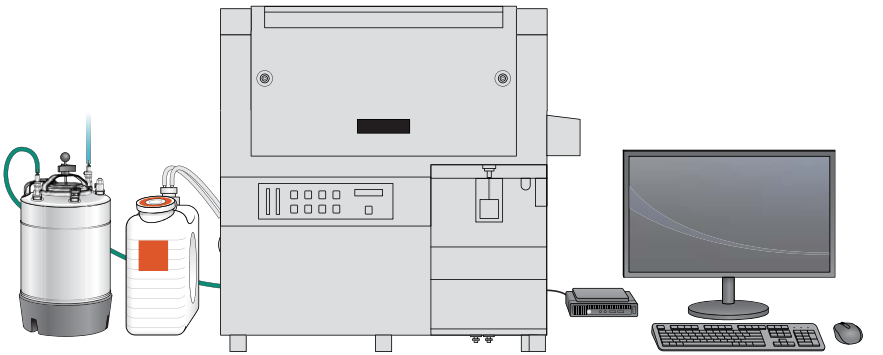
This chapter covers the following topics:

System overview

About the system

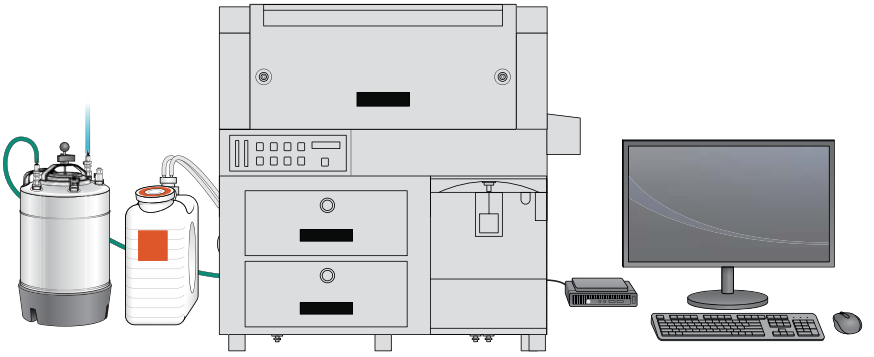
The BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual system includes the flow cytometer, BD FACSDiva software v8.0.1.1 running on the system workstation, the optional BD FACSFlow™ supply system (FFSS), and the optional BD High Throughput Sampler (HTS). Each component is described in detail in the following sections.

BD FACSymphony A3 system



Number	Components
1	Sheath and waste tanks
2	BD FACSymphony A3 flow cytometer
3	Computer workstation

BD FACSymphony A5 system



Number	Components
1	Sheath and waste tanks
2	BD FACSymphony A5 flow cytometer
3	Computer workstation

BD FACSymphony A3 Dual system

1 2 3

Number	Components
1	Sheath and waste tanks
2	BD FACSymphony A3 Dual flow cytometer
3	Computer workstation

Cytometer overview

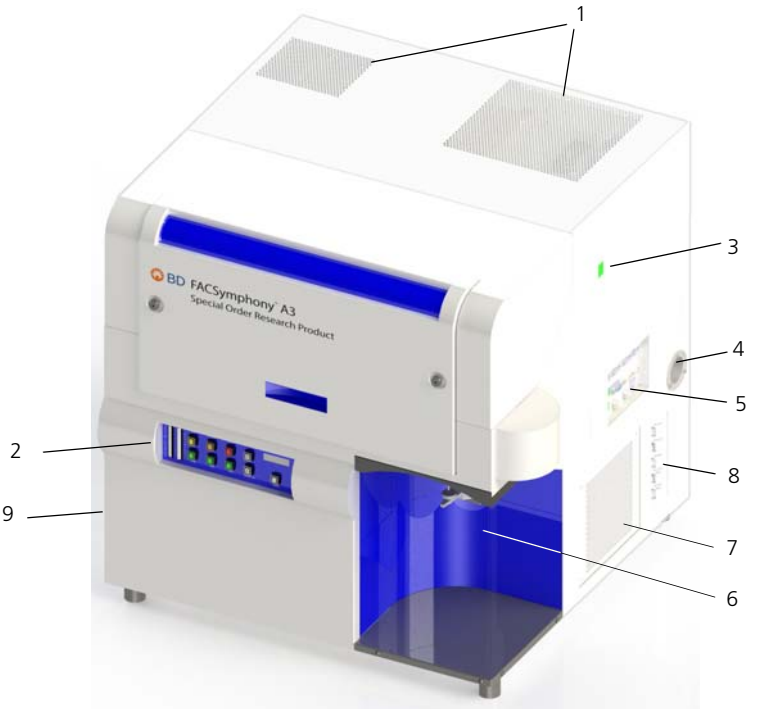
Introduction

The BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual flow cytometers are air-cooled multi-laser benchtop flow cytometers with the ability to acquire parameters for a large number of colors. They use fixed-alignment lasers that transmit light through a flow cell to polygon/decagon detector arrays. These detectors collect and translate the resulting fluorescence signals into electronic signals. Cytometer electronics convert these signals into digital data.

Components

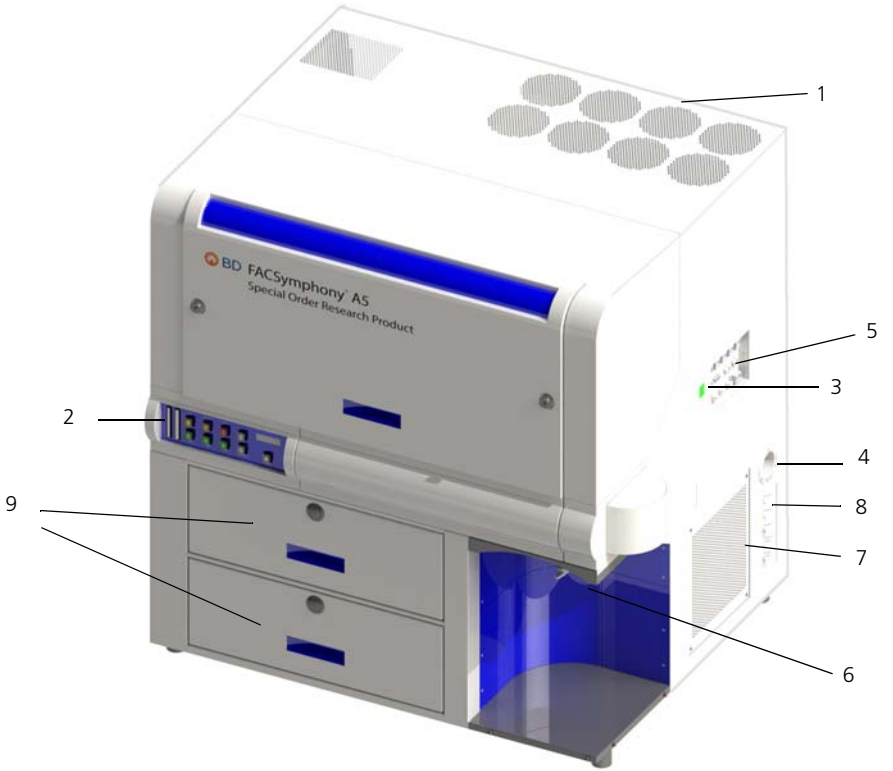
The following figures show the main components of the each instrument. Each component is described in detail in the following sections.

BD FACSymphony A3



Number	Component	Number	Component
1	Heat ventilation slots (top)	6	Sample injection port (SIP)
2	Control panel	7	Heat ventilation slots (side)
3	Power button	8	Air and fluidic ports
4	Electrical plug	9	Optics access door (polygon/ decagon detector arrays) left side
5	Fluidic sensors		

BD FACSymphony A5



Number	Component	Number	Component
1	Heat ventilation slots (top)	6	Sample injection port (SIP)
2	Control panel	7	Heat ventilation slots (side)
3	Power button	8	Air and fluidic ports
4	Electrical plug	9	Optics access doors (polygon/ decagon detector arrays)
5	Fluidic sensors		

BD FACSymphony A3 Dual

Number	Component	Number	Component
1	Heat ventilation slots (top)	6	Sample injection port (SIP)
2	Control panels	7	Heat ventilation slots (side)
3	Power button	8	Air and fluidic ports
4	Electrical plug	9	Optics access door (polygon/ decagon detector arrays)
5	Fluidic sensors		

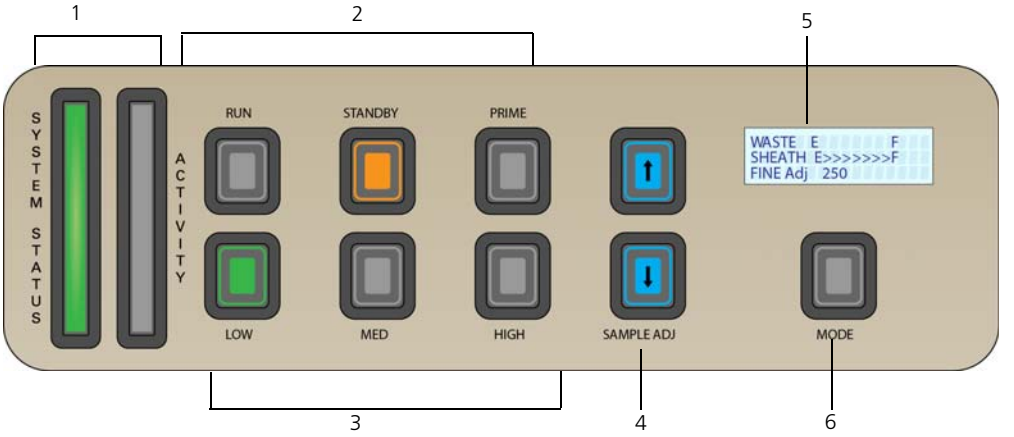
Caution! Do not place any objects on top of the instrument. Blocking the ventilation may cause the instrument to overheat.

Caution: Electrical Hazard! Do not place liquids on top of the instrument. Any spill of liquid into the ventilation openings could cause electrical shock or damage to the instrument.

Control panel

Overview

The following figure shows the components in the control panel, which are listed in the table.



Number	Component
1	System indicators
2	Fluid control buttons
3	Sample flow rate buttons

Number	Component
4	Sample fine adjust buttons
5	Status screen
6	Mode button

More information

- Fluidics system (page 23)
- Optics (page 31)

Fluidics system

Introduction

The fluidics system carries the sample out of the sample tube and into the sensing region of the flow cell. Cells are carried in the sample core stream in single file and measured individually.

System indicators

There are two system indicators (System status and Activity) on the control panel.

- **System status.** Shows the status of the sheath and waste tank levels. The following table describes the LED indicators, conditions that trigger them, and any action that must be taken.

LED color	Status	Action
Green	Good	None
Yellow	**Caution!** Sheath and waste tanks need attention.	Check tank levels
Red	**Caution!** Take immediate action.	• Empty waste tank • Fill sheath tank

System status is also displayed on the Status screen. See Status screen (page 26) for a description of the Status screen.

- **Activity.** Shows whether the cytometer power is on and the status of acquisition. The following table describes the indicator LEDs, and the status that triggers them.

Indicator	LED color	Status
Steady pulse	blue	Cytometer is powered on.
Fluctuates	blue	Cells are passing through the flow cell.

Fluid control

The three fluid control buttons (Run, Standby, and Prime) set the cytometer operation.

- **Run.** Pressurizes the sample tube to transport the sample through the sample injection tube and into the flow cell.

  The Run button is green when the sample tube is on and the support arm is centered. When the tube support arm is moved left or right to remove a sample tube, the cytometer switches to an automatic standby status to conserve sheath fluid, and the Run button changes to orange.

- **Standby.** Stops fluid flow to conserve sheath fluid.

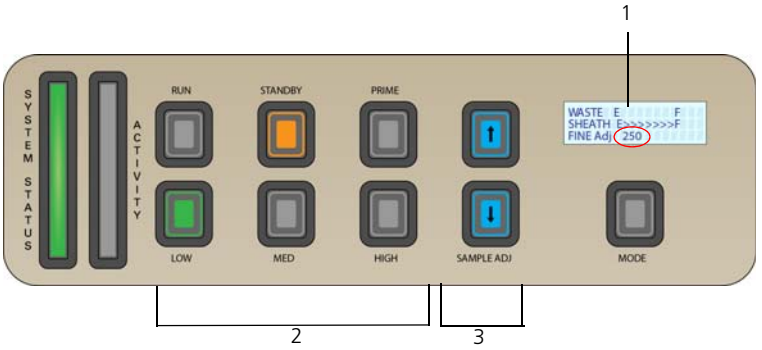
  When you leave the cytometer for more than a few minutes, place a tube containing 1 mL of deionized (DI) water on the sample injection port (SIP) and press Standby.

- **Prime.** Prepares the fluidics system by draining and filling the flow cell with sheath fluid.

  The fluid flow initially stops, and pressure is reversed to force fluid out of the flow cell and into the waste container. After a preset time, the flow cell fills with sheath fluid at a controlled rate to prevent bubble formation or entrapment. At completion, the cytometer switches to standby mode.

Sample flow rate control

The three flow rate control buttons (LOW, MED, HIGH) set the sample flow rate through the flow cell. The SAMPLE ADJ buttons allow you to adjust the rate to intermediate levels.



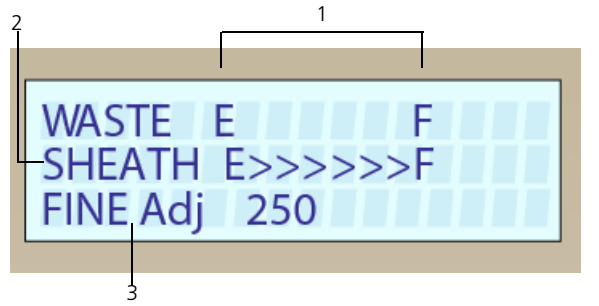
Number	Component
1	Status screen
2	Sample flow rate buttons
3	Sample fine adjust buttons

When sample adj is set to 250 (as shown on the status screen on the control panel) the sample flow rates at the low, med, and high settings are approximately 12, 35, and 60 µL/min of sample, respectively. Each time you press one of the SAMPLE ADJ buttons, the fine adjust of the indicated sample increases or decreases by 10. The following table shows the approximate sample flow rate range for low, medium, and high.

Settings	Sample flow rate (µL/min)
Low	6–24
Med	17.5–70
High	30–120

Status screen

The first two lines of the status screen show the level of the waste and sheath tanks. The third line toggles between three different displays and is described in detail in the following table.



Line	Definition
1	**Waste level.** Shows range (1) from E (empty) to F (full). The display line increases from left to right in sequences of 20%. System status turns yellow at 80%, and red at 100% full.
2	**Sheath level.** Shows range from E to F. The display line decreases from right to left in sequences of 20% from full level. System status turns yellow at 20%, and red at 0%.
3	**Fine Adj.** Shows the current setting of fine adjust. Fine adjust can be set in increments of 10 from 0 to 500. The last value persists, even after cytometer shutdown.
	Air pressure. Shows the air pressure as either greater than (>) 5.4 psi, or less than (<) 5 psi. To display psi, press the Mode button, followed by the Up button.
4	**FFSS Mode.** Shows that the cytometer is in FFSS mode. To enter this mode, press the MODE button 4 times. After you reach FFSS OFF, push the SAMPLE ADJ button once. You should now see the panel display FFSS ON. To exit, press the MODE button 4 times, then SAMPLE ADJ button once. Repeat the process to turn FFSS off.

Fluidic alarms and the Mode button

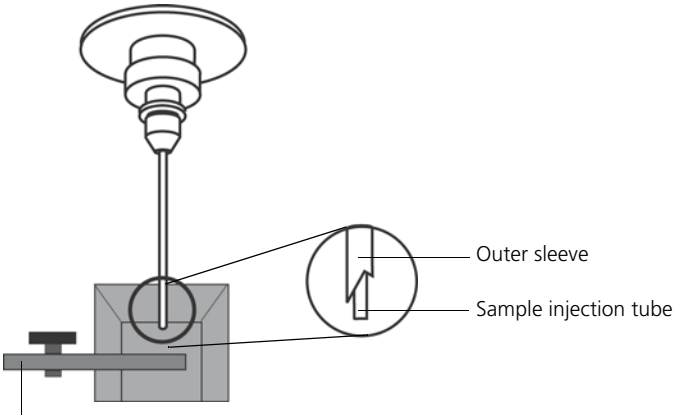
The fluidic alarms are triggered by the waste and sheath fluid levels in the tanks. The alarms sound when the waste tank is nearly 100% full and the sheath tank is empty.

To silence the alarm, press the Mode button, then press the Down button. The Mode button flashes to indicate the cytometer is in silent mode. Repeat this sequence to turn off silent mode.

Note: When the cytometer is in FFSS mode, the alarms are deactivated.

Sample injection port

The SIP is where the sample tube is installed. The SIP includes the sample injection tube and the tube support arm. Samples are introduced through a stainless steel injection tube equipped with an outer droplet containment sleeve. The sleeve works in conjunction with a vacuum pump to eliminate droplet formation of sheath fluid as it backflushes from the sample injection tube.



Sample injection tube. Stainless steel tube that carries sample from the sample tube to the flow cell. This tube is covered with an outer sleeve that serves as part of the droplet containment system.

Tube support arm. Arm that supports the sample tube and activates the droplet containment system vacuum. The vacuum is on when the arm is positioned to the side and off when the arm is centered.

Note: If a sample tube is left on the SIP with the tube support arm to the side (vacuum on), the sample will be aspirated into the waste container.

Cautions when using the HTS option

Caution: Biohazard! When using the flow cytometers with the HTS, ensure that the HTS is completely pushed into the operating position before removing the droplet containment module (DCM) sleeve or disconnecting the sample coupler from the SIP. This is to avoid accidental leakage of potentially biohazardous liquids directly onto the instrument. With the HTS in the proper location, the containment dish with padding is directly below the SIP.

Caution! If you are using the HTS option, always slide the HTS mount slowly to prevent sample cross-contamination when the wells are full. Never move the HTS when it is in operation.

Caution! Do not lean on or put any weight on the HTS as it could damage the instrument.

Droplet containment module

The DCM prevents sheath fluid from dripping from the SIP and provides biohazard protection.

When no sample tube is installed on the SIP, sheath fluid backflushes through the sample injection tube. This backflush helps prevent carryover of cells between samples. The DCM vacuum is activated when the sample tube is removed and the tube support arm is moved to the side. Sheath fluid is aspirated as it backflushes the sample injection tube.

Sheath and waste containers

Introduction

This topic describes the sheath and waste containers. The sheath and waste containers are outside the cytometer and are positioned on the floor.

Note: If you are using the FFSS, see the documentation provided.

Sheath container

The sheath container has a capacity of 10 L. Sheath fluid is filtered through an in-line, interchangeable filter that prevents small particles from entering the sheath fluid lines. An alarm sounds when the container is full.

Caution! Do not fill the sheath tank to its maximum capacity. When an overfull tank is pressurized, erratic cytometer performance can result.

Waste container

The waste container has a capacity of 10 L. An alarm sounds when the container is full.

More information

- Preparing the sheath container (page 37)
- Preparing the waste container (page 42)
- Status screen (page 26)
- Fluidic alarms and the Mode button (page 27)

Optics

Introduction

This topic describes the optical components for the BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual flow cytometers including:

- Detector arrays
- Laser options
- Optical filters
- Signal detectors

Detector arrays

The detector arrays consist of polygons/decagons. Each polygon/decagon can be outfitted with two to 10 PMTs and can detect up to 10 signals.

Laser options

The flow cytometers can be configured with up to five lasers as listed in the following table.

Laser	Wavelength (nm)	Power (mW)
Violet (standard)	405	250
Blue (standard)	488	400
UV (standard)	355	100
Yellow Green (standard)	561	300
Green (optional)	532	1000
Red (optional)	640	100
Red (standard)	637	140
Red (optional)	628	1000

Optical filters

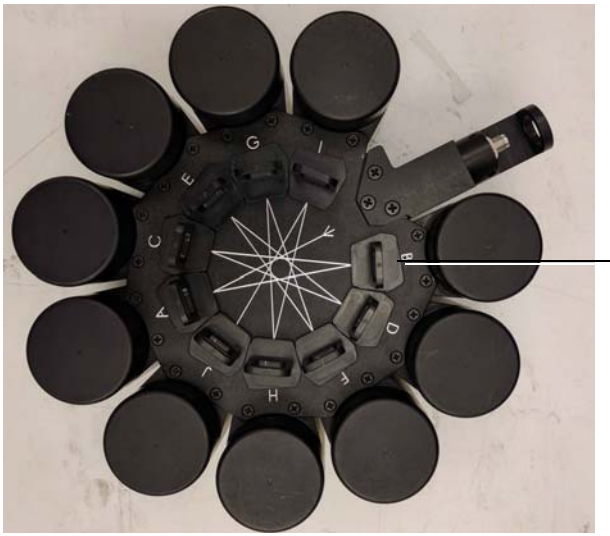
Optical filters attenuate light or help direct it to the appropriate detectors. The name and spectral characteristics of each filter appear on its holder.

There are two types of optical filters in the BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual flow cytometers:

- **Longpass dichroic filters (LPs).** Transmit wavelengths that are longer than the specified value and reflect all light below the specified wavelength.
- **Bandpass filters (BPs).** Pass a narrow spectral band of light.

When dichroic filters are used as steering optics to direct different color light signals to different detectors, they are called dichroic mirrors. LP dichroic mirrors transmit longer wavelengths to one detector while reflecting shorter wavelengths to a different detector.

The flow cytometer polygon/decagon detector arrays use dichroic longpass mirrors on the inside, and bandpass filters on the outside of the filter holders.



Signal detectors

Light signals are generated as particles pass through the laser beam in a fluid stream. When these optical signals reach a detector, electrical pulses are created that are then processed by the electronics system.

There are two types of signal detectors in the BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual flow cytometers:

- **Photomultiplier tubes (PMTs).** Used to detect the weaker signals generated by side scatter and all fluorescence channels. These signals are amplified by applying a voltage to the PMTs.
- **Photodiodes.** Less sensitive to light signals than the PMTs. A photodiode is used to detect the stronger forward scatter (FSC) signal.

Workstation

Introduction

This topic describes the components of the workstations.

Workstation components

Acquisition, analysis, and most instrument functions are controlled by the workstations. They include a PC, one or two monitors, and a printer. Your workstation is equipped with the following:

- Microsoft Windows operating system
- BD FACSDiva software version 8.0.1.1 or later for data acquisition and analysis
- Software documentation including the help system

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3

Cytometer setup

This chapter covers the following topics:

Starting the cytometer and computer

Introduction

This topic describes how to start the cytometer and turn on the computer.

Note: If your system is using the FFSS, make sure that the FFSS is powered on before the cytometer.

Procedure

To start the cytometer:

1. Turn on the power to the flow cytometer.
2. Allow 30 minutes for the optical system temperature to stabilize.

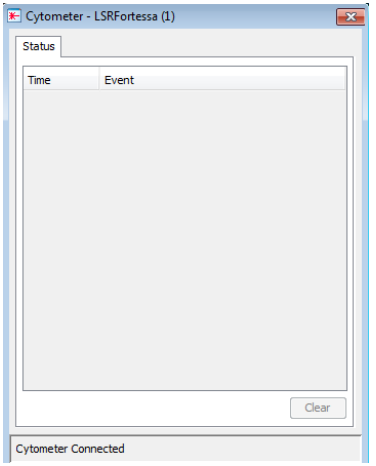
   

   Caution! Failure to warm up and stabilize the instrument could affect sample data.
3. Turn on the computer and log in to Windows.

   Note: You can turn on the power to the flow cytometer and the workstation in any order.
4. Start BD FACSDiva software by double-clicking the shortcut on the desktop, and log in to the software.
5. Check the **Cytometer** window in BD FACSDiva software to ensure that the cytometer is connected to the workstation.

   The cytometer connects automatically. While connecting, the message *Cytometer Connecting* is displayed in the status area

of the Cytometer window. When connection completes, the message changes to *Cytometer Connected*.



If the message *Cytometer Disconnected* appears, see Electronics troubleshooting (page 112).

Preparing the sheath container

Introduction

This topic describes how to prepare the sheath container.

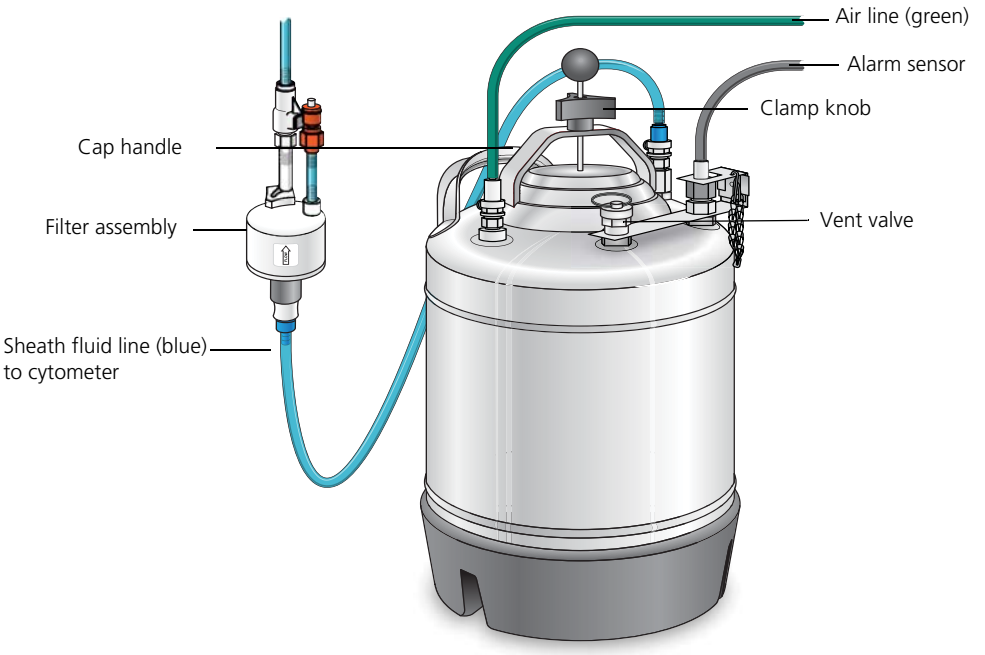
Note: If your system is using the FFSS, see the documentation provided with your system.

When to check the sheath container

Check the fluid levels in the sheath container every time you use the cytometer. This ensures that you do not run out of sheath fluid during an experiment.

Sheath container components

The sheath container components are shown below.



Procedure

To prepare the sheath container:

1. Verify that the flow cytometer is in standby mode.

   Press the STANDBY button on the control panel if necessary.

2. Disconnect the green air line, sheath fluid line, and alarm sensor from the sheath container.

3. Depressurize the sheath container by pulling up on the vent valve.

4. Remove the sheath container lid.

   Unscrew the clamp knob and push down to loosen, if necessary. Tilt the cap to the side to remove it from the tank.

5. Add 10 L of sheath fluid, such as BD FACSFlow™ solution, to the sheath container.

   Note: The 10 L will reach the interior line on the sheath tank. Do not fill the sheath tank further.

6. Replace the sheath container lid.
7. Reconnect the green air line and the alarm line.
8. Make sure the gasket on the inside lip of the sheath lid is seated correctly and has not slipped out of position.

   If the gasket is not seated correctly, the tank will not pressurize properly.

9. Close the sheath lid and tighten the clamp knob to finger-tight. Ensure that the blue sheath fluid line is not kinked.

More information

- Removing air bubbles (page 39)
- Changing the sheath filter (page 58)
- Cleaning or replacing the sheath gasket (page 64)

Removing air bubbles

Introduction

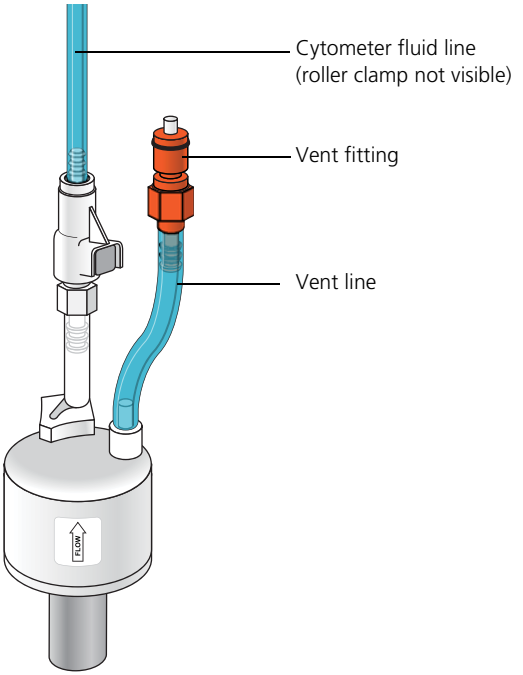
This topic describes how to remove trapped air bubbles in the sheath filter and the sheath line. Air bubbles can occasionally dislodge and pass through the flow cell, resulting in inaccurate data.

Note: Perform this activity every time the sheath tank is refilled.

Procedure

To remove air bubbles:

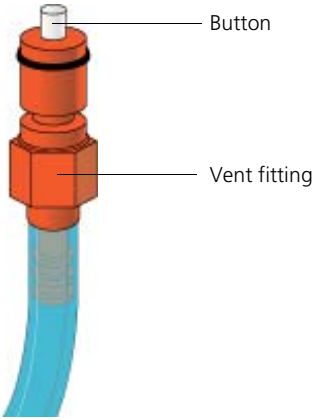
1. Check the sheath filter for trapped air bubbles.



2. If bubbles are visible, gently tap the filter body with your fingers to dislodge the bubbles and force them to the top.

Caution! When removing air bubbles, do not vigorously shake, bend, or rattle the sheath filter or you might damage it.

3. Direct the vent line into a beaker and press the small button at the end of the vent fitting against the side of the beaker until a steady stream of fluid empties from the filter.



4. Tilt the filter and verify that no trapped air remains in the filter.
5. Repeat steps 3 and 4 until no air is observed in the filter.
6. Check the sheath line for air bubbles.
7. Open the roller clamp at the fluidics interconnect (if necessary) to bleed off any air in the line. Collect any excess fluid in a waste container.
8. Close the roller clamp.

Preparing the waste container

Introduction

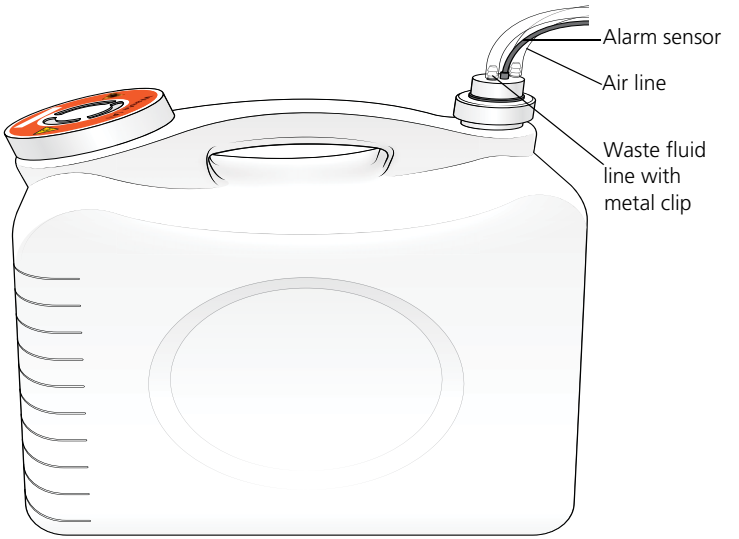
This topic describes how to prepare the waste container. Prevent waste overflow by emptying the waste container daily or whenever the system status indicator turns yellow.

Note: If your system is connected to the FFSS, see the documentation provided with your FFSS.

Caution: Biohazard! All biological specimens and materials coming into contact with them are considered biohazardous. Handle as if capable of transmitting disease. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Waste container components

The following figure shows the main components of the waste container.



Biological precautions

Caution: Biohazard! Contact with biological specimens and materials can transmit potentially fatal disease.

To prevent exposure to biohazardous agents:

- Put the cytometer in standby mode before disconnecting the waste tank to avoid leakage of biohazardous waste.
- Always disconnect the waste container from the cytometer before you empty it. Wait at least 30 seconds for pressure to dissipate before you remove the waste cap or sensor.
- Expose waste container contents to bleach (10% of total volume) for 30 minutes before disposal.
- Do not wet the waste tank cap. If wet, the filter in the cap will cause the tank to malfunction. To keep the cap dry, place it on the bench label side up when it is not on the tank.

Procedure

To prepare the waste container:

1. Verify that the flow cytometer is in standby mode.

   Press the STANDBY button on the control panel if necessary.

2. Disconnect the orange waste tubing and the black alarm sensor line from the waste container.

   Keep the lid on the waste container until you are ready to empty it.

3. Empty the waste container.

   Caution! The waste container is heavy when full. When emptying it, use good body mechanics to prevent injury.

4. Add approximately 1 L of bleach to the waste container and close it.

5. Reconnect the orange waste tubing and make sure it is not kinked.

6. Reconnect the black alarm sensor line.

Priming the fluidics

Introduction

This topic describes how to prime the fluidics system.

When to prime the fluidics

Sometimes, air bubbles and debris may become lodged in the flow cell. This is indicated by excessive noise in the forward and side scatter parameters (FSC and SSC, respectively). In these cases, it is necessary to prime the fluidics system.

Procedure

To prime the fluidics:

1. Move the tube support arm to the side.
2. Remove the tube from the SIP.
3. Press the PRIME fluid control button to force the fluid out of the flow cell and into the waste container.

   Once drained, the flow cell automatically fills with sheath fluid at a controlled rate to prevent bubble formation or entrapment. The STANDBY button turns amber after completion.
4. Repeat the priming procedure, if necessary.
5. Install a 12 x 75-mm tube with 1 mL of DI water on the SIP and place the support arm under the tube. Leave the cytometer in standby mode.

More information

- Cytometer troubleshooting (page 104)

About the optical filters and mirrors

Introduction

This topic provides a description of the optical filters and mirrors.

Filter and mirror configurations

Each PMT has an optic holder in front of it. The optic holders are labeled with numbers indicating the wavelengths of the bandpass filter and longpass dichroic mirror they contain (for example, 780/60 and 750 LP, respectively). The optic holder in front of the last PMT in the detector array contains only a bandpass filter and is marked accordingly.

The filters steer progressively shorter wavelengths of light to the next PMT in the array as indicated by the lines and arrows on the top of the polygon/decagon.

Optic holders, filters, and mirrors

Optic holders house filters and mirrors. Your cytometer includes several blank (empty) optic holders.

Caution! To ensure data integrity, do not leave any slots empty in a detector array when you are using the associated laser. Always use a blank optic holder.

Base configurations

Each flow cytometer has a base cytometer configuration that corresponds to the layout of the installed lasers and optics in your cytometer. This base configuration is set by your field service engineer.

BD FACSDiva cytometer configuration

Before you acquire data using BD FACSDiva software, you must specify a cytometer configuration. The cytometer configuration defines which filters and mirrors are installed at each detector.

BD FACSDiva software provides a BD base configuration for your flow cytometer. Select Cytometer > View Configuration to create, modify, or delete custom cytometer configurations. (See the Cytometer and Acquisition Controls chapter of the *BD FACSDiva Software Reference Manual* for details.)

Custom configurations and baselines

Introduction

This topic describes where to find information on how to create a custom configuration and define a baseline for a performance check.

Overview

BD Cytometer Setup and Tracking (CS&T) software is used to define the baseline performance of your cytometer. A baseline provides a starting point for the tracking of cytometer performance. When running a performance check, you compare the results to the baseline.

See Optimizing cytometer settings (page 65). Please see the latest published filter guides available on our website (bdbiosciences.com) for more information.

See the *BD Cytometer Setup and Tracking Application Guide* for information on creating custom configurations and defining a baseline.

More information

- Running a performance check (page 70)

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4

Maintenance

This chapter covers the following topics:

Maintenance overview

Introduction

This topic provides an overview of the flow cytometer routine maintenance and cleaning procedures.

General use guidelines

Caution: Biohazard! Contact with biological specimens and materials can transmit potentially fatal disease.

Follow these guidelines whenever operating or maintaining the cytometer:

- Wear suitable protective clothing, eyewear, and gloves.
- Handle all biological specimens and materials in accordance with applicable regulations and manufacturer specifications.
- Dispose of waste using proper precautions and in accordance with local regulations.
- Never pipette by mouth.

For fluidics maintenance, we recommend the following cleaning solutions:

- BD™ FACSClean solution
- BD™ FACSRinse solution
- 10% bleach solution

Use DI water to dilute bleach to appropriate concentrations.

Caution! Higher concentrations of sodium hypochlorite and use of other cleaning solutions might damage the cytometer.

When to perform maintenance procedures

Perform maintenance procedures in the following frequencies.

Frequency	Maintenance procedure
Daily	• Cleaning the fluidics (page 51) • Shutting down the cytometer (page 53)
Scheduled (every two weeks)	• Flushing the system (page 54)
Periodic (frequency depends on how often you run the cytometer)	• Changing the sheath filter (page 58) • Changing the Bal seal (page 60) • Changing the sample tube O-ring (page 62) • Cleaning or replacing the sheath gasket (page 64)

Cleaning the fluidics

Introduction

This topic describes how to perform the daily fluidics cleaning.

Overview

Cleaning the fluidics daily prevents the sample injection tube from becoming clogged and removes dyes that can remain in the tubing.

In addition to daily cleaning, follow this procedure immediately after running viscous samples or nucleic acid dyes such as Hoechst, DAPI, propidium iodide (PI), acridine orange (AO), or thiazole orange (TO).

Procedure

To clean the fluidics:

1. Press RUN and HIGH on the cytometer fluid control panel.
2. Install a tube containing 3 mL of a cleaning solution on the SIP with the support arm to the side (vacuum on) and let it run for 1 minute.

For the cleaning solution, use BD FACSClean solution. See Maintenance overview (page 50) for other recommended cleaning solutions.

3. Move the tube support arm under the tube (vacuum off) and allow the cleaning solution to run for 5 minutes with the sample flow rate set to HIGH.
4. Repeat steps 2 and 3 with BD FACSRinse solution.
5. Repeat steps 2 and 3 with DI water.
6. Press the STANDBY button on the fluidics control panel.
7. Place a tube containing no more than 1 mL of DI water on the SIP.

   A tube with 1 mL of DI water should remain on the SIP to prevent salt deposits from forming in the injection tube. This tube also catches back drips from the flow cell.

Caution! Do not leave more than 1 mL of water on the SIP. When the instrument is turned off or left in standby mode, a small amount of fluid will drip back into the sample tube. If there is too much fluid in the tube, it could overflow and affect the cytometer performance.

Shutting down the cytometer

Introduction

This topic describes how to shut down the cytometer.

Before you begin

Each time you shut down the cytometer, perform the daily cleaning as described in Cleaning the fluidics (page 51).

Procedure

To shut down the cytometer:

1. Place a tube of DI water on the SIP.
2. Turn off the flow cytometer.
3. Select **Start > Shutdown** to turn off the computer (if needed).
4. If your system is connected to the FFSS, shut off the FFSS.

If the cytometer will not be used for a week or longer, perform a system flush and leave the fluidics system filled with DI water to prevent saline crystals from clogging the fluidics.

More information

- Cleaning the fluidics (page 51)
- Flushing the system (page 54)

Flushing the system

Introduction

This topic describes how to perform an overall fluidics cleaning to remove debris and contaminants from the sheath tubing, waste tubing, and flow cell. Perform the system flush at least every 2 weeks.

Note: If you are using the FFSS, see the *BD FACSFlow Supply System User's Guide* for instructions on flushing the system.

Cautions

Caution: Biohazard! The cytometer hardware might be contaminated with biohazardous material. Use 10% bleach to decontaminate the instrument.

Procedure

To perform a system flush:

1. Remove the sheath filter.
   a. Press the quick-disconnects on both sides of the filter assembly.
   b. Remove the filter assembly.
   c. Connect the two fluid lines.

Caution! Do not run detergent, bleach, or ethanol through the sheath filter. They can break down the filter paper within the filter body, causing particles to escape into the sheath fluid, possibly clogging the flow cell.

2. Empty the sheath container and rinse it with DI water.
3. Fill the sheath container with at least 1 L of undiluted BD FACSClean solution.
4. Empty the waste container, if needed.
5. Open the roller clamp by the fluidics interconnect, and drain the fluid into a beaker for 5 seconds.
6. Remove the DI water tube from the SIP.

7. Prime the instrument twice:
   a. Press the PRIME button on the fluidics control panel.
   b. When the STANDBY button lights (amber), press the PRIME button again.
8. Install a tube with 3 mL of undiluted BD FACSClean solution on the SIP and put the tube support arm underneath the tube.

   See Maintenance overview (page 50) for other recommended cleaning solutions.
9. Press RUN and HIGH on the cytometer fluid control panel. Run for 30 minutes.
10. Press the STANDBY fluid control button and depressurize the sheath container by lifting the vent valve.
11. Repeat steps 2 through 10 with BD FACSRinse solution.
12. Repeat steps 2 through 10 with DI water.
13. Replace the sheath filter and refill the sheath container with sheath fluid.

Replacing the waste container cap

Introduction

This topic describes how to replace the waste container cap. Replace the cap once a month.

Biological precautions

Caution: Biohazard! Contact with biological specimens and materials can transmit potentially fatal disease.

To prevent exposure to biohazardous agents:

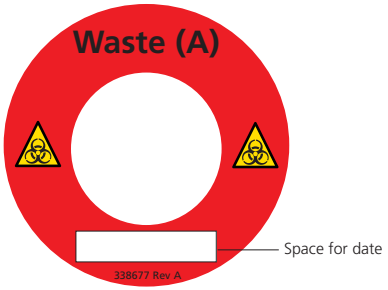
- Put the cytometer in standby mode before disconnecting the waste tank to avoid leakage of biohazardous waste.
- The waste container can become pressurized when the cytometer is running. Always disconnect the waste container from the cytometer before you empty it. Wait at least 30 seconds for pressure to dissipate before you remove the waste cap or sensor.
- Expose waste container contents to bleach (10% of total volume) for 30 minutes before disposal.

Procedure

To replace the cap:

1. Put the cytometer in standby mode.
2. Disconnect the waste container's sensor line and orange waste line from the waste container tank, and wait at least 30 seconds for pressure to dissipate.
3. Remove the waste cap and attached trap from the container and place on the bench label-side up.
4. Detach the cap from the trap.
5. Place a new cap on the trap.

6. Write the date on the cap label.



7. Screw the cap assembly onto the waste container and hand-tighten until it is fully closed.

Caution: Biohazard! To prevent waste container overpressurization, do not overtighten the trap or attached filter cap. Tighten each component only until it is hand-tight. Do not use sealants such as Teflon® tape or other adhesives.

Re-attach the alarm sensor line and waste line to the waste container tank.

Changing the sheath filter

Introduction

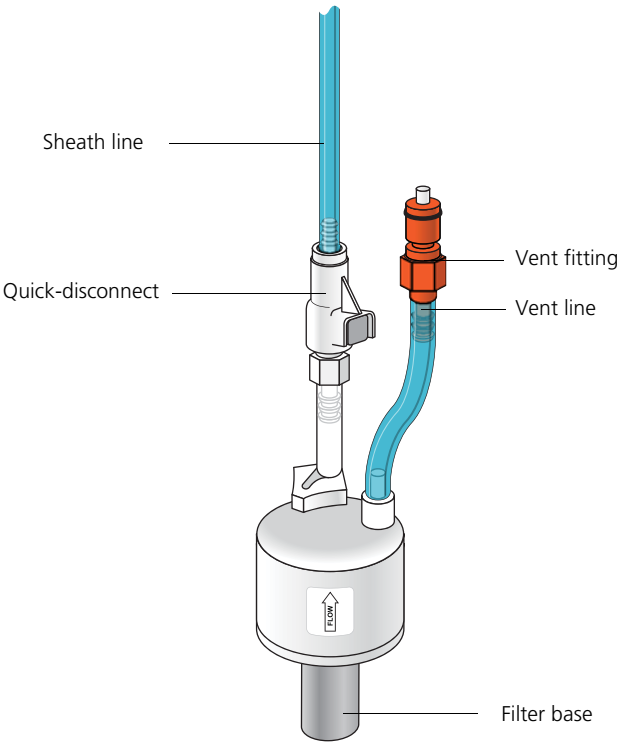
This topic describes how to change the sheath filter. The sheath filter is connected in-line with the sheath line. It filters the sheath fluid as it comes from the sheath container.

When to change the sheath filter

We recommend changing the sheath filter assembly every six months. Increased debris appearing in an FSC vs SSC plot can indicate that the sheath filter needs to be replaced. See Supplies and consumables (page 123) for ordering information.

Sheath filter components

The following illustration shows the sheath filter components.



Removing the old filter

To remove the old filter:

1. Place the cytometer in standby mode.
2. Remove the sheath filter assembly by pressing the quick-disconnect on both sides of the filter assembly.
3. Over a sink or beaker:
   - Remove the vent line from the filter and set it aside.
   - Remove the filter base and set it aside.
4. Discard the used filter assembly in an appropriate receptacle.

Attaching the new filter

To attach the new filter:

1. Connect the vent line to the new filter assembly.

   Twist to attach.
2. Wrap Teflon® tape around the filter threads, then connect the filter to the filter base.
3. Connect the sheath line to the filter assembly by squeezing the quick-disconnect.
4. Attach the cytometer fluid line to the filter assembly via the quick-disconnect.
5. Direct the vent line into a beaker and press the small button at the end of the vent fitting against the side of the beaker until a steady stream of fluid empties from the filter.
6. Tilt the filter and verify that no trapped air remains in the filter.
7. Repeat steps 5 and 6 as necessary to remove all trapped air.

Changing the Bal seal

Introduction

This topic describes how to replace the Bal seal.

The sample injection tube Bal seal is a ring that forms a seal with the sample tube and ensures proper tube pressurization.

When to change the Bal seal

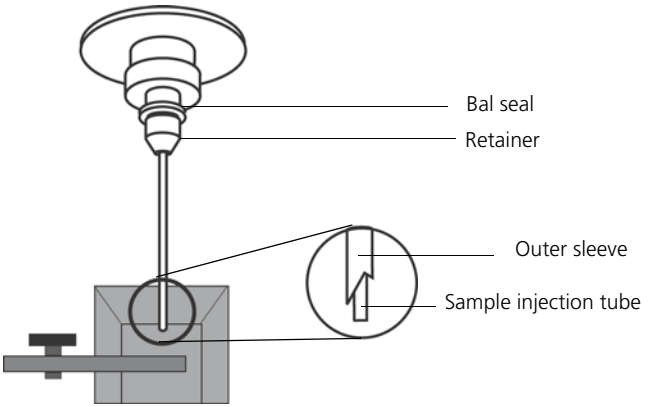
Over time, the Bal seal becomes worn or cracked and requires replacement. Replacement is necessary if a proper seal is not formed when a sample tube is installed on the SIP. Indications that a proper seal has not formed include:

- The tube will not stay on the SIP without the tube support arm.
- When the tube is installed and RUN is pressed on the cytometer, the RUN button is orange (not green).

Procedure

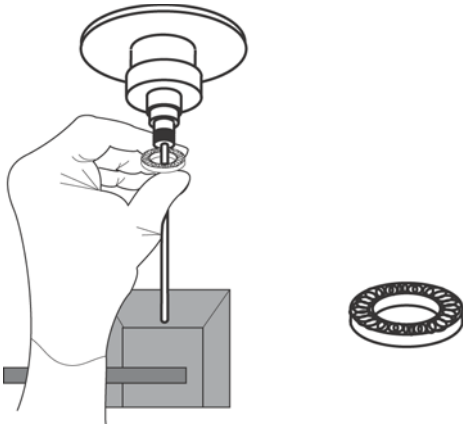
To replace the Bal seal:

1. Remove the outer sleeve from the sample injection tube by turning the retainer counter-clockwise. Slide the outer sleeve down and off of the sample injection tube.



Work carefully. The outer sleeve can fall off as you loosen the retainer.

2. Remove the Bal seal by gripping it between your thumb and index finger and pulling down.



3. Install the new Bal seal spring-side up.

   Ensure that the sample tube O-ring is still in place inside the retainer.

4. Re-install the retainer and outer sleeve over the sample injection tube. Push the outer sleeve all the way up into the sample injection port and then screw the retainer into place and tighten to finger tight. This will seat the Bal seal.

5. Install a sample tube on the SIP to ensure that the outer sleeve has been properly installed.

   If the sleeve hits the bottom of the tube, loosen the retainer slightly and push the sleeve up as far as it will go. Tighten the retainer.

Changing the sample tube O-ring

Introduction

This topic describes how to replace the sample tube O-ring.

The sample tube O-ring, located within the retainer, forms a seal that allows the droplet containment vacuum to function properly.

When to replace the O-ring

Replace the O-ring when droplets form at the end of the sample injection tube while the vacuum is operating.

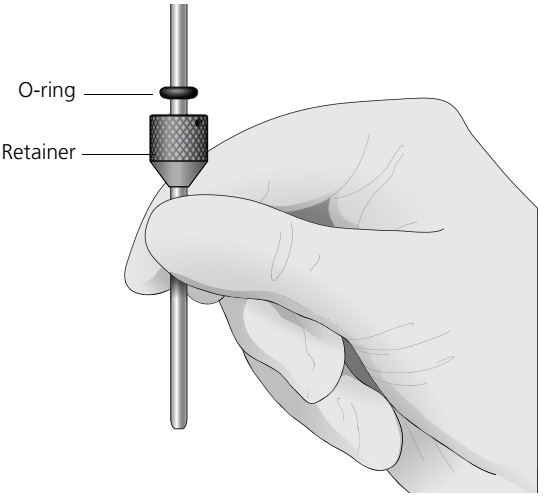
Caution

Caution: Biohazard! Cytometer hardware might be contaminated with biohazardous material. Wear suitable protective clothing, eyewear, and gloves whenever cleaning the cytometer or replacing parts.

Procedure

To change the O-ring:

1. Remove the outer sleeve from the sample injection tube by turning the retainer counter-clockwise.
2. Slide the outer sleeve from the retainer.



3. Invert the outer droplet sleeve and allow the O-ring to fall onto the benchtop.

   If the O-ring does not fall out initially, hold the O-ring with your free hand and slide the outer sleeve to remove the O-ring.

4. Place the new O-ring into the retainer. Make sure the O-ring is seated properly in the bottom of the retainer.

5. Replace the outer sleeve in the retainer.

6. Re-install the retainer and the outer sleeve.

7. Install a sample tube on the SIP to ensure that the outer sleeve has been properly installed.

   If the sleeve hits the bottom of the tube, loosen the retainer slightly and push the sleeve up as far as it will go. Tighten the retainer.

Cleaning or replacing the sheath gasket

Introduction

This topic describes how to clean or replace the gasket of the sheath tank lid.

When to change the sheath filter

We recommend cleaning the sheath gasket when needed.

Procedure

To clean or replace the gasket:

1. Put the cytometer in standby mode.
2. Depressurize the sheath container by pulling up on the vent valve.
3. Remove the lid from the sheath tank.
4. Remove the black gasket from the lid.
5. Rinse it with water to clean the gasket.
6. Place the clean gasket or the new gasket on the lid and make sure the gasket is seated properly on the lid.

5

Optimizing cytometer settings

This chapter covers the following topics:

Cytometer settings workflow

Introduction	This topic describes how to optimize cytometer settings. The optimization is performed using the Cytometer Setup and Tracking, Application Settings, and Compensation Setup features of BD FACSDiva software.
When to optimize settings	Before you record data for a sample, optimize the cytometer settings for the sample type and fluorochromes used.
Manual compensation	Compensation setup automatically calculates compensation settings. If you choose to perform compensation manually, not all of the following instructions apply. For detailed instructions, see the *BD FACSDiva Software Reference Manual*.
First-time users	If you are performing the procedures in this workflow for the first time, you should be familiar with BD FACSDiva software concepts: workspace components, cytometer and acquisition controls, and tools for data analysis. For additional details, see the *BD FACSDiva Software Reference Manual*.
Before you begin	Start the cytometer and perform the setup and QC procedures. See Cytometer setup (page 35).

Workflow for optimizing settings

Cytometer optimization consists of the following steps.

Step	Description
1	Verifying the configuration and user preferences (page 68)
2	Running a performance check (page 70)
3	Setting up an experiment (page 74)
4	Creating application settings (page 80)
5	Recording compensation controls (page 83)
6	Calculating compensation (page 86)

Note: Application settings are optional and do not have to be saved for the experiments. However, they are useful for optimizing cytometer settings.

About the examples

The examples in this chapter use a 4-color bead sample with the following fluorochromes:

- FITC
- PE
- PerCP-Cy™5.5
- APC

If you follow this workflow with a different bead sample (or another sample type), your software views, data plots, and statistics might differ from the example. Additionally, you might need to modify some of the instructions in the procedure.

The information shown in italics is for example only. You can substitute your own names for folders and experiments.

Verifying the configuration and user preferences

Introduction

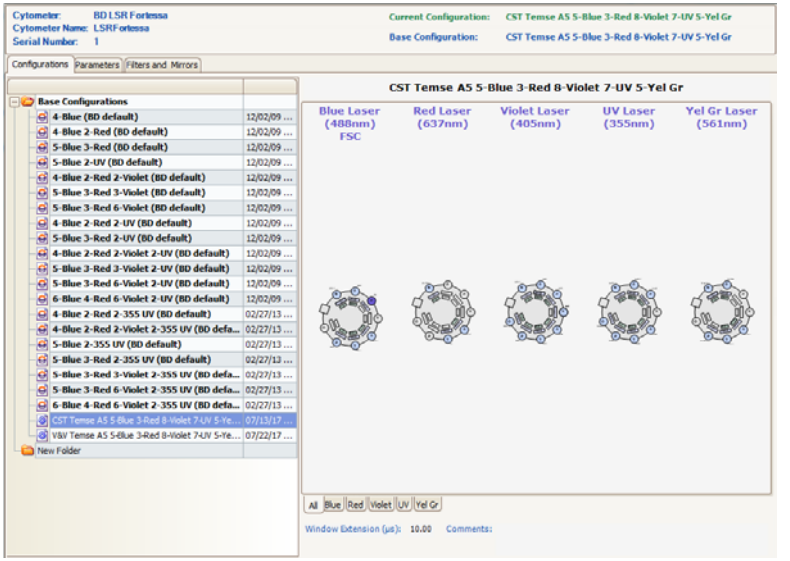
This topic describes how to verify the cytometer configuration and user preferences before you create an experiment.

Caution! To obtain accurate data results, the current cytometer configuration must reflect your flow cytometer optics.

Procedure

To verify the configuration and preferences before you create an experiment:

1. Select **Cytometer > View Configurations** and verify the current configuration.



Your cytometer might include only the base configuration when your cytometer is installed. You can create additional configurations later as needed.

In this example, the cytometer configuration must include the following parameters: FITC, PE, PerCP-Cy5.5, and APC.

2. If you need to select a configuration other than the current configuration:
   a. In the **Configurations** tab, select a configuration.
   b. Click **Set Configuration.**
   c. Click **OK.**
   d. Verify that the configuration you just set matches your flow cytometer optics.
3. Click **OK** to close the **Cytometer Configuration** window.
4. Select **File > Exit** to close CS&T.
5. Select **Edit > User Preferences**.
6. Click the **General** tab and select the **Load data after recording** checkbox.

See the *BD FACSDiva Software Reference Manual* for more information about cytometer configurations and user preferences.

Next step

Running a performance check (page 70)

More information

- Setting up an experiment (page 74)

Running a performance check

Introduction

This topic describes how to run a performance check as part of quality control.

Overview

The CS&T application is designed to monitor performance on a daily basis and to optimize laser delay.

Running a performance check on a regular basis provides a standard for monitoring changes in performance due to degradation of laser power, aging of PMTs, and other potential cytometer service issues. Performance results are also affected by fluidics performance. We strongly recommend following the fluidics maintenance procedures as described in Cleaning the fluidics (page 51).

Considerations

Some BP filters might not be normalized to CS&T settings. In this case, CS&T will generate Qr and Br numbers that are not comparable from instrument to instrument. They are however, still trackable on one cytometer. Part of the process for optimizing cytometer settings includes verifying PMT voltages set by CS&T for all parameters. Carefully examine any channel with a non-CS&T normalized filter.

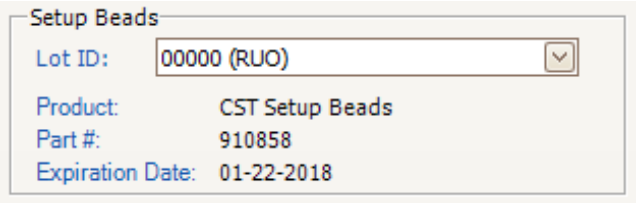
Before you begin

Define the performance baseline for any configuration before running a performance check. See Custom configurations and baselines (page 47).

Procedure

To run a performance check:

1. Select **Cytometer > CST.**
2. Verify that the bead lot information under **Setup Beads** matches the Cytometer Setup and Tracking bead lot.



3. Verify that the cytometer configuration is correct for your experiment.

   If the cytometer is not set to the correct configuration:

   a. Click **Select Configuration** in the **Setup Control** window.

   b. Select the correct configuration from the list.

   c. Click **Set Configuration** and then click **OK**.

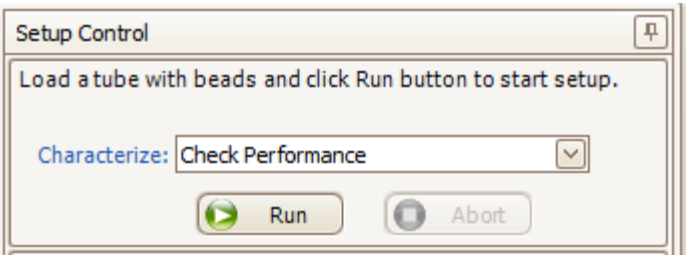
4. Verify that the current configuration has a valid baseline defined.

   If not, see the *BD Cytometer Setup and Tracking Application Guide* for more information on defining a baseline.

5. Prepare the CS&T beads according to the technical data sheet provided with the beads or available on the BD Biosciences website (bdbiosciences.com).

6. Install the bead tube onto the SIP.

7. In the **Setup Control** window, select **Check Performance** from the **Characterize** menu.

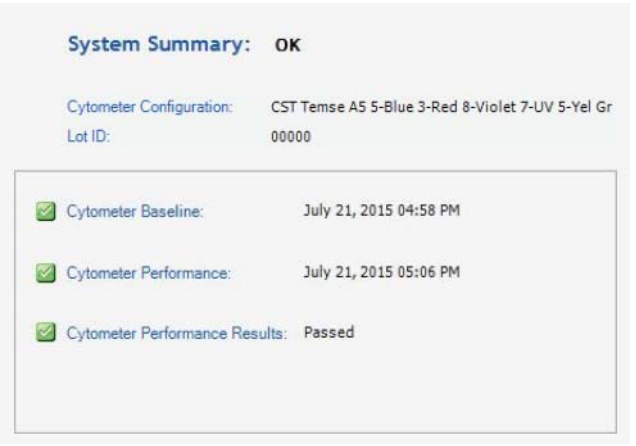


8. Click **Run.**
9. Ensure that Fine Adjust is set to 250, press Run, and Low.

   Plots appear under the Setup tab and the performance check is run. The performance check takes approximately 5 minutes to complete.

10. Once the performance check is complete, click **View Report.**
11. Verify that the cytometer performance passed.

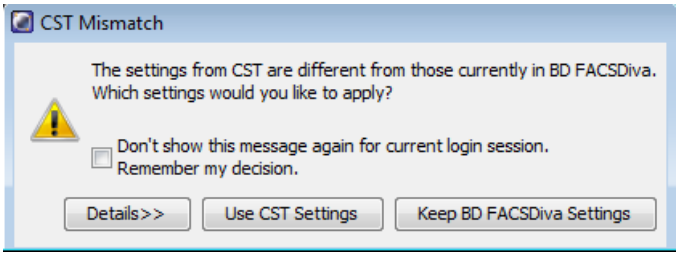
    In the Setup tab, the cytometer performance results should have a green checkbox displayed and the word *Passed* next to it.



If any parameters did not pass, see the *BD Cytometer Setup and Tracking Application Guide* for troubleshooting information.

12. Select **File > Exit** to close the CS&T window and return to the BD FACSDiva interface.

    The CST Mismatch dialog opens.



    Click the **Details** button to verify which cytometer settings will be updated.

13. Click **Use CST Settings**.

    By selecting Use CST Settings, the laser delay, area scaling, and other cytometer settings will be updated to the latest settings from the performance check.

Next step

Continue the optimization of your cytometer for an experiment or sample type as described in Setting up an experiment (page 74).

Setting up an experiment

Introduction

This topic describes how to create an experiment in a new folder, specify the parameters of the experiment, and add compensation tubes.

Creating an experiment

To create an experiment:

1. Click the buttons on the **Workspace** toolbar to display the following windows as needed:

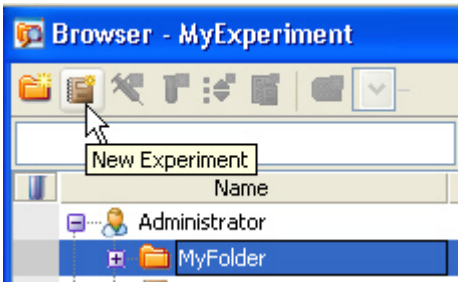
   - Browser
   - Cytometer
   - Inspector
   - Worksheet
   - Acquisition Dashboard

   When you add elements or make selections in the Browser, the Inspector displays details, properties, and options that correspond to your selection.

2. Click the **New Folder** button on the **Browser** toolbar to add a new folder.

3. Click the folder and rename it *MyFolder*.

4. Click *MyFolder*, then click the **New Experiment** button on the **Browser** toolbar.



   a. Click the new experiment in the **Browser** and rename it *MyExperiment*.

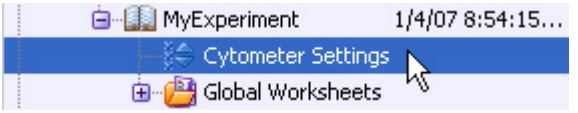
5. Select *MyExperiment* in the **Browser**.

   The Inspector displays details for the experiment.

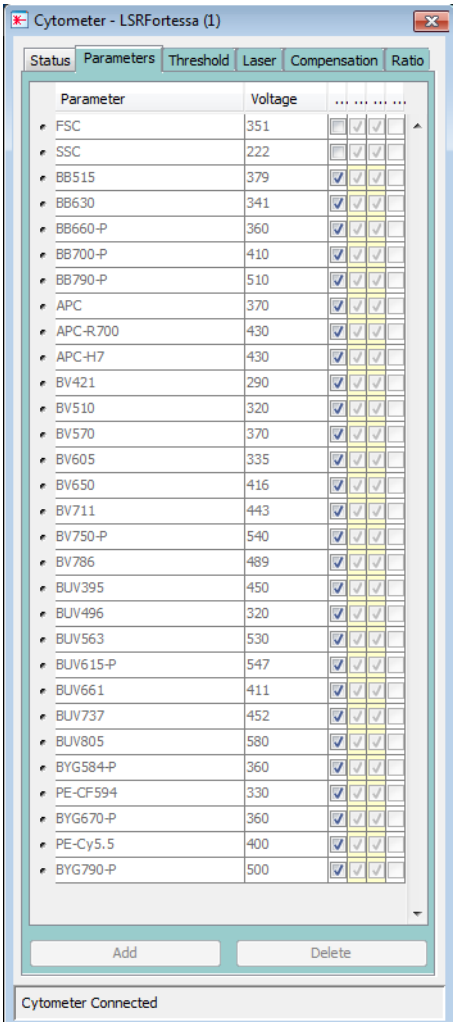
Specifying parameters

To specify the parameters for the new experiment:

1. Select **Cytometer Settings** for the experiment in the **Browser**.



Cytometer settings appear in the **Inspector**.

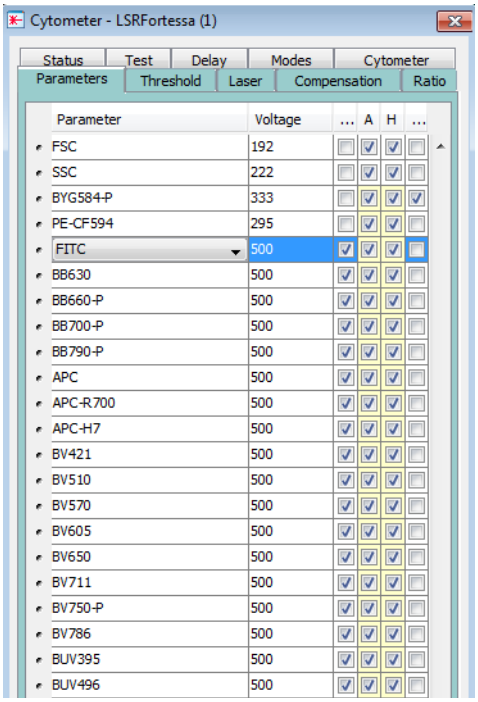


2. Make sure the parameters you need appear on the **Parameters** tab in the **Inspector**.

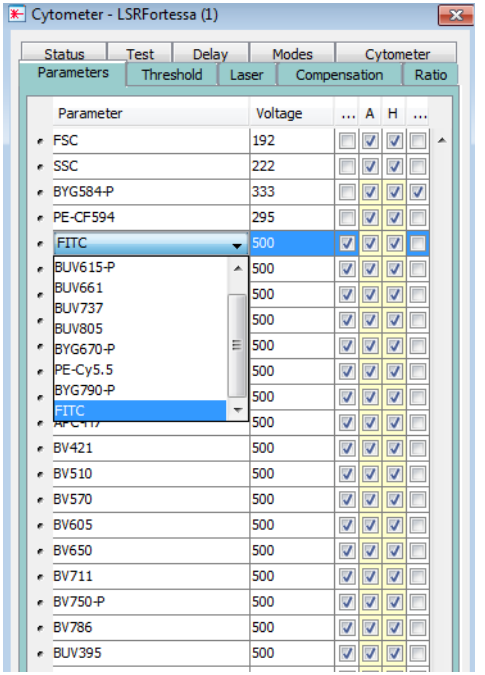
   If more than one parameter is available for a particular PMT, you might have to select the one you need from a menu. For

example, you can set Detector F for the blue laser as FITC or BB515.

a. Click the **Parameter** name to display the available fluorochromes in the **Parameters** list.



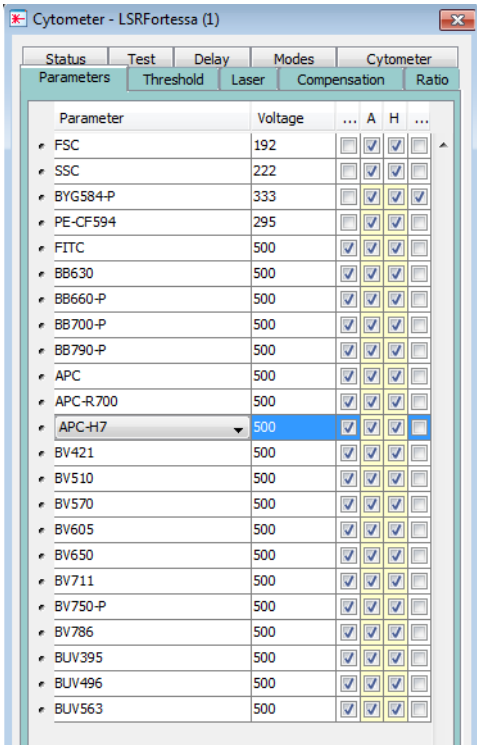
b. Select the specific parameter from the menu. Your selection appears as the selected parameter.



c. For this example, select **FITC** from the menu.

3. Delete any unnecessary parameters.

a. Click the selection button (to the left of the parameter name) to select the parameter.



b. Click **Delete**.

The parameter is deleted.

Creating application settings

Introduction

This topic describes how to create application settings.

About application settings

Application settings are associated with a cytometer configuration and include the parameters for the application, area scaling values, PMT voltages, and threshold values, but not compensation. Each time a performance check is run for a configuration, the application settings associated with that configuration are updated to the latest run.

Using application settings provides a consistent and reproducible way to reuse cytometer settings for commonly used applications.

You can include area scaling adjustment in your application settings. See Adjusting area scaling (page 117) for more information.

Before you begin

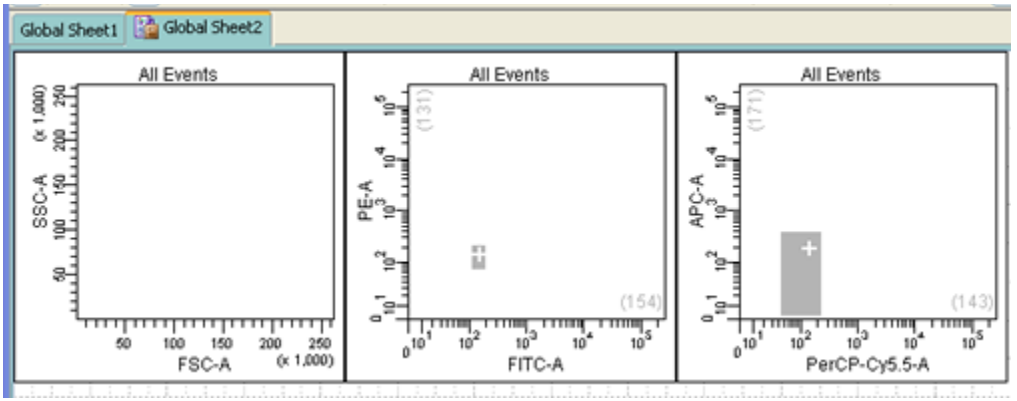
Perform the cytometer setup procedure and run a performance check for the configuration that will be used for the application.

Procedure

To create application settings:

1. In the open experiment, right-click **Cytometer Settings** in the **Browser**, then select **Application Settings > Create Worksheet.**

   A second global worksheet is added with the plots created according to the selections in the Parameters tab.



Use the gray boxes and crosshairs to guide your optimization.

2. Load the unstained control tube onto the cytometer.
3. In the **Cytometer** window, optimize the PMT voltages for the application.
   - Optimize the FSC and SSC voltages to place the population of interest on scale.
   - Optimize the FSC threshold value to eliminate debris without interfering with the population of interest.
   - If needed, increase the fluorescence PMT voltages to place the negative population within the gray boxes. Align the center of the negative population with the crosshair visible in the gray box.

   Note: Do not decrease the fluorescence PMT voltages. Doing so can make it difficult to resolve dim populations from the negative population.
4. Unload the unstained control tube from the cytometer.
5. Load the multicolor sample onto the cytometer or load single-color control tubes and verify each fluorochrome signal separately.

6. Verify that the positive populations are on scale.

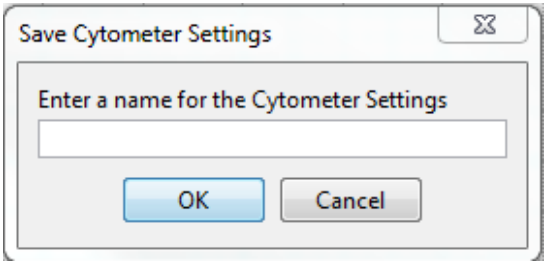
   If a positive population is off scale, lower the PMT voltage for that parameter until the positive population can be seen entirely on scale.

7. Unload the multicolor sample.

8. Place a tube containing DI water on the SIP and put the cytometer on standby.

9. (Optional) Save the application settings by right-clicking **Cytometer settings** in the **Browser**, then selecting **Cytometer Settings > Save.**

10. In the **Save Cytometer Settings** dialog, enter a descriptive name for the settings.



11. Click **OK**.

    The application settings are saved to the catalog.

Next step

Recording compensation controls (page 83)

Recording compensation controls

Introduction

This topic describes how to create and record compensation controls using the Compensation Setup feature of BD FACSDiva software and an experiment with optimized settings.

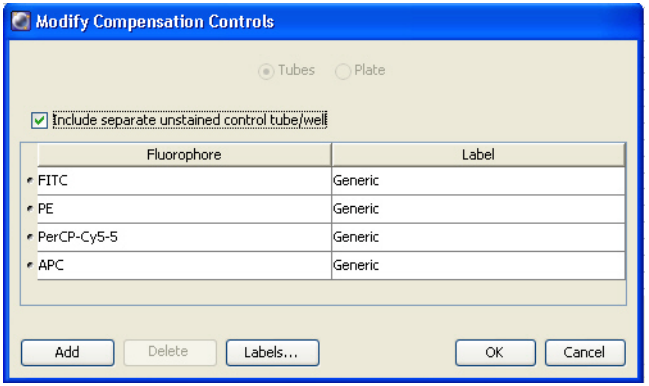
Creating compensation tubes

To create compensation control tubes:

1. Select **Experiment > Compensation Setup > Create Compensation Controls**.

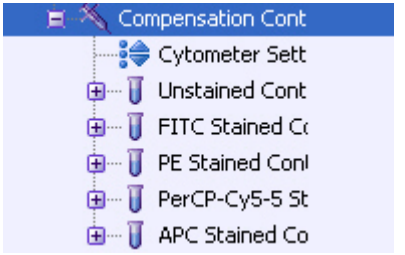
   The Create Compensation Controls dialog opens.

   For this bead example, you do not need to provide non-generic tube labels.



2. Click **OK**.

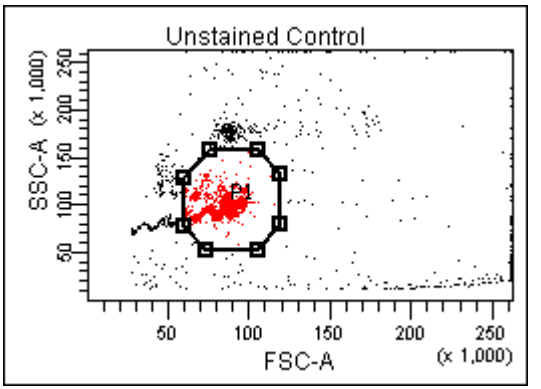
Compensation control tubes are added to the experiment. Worksheets containing appropriate plots and gates are added for each compensation tube.



Recording compensation settings

To record compensation settings:

1. Press RUN and HIGH on the cytometer fluid control panel.
2. Install the unstained control tube onto the SIP.
3. Expand the **Compensation Controls** specimen in the **Browser**.
4. Set the current tube pointer to the unstained control tube (it becomes green), then click **Acquire Data** in the **Acquisition Dashboard**.
5. Verify that the population of interest is displayed appropriately on the FSC vs SSC plot and adjust voltages if necessary.



Since the application settings have been optimized for your sample, the cytometer settings should not require adjustment other than the changing of FSC and SSC voltages to place the beads on scale.

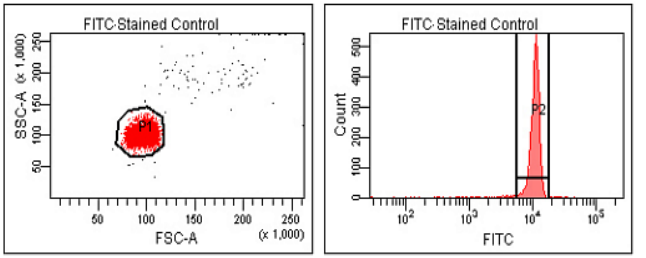
6. Adjust the P1 gate to surround only the singlets.
7. Right-click the P1 gate and select **Apply to All Compensation Controls**.

   The P1 gate on each stained control worksheet is updated with your changes.
8. Click **Record Data.**
9. When recording is finished, remove the unstained control tube from the cytometer.
10. Click **Next Tube**.

Caution! Do not change the PMT voltages after the first compensation control has been recorded. In order to calculate compensation, all controls must be recorded with the same PMT voltage settings. If you need to adjust the PMT voltage for a subsequent compensation control, you must record all compensation controls again.

11. Install the next tube onto the cytometer and repeat steps 8 through 10 until data for all stained control tubes has been recorded.
12. Double-click the first stained control tube to display the corresponding worksheet.

13. Verify that the snap-to interval gate encompasses the positive population.



14. Repeat steps 12 and 13 for the remaining compensation tubes.

Next step

After you have recorded data for each single-stained control, calculate compensation as described in Calculating compensation (page 86).

Calculating compensation

Introduction

This topic describes how to calculate compensation.

Before you begin

Before you can calculate compensation, you need to record the data for each single-stained control.

Procedure

To calculate compensation:

1. Select **Experiment > Compensation Setup > Calculate Compensation.**

   Note: If the calculation is successful, a dialog prompts you to enter a name for the compensation setup. The default name is year/month/day/time.

2. Enter a setup name and click **Link & Save.**

   The compensation is linked to the cytometer settings and saved to the catalog.

   To help track compensation setups, include the experiment name, date, or both in the setup name.

   The compensation setup is linked to the *MyExperiment* cytometer settings, and subsequent acquisitions in *MyExperiment* are performed with the new compensation settings.

We recommend that you always visually and statistically inspect automatically calculated spectral overlap values. The means of the positive controls should be aligned with the means of the negative controls.

More information

- Recording compensation controls (page 83)

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6

Recording and analyzing data

This chapter covers the following topics:

Data recording and analysis workflow

Introduction

This topic outlines the basic acquisition and analysis tasks using BD FACSDiva software.

About the examples

The examples in this chapter are from two 4-color bead samples with the following fluorochromes:

- FITC
- PE
- PerCP-Cy5.5
- APC

If you use a different sample type or if you have skipped the optimization steps in Optimizing cytometer settings (page 65), your software window content, names of folders and experiments, and your data plots and statistics might differ from those shown here. You might also need to modify some of the instructions in the procedure.

For additional details on completing some of the following steps, see the *BD FACSDiva Software Reference Manual*.

This procedure builds on the results obtained in Optimizing cytometer settings (page 65).

Workflow for recording and analyzing data

Recording and analyzing data consists of the following steps.

Step	Description
1	Preparing the workspace (page 91)
2	Recording data (page 92)
3	Analyzing data (page 95)
4	Reusing an analysis (page 101)

Preparing the workspace

Introduction

This topic describes how to prepare the workspace and apply application settings to your experiment before recording data.

Procedure

To prepare the workspace:

1. Using the **Browser** toolbar, create a new specimen in *MyExperiment* and rename it *FourColorBeads*.
2. Create two tubes for the *FourColorBeads* specimen. Rename the tubes *Beads_001* and *Beads_002*.
3. Expand the **Global Worksheets** folder in *MyExperiment* to access the default global worksheet, and rename the worksheet *MyData*.
4. On the *MyData* worksheet, create the following plots for previewing the data:
   - FSC vs SSC
   - FITC vs PE
   - FITC vs PerCP-Cy5.5
   - FITC vs APC

Applying saved application settings to a new experiment

When applications settings are applied to an experiment, the cytometer settings are updated with the parameters included in the application settings, optimized PMT voltages, threshold settings, area scaling factors, and window extension values.

To apply saved application settings to your experiment:

1. Right-click the experiment-level Cytometer Settings and select **Application Settings > Apply.**

2. In the **Application Settings** catalog, select the application settings file you saved previously and click **Apply.**

   If the parameters are not the same, a mismatch dialog opens.

   - Click **Overwrite** to update all settings.
   - Click **Apply** to change only the common parameters.

   For more information, see the *BD FACSDiva Software Reference Manual*.

The cytometer settings are renamed application settings and the cytometer settings icon in the Browser changes.

More information

- Creating application settings (page 80)
- Recording data (page 92)

Recording data

Introduction

This topic provides an example of how to preview and record data for multiple samples.

Before you begin

Prepare the sample tubes.

Recording data

To record data:

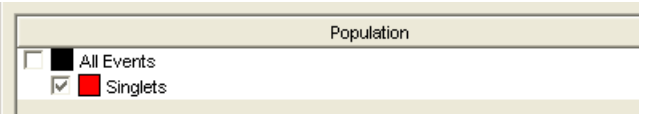
1. Press RUN and HIGH on the cytometer fluid control panel.
2. Install the first sample tube onto the SIP.
3. Set the current tube pointer to *Beads_001*.
4. Click **Acquire Data** in the **Acquisition Dashboard** to begin acquisition.

5. While data is being acquired:

   a. Draw a gate around the singlets on the FSC vs SSC plot.

   b. Rename the P1 gate to *Singlets*.

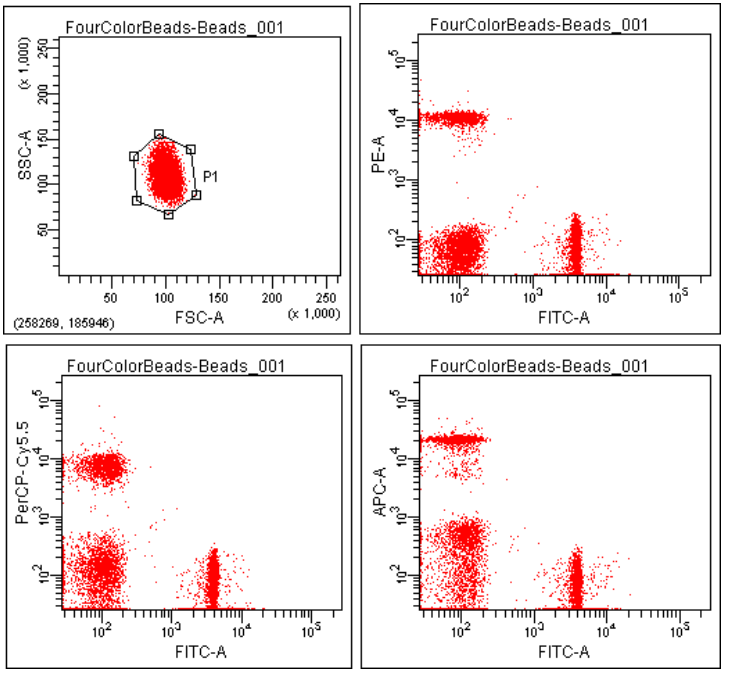
   c. Use the **Inspector** to set the other plots to show only the singlet population by selecting the Singlets checkbox.



6. Click **Record Data.**

7. When event recording has completed, remove the first tube from the cytometer.

   The *MyData* worksheet plots should look like the following.



8. Install the second sample tube onto the SIP.
9. Set the current tube pointer to *Beads_002*.
10. Click **Acquire Data** to begin acquisition.
11. Before recording, preview the data on the *MyData* worksheet to verify that all expected populations are visible and the data is similar to the previous sample.
12. Click **Record Data.**
13. When event recording has completed, remove the second tube from the cytometer.
14. If you are recording more than two tubes, repeat steps 8 through 13 for the remaining tubes.
15. Print the experiment-level cytometer settings by right-clicking the **Cytometer Settings** icon in the **Browser** and selecting **Print**.
16. Install a tube of DI water onto the SIP.
17. Place the cytometer in standby mode.

More information

- Analyzing data (page 95)

Analyzing data

Introduction

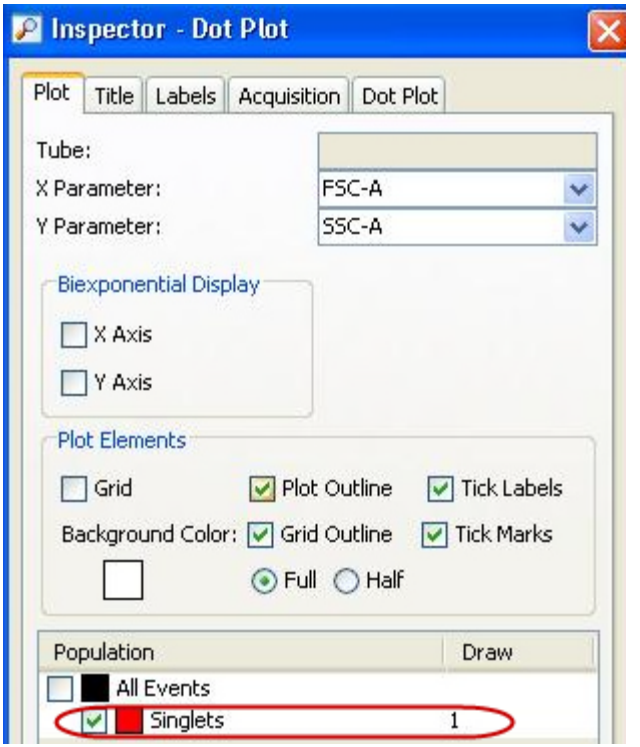
This topic describes how to analyze recorded tubes by creating plots, gates, a population hierarchy, and statistics views on a new global worksheet.

Analyzing data

To analyze data:

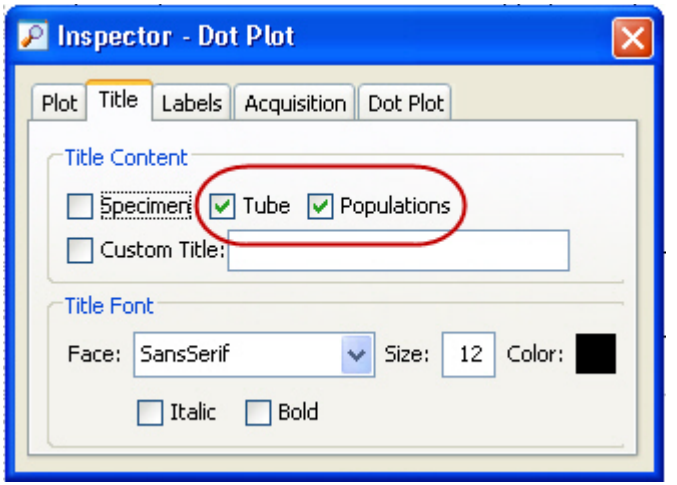
1. Use the **Browser** toolbar to create a new global worksheet. Rename it *MyDataAnalysis*.
2. Create the following plots on the *MyDataAnalysis* worksheet:
   - FSC vs SSC
   - FITC vs PE
   - FITC vs PerCP-Cy5.5
   - FITC vs APC
3. Create a population hierarchy and a statistics view, and set them below the plots on the worksheet.
   - Right-click any plot and select **Show Population Hierarchy.**
   - Right-click any plot and select **Create Statistics View.**
4. Set the current tube pointer to *Beads_001*.
5. Draw a gate around the singlets on the FSC vs SSC plot.
6. Use the population hierarchy to rename the population *Singlets*.

7. Select all plots except the FSC vs SSC plot, and use the **Plot** tab in the **Inspector** to specify to show only the singlet population.



8. Select all plots, and click the **Title** tab in the **Inspector**.

9. Select the **Tube** and **Populations** checkboxes to display their names in plot titles.



10. On all fluorescence plots:

    - Make all plots biexponential. Select all fluorescence plots and select the **X Axis** and **Y Axis** checkboxes in the **Plot** tab of the **Inspector**.

- In the FITC vs PE plot, draw a gate around the FITC-positive population. Name the population *FITC positive* in the population hierarchy.
- In the FITC vs PE plot, draw a gate around the PE-positive population. Name the population *PE positive* in the population hierarchy.
- In the FITC vs PerCP-Cy5.5 plot, draw a gate around the PerCP-Cy5.5-positive population. Name the population *PerCP-Cy5.5 positive* in the population hierarchy.
- In the FITC vs APC plot, draw a gate around the APC-positive population. Name the population *APC positive* in the population hierarchy.

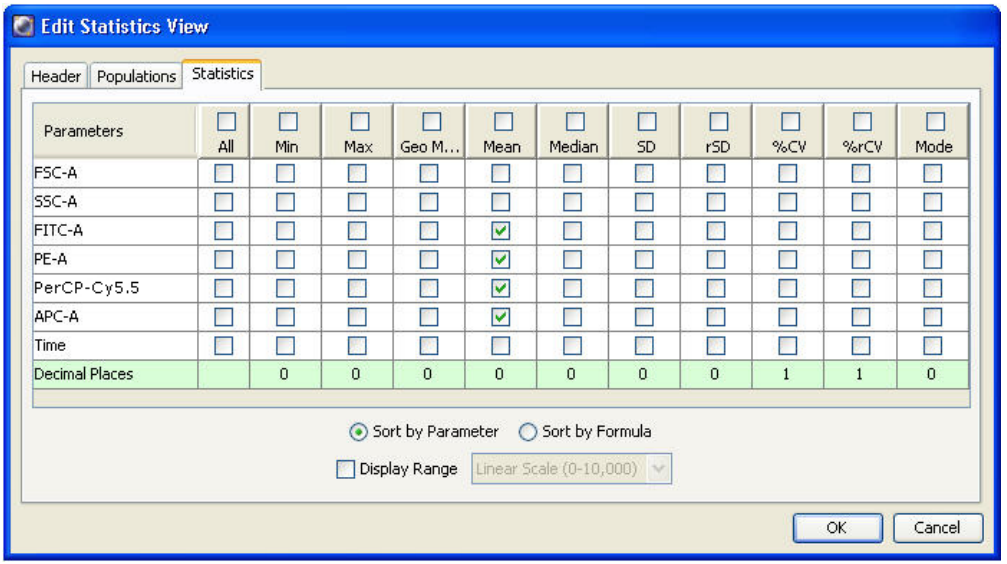
11. Format the statistics view.

    a. Right-click the statistics view and select **Edit Statistics View.**

    b. Click the **Header** tab and select the **Specimen Name** and **Tube Name** checkboxes.

    c. Click the **Populations** tab and select all populations except **All Events.** Clear the **%Parent**, **%Total**, and **#Events** checkboxes.

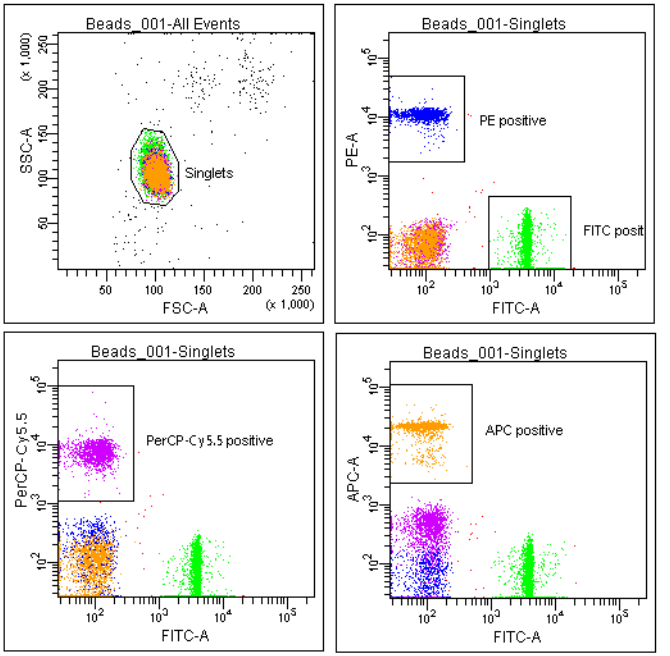
    d. Click the **Statistics** tab and select the mean for each of the fluorescence parameters.



e. Click **OK**.

12. Print the analysis.

Your global worksheet analysis objects should look like the following.



Tube: Beads_001

Population	#Events	%Parent	%Total
All Events	10,000		100.0
Singlets	9,675	96.8	96.8
FITC positive	4,774	49.3	47.7
PE positive	1,499	15.5	15.0
PerCP-Cy5.5 positive	1,529	15.8	15.3
APC positive	1,829	18.9	18.3

Specimen Name: FourColorBeads
Tube Name: Beads_001

Population	FITC-A Mean	PE-A Mean	PerCP-Cy5.5-A Mean	APC-A Mean
Singlets	1,969	1,727	1,257	3,956
FITC positive	3,880	40	43	39
PE positive	95	10,858	71	16
PerCP-Cy5.5 positive	106	66	7,639	390
APC positive	91	59	84	20,482

More information

- Reusing an analysis (page 101)

Reusing an analysis

Introduction

This topic describes how to use a global worksheets to apply the same analysis to a series of recorded tubes. Once you define an analysis for a tube, you can use it to analyze the remaining tubes in the experiment. After viewing the data, print the analysis or save it to a normal worksheet.

Reusing an analysis

To reuse the analysis:

1. Set the current tube pointer to the *Beads_002* tube.
2. View the *Beads_002* data on your analysis worksheet. Adjust the gates as needed.

   Adjustments apply to subsequent tubes viewed on the worksheet. To avoid altering a global worksheet, save an analysis to a normal worksheet, then make adjustments on the normal worksheet.
3. Print the analysis.

Saving the analysis

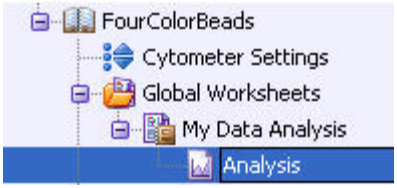
When you perform analysis with a global worksheet, the analysis does not save with the tube.

If you define your analysis on a global worksheet before recording data, you can specify to automatically save the analysis after recording data. You set this option in User Preferences.

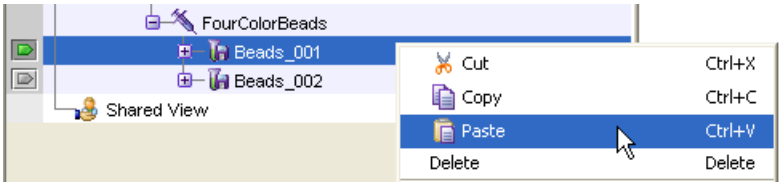
To save a copy of the analysis with a tube:

1. Expand the *MyDataAnalysis* global worksheet icon in the **Browser**.

2. Right-click its analysis and select **Copy.**



3. Click the **Worksheets View** button on the **Worksheet** toolbar to switch to the normal worksheet view.
4. Select **Worksheet > New Worksheet** to create a new normal worksheet.
5. Right-click the *Beads_001* tube icon in the **Browser,** and select **Paste.**



The analysis objects from the *MyDataAnalysis* global worksheet are copied to the *Beads_001_Analysis* normal worksheet. Double-click the *Beads_001* tube in the Browser to view the analysis.

Applying an analysis to normal worksheets

You can apply the global worksheet analysis to multiple tubes (on a single normal worksheet) by selecting multiple tubes before pasting the analysis. Ensure that you collapse all tube elements in the Browser before you paste them to multiple tubes.

More information

- Analyzing data (page 95)

7

Troubleshooting

This chapter covers the following topics:

Cytometer troubleshooting

Introduction

This topic describes possible problems and recommended solutions for BD LSRFortessa X-30, BD LSRFortessa X-50, BD LSRFortessa X-30 Dual, BD FACSymphony A3, BD FACSymphony A5, and BD FACSymphony A3 Dual flow cytometer issues.

Droplets are visible on the SIP

Possible causes	Recommended solutions
Worn O-ring in the retainer	Replace the O-ring. See Changing the sample tube O-ring (page 62).
Outer sleeve is not seated in the retainer	1. Loosen the retainer. 2. Push the outer sleeve up into the retainer until seated. 3. Tighten the retainer.
Outer sleeve is not on the sample injection tube	Replace the outer sleeve. 1. Loosen the retainer. 2. Slide the outer sleeve over the sample injection tube until it is seated. 3. Tighten the retainer.
Waste line is pinched, preventing proper aspiration	Check the waste line.
Waste tank is full	Empty the waste tank.
Droplet containment vacuum is not functioning	Call your BD service representative.
The mode is not set to HTS.	Change the mode to HTS by pressing down the Mode button for more than 3 seconds.

Sample tube not fitting on SIP

Possible causes	Recommended solutions
Sample tube other than Falcon® tubes used	Use Falcon 12 x 75-mm sample tubes. See Equipment (page 126).
Worn Bal seal	Replace the Bal seal. See Changing the Bal seal (page 60).
Sample tube is cracked	Transfer contents to a new tube.

Rapid sample aspiration

Possible causes	Recommended solutions
Support arm is to the side	Place the support arm under the sample tube.
Droplet containment module is failing	Try the solutions in Droplets are visible on the SIP (page 104). If the issue is not resolved, call your BD service representative.

No events in acquisition display and RUN button is green

Possible causes	Recommended solutions
Threshold is not set to the correct parameter (usually FSC)	Set the threshold to the correct parameter for your application.
Threshold level is too high	Lower the threshold level.
PMT voltage for threshold parameter is set too low	Set the PMT voltage higher for the threshold parameter.
Gating issue	See the *BD FACSDiva Software Reference Manual* for information on setting gates.
Air in the sheath filter	Purge the filter. See Removing air bubbles (page 39).
Air bubble or debris in the flow cell	Prime the fluidics system. See Priming the fluidics (page 44).

Possible causes	Recommended solutions
No sample in the tube	Verify that sample remains in the tube and if necessary, add sample to the tube or install a new sample tube.
Sample is not mixed properly	Mix the sample to suspend the cells.
Waste tank is full	Empty the waste tank.
PMT voltages set too low or too high for display parameter	Adjust the PMT voltages.
Too few events are displayed	Increase the number of events to display.
Sample injection tube is clogged	Remove the sample tube to allow backflushing. If the event rate is still erratic, clean the sample injection tube. See Cleaning the fluidics (page 51).
Bal seal is worn	Replace the Bal seal. See Changing the Bal seal (page 60).
Instrument is not warmed up	Wait 30 minutes for the instrument to warm up.
Laser delay is set incorrectly	Adjust the laser delay settings. See Manual settings (page 113).
Laser is not functioning	Verify the malfunction by changing the threshold to an alternative laser while running the appropriate sample. If unsuccessful, contact BD Biosciences.
Tube is cracked or misshapen	Replace the sample tube.

No events in acquisition display and RUN button is orange

Possible causes	Recommended solutions
RUN is not activated	Press the RUN button.
Sample tube is not installed or is not properly seated	Install the sample tube correctly on the SIP.
Sample tube is cracked	Replace the sample tube.
Waste tubing line is disconnected from the waste container tank	Connect the waste tubing line to the waste container tank.
Sheath container is not pressurized	• Ensure that the sheath container lid and all connectors are securely seated. • Inspect the sheath container O-ring inside the lid and replace it if necessary. See Cleaning or replacing the sheath gasket (page 64)
Bal seal is worn	Replace the Bal seal. See Changing the Bal seal (page 60).
Air leak at sheath container	Ensure that the sheath container lid and all connectors are securely seated.
Sheath container is empty	Fill the sheath container.
Air in sheath filter	Purge the filter. See Removing air bubbles (page 39).

No fluorescence signal

Possible causes	Recommended solutions
Incorrect fluorochrome assignment	Make sure that the cytometer configuration in the software matches the optical filters in the cytometer and the configuration is as expected.
Laser is not functioning	Call your BD service representative.

No signal in red laser channels (when red laser is installed)

Possible causes	Recommended solutions
Incorrect laser delays due to a change in the sheath tank fluid level	• Check the fluid level in the sheath tank and refill if necessary. • Adjust the laser delay settings. See Manual settings (page 113).

High event rate

Possible causes	Recommended solutions
Air bubbles in the sheath filter or flow cell	Remove the air bubbles. See Removing air bubbles (page 39).
Threshold level is too low	Increase the threshold level. See the *BD FACSDiva Software Reference Manual* for instructions.
PMT voltage for the threshold parameter is set too high	Set the PMT voltage lower for the threshold parameter. See the *BD FACSDiva Software Reference Manual* for instructions.
Sample is too concentrated	Dilute the sample.
Sample flow rate is set to HIGH	Set the sample flow rate to MED or LOW.

Low event rate

Possible causes	Recommended solutions
Threshold level is too high	Lower the threshold level. See the *BD FACSDiva Software Reference Manual* for instructions.
Air bubble or debris in the flow cell	Prime the fluidics system. See Priming the fluidics (page 44).
PMT voltage for the threshold parameter is set too low	Set the PMT voltage higher for the threshold parameter. See the *BD FACSDiva Software Reference Manual* for instructions.

Possible causes	Recommended solutions
Sample is not adequately mixed	Mix the sample to suspend the cells.
Sample is too diluted	Concentrate the sample. If the flow rate setting is not critical to the application, set the flow rate switch to MED or HIGH.
Sample injection tube is clogged	Remove the sample tube to allow backflushing. If the event rate is still erratic, clean the sample injection tube. See Cleaning the fluidics (page 51).

Erratic event rate

Possible causes	Recommended solutions
Sample tube is cracked	Replace the sample tube.
Air bubble or debris in the flow cell	Prime the fluidics system. See Priming the fluidics (page 44).
Bal seal is worn	Replace the Bal seal. See Changing the Bal seal (page 60).
Sample injection tube is clogged	Remove the sample tube to allow backflushing. If the event rate is still erratic, clean the sample injection tube. See Cleaning the fluidics (page 51).
Contaminated sample	Prepare the specimen again. Ensure that the tube is clean.
Sheath filter is dirty	Replace the filter. See Changing the sheath filter (page 58).

Distorted scatter parameters

Possible causes	Recommended solutions
Cytometer settings are improperly adjusted	Optimize the scatter parameters. See the *BD FACSDiva Software Reference Manual* for instructions.
Air bubble in the sheath filter or flow cell	Purge the air from the filter. See Removing air bubbles (page 39).
Flow cell is dirty	Flush the system. See Flushing the system (page 54).
Air leak at sheath container	Ensure that the sheath container lid is tight and all connectors are secure.
Hypertonic buffers or fixative	Replace the buffers or fixative.

Excessive amount of debris in display

Possible causes	Recommended solutions
Threshold level is too low	Increase the threshold level.
Sheath filter is dirty	Replace the filter. See Changing the sheath filter (page 58).
Flow cell is dirty	Flush the system. See Flushing the system (page 54).
Dead cells or debris in the sample	Examine the sample under a microscope.
Sample is contaminated	Re-stain the sample. Ensure that the tube is clean.
Stock sheath fluid is contaminated	Rinse the sheath container with DI water, then fill the container with sheath fluid from another (or new lot) bulk container.

High CV or poor QC results

Possible causes	Recommended solutions
Air bubble in sheath filter or flow cell	• Purge the filter. See Removing air bubbles (page 39). • Prime the fluidics system. See Priming the fluidics (page 44).
Sample flow rate is set too high	Set the sample flow rate lower.
Air leak at sheath container	Ensure that the sheath container lid is tight and all connectors are secure.
Flow cell is dirty	Flush the system. See Flushing the system (page 54).
Waste tank is pressurized	Replace the waste container cap. See Replacing the waste container cap (page 56).
Poor sample preparation	Repeat sample preparation.
Sample was not diluted in the same fluid as the sheath fluid	Dilute the sample in the same fluid as you are using for sheath.
Old or contaminated QC particles	Make new QC samples and perform the quality control procedure again.
Instrument is not warmed up	Wait 30 minutes for the instrument to warm up.
Laser is not functioning	Contact BD Biosciences.
Optical alignment problem	Contact BD Biosciences.

Electronics troubleshooting

Introduction

This topic describes possible problems and recommended solutions for electronics issues.

"Cytometer Disconnected" in cytometer window

Possible causes	Recommended solutions
Cytometer power is off	Turn on the cytometer main power.
Communication failure between workstation and cytometer	1. In BD FACSDiva software, select **Cytometer > Connect.** 2. If connecting does not work, restart the cytometer. Turn the cytometer off, wait 1 minute, and turn on the cytometer main power. 3. If connecting still does not work, contact BD Biosciences.

8

Manual settings

This chapter covers the following topics:

About laser delay

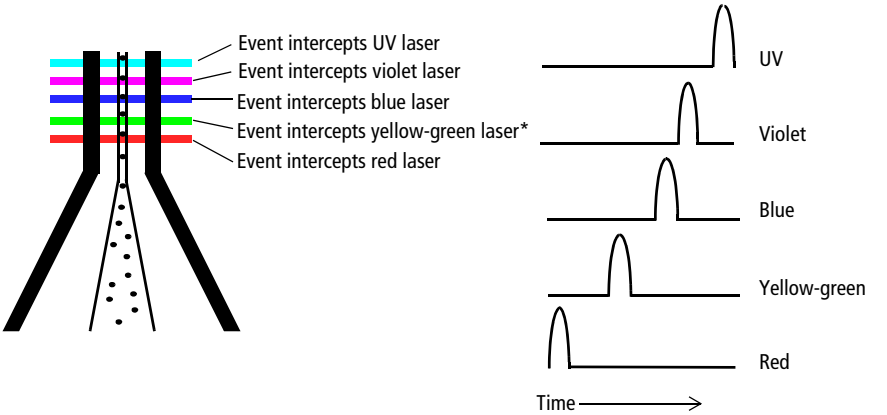
Introduction

This topic describes how to manually set the laser delay if you are not using CS&T for cytometer setup and tracking.

About laser signal delay

Sample interrogation takes place within the cuvette flow cell. Laser light is directed through a series of prisms that focus multiple lasers on the event stream at different positions. This allows optimal detection of fluorescent signals from each laser with minimal cross-contamination from the other beams.

For example, in a four-laser system, the blue laser intercepts the stream first, followed by the violet, UV, and red lasers. Because the laser signals are spatially separated, there is a slight delay between the detection of each laser's signal.



*The yellow-green laser is only available through the BD special order research program.

The laser delay setting in BD FACSDiva software is used to re-align the signals so they can be measured and displayed on the same time scale. Signals are aligned with respect to the blue laser, so the blue laser will have a 0 delay value, and the red laser will have the longest delay.

Optimizing laser delay

Introduction

This topic describes how to optimize the laser delay using BD FACSDiva software.

Before you begin

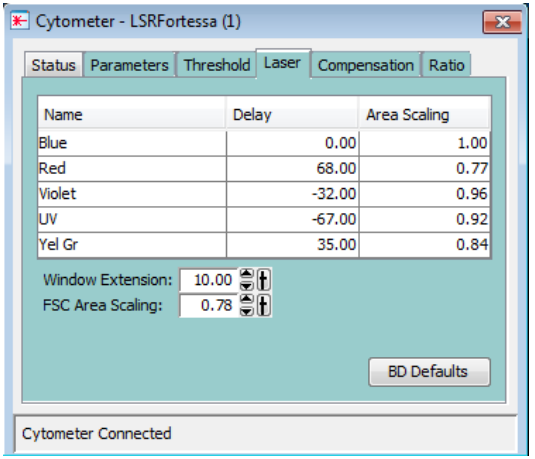
To optimize the delay for a given laser, you must acquire events from a sample with a fluorescence signal excited by that laser. Follow the procedures in Recording and analyzing data (page 89), for sample optimization and acquiring data.

Procedure

To optimize laser delay:

1. While acquiring data from your sample, create a histogram to show the fluorescence signal excited by the laser for which the delay is to be optimized.
2. In the **Acquisition Dashboard**, set the **Events to Display** to 500 events.
3. Click the **Laser** tab in the **Cytometer** window.

   Window extension and laser delay values are displayed in microseconds (µs).



4. Set the **Window Extension** value to 0 µs.

5. Set an initial laser delay value **only** for the laser you are optimizing.
   - If you are optimizing the violet laser, set its delay to –35 µs.
   - If you are optimizing the UV laser, set its delay to –65 µs.
   - If you are optimizing the red laser, set its delay to 65 µs.
   - If you are optimizing the blue laser, set its delay to 0 µs.
   - If you are optimizing the yellow-green laser, set its delay to 35 µs.
6. While observing the positive events on the histogram, adjust the laser delay in 1-µs increments. You might need to adjust the delay above or below the initial setting.

   Choose the setting that moves the events farthest to the right (highest fluorescence intensity).
7. Draw an interval gate on the histogram for the positive events.
8. Create a statistics view to display the mean fluorescence intensity (MFI) of the gated population.
9. While observing the MFI for the gated population, adjust the laser delay in 0.1-µs increments within a range of 2.0 µs of the setting obtained in step 6.

   Preserve the setting that maximizes the fluorescence intensity.
10. Reset the **Window Extension** to 10 µs.

Adjusting area scaling

Introduction

This topic describes how to manually adjust the area scaling on your cytometer if necessary for your application. The area scaling is automatically set in CS&T. Depending on the size of your target particle, you might need to adjust the area scaling manually. Larger particles are more likely to require an area scaling adjustment.

About area scaling

The area of a pulse is calculated by BD FACSDiva using measured height and width measurements. It is sometimes important to verify that the area calculation and the height measurement are equivalent by adjusting the factor applied to the area. The required area scaling factor changes based on sheath pressure and particle size.

About this example

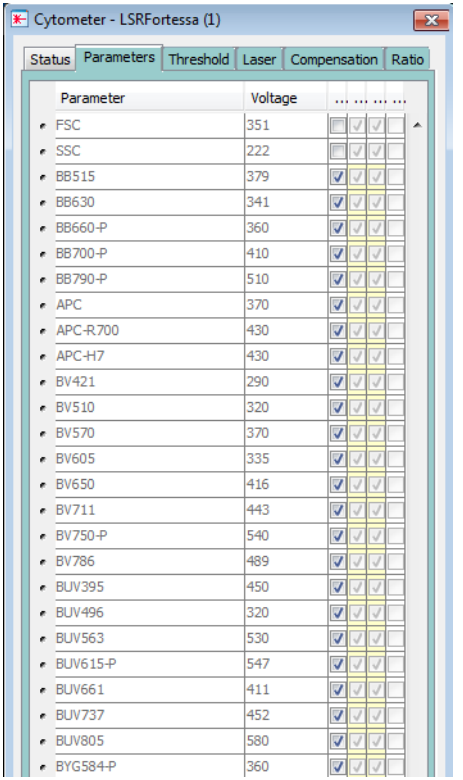
The following example describes how to adjust area scaling for an experiment which uses only the 488-nm laser and the 640-nm laser. You must adjust area scaling for all lasers used in your experiment. To adjust the other lasers, add a parameter and the corresponding plots from that laser to the procedure.

Procedure

To adjust area scaling:

1. Open an existing experiment, or create a new experiment in the **Browser**.
2. Create a new specimen by clicking the **New Specimen** button on the **Browser** toolbar.

3. In the **Inspector**, click the **Parameters** tab and select the **H** checkbox to select the height for each parameter.



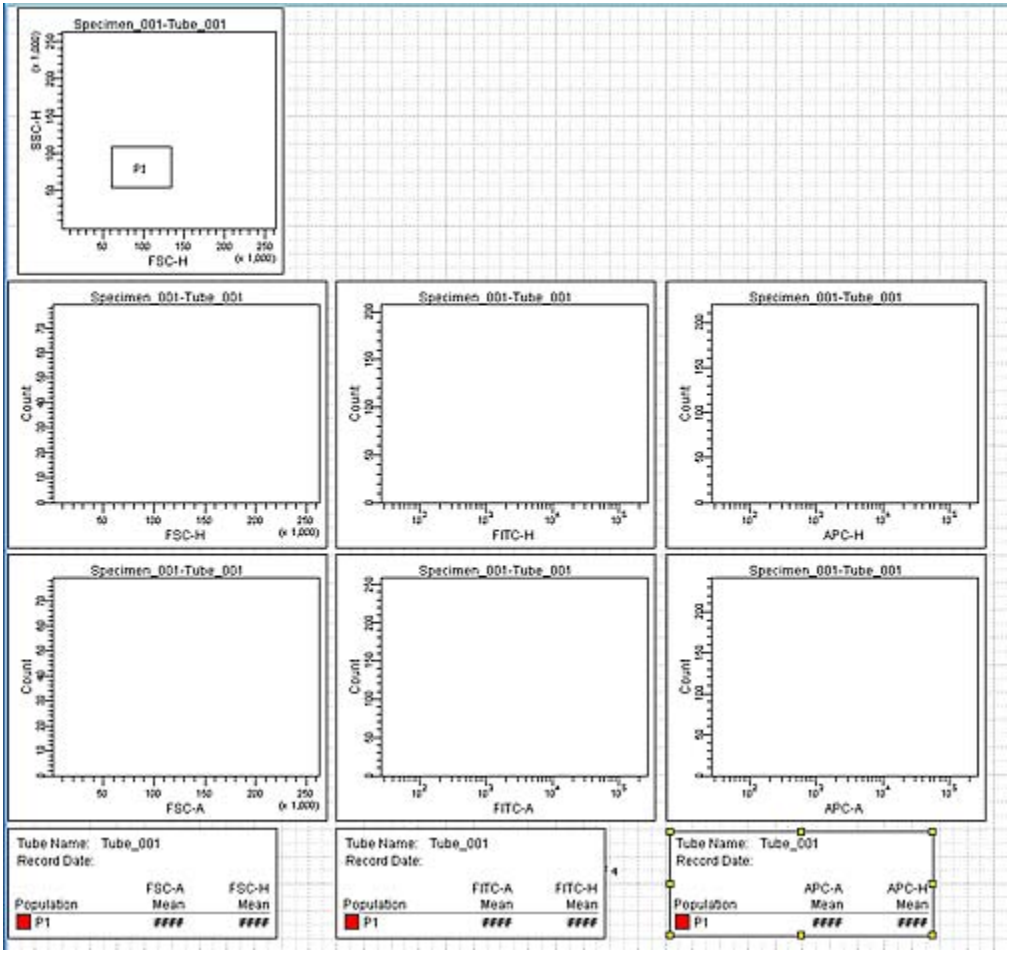
4. On the global worksheet, create the following plots and histograms:

   - FSC vs SSC dot plot
   - FSC-H and FSC-A histogram
   - FITC-H and FITC-A histogram
   - APC-H and APC-A histogram

5. Create a P1 gate in the FSC vs SSC plot, and show only the P1 population in all histograms.

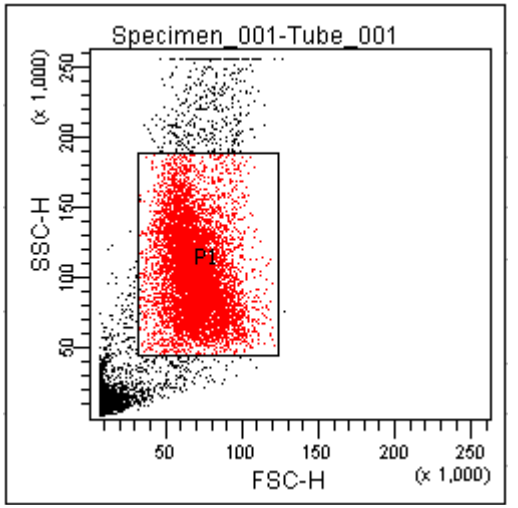
6. Create three statistic views showing the following:
   - FSC-H and FSC-A means for P1
   - FITC-H and FITC-A means for P1
   - APC-H and APC-A means for P1

   Your worksheet should look similar to the following figure.



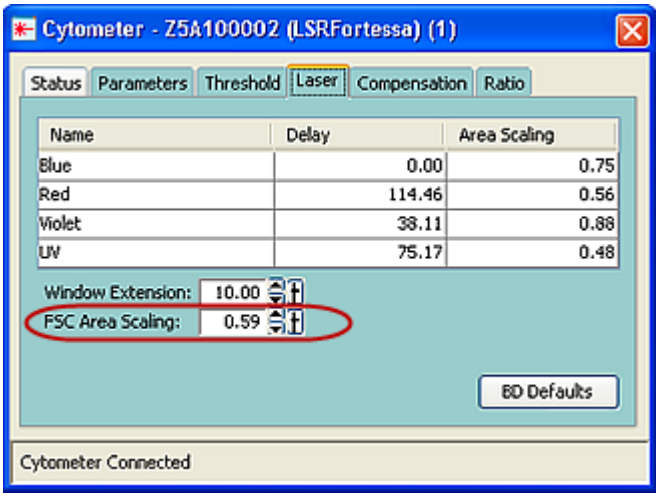
7. Expand the new specimen, then set the current tube pointer to *tube_001*.

8. Install the FITC-positive control tube onto the loading port and click **Load** in the **Acquisition Dashboard.**
9. Adjust the FSC and SSC voltages to place the particles on scale.



10. Adjust the P1 gate around the population of interest.
11. Adjust the FSC area scaling.
    a. Click the **Laser Tab** in the **Cytometer** window.
    b. Adjust the FSC area scaling factor until the FSC-A signal matches the FSC-H signal:
        - Increase the area scaling factor if the FSC-A signal is lower than FSC-H.

- Decrease the area scaling factor if the FSC-A signal is higher than FSC-H.

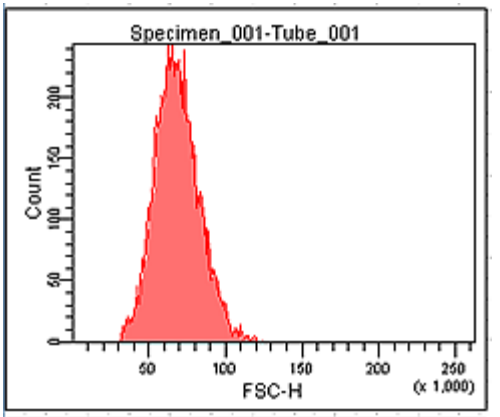


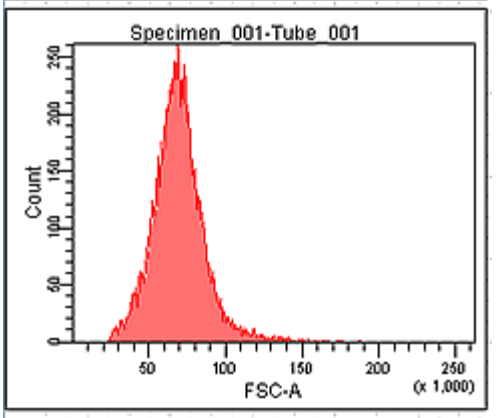
c. View the result of your change in the histograms and statistics views.

12. Adjust the blue laser area scaling factor until the FITC-A signal matches the FITC-H signal, if needed.

13. Unload the FITC-positive control tube, then load the APC-positive control tube.

14. Adjust the red laser area scaling factor until the APC-A signal matches the APC-H signal, if needed.





Tube Name: Tube_001
Record Date: Dec 1, 2009 10:57:48 AM

Population	FSC-A Mean	FSC-H Mean
P1	68,989	68,339

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Supplies and consumables

This chapter covers the following topics:

Ordering information

To order spare parts and consumables from BD Biosciences:

- Within the US, call (877) 232-8995.
- Outside the US, contact your local BD Biosciences customer support representative.

Worldwide contact information can be found at bdbiosciences.com.

Beads

Introduction

This topic lists the QC and CS&T beads available.

QC particles

Particle	Laser	Supplier	Catalog No.
SPHERO™ Rainbow Calibration Particles (8 peaks)	All	BD Biosciences	559123
SPHERO™ Ultra Rainbow Fluorescent Particles (single peak)	All	Spherotech, Inc.	URFP-30-2
BD™ DNA QC Particles	Blue (488 nm)	BD Biosciences	349523

CS&T beads

Bead	Laser	Supplier	Catalog No.
BD FACSDiva™ CS&T research beads	• UV (355 nm) • Violet (405 nm) • Blue (488 nm) • Red (637 nm) • Yellow-green (561 nm)	BD Biosciences	• 655050 (50 tests) • 655051 (150 tests)

Reagents

Reagent	Supplier	Catalog No.
BD FACSFlow™ sheath fluid	BD Biosciences	342003
BD FACS™ sheath solution with surfactant (recommended for use with the HTS option)	BD Biosciences	336524
Monoclonal antibodies	BD Biosciences	See the BD Biosciences Product Catalog or the BD Biosciences website (bdbiosciences.com)
BD FACS™ lysing solution	BD Biosciences	349202
BD™ FACSRinse solution	BD Biosciences	340346

Reagent	Supplier	Catalog No.
BD FACSClean solution	BD Biosciences	340345
Dyes and fluorochromes	BD Biosciences, Life Technologies, or Sigma	–
Chlorine bleach (5% sodium hypochlorite)	Clorox® or other major supplier (to ensure that the bleach is at the correct concentration and free of particulate matter)	–

Equipment

Equipment item	Supplier	Catalog No.
Bal seal	BD Biosciences	343509
O-ring, sample tube		343615
Sheath filter assembly		34364507
Falcon polystyrene test tubes, 12 x 75-mm	Corning	352008

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